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# BC biochimica clinica

## RIASSUNTI 51° CONGRESSO NAZIONALE SIBioC 2019

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# 51° Congresso Nazionale della Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica (SIBioC - Medicina di Laboratorio)

**Padova, 20-22 novembre 2019**

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

SS01 - La fragilità dell'informazione: internet e Medicina di laboratorio

SS01-01

## INFORMATION, MISINFORMATION AND DISINFORMATION ON THE WEB

**P. Pezzati**

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Social media, the collective of online communications channels dedicated to community-based input, interaction, content-sharing and collaboration, has the ability to combine the credibility of interpersonal persuasion with mass media, resulting in desired attitude or behavior among a large group of people. However, concerns have been raised as to the safety of social media MySpace, Facebook, Twitter, Cyworld, Pinterest and the like, since they appear to be a particularly fertile breeding ground for "fake news". While a fake news refers to news that has been entirely fabricated or made up, there are others way to mislead audiences, such as practicing "disinformation" (incorrect information) and "misinformation" (deceiving information).Both works because many people fail to recognize false information when it's presented to them. In a 2018 survey (1),organized by The UK National Literacy Trust, aimed to test the critical literacy skills, only 1.9% of children and young people overall were able to identify all news stories correctly as either real or fake (3.1% of primary pupils, 0.6% of secondary students). This is comparable to a 2017 survey of adults (4%). The tendency to readily accept falsehood can have serious consequences for evidence based decision making on a variety of social issues including health related questions. Patients use social media to research health information and/or health-related products, to communicate with providers and to look for peer group support. All these activities may lead to make dangerous decisions, or, on the opposite, may represent a powerful way to spread knowledge and health recommendations, according to the quality of web content. Even the way in which the content is presented and spread, can have different consequences. A recent study conducted on social media messaging on HPV vaccination(2) showed that provaccine messages report factual information, while antivaccine message appeal to emotion and may overrule logical reasons. Emotional content deliberately playing into people's basic emotions such as fear, anger or empathy ,unable to rapidly gain followers. Other social media strategies to attract followers includes: impersonation, group polarization, conspiracy, discrediting opponents and "trolling people online". In addition, social media have the unique capacity of amplifying news through phenomena such as echo chamber, filter bubble and moral tribes (3). All these have, as a consequence for users, a limited exposure to different views and possibly high risk of cognitive biases. Initiatives aimed to increase the quality of evidence available on web are desirable and praiseworthy, however, patients and health professional critical thinking and evidence based reasoning need to be promoted and sustained as a way to guard against the spread of misinformation in society.

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SS01-02

## DIAGNOSTIC ALGORITHMS ON THE WEB: HOW RELIABLE ARE THEY?

**D. Giavarina**

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The concept of algorithm has existed for centuries. An algorithm is a step-by-step method of solving a problem. Diagnosing is a problem to solve, and it is helpful to have unambiguous specifications for performing an automated reasoning to achieve the solution: the diagnosis. For decades, algorithms have been proposed in many fields of diagnostic and therapeutic medicine. In Laboratory Medicine their use are particularly frequent, given the availability of quantitative data, reference limits and cut-off. Only for instance, the Mayo Clinic publish more than 120 different diagnostic algorithms, in its website (1). Many websites offer reliable algorithms, most of them under the endorsement of scientific societies.

Nevertheless, Web is a democratic area, where everyone can write, load and propose everything. However, science is not democratic, nor is medicine, laboratory too. Therefore, the first problem is the same for the scientific information: how recognizing solid and reliable diagnostic algorithms on the Web, and which algorithms to choose between many options. The solutions are the usual ones: Evidence Base Medicine, involving experts, evidences and patients. A second problem is the new social nature of the Web, the evolution of the interactions between Web and users. We are now in a transition era, the 2.0 Web, moving towards the Web 3.0, the so-called semantic Web. The most significant difference between Web 2.0 and 3.0 in the World Wide Web is greater collaboration among Internet users, content providers and enterprises. Users have now more input into the nature and scope of Web content and in some cases exert real-time control over it. The problem is that, to obtain a good output from algorithms, or from an artificial intelligence (AI), a good input needs. Algorithms used by experts could perform better that the same ones used by patients. An expert mediation between patient and web may be the winning move, as shown by the information sites, like Lab Tests Online (LTO), Mayo Clinic Laboratories, etc.

The third problem is the incoming of AI. Web 4.0 coming, in which intelligent agents and smart things will be

connected with the social environment. A learning machine, reading data from the same patients, big data and any available network data, could develop new diagnostic algorithms (2,3). Probably human intelligence will be not able to read or understand the “steps” of those algorithms; it can only look at the input and the output. All this can be a little distressing. Nevertheless, the main question is “are or will be these algorithms reliable for medical application and diagnosis”?
The preliminary data seem to confirm (4) this statement. Furthermore, a huge amount of data and information actually available require information technologies to be analyzed, processed and evaluated quickly. Not just Information Technology (IT), but AI will be needed for more and more personalized medicine.
Challenging the efficacy of this or that automatic algorithm, or holding that human thought can still bring to synthesis a constantly growing amount of data and knowledge is at least unrealistic, if not fool. Like Web 4.0, we are moving towards a Medicine 4.0 (5). This is probably a potential risk to the role of "human" in medicine, as it will be in all other intellect activities of our species. Nevertheless, it is also an incredible opportunity to think beyond the limits of our brain and to demonstrate the superiority of humans over machines.

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SS01-CO01

**HAEMATOLOGICAL MULTIDISCIPLINARY DIAGNOSTIC TEAM (H-MDT): A NOVEL INTEGRATED OPERATIVE MODEL TO IMPROVE LABORATORY DIAGNOSTIC SERVICE FOR HAEMATOLOGIC DISEASES**

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Since early in the past decade, the U.K. National Institute for Health and Care Excellence (NICE) recommended the development of centralised laboratory services based on an integrated specialised team approach (MDT), aiming to establish effective diagnostic workflow and provide accurate multidisciplinary diagnosis for hematologic patients (as also reported in the latest NICE guidance Improving outcomes in haematological cancers, 2016).In line with this suggestion, our laboratory service has recently implemented a new functional model based on the integration of different laboratory specialists playing a role in the diagnostic process of haematological disorders. Indeed, in 2018, we defined a multidisciplinary group named “H-MDT”, which is formed by clinical pathologists, haematologists and (thus including cytometry, morphology and histopathology), with the final aim to provide a combined cyto-histo-morphological diagnostics. All professionals involved in the H-MDT are able to cooperate directly, by discussing clinical cases with complete sharing of patient information and results deriving from different of analysis. Of note, such coordinated teamworking is strategically supported by a shared Informative Laboratory System (ILS), particularly useful for rapid crosscheck of results, request of further investigations in case of discrepancies, and achievement of integrated diagnostic reporting. In addition, our H-MDT is strongly connected with the local laboratory network, in terms of technologies and ILS, as well as of shared criteria for case sorting and rule-in algorithms, thus allowing a prompt taking charge of haematological patients since their first access to the health system. Moreover, a continuous training and meeting programme promotes harmonization of behaviour and sharing of expertise among involved professionals. Potentials of our H-MDT approach are already evident in the daily laboratory practice and are going to be measured by novel indicators of process, outcome and impact on health system. In conclusion, this project aims to guarantee clinical effectiveness, efficiency and accuracy of haematological diagnostics, possibly improving local laboratory work-flow and contributing to the best management of haematological patients.

SS02 - Cannabis: stupefacente, medica e light. Aspetti di Medicina di laboratorio

SS02-01

**PHARMACOKINETICS OF CANNABINOIDS IN CONVENTIONAL AND NON CONVENTIONAL MATRICES. LABORATORY TESTS**

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Pharmacokinetics data on cannabinoids in different biological fluids are obtained by hyphenated techniques that identify and quantify each different drug and/or

metabolite. Specifically in our laboratory we are applying ultra-high performance liquid chromatography coupled to tandem mass spectrometry due to the high sensitivity and specificity of this technique. In this concern, we have developed assays to identify and quantify cannabinoids, their metabolites and their precursors of therapeutic cannabis studied in blood, urine, oral fluid and sweat. It is possible to applied a fast and sensitive method using ultra-high performance liquid chromatography coupled with tandem mass spectrometry for analysis of Δ-9-tetrahydrocannabinol, cannabidiol, their acidic precursors Δ-9-tetrahydrocannabinolic acid A and cannabidiolic acid, cannabinol and some major metabolites of THC such as 11-nor-9-carboxy-THC, 11-hydroxy-THC, Δ-9-THC-Glucuronide and THC-COOH-Glucuronide in conventional (whole blood and urine) and non-conventional (oral fluid and sweat) matrices in individual using recreational cannabis, medical cannabis or light cannabis. The advantage of using UHPLC–MS/MS is that together with THC and cannabidiol also their carboxylated acid tetrahydrocannabinol and cannabinoid acid can be detected and this is very important in case of medical cannabis. The kinetic of recreational cannabis is a very fast kinetic where cannabinoids are detected in the first minutes after smoking because recreational cannabis is only used by smoking or vaporization. THC, its glucuronide, 11-hydroxy-THC is present in blood in saliva but noting on sweat because sweat is an acidic medium and cannabinoids do not preferentially are excreted in this matrix. In case of medical cannabis since the used preparations are decoction and tea, peak of different cannabinoids is around one hour, delayed with respect to smoked cannabis. Conversely, in case of light cannabis, since this product is also smoked, the kinetics is similar to that of recreational cannabis. Another possibility to distinguish recreational cannabis from medical cannabis and light cannabis is the THC/CBD concentration ratio which is more than 10 in case of recreational cannabis, greater than 2 in case of medical cannabis and it is less than one in case of light cannabis.

SS02-02

**LABORATORY MEDICINE APPLIED TO THE USE OF MEDICAL AND LIGHT CANNABIS**

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From an analytical point of view, the difference between medical cannabis and cannabis light is in the percentage of the pharmacologically active cannabinoids. The Italian FM 2 medical product contains about 6% $\Delta^9$-tetrahydrocannabinol (THC) and 8% cannabidiol (CBD). Conversely, in the light cannabis THC is generally less than 0.2% or in some specific cases less than 0.4% while CBD can range from 2% to 40%. The use of these two products is completely different. Medical cannabis is a symptomatic treatment mainly used for neuropathic pain in multiple sclerosis as well as peripheral neuropathic pain, rheumatoid arthritis, sleep disturbances and depression and intractable cancer pain. Light cannabis is currently a free sale product, used principally for the properties of CBD, since THC is quite absent. Indeed, CBD is not psychotropic but it is psychoactive because it is a major miorelaxant and humour tranquillizer relaxant with no abuse liability. Nevertheless, it causes drowsiness, a symptom that should dangerous in normal daily activities such as driving or working.
Screening tests on biological fluids from consumers of medical cannabis clearly give a positive result, which goes to confirmatory chromatographic analysis. This latter provides cannabinoids concentrations which cannot be distinguished by those obtained after consuming recreational cannabis. In medico-legal situations (eg. workplace drug testing, driving under the influence of drug, etc.) only medical prescription can exonerate an individual from the charge of illegal cannabis use.
Conversely, due to the very low THC concentration achieved after consuming light cannabis, typical screening tests can result either negative (eg. after a single product consumption) or positive (eg. after repeated consumption) as a function of test cut–off. In case of positivity to these test, the confirmatory chromatographic analysis will disclose very low THC concentration in any of eventual analysed biological fluid (oral fluid, blood or urine) and higher concentrations of CBD.
For this reason, THC/CBD concentration ratios in serum, blood and oral fluid or THC-COOH/CBD concentration ratio in urine never exceeding the mean value of 0.9 might be a useful biomarker to identify use of light cannabis vs that of illegal THC cannabis, where the THC/CBD concentration ratios are generally greater than 10 or vs that of medical cannabis where ratios are greater than 2.

SS02-CO02

**TITOLAZIONE DEI PREPARATI GALENICI OLEOSI A BASE DI CANNABIS IN REGIONE LIGURIA: PROGETTO SPERIMENTALE DEI LABORATORI DI RIFERIMENTO REGIONALE**

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L'uso medico della cannabis trova sempre maggiori applicazioni nella cura e nel supporto di numerose patologie. In Liguria le preparazioni galeniche vengono effettuate principalmente dalle farmacie ospedaliere, secondo gli "standard tecnici ALISA " (delibera n.90 del 10 agosto 2017). I preparati devono essere titolati,

per legge, al fine di stabilire la concentrazione dei principi attivi (d9THC e CDB) e consentire l'impostazione corretta del protocollo terapeutico. La Regione Liguria è all'avanguardia nell'uso della cannabis medica con un numero di pazienti trattati superiore a 1000. Le procedure di titolazione dei preparati galenici hanno previsto, sino ad oggi, l'invio ad un laboratorio certificato esterno alla Regione. Lo scopo di questo progetto è di organizzare all'interno della Regione Liguria l'attività di titolazione presso i due laboratori di riferimento regionale (Laboratorio Analisi dell'Istituto Giannina Gaslini e Laboratorio di Tossicologia di Sarzana) sviluppando al contempo un protocollo di gestione del processo secondo criteri di qualità e gestione del rischio che possano essere proposti come modello organizzativo anche in contesti diversi da Regione Liguria. Le fasi del processo (preanalitica, analitica e post-analitica) sono state identificate e descritte. E' stata effettuata una valutazione dell'attività delle diverse farmacie produttive, degli intervalli e dei volumi di produzione, dei requisiti medico-legali di manipolazione e movimentazione della sostanza stupefacente e sono state identificate le responsabilità operative. La fase sperimentale prevede la valutazione puntuale degli effettivi carichi di lavoro e la distribuzione ai laboratori di riferimento, la gestione dei controlli di qualità interni ed esterni, la sincronizzazione degli invii dalle diverse farmacie produttive. La definizione della fase preanalitica prevede le modalità di trasporto e accettazione dei campioni e relative responsabilità. La fase analitica prevede la messa a punto e la validazione del metodo analitico (mediante UHPLC-MS/MS) nei due laboratori su calibratori, controlli e campioni reali di preparati galenici oleosi a partire da differenti prodotti (FM2, FM1, bediol,bedrobinol, cannabis ad elevato contenuto di CBD). I risultatisono stati poi comparati ed è in corso una armonizzazione per minimizzare la variabilità analitica tra i due laboratori stessi.La definizione della fase post-analitica include le modalità e le tempistiche refertazione alle varie farmacie richiedenti. La fase sperimentale, attualmente in corso, terminerà a fine anno e da gennaio 2020 inizierà la fase attuativa del progetto.

SS03 - La valutazione della fragilità nelle discrasie plasmacellulari

SS03-01

**THE CLINICAL LABORATORY IN PLASMA CELL DYSCRASIAS**

**G. Palladini**

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Plasma cell dyscrasias (PCD) range from asymptomatic, pre-malignant conditions, such as monoclonal gammopathy of undetermined significance (MGUS), found in approximately 4% of the general population >50 years, and smoldering multiple myeloma, to overtly malignant diseases. Progression from MGUS to symptomatic conditions can be due to increase in clonal burden giving rise to overt multiple myeloma (MM) or to the ability of the monoclonal component (MC) to directly cause organ damage in monoclonal gammopathies of clinical significance (MGCS). The MC is the specific hallmark of PCDs and its identification and measurement is crucial in diagnosis, staging, assessing response to therapy and identifying relapses. Biomarkers of organ dysfunction and damage are used to identify symptomatic disease requiring chemotherapy. Thus, the clinical laboratory plays a key role in the diagnostic workup and management of PCDs. Most MCs are detected incidentally by serum protein electrophoresis (SPEP) during the investigation of other conditions. A MGUS phase consistently precedes MM and AL amyloidosis. Thus, when a MGUS is identified the MC should be quantified and typed and circulating free light chains (FLC) should be measured in order to assess the risk of progression. Patients with intermediate and high risk MGUS (having either non-IgG isotype, a MC concentration >15 g/L, or abnormal FLC ratio) should be addressed to specialized hematological follow-up, aimed at timely detection of organ damage by MM (measuring hemoglobin, calcium and serum creatinine) or AL amyloidosis (measuring natriuretic peptides and albuminuria). When MM or AL amyloidosis are suspected a MC should be searched. The combination of SPEP and FLC quantification grants sufficient sensitivity to identify MCs in multiple myeloma, while in AL amyloidosis FLC measurement needs to be combined with immunofixation (IFE) of both serum and urine. Since MGCS are dreadful but treatable diseases usually caused by small plasma cell clones with low MC concentrations, the clinical laboratory should report all the monoclonal components identified, irrespective of their concentration. The ability of measuring FLC greatly increased our ability of monitoring PCD. Quantification of FLC identifies a MM defining event (ratio of involved / uninvolved FLC >100) and is used in staging AL amyloidosis. Assessment of response to chemotherapy in PCDs requires serum and urine IFE and FLC measurement (plus plasma cell count and evaluation of clonality in the bone marrow in MM and biomarkers of organ involvement in AL amyloidosis), and the same tests are used to identify relapse. Monoclonal antibody immunotherapy may interfere with response assessment and specific tools are needed to address this problem. More profound hematologic responses are associated with better outcomes in MM and AL amyloidosis, and new tools based on mass spectrometry identification of MCs in serum and urine and next generation sequencing or next generation flow cytometry to identify clonal disease in the bone marrow are being tested and will enter clinical practice to define minimal residual disease.

SS03-02

**THE ROLE OF CLINICAL LABORATORY IN THE ASSESSMENT OF FRAILTY IN MULTIPLE MYELOMA AND AL AMYLOIDOSIS**

**P. Milani**

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Multiple myeloma (MM) and light chain (AL) amyloidosis manifest with organ damage. This is caused by the tumor bulk and the organ toxicity of the monoclonal component in multiple myeloma and AL amyloidosis respectively. Organ damage makes patients particularly susceptible to the toxicity of chemotherapy. Thus, very effective agents are changing the therapeutic armamentarium for these diseases, we still need simple, objective tools to assess frailty in those subjects towards tailored, and risk adapted therapy. In multiple myeloma, the biologic and genetic prognostic factors are useful predictors of prognosis and are combined in the International Staging System, but are still insufficient to clearly identify "frail subjects". In this context, the International Myeloma Working Group (IMWG) defined a "frailty score" based on the combination of age, functional status and comorbidities that was able to predict survival and toxicity, and determine the tolerability of therapy (1). Subsequently, the Revised Myeloma Comorbidity Index (R-MCI) provided an alternative tool for assessing frailty considering renal and lung functions, age, Karnofsky index and cytogenetics data (2). Those scores are characterized by the lack of objectives measures, they are time consuming and it could be a challenge in a busy clinical practice to incorporate all of the frailty assessments proposed. Thus, we proposed a frailty score based on the N-terminal fragment of natriuretic peptide type B (NT-proBNP) in combination with performance status (ECOG) and age (3). The use of the biomarker based approach was also showed in different subsequent studies in newly diagnosed multiple myeloma patients.

In AL amyloidosis the possible contribution of geriatric assessment scores has not yet been validated. However, the choice of anti-plasma cell therapy is based on the evaluation of patient's fitness, mostly based on cardiac biomarkers staging. Serum levels of NT-proBNP and cardiac troponins form the basis for a staging system introduced in 2004 by the Mayo group (4) that allowed the identification of three groups with a significant difference in survival outcome and it is used for risk stratification in clinical trials. Subsequently, an European collaboration defined "extremely-fragile" patients (NT-proBNP >8500 ng/L and systolic blood pressure <100 mmHg at diagnosis) who still represent an unmet need for treating physicians (5).

In conclusion, in both multiple myeloma and AL amyloidosis a biomarker-based approach for the identification of frail subjects has been proposed. Further studies are needed in order to create a uniform definition for frailty in multiple myeloma and identify the most effective combination of the actual proposed scores.

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SS03-CO03

## HEMATOLOGIST AND CLINICAL BIOCHEMIST: AN ENDURING MARRIAGE IN PLASMA CELL DYSCRASIAS – 30 YEARS OF EXPERIENCE AT THE UNIVERSITY OF NAPLES FEDERICO II HOSPITAL

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The approach to the study of plasma cell dyscrasias starts from two "singles" who inevitably encounter each other in an unbreakable and lasting bond. The hematologist is the one who always makes the first move with his "suspect" ways but after the "yes", if the relationship goes forward, it is in the laboratory scientist's hands. If the game on each side becomes inverted, at the beginning of the relationship the hematologist might not be considered "indispensable" but, even if the relationship starts off quietly, it nevertheless starts. If there is trust then there are no rivals! How many times have we heard "you are the only one I trust!" The variability that occurs in each phase: preanalytical, analytical, laboratory report and its interpretation of results inevitably arrives at dialogue and continuous comparison and experience suggests that "betrayals" are to be avoided. For 30 years our Laboratory has autonomously managed each sample that could have been suspect to serum protein electrophoresis (SPE) for the presence of a Monoclonal Component (MC), directly proceeding to Immunotyping techniques (IFE, ISE). At the same time clinical suspicious samples were typed even if they did not show the possible presence of MC at SPE. From the decades 1989-99 and 1999-2009 SPE (high resolution with

visible interpretation) was carried out on #55,000 samples/y (53,100-61,000) of which #1,480 (2.7%), were submited to IFE. In 2009-2019 SPE (capillary electrophoresis) was slightly reduced, #50,000 (48,050- 51,100), with the number of immunotypings carried out remaining unvaried and immunotyping rising to 3% (#85% positive) which highlights a slight trend of improvement in the prescribed appropriateness of the SPE. The evaluation of Bence Jones Protein, #400 IFE/y, and the serum Free Light Chains measurement, #2000/y, have contributed to reinforcing the “marriage” over time. Today the Hematology follows #1500 patients per year (MGUS, MM, Amyloidosis AL, etc.) at diagnosis and follow-up on an inpatient or DH basis. What can we hope for? That the relationship always remains alive and healthy through the continuous renewal of two partners of their ongoing and intense comparison.
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SS04 - Malattie cardiache: differenti aspetti di una fragilità

SS04-CO04

**THE HIDDEN FRAGILITY IN THE HEART OF THE ATHLETES: SEARCHING FOR GENETIC BIOMARKERS OF CARDIAC RISK**

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Introduction: Intense physical activity during sports performances can lead to syncope or even Sudden Cardiac Death (SCD) in asymptomatic athletes, unknowingly suffering from cardiac disease. The prevention of SCD in athletes is a topical issue that involves many fields of the healthcare systems. Clinical molecular biology in the evaluation and prevention of cardiological risk in these athletes allows identifying pathological cardiac abnormalities, which could otherwise remain unknown.
Aim: To identify predictive markers of cardiac diseases and to know the frequency of inherited cardiac mutations potentially at risk of SCD, in subjects playing competitive sports. Materials and Methods: We performed genetic analysis in athletes showing borderline ECG and/or echocardiographic findings or with family history of cardiac diseases. A total of 61 athletes were recruited from CONI (Rome) and from the Cardiology Unit of the Monaldi Hospital (Naples). We analyzed 24 athletes by Sanger sequencing (SS), including the 8 sarcomeric, 5 desmosomal and 5 ionic channel genes; 14 by nextgeneration sequencing (NGS), using a home-made gene panel of 138 SCD-related genes; and 23 athletes by both methodologies. Results: We found 190 rare variants (MAF ≤0.01), spanning over 64 different genes and including 51 novel ones. Sarcomeric genes are the most frequentlymutated. We globally identified 8 pathogenic/likely pathogenic variants and 9 variants reported as "conflicting interpretations of pathogenicity" in 15 independent athletes (24,6%). The remaining variants were classified as variant of unknown significance. All variants found with SS were called also using NGS approach. However, the NGS identified an in-frame insertion in the RyR2 gene, which was lost by SS. Conclusions: In conclusion, this study highlights that molecular analysis can reveal DNA alterations in asymptomatic athletes with family history of cardiac diseases or borderline signs of heart dysfunction, which could cause SCD. Consequently these athletes were disqualified from competitive sport activity. Therefore, we recommend that molecular analysis is included in sport pre-participation screening when a family history of even mild heart dysfunction or symptoms/signs of cardiac dysfunction in the athlete suggest a cardiac disease.

SS05 - Ruolo dell’esercizio fisico nella prevenzione della fragilità

SS05-01

**EXERCISE PRESCRIPTION FOR CHRONIC CONDITIONS**

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Exercise prescription commonly refers to the specific plan of fitness-related activities that are designed in order to accomplish the needs of people affected by chronic conditions. The exercise plan relies upon exercise testing, for the objective measurement of fitness components (i.e. body composition, aerobic power, muscular strength and endurance, flexibility, coordination and balance).The customized exercise training program defines exercise Frequency, Intensity, Time, Type, total Volume and Progression (FITT-PV principle) (1). There is strong evidence that to comply with exercise prescription improves biochemical parameters, quality of life and survival in helathy people as well as in all frailty conditions and several chronic illness, including ischemic cardiomyopathy and hearth failure, diabetes, chronic obstructive pulmonary disease, osteoporosis, breast cancer. However, in order to obtain compliance, the goal of exercise prescription must be focused on patients motivation. Due to its potential to improve health conditions and reduce healthcare costs, the exercise referral promotion is now recognized as an essential assistance level by the Italian national healthcare system

(2), but its implementation is still problematic. Public health programs need exercise testing and prescription schemes sustainable and universally available. Testing must be economic, convenient for patients and easy to repeat along a several years follow up. Exercise facilities must be near to patient's home, and must include public spaces and green areas. Counseling approach must be tailored in order to sustain individual motivation. There is now the need of: research that focuses on economic, simple, ambulatory, long term, exercise prescription programs, that might include inexpensive and easy to perform exercise testing to evaluate and feedback patients about their fitness level. Research design should be intention to treat
- sharing of practices and policies that aim to make exercise prescription universally available
- promote knowledge and attitudes towards exercise prescription among healthcare providers
The Emilia-Romagna regional council act D.G.R. n. 2127/2016, and subsequent operational protocols, design a framework for exercise prescription and referral that involves public healthcare system and recognized private gyms with a kinesiologist with a specialist graduate degree in preventive and adapted motor activity. Outpatients with neurological or osteoarticular disorders are referred to group exercise sessions. Those with cardio respiratory or metabolic conditions are evaluated in sports services and then are prescribed with tailored supervised gym or autonomous open air exercise programs. An easy to perform, perception based submaximal walking test has been developed. Effectiveness of this scheme is supported by observational studies (3) and preliminary data from RCTS (4) performed by regional universityiesand health institutions.

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SS06 - Sindrome metabolica, infiammazione e frgilità

SS06-CO05

**STOOL PROTEOMICS FOR DIAGNOSING INFLAMMATORY BOWEL DISEASES**

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Backgrounds: For diagnosing and monitoring Inflammatory bowel diseases (IBD), blood ERS, CRP and fecal calprotectin (fCal) are recommended. These inflammatory biomarkers, however, have limitations in sensitivity and specificity. The aim of this study was to discover new stool protein/peptide biomarkers for IBD diagnosis. Methods: Within a 1000-4000 m/z range, MALDI-TOF/ MS analyses of stool samples from an exploratory cohort, made of 34 healthy subjects (HC), 72 patients with Crohn's disease (CD) and 56 patients with ulcerative colitis (UC) were performed. LTQOrbitrap XLAPC (Thermo Fisher Scientific) was used for proteomic analyses. Immunohistochemistry was performed with anti-APC antibody (Sigma-Aldrich). Stata v13.1(StataCorp, LakeWay Drive, TX, USA) was used for statistical analyses. Results: MALDI-TOF/MS spectra were highly enriched in features in IBD; in HC, features were few or even absent. A total of 426 features were identified, 67 HC-associated and 359 IBD-associated features. A classification algorithm, derived from MALDI-TOF/MS spectra, allowed to obtain a sensitivity of 81% and a specificity of 97%. Blind analysis of a validation cohort (28 HC, 27 CD and 15 UC) confirmed the high specificity of MALDI-TOF/MS spectra, although sensitivity was lower (55%). Logistic regression showed that MALDI/TOF/
MS spectra were independently correlated with IBD ($p<0.0001$), while fCal was a less significant predictor ($p=0.029$). MALDI-TOF/MS-MS of the m/z 1810.8, highly correlated with IBD, resulted in Adenomatous polyposis coli 2 protein, homologous of APC. Immunohistochemistry of IBD tissues reveals that APC was over-expressed in mucosa infiltrating inflammatory cells. APC+ cells were polarized towards the surface mucosa in UC and the muscolaris mucosae in CD. IBD stool proteomic analyses showed that Ig, proteins of neutrophil mediated cytotoxicity and neutrophil mediated killing of symbiont cells were over expressed; proteins involved in nucleic acid assembly and organization were mainly downexpressed. Conclusions: This study provides evidences of the clinical utility of a new proteomic method for diagnosing IBD and offers insights on the pathogenetic role of APC and other newly described proteins, which might become new clues for diagnosis and cancer risk assessment.

SS07 - Invecchiamento, demenza e fragilità

SS07-CO07

**EFFICIENT RT-QuIC SEEDING ACTIVITY FOR α-SYNUCLEIN IN OLFACTORY MUCOSA SAMPLES OF PATIENTS WITH PARKINSON'S DISEASE AND MULTIPLE SYSTEM ATROPHY**

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Parkinson's disease (PD) is a neurodegenerative disorder (ND) whose diagnosis is often challenging because symptoms may overlap with neurodegenerative parkinsonisms. PD is characterized by intraneuronal accumulation of abnormal α-synuclein in brainstem while neurodegenerative parkinsonisms are associated with accumulation of either α-synuclein, as in the case of Multiple System Atrophy (MSA) or tau, as in the case of Corticobasal Degeneration (CBD) and Progressive Supranuclear Palsy (PSP), in other diseasespecific brain regions. Their definite diagnosis can be formulated only at neuropathological level by detection and localization of these aggregates, considered diseasespecific biomarkers (DSB), in the brain. Compelling evidence shows that trace amount of DSB can appear in cerebrospinal fluid (CSF) or peripheral tissues (e.g. olfactory mucosa and urine) of patients in the early stages of the disease. However, their concentration remains well below the limits of detection of the modern diagnostic techniques. With the advent of an innovative and ultrasensitive technique named Real Time Quaking Induced Conversion (RT-QuIC) it has been demonstrated the presence of DSB in CSF and peripheral tissues of patients with NDs. For instance, trace-amount of abnormal α-synuclein were detected in CSF samples of patients with PD, with high sensitivity and specificity. We have therefore decided to extend this technique for the analysis of olfactory mucosa (OM) and urine samples collected from patients with a clinical diagnosis of PD (n=18), MSA (n=11), CBD (n=6) and PSP (n=12). Results of OM analysis showed that PD and MSA samples induced stronger RTQuIC seeding activity for α-synuclein compared to CBD and PSP. Moreover, MSA and PD reaction products acquired peculiar biochemical and morphological features eventually useful for discriminating between diseases. RT-QuIC analysis of urine-derived exosomes is currently ongoing. Taken together these data suggest that RT-QuIC can be exploited for identifying peripheral DSB associated with PD and other neurodegenerative parkinsonisms that might be useful for predicting disease onset, diagnosis, and progression as well as for evaluating the response to treatment in patients enrolled in clinical trials.

SP01 - La fragilità

SP01-01
**LABORATORY MEDICINE AND FRAILTY**

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The FRAILOMIC consortium, an international coalition established for identifying predictors of frailty and disability, has endorsed a concrete definition of frailty, as "age-associated syndrome, characterized by decreased biological reserve and resistance to stressors due to functional decline of several physiological systems, which places the individual at enhanced risk of disability, hospitalization and death" (1). This straightforward definition includes essential psychological, social and cognitive factors, along with additional issues related to lifestyle factors, comorbidities and interplays among all variables. It is now clear that frailty can be efficiently prevented or delayed by establishing appropriate interventions. Like many other human diseases, laboratory medicine can play a substantial role in this clinical setting, by providing an armamentarium of genetic, epigenetic and phenotypic tests which would help identifying subjects at higher risk of becoming frail, even at the pre-frail stage (2). Many important investigations have contributed to dissect predictive, diagnostic and even prognostic biomarkers of frailty, such as vitamin D, cortisol, testosterone, troponin, creatinine, cytokines and other inflammatory mediators. The FRAILOMIC consortium (available at: http://www.frailomic.org/) has then been created and funded under the European FP7 framework for providing further insights into the relationship between laboratory medicine and frailty (3). Briefly, FRAILOMIC encompasses many universities and clinical or research laboratories, engaged to create an European network which will define clinical instruments and validate measurable biomarkers for prevention, diagnosis and management of frailty. A paramount number of genetic, epigenetic and phenotypic biomarkers have been measures in several thousands of individuals. The clinical performance of these biomarkers has then been evaluated against analytical specifications and clinical outcomes. A selected set of these biomarkers has then been validated prospectively, by means of a best fit model, which enables to drive the further definition of discrete test panels that could be employed in clinical practice, with expectations to lower the clinical, social and economic impact of frailty.

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SP02 - La fragilità della Medicina di laboratorio nel NNS

SP02-01

## BENEFITS AND RISKS OF STANDARDIZATION, HARMONIZATION AND CONFORMITY TO OPINION IN CLINICAL LABORATORIES

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The trend toward increasingly larger laboratory networks has resulted in the desire to standardize platforms and procedures among laboratories. This trend is also driven by administrators seeking to achieve resource efficiencies, and to meet the changes brought about by computerization and the adoption of electronic medical records (1). Although conformity and standardization of laboratory networks may improve testing efficiencies and patient care outcomes, this approach can also have serious drawbacks. In most instances standards do not exist and so 'standardization' becomes conformity to an arbitrary opinion and can predispose to system failure, reduced competitiveness, constraint of innovation and diminished patient care quality and outcomes (1-3). The application of standardization in clinical laboratories has also unmasked the distinct philosophical approaches of laboratory physicians and scientists (i.e., "professionalism") towards standardization initiatives compared to laboratory administration and managers (i.e., "managerialism"). Managerialism argues that administrative oversight of laboratories is needed to ensure proper 'control' of the system, which cannot be entrusted to individuals. The social purpose of managerialism is therefore not primarily patient-centered. In contrast, professionalism is a patient-centered approach whose social purpose is primarily focussed on patient outcomes. Professionalism is more closely aligned with right-brain thinking, creativity, emotional intelligence, critical thinking and external motivators, all attributes required for innovation (4,5).

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SP03 - Fragilità in età pediatrica: il valore dell'esame emocromocitometrico

SP03-CO06

## THE ADDED VALUE OF CYTOMETRIC ANALYSIS BY FC500 AND CYTODIFF FOR DIAGNOSIS OF ACUTE LEUKAEMIA IN ROUTINE AND EMERGENCY SETTING

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Introduction: Within the clinical laboratory, the hematological diagnosis is based on high-performance technologies and hematopathologist skills. Microscopic professional review is experience based and timeconsuming. The availability of a rapid and standardized WBC differential count through flow cytometry analysis with Cytodiff reagent represents a strong opportunity for laboratories. Materials and methods: The study was conducted on 69 peripheral patient blood samples suspected of haematological acute pathology collected between October 2016-October 2018. All sample were valued for a complete blood count (CBC-DIFF) by Beckman Coulter LH 750 and DxH 800, peripheral blood smear for morphological evaluation by Beckman Coulter Wright staining, FC500 analysis with pre-mixed Cytodiff reagent including 6 antibodies, morphological analysis in a specialized laboratory by MGG staining and indicated cytochemistry, second-level flow cytometric analysis by BD FACSCanto II. Results: On 69 samples with suspected Acute Leukemia (AL), further confirmed in the reference laboratory and by second-level flow cytometric analysis, the comparison between microscopic WBC differential count and FC500 analysis resulted in an agreement in 66 samples for blasts number (90%) and a discordance in 3 samples (10%). Among the 66 samples in agreement, in 17 (27%) we reviewed the gating proposed by the flow cytometer's software to get the final number of blasts.
Conclusions and disussion: Given the urgency diagnosis of acute newly blood disorders, the confirmation of both presence and number of blasts is important for right management of the patient. The clinical laboratory professional staff does not always foresees the presence of hemopathologists over the 24 hours. Our study demonstrates the efficiency and effectiveness of the integration of FC500 into a WBC differential workflow in a routine laboratory. This approach can be used to confirm suspected acute leukaemia not only when skilled morphologist haemathopathologist is present but also

when other professionals are called for acute leukaemia suspect. The availability of FC500 analysis with Cytodiff can be an important support for short time analysis, automatic gating, standardization and reliability of results.

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SP04 - Sessione SIBioC Young Scientist: Biomarcatori di fragilità nei pazienti con patologia acuta e cronica

SP04-01

**DEMOGRAPHIC FRAGILITY AND INFERTILITY: THE CONTRIBUTION OF LABORATORY MEDICINE**

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Today, infertility is considered a major public health issue: it has been estimated that approximately 1 out of 6 people worldwide suffers from infertility during the reproductive lifespans. This number is expected to grow up in a near future due to the increased age of both partners at first pregnancy and also to unhealthy lifestyle habits. Thus, the management of health reproduction will be ever more important for healthcare providers.
As with all areas of health - to stay healthy people needs to be empowered with knowledge - the provision of accurate fertility information must become mandatory to increase fertility awareness. Women and men are unaware of their own fertility potential, the constraints on their fertility, the signs, symptoms or preventable causes of fertility problems (1). In particular, there is a lack of awareness of individual variation in terms of how fertility declines with age; globally, people are delaying the age of first conception for several reasons (2), but female and male fertility declines with age based on a combination of genetic and lifestyle factors (3,4). Understanding how individual fertility may decline with age and the modifiable lifestyle factors, such as diet and alcohol consumption, is a huge opportunity for increasing fertility awareness and ensuring people are able to make well informed reproductive choices.
In this context, it is important to underline that the reproductive systems of both partners have to work properly and in a precise combination to get a pregnancy; thus, infertility assessment requires the simultaneous evaluation of the two members of a couple to be effective. Considering all the above, laboratory medicine is expected to have a key role in reproductive-related issues. Indeed, thanks to the availability of sensitive and highly performing technics for the detection of DNA alterations in several genes simultaneously and starting from low amount of DNA, molecular analyses are becoming most widely used, also in reproductive medicine. First of all, a molecular evaluation of both partners can be useful to identify the causes of male and/or female infertility, thus providing useful information for the clinical management of the couple. In addition, it is possible to identify genetic diseases transmissible to the offspring and, consequently plan antenatal testing in order to avoid the birth of affected child. Finally, molecular testing can support the decision for the most proper assisted reproductive technique. Thus, the integration of molecular analyses at different steps of the infertile couples journey, based on couples personal and familial history, is able to improve their reproductive outcomes. Nevertheless, to date the use of molecular analyses in this field is still fragmented and cumbersome. However, stratifying the population, through the identification of risk factors and diseases that may be present, allows the planning of targeted diagnostic-therapeutic programs (5).

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SP04-CO07

**MOLECULAR PROFILE OF BRCA-NEGATIVE LONG SURVIVOR HIGH GRADE OVARIAN CANCER PATIENTS USING A SOMATIC MULTIGENE PANEL**

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Introduction: High Grade Serous Ovarian Cancer

(HGSOC) is the commonest subtype of ovarian cancers. Familial cases usually are found to be due to mutations in BRCA1/2 genes. The contribution of these alleles can only explain a small part of the heritability. Many other genes are thought to play an important role. Next Generation Sequencing (NGS) provides efficient method or the evaluation of multiple genes simultaneously. In this context, the relevance of tumor tissue evaluation emerges. In order to investigate the contribution of several desease-associated genes, we selected BRCA-negative HGSOC patients to be genotyped by mean of a new multigene panel.Method. A total of 22 fresh frozen tumor samples obtained from long survivor BRCA-negative patients with a diagnosis of HGSOC were investigated using a new 12-genes panel (Devyser HBOC). Somatic mutations and copy number variations of ATM, BARD1, BRIP1, CDH1, CHEK2, NBN, PALB2, PTEN, RAD51C, RAD51D, STK11 and TP53 genes were tested. Results: In this cohort we found: n=18 mutated subjects (82% of the 22 patients) with 14 pathogenic/ variant of unknown clinical significance (VUS) in TP53; n=1 VUS in BRIP; n=1 VUS in PALB2; n=1 VUS in NBN and n=1 VUS in ATM. Among the patients affected by TP53 alterations, we additionally found n=1 VUS in BARD1 and n=1 VUS in APC.
Discussion: The hereditary risk associated with HGSOC is emerging as a consequence of the contribution of several genes beyond BRCA1/2.An extended investigation using multigene panels is mandatory to gain a more comprehensive overview on the genetic status of these patients. In this study we assayed a new multigene NGS panel in order to characterize the somatic mutational landmarks of HGSOC BRCA-negative patients. As expected, TP53 gene was found to be the principal genetic driver of the disease with a prevalence of missense mutations. Other gene variations in BRIP, PALB2, NBN, ATM, APC and BARD1 were identified. We conclud that querying other gene associated with the disease, would be of an extremely important benefit for the patients evaluation, allowing the clinicians to acquire not only a more appropriate information about the genetic status but also to evaluate the correlation between molecular and clinical features.

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SP04-CO08

## THE CLINICAL BIOCHEMISTRY LABORATORY AND ITS ROLE ON THE OCCASION OF THE XXX SUMMER UNIVERSIADE HELD IN NAPLES

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Introduction: During their training and competitions, athletes can feel sick, be injured or diseased; which dramatically impairs their performance. The staff working at a clinical biochemistry laboratory is responsible for determining which are the lab tests relevant to the most frequent athletes' diseases, collecting samples as well as processing and reporting the results. Methods and results: The Clinical Biochemistry Laboratory of our Department – DAIMELAB – was in charge of healthcare on the occasion of the XXX Summer Universiade. The equipment for blood sampling was promptly provided and an effective transport system was arranged, including the storage of test tubes vertically oriented and kept at a temperature of 2-8°C. Three after blood drawing, centrifugation of the specimen was required before delivery. Insulated tertiary containers and secondary containers were used in compliance with the current regulation. First level laboratory tests were performed according to the official guidelines and divided into four analytic panels: Biochemical Panel Glucose, Urea, Creatinine, Ca, Na, K, Cl, Mg, CRP, Total and Direct Bilirubin, Amylase, Lipase, AST, ALT, LDH, CK, Total Protein, Albumin, Iron), Cardiac Panel (I-Troponin), Hematologic panel (Blood Cell Count) and Coagulation Panel (PT, aPTT, Fibrinogen, D-Dimer). The staff of the laboratory was always available to carry out further analyses when necessary. We provided an application form reporting the ethnic group, the type of blood test to be performed and the relevant regulation. Each form included personal data, data processing policy and medical informed consent, even in view of future investigations. A preferencial analytic route was developed in order to manage each individual sample separately. Medical reports were adjusted to the different ethnic groups of the athletes and their reference values. An online platform was also set up for processing and displaying the reports. Conclusions: Our department worked in close cooperation with the Organizing Committee of the Event, allowing for the most appropriate planning of specimens procedures in terms of transport, check-in, analysis and reporting.

CC01

**Rischio clinico ed ematologia di laboratorio: è possibile affidarsi al solo MCV per scoprire l'errata identificazione del paziente?**

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Tra gli errori preanalitici, quelli di errata identificazione del paziente (wrong blood in tube, WBIT), sono difficilmente eliminabili. È stato stimato un tasso di errore WBIT pari allo 0.6% circa, identificabile dai laboratoristi con la valutazione dei limiti definiti dalla variabilità biologica (delta check limits). Presentiamo un caso di un uomo di 86 anni, ricoverato in seguito a caduta accidentale e trauma cranico e affetto da mielodisplasia e da decadimento cognitivo, con alterazione dell'orientamento spazio-temporale e ipoacusia. L'esame emocromocitometrico (XN-2000, Sysmex Co. Kobe, Japan) all'ingresso mostrava anemia (HBG 88 g/L) e trombocitopenia (PLT 83*10^9/L) con WBC 6.24*10^9/L, RBC 3.19*10^12/L, HCT 0.289 L/L, MCV 90.6 fL, MCHC 304 g/L, NRBC 0% e allarmi strumentali (linfopenia, cellule immature, blasti?, left shift?). La revisione microscopica evidenziava 4% di blasti, 4% di mileociti e 2 % di metamielociti neutrofili). Il giorno seguente la risoluzione del quadro ematologico (WBC 7.14*10^9/L, RBC 3.78*10^12/L, HBG 117 g/L, HCT 0.348 L/L, MCV 92.1 fL, MCHC 336 g/L, PLT 242*10^9/L) e l'assenza di allarmi leucocitari hanno suggerito la verifica dell'attribuzione del campione e di eventuali procedure cliniche instaurate: è stato così riscontrato un errore di tipo WBIT. L'analisi di un nuovo prelievo ha evidenziato la variazione significativa di quasi tutti i parametri (WBC 5.02*10^9/L, RBC 2.61*10^12/L, HBG 72 g/L, HCT 0.231 L/L, MCV 88.5 fL, MCHC 312 g/L, PLT 61*10^9/L) con grave anemia e necessità di trasfusione. Nonostante, in assenza di trasfusioni e di variazioni dello stato clinico, alcuni parametri siano relativamente stabili nello stesso paziente, nel caso riportato, la valutazione dei soli parametri usati comunemente per la valutazione dell'errore WBIT, non avrebbe permesso la rilevazione dell'errore, poiché entro i limiti predefiniti: 2.01*10^9/L per WBC e 3.97 fL per MCV al 95%. La variazione del quadro morfologico e la comunicazione con i clinici hanno permesso di rilevare l'errore che, se non riscontrato, avrebbe portato alla mancata trasfusione del paziente. Il presente caso sottolinea la necessità d'implementare un protocollo multiparametrico per la rilevazione degli errori WBIT, efficace ma di facile applicabilità clinica.

CC02

**Una variante emoglobinica co-migrante con le normali frazioni emoglobiniche in HPLC**

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I diversi metodi separativi disponibili in commercio per la valutazione degli assetti emoglobinici consentono di identificare la maggior parte delle varianti con alcune differenze dovute al diverso principio chimico-fisico utilizzato.Conseguentemente, per la valutazione degli assetti, è raccomandato l'utilizzo di due metodi per l'ottimizzazione della sensibilità diagnostica e per poter indirizzare in maniera puntuale le indagini di 2° livello a vantaggio del tempo e dei costi diagnostici.Riportiamo un caso clinico relativo ad una paziente italiana il cui assetto mostrava in cromatografia a scambio ionico (Bio-Rad Variant II$^{TM}$ nella modalità "HbA2/HbA1c dual program) la presenza di HbF al 19,3% ed HbA2 al 0,7%. I parametri ematologici risultavano invece nella norma e conseguentemente l'unica ipotesi che era possibile formulare era quella della presenza di una persistenza dell'emoglobina fetale (che giustificava l'aumento di HbF) a cui era associata una delta talassemia eterozigote (che giustificava la riduzione di HbA2). Vista la disponibilità nel laboratorio del metodo in elettroforesi capillare (Capillarys 3 Tera, Capi3 Hemoglobine) e considerata la particolarità del campione si è proceduto ad ulteriori approfondimenti al fine di confermare l'ipotesi diagnostica iniziale. Il campione ha mostrato in elettroforesi capillare (CE) un profilo atipico per la presenza di una variante emoglobinica (22%) chiaramente identificata in zona Z14 mentre il valore di HbA2 risultava 1,7%. L'ipotesi diagnostica formulata sulla base delle evidenze dell'HPLC appariva quindi errata poiché basata sulla non corretta identificazione delle frazioni emoglobiniche (la variante emoglobinica veniva erroneamente identificata come HbF). Vista la particolarità del difetto il campione è stato inviato ad un laboratorio di secondo livello per la caratterizzazione dei geni globinici e l'approfondimento diagnostico molecolare ha confermato la presenza della mutazione c.271 A>G del gene $\alpha$1 globinico. Tale variante (Hb Sudbury) è riportata nel database HbVar ma non documentata in letteratura e mai riscontrata in Italia. Occorre precisare che la corretta identificazione del difetto dei geni globinici è cruciale ai fini del "counselling" ed in questo caso è stata possibile solo grazie alla CE.

CC03
**Una variante emoglobinica rara**

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BS è un neonato di origine africana di pochi giorni, che si presenta alla prima osservazione con un emocromo nella norma, ma con richiesta di screening neonatale per ricerca di varianti emoglobiniche per la presenza di rischio di doppia eterozigosi SC (SCD) poiché i genitori sono portatori di trait HbS (madre) e HbC (padre). La richiesta è appropriata perché in presenza di SCD viene suggerita terapia profilattica per evitare infezioni neonatali. Lo screening, eseguito in elettroforesi capillare (Capillarys TERA, SEBIA), evidenzia presenza di HbA (10%), HbF (66%) e una variante emoglobinica di incerta identificazione (HbX circa 25%) preceduta da una piccola spalla. L'elettroforesi emoglobinica su gel di agarosio a pH alcalino e acido (G26 INTERLAB) conferma la presenza di HbF e di una banda variante con caratteristiche di migrazione diverse sia da HbC che da HbS. Si richiede nuovo campione, che perviene quando BS ha circa 20 giorni. Il quadro elettroforetico è confermato ma ora si evidenza anche un piccolo picco prima della variante X che sembra eluire in zona HbS. All'analisi molecolare, eseguita in altro laboratorio (Ospedale Maggiore Milano), vengono sequenziate le catene beta e gamma globiniche e risulta una doppia eterozigosi con la presenza di HbS trait ma anche di una mutazione in eterozigosi a carico della catena gamma globinica compatibile con HbF Granada.Le varianti gamma globiniche si possono vedere solo alla nascita ma sono nella maggior parte dei casi asintomatiche e spariscono quando nei primi mesi dopo la nascita la produzione di catene gamma viene sostituita dalla produzione di catene beta ( switch). In qualche caso possono causare una emolisi neonatale che comunque si risolve con lo switch.
Conclusioni: lo screening neonatale è appropriato per escludere condizioni riconducibili a SCD, quando serve per il bancaggio del cordone ombelicale, quando occorre escludere cause di ittero marcato prodotto da emoglobinopatie, oppure nel caso di genitori a rischio per beta talassemia o altro, non esaminati oppure che non hanno fatto diagnosi prenatale pur essendo a rischio. Nelle situazioni più complesse è necessario ricorrere all'analisi molecolare.

CC04
**Un caso di falsa neutropenia**

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Si presenta il caso clinico di una neonata con ritardo di crescita, febbre, candidosi muco cutanea, ascesso perianale, trasferita all'età di due mesi nel centro di Immunologia pediatrica per sospetta immunodeficienza. Un primo esame emocromocitometrico eseguito con strumentazione Beckman Coulter DxH-800 rilevava una neutropenia severa associata a monocitosi con un'anomala distribuzione degli eventi nel grafico strumentale; lo stesso esame ripetuto su analizzatore Sysmex XN-9000 mostrava invece la presenza di una normale quantità di neutrofili. La revisione microscopica su striscio di sangue periferico confermava trattarsi di una falsa neutropenia, dovuta all'errata classificazione dei neutrofili come monociti da parte del primo strumento, a causa della totale assenza di granuli citoplasmatici e nucleo ipolobato (neutrofili pseudo Pelger- Huët). Tali caratteristiche morfologiche, nel contesto del quadro clinico, suggerivano una possibile diagnosi di immunodeficienza primitiva da assenza di granuli specifici. Dopo aver escluso mutazioni a carico di CEBPE, fattore di crescita coinvolto nell'espressione dei granuli specifici, il sequenziamento del DNA con metodica di Sanger mostrava una delezione di 4 basi nel gene SMARCD2, alterazioni del quale sono state recentemente correlate a una sindrome caratterizzata da difetti di maturazione dei precursori mieloidi, assenza di granuli specifici dei neutrofili e dismorfismo corporeo. Nel presente caso, l'esame radiografico dello scheletro aveva effettivamente mostrato una anomalia di lunghezza degli arti superiori, mentre l'esame morfologico dell'aspirato midollare confermava la displasia e la rallentata maturazione dei neutrofili. La paziente era quindi candidata a un trapianto allogenico di cellule staminali ematopoietiche, poi eseguito all'età di 6 mesi da donatore familiare HLA-identico (fratello) non portatore della mutazione. Dopo il trapianto, caratterizzato da attecchimento completo, le lesioni perianali si risolvevano e la paziente veniva dimessa. Se i risultati degli analizzatori automatici presentano anomalie, la revisione microscopica o l'esame morfologico possono consentire al Laboratorio di fornire in tempi brevi indicazioni utili a orientare correttamente il percorso diagnostico di secondo livello.

CC05
**Un linfoma di difficile inquadramento**

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Premessa: Presentiamo il caso di un paziente con linfoma triplo hit, una rara forma di linfoma non Hodgkin a cellule B altamente aggressivo, con caratteristiche morfologiche, fenotipiche e genetiche intermedie tra linfoma diffuso a grandi cellule B (DLBCL) e linfoma di Burkitt (LB), data l'importanza di un corretto e precoce inquadramento ai fini terapeutici e prognostici.
Caso clinico: Paziente di 78 anni, sesso maschile, presentatosi in Pronto Soccorso per iporessia, mialgie e malessere generale. All'esame obiettivo si riscontrano polimicroadenopatie laterocervicali e inguinali bilaterali, modesta splenomegalia. Gli esami ematochimici evidenziano anemia (Hb=8.6 g/dL), piastrinopenia (Piastrine=12 10^9/L), marcato aumento della lattato deidrogenasi (LDH=12411 UI/L). L'analisi dei citogrammi mostra uno scattergram, relativo alla differenziazione leucocitaria, anomalo con un cluster monocitario con distribuzione alterata, che pone il sospetto della presenza di blasti. L'esame microscopico dello striscio di sangue periferico evidenzia la presenza di elementi cellulari mononucleati medio grandi (30%), con citoplasma intensamente basofilo e presenza di vacuoli a stampo, nucleo tondeggiante. Nel sospetto di linfoma di Burkitt è stato eseguito immunofenotipo su sangue venoso periferico che evidenzia un incremento della popolazione B linfocitaria con caratteristiche immunofenotipiche e dimensionali compatibili con leucemizzazione da malattia linfoproliferativa/linfoma. L'immunofenotipo, la citogenetica e l'esame istologico del midollo risultano compatibili con linfoma B di alto grado con traslocazione di MYC, BCL-2, BCL-6 (secondo WHO 2017) (c.d. triplo hit).
Discussione: Il linfoma triplo hit T è una forma di linfoma caratterizzato dalla presenza di tre riarrangiamenti genici: c-MYC, BCL-2 e BCL-6 ed è associato a decorso clinico aggressivo poichè ha la tendenza a diffondere in siti extranodali, con possibilità di invasione del midollo osseo e del sistema nervoso centrale. E' inoltre caratterizzato da scarsa risposta al trattamento chemioterapico, alla eventuale terapia di salvataggio con trapianto di cellule staminali autologo in caso di recidiva e a ridotta sopravvivenza.

CC06
**Una complicata valutazione della risposta alla terapia in un paziente con malattia da deposito da catene leggere libere**

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Il paziente è un uomo di 28 anni giunto alla diagnosi di malattia da deposito di catene leggere (LCDD) nell'aprile 2014. All'esordio l'elettroforesi (EF) capillare del siero ha mostrato una componente monoclonale (CM) di 0.87 g/dL in regione γ anodica, tipizzata all'immunofissazione (IF) come IgGκ. Le catene leggere libere (FLC) κ erano 105 mg/L con rapporto κ/λ 3.5 (v.r. 0.26-1.65). La creatinina era 1.55 mg/dL (eGFR 60 mL/min) e la proteinuria delle 24 ore era di 1.68 g/24 ore. È stata iniziata una terapia con bortezomib e desametasone, seguita da un trapianto autologo di cellule staminali e da un trattamento con lenalidomide e desamentasone, concluso nel febbraio 2016. Il paziente non è risultato responsivo a nessuna di queste linee di terapia (CM 0.79 g/dL, FLC κ 92 mg/L e rapporto κ/λ 3.2) e per il peggioramento della funzionalità renale, è stata iniziata la dialisi. Al fine di ottenere la remissione ematologica completa (RC), che avrebbe permesso l'inserimento del paziente in lista di trapianto di rene, è stata iniziata una terapia con daratumumab, un anticorpo monoclonale (Ab) IgGκ anti-CD38. Dopo 8 infusioni le FLC κ erano 15 mg/L, con rapporto κ/λ nella norma (0.93). All'EF capillare era presente un picco monoclonale (0.50 g/dL), tipizzato all'IF come IgGκ. Dopo altre 8 infusioni, il rapporto κ/λ era nella norma (1.01) e persisteva il picco monoclonale (0.53 g/dL) all'EF capillare del siero. Per verificare l'ottenimento della RC, data la possibile interferenza del daratumumab (Ab monoclonale IgGκ), è stata eseguita l'IF con Hydrashift, che utilizza un Ab anti-daratumumab. Questo esame non ha rilevato la CM IgGκ dell'esordio. È stato concluso che il picco monoclonale osservato all'EF capillare del siero era riferibile al daratumumab e, vista la normalizzazione del rapporto κ/λ e l'assenza della CM IgGκ, è stato possibile confermare l'ottenimento della RC e il paziente è stato inserito in lista di trapianto renale. L'utilizzo del daratumumab ha reso difficile la valutazione della RC, nonostante la profonda riduzione delle FLC κ. L'impiego di Hydrashift è stato determinante per la valutazione della qualità della risposta ematologica.

CC07

## Inspiegabili alterazioni dei test coagulativi

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Case description: before a minor orthopaedic surgery, a 72 years-old female, with no relevant comorbidities, was screened with routine coagulation tests. Prothrombin time (PT) and Activated Partial Thromboplastin Time (aPTT) showed altered results: the system registered a biphasic curve and thus was unable to calculate coagulation time. The anamnesis of the patient was negative for any major pathology or any relevant haemorrhagic events. Bleeding score according to the ISTH-SSC Bleeding Assessment Tool was 0. Blood cells count and fibrinogen were normal, as well as the levels of all coagulation factors, whereas protein C (PC) and protein S (PS) levels could also not be calculated. After reviewing previous laboratory findings, we discovered a serum protein electrophoresis of three months before that showed the presence of a small gamma component, which the patient had never investigated further. Therefore, we hypothesized the presence of an abnormal protein that could interfere with coagulation tests. We performed the serum immunofixation, finding a monoclonal component IgM Kappa type. The detection of Bence-Jones protein in the urine was positive for a Kappa type present in traces. The phenotypical characteristics of the monoclonal component (milky white, adhesive) were similar to those of the cryoglobulins. In fact, the research for cryoglobulins was positive for cryoglobulin type II. Therefore, we repeated the coagulations assay on the patient sample conserved at 37 °C, obtaining no variation. In order to confirm our hypothesis, we diluted a pool of normal plasmas (with normal value of coagulation tests) with the isolated IgM component at progressively higher concentrations. We were not able to reproduce the biphasic curve, but we registered a prolongation of PT and aPTT and a slight reduction of PC and PS. We also tested for the presence of antiphospholipid antibodies: the results showed extremely high levels of anti β2-glycoprotein-I and anti-cardiolipin IgM antibodies with positive lupus anticoagulant and no variation when tested at 37°C. Finally, we assessed the coagulation function through thromboelastography, obtaining normal value.
Conclusion: the presence of an IgM monoclonal component, although not quantitatively relevant, was functionally able to interfere with the coagulation time calculation system.

CC08

## Il valore aggiunto della diagnostica molecolare nelle forme monogeniche di diabete mellito

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Monogenic Diabetes (MD) represents a frequent cause of diabetes in pediatrics (2-6%) and Maturity Onset Diabetes of the Young (MODY) is the most common form. MODY comprise heterogeneous disorders, characterized by not autoimmune diabetes due to mutations of at least 14 different genes involved in the homeostasis of glucose and in pancreatic development. Molecular diagnosis of MODY is very important to highlight the specific genetic alteration and to perform a definitive diagnosis that allows to guide clinicians towards the best treatment for the patient, to predict clinical evolution of the disease and to identify other at-risk members in the family. We describe the case of a woman who came to the attention of clinicians for the early onset of diabetes and the absence of autoimmunity; she was then referred to genetic testing for MD. We identified a heterozygous deletion of the entire HNF1b gene suggesting a MODY5 diagnosis. To define the extension of the deletion, we performed an array-Comparative Genomic Hybridization analysis that revealed a large 1.67 Mb deletion on chromosome 17q12 that includes 23 OMIM genes. A more careful evaluation of the patient revealed additional clinical features such as kidney cysts, intellectual disability, minor skeletal malformations and facial dysmorphisms. A 17q12 deletion syndrome has been previously reported in the literature with variable signs and symptoms. The more common features are kidneys abnormalities, diabetes and intellectual disability, or behavioral or psychiatric disorders. Deletion of HNF1b is a known cause of kidney abnormalities, as well as of diabetes, while loss of LHX1 could contribute to intellectual disability. This case supports the notion that diabetes when caused by large deletions including many genes, may be considered just one of the symptoms of a "clinical syndrome" that includes other features due to alteration of neighboring genes. Our case strongly supports the need for an extremely accurate clinical evaluation of diabetic patients in order to choose the most appropriate genetic test for a correct diagnosis; this is of paramount relevance to correctly classify the molecular etiology of patients' clinical signs, to offer appropriate genetic counseling and to guide therapeutic strategies.

CC09

**Assenza di bande monoclonali liquorali e positività del relativo indice siero-liquor: una dissociazione da indagare attentamente**

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Background: The immune intrathecal response in patients with neurological disorders has been employed as the pathophysiological background for the development of biochemical assays in cerebro-spinal fluid (CSF). They included: IgG index (known as Link index), detection of IgG oligoclonal bands (OCB) by isoelectrofocusing (IEF) and the Kappa Free Light Chain (KFLC) index (which measures intrathecal KFLC production). Patient and Methods: a 13 year-old child was hospitalized for appearance of frontal and bitemporal headache with poor responsiveness to paracetamol, associated with nuchal pain and photophobia. He also reported nausea and repeated episodes of vomiting. Entrance blood tests showed a slight increase in WBC and normal CRP. A lumbar puncture was performed and biochemical examinations on CSF including OCB. Kappa and Lambda KFLC and LFLC (lambda free light chain) as well as IgG were measured in serum and CSF by nephelometry. OCBs were measured by IEF followed by immunofixation (Hydragel9 CSF Isofocusing; Sebia, Bagno a Ripoli, FI, Italia) on agarose electrophoresis system (Sebia Hydrasys). Results: the biochemical result on CSF showed a negative IgG index (0.5), no detection of OCB IgG (type I), but a positive KFLC index (24) with a CSF concentration of KFLC of 0.14 mg/dL and LFLC of 0.14 mg/dl. The free light chain data suggested an intrathecal synthesis. A CSF IEF followed by immunofixation using anti-IgM was then performed together with the IgM index using Reiber's hyperbolyc graphic. The result was a presence of a suspected monoclonal band of IgM and a 76% of intrathecal synthesis of IgM. A subsequent molecular biology investigation on CSF was then performed on CSF to find bacterial and/or viral infection and a positivity for enterovirus was found.Conclusion: the diagnosis of enterovirus encephalitis was made possible by molecular biology investigation, but the early suspicion of intrathecal synthesis was suggested above all by KFLC index and confirmed by IgM index and Reiber analysis, rather than the gold standard IEF for IgG OCB.

CC10

**Le malattie rare richiedono una attenta verifica degli intervalli di riferimento e terapeutici**

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Background: La Malattia di Wilson (MW) è una malattia autosomica recessiva, causata da mutazioni del gene ATP7B, con conseguente disordine del metabolismo del rame e manifestazioni cliniche multiorgano. Le terapie disponibili al momento sono chelanti (Penicillamina e Trientina) e zinco acetato. La cupruria (uCu), misurata in spettrofotometria di assorbimento atomico (v.r.< 40 µg/24h), viene impiegata per valutare il trattamento con Penicillamina (Pe), considerato adeguato quando in fase di mantenimento la uCu è stabilmente compresa tra i 200 e i 500 µg/24h[1,2]. Valori al di fuori del range atteso sono considerati indicativi di scarsa compliance o sovradosaggio del farmaco.
Caso clinico: Maschio, 30 anni, affetto da MW diagnosticata all'età di 5 anni e da allora in terapia con Pe al dosaggio standard di 20 mg/Kg/die. L'esordio della MW era esclusivamente epatico: AST 130 U/L (v.r.<34 ); ALT 395 U/L (v.r.<55); GGT 187 U/L (v.r. 12-64). A diagnosi i principali marcatori biochimici della MW, uCu e ceruloplasmina sierica (v.r. 0.2-0.4), erano rispettivamente 270 µg/24h e 0.16 g/L. Dopo inizio della terapia chelante, a partire dai 24 mesi di follow-up si assisteva a completa normalizzazione di AST, ALT e GGT, persistente fino all'ultima osservazione, avvenuta dopo oltre 20 anni. Nel follow-up il livello medio di uCu(±2SD) era 746.7(±467.1) µg/24h, mediana 662(52-1536) µg/24h. Il valore non è mai rientrato nel range atteso (200-500 µg/24h), ma, il paziente non ha mostrato evolutività dell'epatopatia, non si è assistito a comparsa di segni/sintomi neuropsichiatrici, nè a altri clinico-laboratoristici della MW.
Conclusione: Nell'ambito delle malattie rare, con l'ampliarsi delle casistiche e l'allungarsi dei tempi di follow-up, è necessaria una frequente rivalutazione dei range di normalità e/o terapeutici, da raggiungere attraverso una continua interazione tra clinica e laboratorio. All'AOU Federico II di Napoli è attivo dal 2017 il Percorso Diagnostico Terapeutico Assistenziale (PDTA) della MW, costituito da un team multidisciplinare: pediatri; epatologi dell'adulto; neurologi; radiologi; psichiatri; oculisti e laboratoristi, per un'integrata e completa presa in cura del paziente con MW. Referenze: [1]Socha P et al. JPGN 2018;66:334-442. [2]EASL. J. Hepatol. 2012;56:671-85

CJ01

## MOLECULAR PROFILE OF BRCA-NEGATIVE LONG SURVIVOR HIGH GRADE OVARIAN CANCER PATIENTS USING A SOMATIC MULTIGENE PANEL

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Introduction: High Grade Serous Ovarian Cancer (HGSOC) is the commonest subtype of ovarian cancers. Familial cases usually are found to be due to mutations in BRCA1/2 genes. The contribution of these alleles can only explain a small part of the heritability. Many other genes are thought to play an important role. Next Generation Sequencing (NGS) provides efficient method for the evaluation of multiple genes simultaneously. In this context, the relevance of tumor tissue evaluation emerges. In order to investigate the contribution of several desease-associated genes, we selected BRCA-negative HGSOC patients to be genotyped by mean of a new multi-gene NGS panel.Method. A total of 22 fresh frozen tumor samples obtained from long survivor BRCA-negative patients with a diagnosis of HGSOC were investigated using a new 12-genes panel (Devyser HBOC). Somatic mutations and copy number variations of ATM, BARD1, BRIP1, CDH1, CHEK2, NBN, PALB2, PTEN, RAD51C, RAD51D, STK11 and TP53 genes were tested.
Results: In this cohort we found: n=18 mutated subjects (82% of the 22 patients) with 14 pathogenic/ variant of unknown clinical significance (VUS) in TP53; n=1 VUS in BRIP; n=1 VUS in PALB2; n=1 VUS in NBN and n=1 VUS in ATM. Among the patients affected by TP53 alterations, we additionally found n=1 VUS in BARD1 and n=1 VUS in APC.
Discussion: The hereditary risk associated with HGSOC is emerging as a consequence of the contribution of several genes beyond BRCA1/2.An extended investigation using multigene panels is mandatory to gain a more comprehensive overview on the genetic status of these patients. In this study we assayed a new multigene NGS panel in order to characterize the somatic mutational landmarks of HGSOC BRCA-negative patients. As expected, TP53 gene was found to be the principal genetic driver of the disease with a prevalence of missense mutations. Other gene variations in BRIP, PALB2, NBN, ATM, APC and BARD1 were identified. We conclude that querying other gene associated with the disease, would be of an extremely important benefit for the patients evaluation, allowing the clinicians to acquire not only a more appropriate information about the genetic status but also to evaluate the correlation between molecular and clinical features.
Reference: Michael-Antony Lisio et al., High-Grade Serous Ovarian Cancer: Basic Sciences, Clinical and Therapeutic Standpoints. Int. J. Mol. Sci. 2019, 20, 952

CJ02

## THE CLINICAL BIOCHEMISTRY LABORATORY AND ITS ROLE ON THE OCCASION OF THE XXX SUMMER UNIVERSIADE HELD IN NAPLES

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Introduction: During their training and competitions, athletes can feel sick, be injured or diseased; which dramatically impairs their performance. The staff working at a clinical biochemistry laboratory is responsible for determining which are the lab tests relevant to the most frequent athletes' diseases, collecting samples as well as processing and reporting the results.
Methods and results: The Clinical Biochemistry Laboratory of our Department – DAIMELAB – was in charge of healthcare on the occasion of the XXX Summer Universiade. The equipment for blood sampling was promptly provided and an effective transport system was arranged, including the storage of test tubes vertically oriented and kept at a temperature of 2-8°C. Three hours after blood drawing, centrifugation of the specimen was required before delivery. Insulated tertiary containers and secondary containers were used in compliance with the current regulation. First level laboratory tests were performed according to the official guidelines and divided into four analytic panels: Biochemical Panel (Glucose, Urea, Creatinine, Ca, Na, K, Cl, Mg, CRP, Total and Direct Bilirubin, Amylase, Lipase, AST, ALT, LDH, CK, Total Protein, Albumin, Iron), Cardiac Panel (I-Troponin), Hematologic panel (Blood Cell Count) and Coagulation Panel (PT, aPTT, Fibrinogen, D-Dimer). The staff of the laboratory was always available to carry out further analyses when necessary. We provided an application form reporting the ethnic group, the type of blood test to be performed and the relevant regulation. Each form included personal data, data processing policy and medical informed consent, even in view of future investigations. A preferencial analytic route was developed in order to manage each individual sample separately. Medical reports were adjusted to the different ethnic groups of the athletes and their reference values. An online platform was also set up for processing and displaying the reports.
Conclusions: Our department worked in close cooperation with the Organizing Committee of the Event, allowing for the most appropriate planning of specimens procedures in terms of transport, check-in, analysis and reporting.

CJ03

## DIAGNOSTIC POTENTIAL OF A NOVEL REAL-TIME QUANTITATIVE PCR-BASED ASSAY TO MEASURE CYCLIN D1/CCND1 MRNA EXPRESSION LEVELS IN BONE MARROW-DERIVED PLASMA CELLS FROM PATIENTS WITH AL AMYLOIDOSIS

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AL amyloidosis is a life-threatening plasma cell (PC) tumor. A significant proportion of AL amyloidosis patients do not respond to frontline anti-plasma cell drugs and die before being offered second-line therapies. Hence, predicting which patients are more likely to resist frontline drugs has the potential to change the natural history of this disease.

Recent studies showed that, both in AL and in multiple myeloma, the presence of the t(11;14)(q13;q32) or the consequent overexpression of the proto-oncogene CCND1, encoding cyclin D1, is associated with lower response rates towards bortezomib, and higher rates of response towards melphalan. As t(11;14) and CCND1 expression levels are not invariably associated, with a non-negligible proportion of t(11;14)-negative clones which overexpress CCND1, we reasoned that a molecular diagnostic assay for measuring CCND1 levels in bone marrow (BM)-derived PCs may complement cytogenetics to guide treatment choices.

We designed a TaqMan-based, multiplexed assay for reliable CCND1 expression level measurements in purified BM-PCs. ALG9 was selected as normalizing gene based on meta-analyses of transcriptomic datasets and subsequent experimental validation.

The assay showed a good PCR efficiency, a dynamic range spanning at least 5 orders of magnitude of cDNA dilution, and low intra- and inter-assay variation. Moreover, the assay was compatible with low input RNA (obtained from 10E-4 immunopurified CD138+ cells).

We then applied the assay to measure CCND1 expression levels in BM plasma cells of 16 patients with AL with known t(11;14) status at diagnosis. CCND1 expression was detected in 7 out of 8 t(11;14) positive cases and in 2 out of 6 t(11;14) negative cases.

Our results show that t(11;14) positivity as assessed by interphase FISH does not intercept all CCND1-expressing PCs in AL, strengthening the rationale for the development of a complementary test to measure CCND1 levels in these tumors.

Further studies will be needed to fully explore the potential clinical utility of a TaqMan-based assay for CCND1 measurements in AL, especially in terms of prognostication of response to novel agents and guidance for treatment choices, towards a precision medicine approach.

CJ04

## THE CORRELATION BETWEEN INFLAMMATORY BIOMARKERS, OMEGA-3 INDEX, AND ARACHIDONIC ACID (AA)/EICOSAPENTAENOIC ACID (EPA) RATIO: A STUDY IN WELL-TRAINED RUNNERS

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Recent data have expanded the concept that inflammation may be a critical component of myocardial dysfunction, particularly in highly trained athletes. Moreover, biomarkers associated with polyunsaturated fatty acids (PUFA) metabolism may provide valuable information on health outcomes and long-term disease risk. For example, it was proposed that a low omega-3 index (eicosapentaenoic acid [EPA, C20:5n-3] plus docosahexaenoic acid [DHA, C22:6n-3]) may be considered as a potential risk factor for cardiovascular disease, especially sudden cardiac death. Additionally, although the interaction between arachidonic acid (AA, C20:4n-6) and EPA is complex and still not properly understood, several findings support the hypothesis that the balance between AA and EPA is important to regulate the synthesis of inflammatory mediators, especially during high-intensity exercise. However, there are no studies on the link between kilometers (Km) run per week, PUFA indices, and changes in inflammatory mediators. We conducted an observational, cohort study of 257 non-elite runners (mean age: 40.85 ± 12.17 years) who provided a blood sample for analysis. PUFA levels were quantified by gas chromatography (GC) and then omega-3 index and AA/EPA ratio measured. C-reactive protein (CRP), prostaglandin E2 (PGE2), and cytokines (IL-1β, IL-12, TNF-α) were measured by standard kit methods (ELISA). Data on running habits were collected: weekly running distance (Km/week). After adjusting for major confounders, a gradual decrease of the ω-3 index was observed with higher weekly running distance ($\beta$ = #0.033; 95% CI #0.039 to #0.026; R = 0.447; $p < 0.0001$). We also found a progressive increase of the AA/EPA ratio in subjects who ran greater weekly distances ($\beta$ = 0.092; 95% CI 0.038 to 0.146; R = 0.320; $p = 0.001$). Finally, as expected, a lower ω-3 index and a higher AA/EPA ratio were correlated with a greater expression of the pro-inflammatory mediators in individuals who ran greater weekly distance: CRP ($p < 0.001$), PGE2 ($p = 0.0012$) IL-1β ($p = 0.024$), IL-12 ($p = 0.026$), TNF-α ($p < 0.001$). These findings suggest that distance running training and its weekly volume may negatively contribute to changes of the ω-3 index, AA/EPA ratio, and inflammatory biomarkers.

CJ05

## A DISTINCTIVE MIRNA EXPRESSION PROFILE CHARACTERIZES AGGRESSIVE PANCREATIC CANCER

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Background: Pancreatic Cancer (PDAC) remains one of the most dismal diseases worldwide, with poor prognosis and a 5-year survival rate of only 5%. Although survival is mainly related to tumor stage, very long as well as very short survival have been described in patients with early stage tumors. Recent studies have reported the prognostic utility of circulating microRNA (miRNA) profiling in several malignancies, supporting their potential role as an emerging class of suitable biomarkers also in PC setting. Aim of this work is to perform a whole miRNA microarray analysis in sera of highly selected stage I-IIA PDAC patients, with different survival rates (less or more than 24 months after surgery) in order to identify whether miRNAs predict survival.
Methods: Total RNA was extracted from archival sera of 15 patients diagnosed with stage I-IIA PDAC and subjected to curative surgery. The patients were selected from a retrospective cohort on the basis of overlapping characteristics regarding age, gender, diabetes, jaundice, surgery and classified on the basis of different survivals: group A) less than 24 months (7/16 pts) and group B) more than 24 months (8/16 pts). miRNAs expression profile was generated using Agilent SurePrintG3 Human miRNA v.21 (8x60K) microarrays. Data were analysed bioinformatically to extract significantly deregulated candidates.
Results: A total of 2.549 human miRNAs were screened out. Unsupervised cluster analysis did not reveal a clear distinction between the two patients' groups. However the SAM two class analysis allowed identifying 77 miRNAs ($p < 0.05$) differentially expressed between groups A) and B) patients. Among the most significantly de-regulated miRNAs, hsa-miR-6800-5p, 3665, 1246 and 940 were down-regulated in group A) with respect to group B) patients. Of particular interest also hsa-miR-451a and -5100 previously correlated with survival in PDAC and hsa-miR-320d correlated with survival in rectal cancer.
Conclusions: Although data should be confirmed in a larger group of patients and validated by another molecular technique (e.g. RT-PCR or ddPCR), these preliminary results demonstrate that the identification in sera of miRNAs in a highly selected series of patients might represent a challenge for predicting PDAC survival.

# 51° Congresso Nazionale della Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica (SIBioC - Medicina di Laboratorio)

**Padova, 20-22 novembre 2019**

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| • | **P002, P065, P067-P071, P073** | Analisi Decentrate, Controllo di qualità, standardizzazione, tracciabilità |
| • | **P036-P041, P047-P048** | Casi clinici |
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*Nota dell'Editore: i riassunti sono stati riprodotti senza alcuna revisione dal materiale direttamente fornito dagli autori.*

P001

## FECAL EOSINOPHIL CATIONIC PROTEIN AS POTENTIAL MARKER OF DISEASE ACTIVITY IN PATIENTS WITH EOSINOPHILIC ESOPHAGITIS

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Backgrounds: Eosinophilic Esophagitis (EoE) is a chronic disorder, characterized by symptoms of esophageal dysfunction and, histologically, by eosinophilic-infiltration. Endoscopic and histological examination is required for diagnosis and follow-up, since a reliable marker has not been identified yet. We evaluated the potential use of Eosinophil cationic protein (ECP) in EoE patients' faeces (F-ECP) as a potential marker of disease activity.
Methods: Twenty-nine consecutive EoE-patients assessed at Gastroenterological Unit between January 2018 to January 2019 for endoscopic follow-up were included. Faecal and histological specimens were collected on the same day. Histological activity was staged considering the number of eosinophils for high-power field (Eos/HPF). A control-group (HC) of 71 patients, in whom EoE had been excluded according to International criteria, was included. F-ECP was evaluated with a fluorescence-enzyme immunoassay (PHADIA, Thermo Fisher Scientific). Spearman's rank correlation was applied and F-ECP predictive positive and negative values (PPV, NPV) were calculated.
Results: EoE-study-population consisted of 29 patients affected by EoE (mean age 32-yo, range 18-62yo, 24 M), showing F-ECP values ranging from 0 to 172 ug/L (mean value 17,3 ug/L). In the HC (mean age 52-yo, range 35-64, 38 Male), F-ECP values ranging from 0 to 32 ug/L (mean value 8.1 ug/L). A statistically significant relationship between F-ECP and Eos/HPF was observed ($rs = 0,4172$, $p = 0.02438$). PPV and NPV of F-ECP were evaluated using two cut-off values, F-ECP < 2 ug/L (as negative) and F-ECP < 8.1 ug/L (as mean value in the control group). Among the study group, 21 patients (72%) showed histological remission, but only 8 (38%) had F-ECP negative values. Setting a cut-off value of 8.1 ug/L, F-ECP NPV and PPV for histological remission were 86% and 40%, respectively, while they were 100% and 38% with cut-off value of 2 ug/L.
Conclusions: Our preliminary data show a good correlation of F-ECP values with histology activity and a clear difference in terms of levels between EoE patients and controls. However, the wide variability of our ECP levels evaluated, requires further cases and more histologically-active patients, in order to assess its usefulness in clinical practice.

P002

## PROGRAMMA DI VALUTAZIONE ESTERNA DI QUALITÀ PER LA DETERMINAZIONE DELLA GLICEMIA: VERIFICA DELLA VARIABILITA' ANALITICA DI TRE GLUCOMETRI "POCT" PER L'UTILIZZO IN AMBITO OSPEDALIERO

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L'utilizzo dei "point-of-care-testing" (POCT) per la determinazione della glicemia in ambito ospedaliero può rendersi necessario in situazioni in cui occorre prendere decisioni terapeutiche in tempi non compatibili con il "turnaround time" del laboratorio. E' quindi fondamentale sottoporre i glucometri dislocati nei vari reparti, oltre che al controllo di qualità interno, ad un programma di Valutazione Esterna di Qualità (VEQ). Il Centro di Ricerca Biomedica dal 2016 gestisce uno specifico Schema di VEQ che prevede 4 esercizi di due campioni ciascuno, al quale nel 2019 aderiscono 20 laboratori, 164 POCT.
Scopo: Valutare la variabilità analitica ed il livello di armonizzazione di tre POCT: Nova Biomedical Nova Pro (NP), Roche Accu-Check Inform (ACI), Abbott Freestyle Optium (FO). Esula da questo studio la valutazione dell'accuratezza.
Materiali e Metodi: Sono stati analizzati i dati relativi a 24 campioni di controllo liquidi preparati da siero umano, con il seguente intervallo di concentrazione: NP (n=17±11) 65-226 mg/dL; ACI (n=81±8) 77-274 mg/dL; FO (n= 38±10) 77-284 mg/dL. E' stato calcolato il CV% medio per POCT, considerando le specifiche di qualità per l'imprecisione della glicemia (<2,8% livello desiderabile, des. e <4,2% livello minimo, min). E' stato inoltre calcolato il n. di prestazioni accettabili riscontrate in base ai Limiti di Accettabilità (LA) scelti nella VEQ: per valori <100 mg/dL LA= 12 mg/dL, per valori >100 mg/dL LA =12,5%.
Risultati: CV% medio e (range): NP= 3,97 (0,41-11,5), 24 campioni: 11 des., 3 min., 10 non accettabile (na); ACI= 2,07 (1,30-3,12): 24 campioni: 20 des., 4 min.; FO= 8,55 (0,55-14,7), 18 campioni: 4 des., 1 min., 13 na.
Prestazioni accettabili: NP= 95,1%; ACI= 99,9%; FO=84,5%.
Differenze mediane tra i POCT: NP vs ACI= -18,5±10,1 mg/dL (-12,4±2,9%); FO vs ACI= 10,3±10,5 mg/dL (6,4±6,8%); NP vs FO= -30,1±17,9 mg/dL (-18,0±5,6%).
Discussione e Conclusioni: La variabilità analitica è risultata molto contenuta per ACI, mediamente accettabile per NP e molto ampia per FO. NP tende a sottostimare i valori di glicemia vs ACI mentre FO tende a sovrastimarli. Il Programma di VEQ permette di definire lo stato dell'arte delle prestazioni dei glucometri ed è uno strumento indispensabile al Laboratorio per la loro "gestione centralizzata".

P003

## DIAGNOSTIC AND PROGNOSTIC ROLE OF eEF1A IN CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background: Chronic lymphocytic leukaemia (CLL), the most common form of leukaemia in adults in Western countries, is characterized by the clonal expansion of B cells. Despite major advances in CLL therapy/diagnosis, the medical approach to CLL can be further improved. Here we explore the potential diagnostic/therapeutic role of the elongation factor 1 A (eEF1A) in CLL. Two major isoforms of eEF1A proteins exist: the ubiquitous eEF1A1 and the tissue-specialized eEF1A2. Beside their role in the elongation step of translation, both isoforms are involved in different cellular processes such as cell proliferation and apoptosis. Whereas both eEF1A isoforms play a role in solid and hematologic human tumors, nothing is known in CLL.

Methods: eEF1A1/eEF1A2 amounts were quantitated by quantitative real time PCR and western blotting in the lymphocytes of 46 CLL patients vs 26 normal control. eEF1A1 functional role in CLL was investigated in a cellular (MEC-1) and in a subcutaneous xenograft animal model of CLL via its targeting by an aptamer (GT75) or a siRNA (siA1), we previously developed. As control molecules an inactive aptamer (CT75) or siRNA (siGL2) were used.

Results: At the mRNA level, eEF1A1 but not eEF1A2 was significantly (p=0,0081) more elevated in CLL lymphocytes compared to control. At the protein level, both eEF1A1 and eEF1A2 were more elevated (p=0,028) in CLL lymphocytes compared to control. Moreover, eEF1A1 but not eEF1A2 protein levels were higher (p=0,0042) in patient which died during the study compared to those surviving. Finally, eEF1A1 targeting by either GT75 or siA1 resulted in MEC-1 viability down regulation (p=0,04) mostly due to autophagy stimulation.

In vivo, GT75 or siA1 resulted in tumor growth down-regulation (p=0.014) and extension of animal survival (p=0.014), demonstrating the functional role of eEF1A1 in CLL.

Conclusions: The increase of eEF1A1/eEF1A2 protein in lymphocytes of CLL patient cells suggests a role as possible novel CLL markers. The increase of eEF1A1 protein in dead vs surviving patients may confer to eEF1A1 also the role of a novel prognostic marker. This, together with the involvement of eEF1A1 in MEC-1 survival in vitro and in vivo, opens the possibility to consider eEF1A1 also as a novel therapeutic target in CLL.

P004

## KLK3, RASA1 AND NAALADL2 GENES POLYMORPHISMS: PROSTATE CANCER RISK, AGGRESSIVENESS OF NEOPLASIA AND SERUM PSA LEVELS

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Background: prostate cancer (PCa) represents the third cause of death for cancer among males.We aimed to verify if serum PSA levels and PCa risk/aggressiveness are modulated by polymorphisms of KLK3, RASA1 and NAALADL2 genes.

Methods: we studied 1.058 men who consecutively underwent prostate biopsy for clinical suspicion of prostate cancer. At histology PCa was present in 401 cases and absent in 657. Gleason score distribution in PCa patients was: ≤ 6 (n=261); = 7 (n=83) and >7 (n=57). Serum tPSA and f/tPSA levels were determined. Four polymorphisms were studied: rs35148638 (RASA1), rs78943174 (NAALADL2), rs2735839 and rs17632542 (KLK3).

Results: All polymorphisms were in Hardy-Weinberg equilibrium. PCa diagnosis at logistic regression model was significantly predicted by the KLK3 rs17632542 polymorphism (p<0.001) and tPSA (p<0.001) and f/tPSA (p<0.001). Patients bearing the KLK3 rs17632542C rare allele had a significantly higher risk of PCa diagnosis (p<0.001) (OR 2.1, 95% CI 1.40-3.19). Gleason score ³7 was associated with increased tPSA (p<0.001), decreased f/tPSA (p<0.003) and the KLK3 rs2735839 polymorphism: the rs2735839A rare allele was present in 22.3% and 37.9% of patients with Gleason score=6 and Gleason score ³7, respectively. In controls, tPSA was significantly lower in subjects bearing NAALADL2 rs78943174T rare allele (p<0.05). f/tPSA was higher in subjects with the KLK3 rs17632542C or rs2735839A rare alleles (p<0.001) and with the RASA1 rs35148638 C/C genotypes (p<0.01).In PCa subjects, tPSA was not associated with polymorphisms studied.

Conclusions: KLK3 rs17632542 and rs2735839 polymorphisms were significantly associated with the risk and aggressiveness of PCa respectively. tPSA and f/tPSA serum levels are genetically modulate respectively from NAALADL2 polymorphism and from KLK3 rs17632542 and RASA1 polymorphisms, in absence of tumour. These results suggest a potential role of these polymorphisms as biomarkers for PCa in association with the diagnostic and prognostic indexes currently recognized.

P005
**EMERGING ROLE OF MONOCYTES AND OF THEIR INTRACELLULAR CALCIUM CONTENT IN SPONDYLOARTHRITIS**

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Background. The Spondyloarthritis (SpA) are a group of a multifactorial diseases characterised by a complex interplay between an inherited background and environmental factors that lead to immune response dysregulation and inflammation of the joints. In SpA patients, macrophages infiltrating the inflamed joints, express inflammatory cytokines, enzymes causing tissue remodelling and other inflammatory molecules. The SpA synovial tissue is characterized by an increased vascularization and an infiltrate composed of nucleated polymorphs, macrophages and lymphocytes. In these cells calcium signals are essential for various cellular functions.
Aims: The aims of this work are to investigate whether TNF-$\alpha$, IL-1$\beta$, TGF-$\beta$, S100A8, S100A9, MMP3, MMP8 and MMP9 mRNA expression levels and intracellular calcium ($[Ca^{2+}]_i$) fluxes variations in PBMCs might be associated with SpA.
Methods: The study population comprised 64 patients with a diagnosis of SpA (26 had diagnosis of Ankylosing Spondylitis (AS), and 38 had a diagnosis of Psoriatic Arthritis (PsA)) and 100 healthy controls. Relative quantification (Real Time PCR) of TNF-$\alpha$, IL-1$\beta$, TGF-$\beta$, S100A8, S100A9, MMP3, MMP8 and MMP9 mRNA were performed. $[Ca^{2+}]_i$ fluxes were studied in patients and controls monocyte cells by a fluorescent microscope.
Results: mRNA expression levels in PBMCs of TNF-$\alpha$, IL-1$\beta$, TGF-$\beta$, MMP8 and MMP9 were similar in AS and PsA patients when compared to controls. S100A9 mRNA expression did not vary, the expression of S100A8 ($F=3.29$, $p=0.039$) was reduced in PsA patients. S100A8 and S100A9 expression levels were significantly correlated with circulating inflammatory cells and S100A8 was correlated with CRP and ESR. Monocytes from healthy controls had evident and frequent ($[Ca^{2+}]_i$) oscillations, while SpA patients monocytes did not. The percentage of cells exhibiting ($[Ca^{2+}]_i$) oscillations profile was significantly lower in AS with respect to controls ($F=6.15$, $p=0.003$). Conclusions: SpA associates with a reduced expression of the inflammatory S100A8 calcium binding protein and with a decreased intracellular calcium fluxes in patients' cells compared to healthy subjects, suggesting that the presence of the disease affects the "on-off" mechanisms that regulate the concentration of intracellular calcium.

P006
**VITAMIN D PREVENTS PANCREATIC CANCER-INDUCED APOPTOSIS SIGNALLING OF INFLAMMATORY CELLS**

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Background: Epidemiological studies suggest a potential role of Vitamin D (Vit D) in decreasing PDAC incidence and this might be consequent to Vit D regulation of anticancer immune response. In PDAC microenviroment cancer cells might shape immune cells, this being partly correlated with the cancer cells mutational status, mainly with SMAD4 deletion.
Aims. To verify whether VitD has any impact on intracellular signalling pathways of cancer cells and of cancer cells-shaped immune cells focusing on inflammation (NF-kB, STAT), proliferation (Akt, MAPK), apoptosis (Caspases) and TGF-beta pathways.
Methods: The PDAC cell lines BxPC3 (HD for SMAD4) and SMAD4 transfected BxPC3 (BxPC3-SMAD4+) were used. PBMCs were cultured for 24 hours in control medium (RPMI + 10 % FCS), in BxPC3 and BxPC3-SMAD4+ conditioned media (CM). Both cancer cell lines and PBMCs were cultured in the presence or absence of VitD (Calcipotriol, 100 nM). Total protein lysates were used for western blot analysis of: p-Akt (Ser473,Thr308), Caspase (Cleaved Caspase 3 and Cleaved Caspase 8), NF-kB (p-p65 (Ser536), p-IkB-a (Ser32)), MAPK (p-Erk 1/2(Thr202/Tyr204)), STAT-3 (p-STAT-3(Tyr705)) and TGF-beta.
Results: VitD did not exert any effect on the studied signalling pathways when PDAC cell lines were examined. Both BxPC3 and BxPC3-SMAD4+ CM reduced in PBMCs the NF-kB and STAT inflammatory pathways (reduced IkB-a, reduced p65 and STAT-3 phosphorylation), this effect being partly reverted by VitD, which targeted the NF-kB pathway. In PBMCs, the Akt and MAPK pathways, involved in cellular proliferation, were not influenced by PDAC CM nor by VitD. In PBMCs, BxPC3 and BxPC3-SMAD4+ CM caused a significant activation of apoptosis signalling by activating Caspase 8, not Caspase 3. VitD treatment inhibited this effect. In PBMC, the expression of TGF-beta was not affected by CM or VitD.
Conclusions: Pancreatic tumor CM, independently from genetic mutations, inhibit inflammatory pathways while favouring the induction of the apoptosis signalling pathways in immune cells. Vitamin D treatment might reduce this cancer related effect which results in the Vitamin D potential therapeutic role.

P007
**JAK2 LOW ALLELE BURDEN: A MONOCENTRIC EXPERIENCE**

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Introduction: The JAK2V617F mutation is detected in most (95%) of patients with polycythemia vera (PV) and in about 60% of myelofibrosis (MF) and essential thrombocythemia (ET) patients. We tested 749 subjects for JAK2V617F mutation between 2009 and 2019. We submit clinical and biological data from 26 patients with low (0.05-1.8%) JAK2V617F allele burden (AB).Prospective molecular monitoring was performed in a low AB-patient at a 12-month and 24-month follow-up (FU)
Matherials and methods: DNA was extracted from granulocytes of peripheral blood using QIAmp DNA Blood Minikit (Qiagen). JAK2V617F allele was detected and quantified with Ipsogen JAK2 MutaQuantKit (Qiagen). The RQ-PCR was performed with Applied Biosystem 7500 RT-PCR System using TAQman Universal PCR Master Mix (Thermo Fisher). The results can be: POSITIVE: beyond the critical threshold for positivity (0.50% V617F) and beyond LOQ (Limit of Detection, 0.1%) threshold. NEGATIVE: if a case underneath the critical threshold for positivity is encountered (0.05%).
Results: a low AB (%) resulted in 26 patients (3,5%) in our Centre: 21 with 0.1<AB<1.8 and 5 with 0.05<AB<0.08. 19 patients having 0.1<AB<1.8 received a diagnosis of MPN according to WHO 2008 criteria (3 MF, 11 ET, 5 PV), and 2 a diagnosis of Myelodysplastic Syndrome. We study particularly patients with 0.05<AB<0.08. Among these, 3 patients had a diagnosis of ET by bone marrown histology; 1 of them had CALR mutation. Another patient evolved in MF after 7 years. In this patient, the JAK2 FU showed a decreased AB (0.05%), even if the patient was treated with Anagrelide. The fourth patient was studied with 2 FU (12-24 months) of JAK2 and an increased ratio to 0.65%. No mutation in CALR and MPL genes was found; the Next Generation Sequencing detected JAK2 GGCC (46/1) haplotype. The last patient had a diagnosis of non-Hodgking Lymphoma; a new evaluation had determined a reduction of AB.
Conclusions: Literature data and our experience suggest the need to study the patients whose AB JAK2 falls beyond the threshold for analytical positivity, but underneath LOQ (0.05<AB<0.1), because the repetition of the molecular test over time oriented the diagnostic process with a non invasive method.

P008
**IDENTIFICAZIONE DEI SOGGETTI CON TRAIT TALASSEMICO: NON SEMPRE L'INDAGINE DI 1° LIVELLO È SUFFICIENTE**

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È pervenuta alla nostra osservazione una donna (A.A.) di 30 anni, gravida alla 11$^{a}$ settimana, per sottoporsi a diagnosi prenatale da villi coriali in quanto dalle indagini biochimiche è risultato che la suddetta presenta un trait beta talassemico (eritrociti: 5,45x10$^{6}$/ul, emoglobina: 11,9 g/dl, MCV: 68,8 fl, MCH: 21,7 pg, HbA2: 5,2%, HbF: 2,3%); mentre il marito mostra un quadro biochimico (eritrociti: 5,81x10$^{6}$/ul, emoglobina: 14,9 g/dl, MCV: 77,4 fl, MCH: 25,6 pg), ma soprattutto un tracciato elettroforetico dell'emoglobina che, in corrispondenza del picco dell'HbA2, presenta una variante presumibilmente riferibile all'Hb Lepore (HbA2: 9,5%, HbF: 2,5%).
L'analisi molecolare dei geni beta globinici effettuata sulla signora A.A. ha messo in evidenza la mutazione IVS1.110 G>A (c.93-21G>A) in eterozigosi, mentre il marito presenta la mutazione Hb Lepore-BW in eterozigosi. In questi casi, onde evitare la presenza concomitante di mutazioni dei geni alfa e beta globinici che possono bilanciare il quadro biochimico, è nostra consuetudine eseguire anche lo studio dei geni alfa globinici. Lo studio molecolare dei geni alfa globinici ha accertato che il signor M.P. presenta la delezione -4.2 che determina la perdita di un solo gene $\alpha$ ed un fenotipo $\alpha$+ ($\alpha^{-4.2}\alpha/\alpha\alpha$) detto anche $\alpha$2Tal; mentre la signora A.A. non presenta mutazioni nei geni alfa globinici. In conclusione possiamo affermare che il caso presentato esprime i limiti degli esami di 1° livello. Quindi, vi sono casi specifici dove risulta indispensabile lo studio molecolare dei geni alfa e beta globinici. Nel nostro caso lo studio molecolare dei geni alfa e beta globinici ha permesso prima di tutto di porre una corretta correlazione genotipo-fenotipo ed in secondo luogo ha permesso di poter calcolare l'esatto rischio di ricorrenza di procreare non solo figli affetti da beta talassemia ma anche di comprendere la possibilità di avere figli che presentano entrambi i deficit molecolari (probabilità: 12,5% $\alpha\alpha\alpha\alpha/\beta\beta$; 25% $\alpha\alpha\alpha\alpha/-\beta$; 12,5% $\alpha$-$\alpha\alpha/\beta\beta$; 25% $\alpha$-$\alpha\alpha/-\beta$; 12,5% $\alpha\alpha\alpha\alpha/$--; 12,5% $\alpha$-$\alpha\alpha/$--).

P009
## LE IPERCOLESTEROLEMIE, UTILITÀ DELLA ESATTA CORRELAZIONE GENOTIPO-FENOTIPO

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È pervenuto alla nostra osservazione un giovane ragazzo (M.G.) di 13 anni, che presentava in maniera persistente i seguenti valori biochimici: colesterolemia 292,0 mg/dl, colesterolemia HDL 48,0 mg/dl, colesterolemia LDL 226,8 mg/dl e trigliceridemia 63,0 mg/dl. Inoltre, da una anamnesi effettuata è risultato che il padre del ragazzo presentava una ipercolesterolemia di n.d.d. In passato il ragazzo pur avendo eseguito una terapia dietetica non aveva avuto una riduzione significativa della colesterolemia. Da quando osservato si era postulato che, molto probabilmente il giovane M.G. fosse affetto da "ipercolesterolemia poligenica comune". Per comprendere l'esatta correlazione genotipo-fenotipo si è proceduto all'amplificazione e sequenziamento high-throughput con NimbleGen SeqCap Target Enrichment kit (Roche) su piattaforma illumina dei seguenti geni: gene del recettore delle lipoproteine a bassa densità (LDL-R) associato ad ipercolesterolemia familiare autosomica dominante tipo1 (FH-1), gene dell'apolipoproteina B-100 (ApoB) associato a ipercolesterolemia familiare da difetto di ApoB-100 (FDB o FH-2), gene di PCSK9 con alterazioni della normale funzione dell'enzima proteolitico PCSK9 (guadagno di funzione) associato a ipercolesterolemia familiare autosomica dominante tipo 3 (FH-3), gene LDLRAP1 o ARH (LDL receptor adaptor protein) associato a ipercolesterolemia familiare Autosomica recessiva. L'analisi genomica ha rilevato la presenza della variante genomica c. 1694G>T in condizione di eterozigosi nel gene LDL-R che a livello proteico determina la variante p. Gly565Val (rs28942082). La variante genomica identificata è descritta in letteratura scientifica come associata ad "ipercolsterolemia familiare autosomica dominante tipo1 (FH-1)" ed è classificata nei database di riferimento come variante patogenetica (Clin Var ID-3688). Tale alterazione genetica si trasmette con modalità autosomica dominante. La conseguenza di questo difetto è rappresentata dalla riduzione dell'uptake cellulare delle LDL con il conseguente aumento delle concentrazioni plasmatiche del colesterolo LDL (C-LDL). L'identificazione precoce dei pazienti con FH1 può essere utile per stabilire una adeguata terapia e prevenire complicanze cardiovascolari. La terapia di scelta prevede l'utilizzo delle statine (atorvastatina e rosuvastatina) alla massima dose, in monoterapia, od in associazione ad ezetimibe se necessario per avvicinarsi al target di C-LDL (riduzione del C-LDL almeno del 50%). Nel nostro caso, lo studio molecolare dei geni implicati nel metabolismo del colesterolo ha permesso prima di tutto di porre una corretta diagnosi differenziale ed in secondo luogo ha permesso di poter correlare il difetto molecolare con il quadro clinico osservato in modo tale da stabilire l'esatto follow up da seguire ed il calcolo esatto del rischio di ricorrenza.

P010
## MUTATIONS IN CALRETICULIN GENE (CALR) : MOLECOLAR CHARACTERIZATION AND CLASSIFICATION OF MYELOPROLIFERATIVE NEOPLASM (MPN)

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In the last years there has been an improved interest in molecolar characterization and classification of myeloproliferative neoplasm (MPN) and target therapy. Mutations in Calreticulin gene (CALR) could be considered as a new marker in diagnosis of MPN, have been reported in most patients with essential thrombocytemia (ET) and idiopathic myelofibrosis (MF). It's important to underline that, patients with negative JAK2 V617F mutations, are often associated mutations with CALR ans MPL genes, particulary in the receptor binding region. Therefore, detecting both mutations in negative JAK2 patients can allow a correct diagnosis of MPL.

In this study, we plan to analyze CALR and MPL mutations in suspect myeloproliferative neoplasm with negative BCR-ABL (Philadelphia chromosome) and JAK2 mutations. A second purpose is to identify a possible association between mutations types ; type1 and type 2 for CALR mutation, allelic status for MPL positive patients and the risk of hematologic malignancy progression.

We have been enrolled 98 patients, 22 of them have the BCR-ABL rearrangement (22,4%) and were diagnosed as CML (Chronic Myloide Leukemia) which is not included in this evaluation. 76 patients (32 males and 44 females, age: 5-82 ; average: 53,43 ; median: 58) are subjects for a molecular analysis of JAK2, CALR ans MPL genes.

From peripherical blood samples, DNA and RNA were extracted and used for molecolar studies. BCR-ABL transcript and JAK2 mutation analysis had already been done for all samples, by qualitative and quantitative PCR (Polymerase Chain Reaction) with allelic discrimination. Other cases were analyzed for CALR type 1 and type 2 mutation using a homemade kit and a commercial kit for W515 L/K MPL mutations. Only negative samples for JAK2 mutation were examined for CALR and MPL mutations.

Among 76 cases which don't carry BCR-ABL rearrangement, we found 21 cases hearboring JAK2 V617F mutation (29,57%) ; 7 cases (8,21 %) have type 1 CALR mutation, and one case carry type 2 CALR mutation; Furthermore two cases with (2,05%) W515 L MPL mutation.

The CALR gene screening, in qualitative PCR is practically rapid, sensitive and can show all deletions and insertions of CALR gene, later confirmed with sequencing analysis. The evaluation of quantitative allelic discrimination in MPL (ratio FAM/VIC) could be used in the monitoring of minimal residual diseases (MDR). The proliferative increase of bone morrow, complications and risk of thrombotic and embolic occurrence are lower in CALR mutated patients than those in JAK2 V617F mutated patients.

P011

## IDENTIFICATION OF BIOLOGICAL MARKERS IN PATIENTS WITH CHRONIC LYMPHATIC LEUKEMIA: MOLECULAR ANALYSIS AND PROGNOSTIC IMPLICATIONS

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Chronic lymphocytic leukemia (CLL) is a genetically heterogenous disease characterised by genomic alterations and gene mutation that may portend worse survival or resistence to treatments. We analyzed a monocentric real life cohort of 211 unselected CLL patients with a long term follow up (median: 10 yrs; range: 1-29). All patients, have been observed in our institution from 01/1988 to 07/2018; 136 were male and 75 female; median age at diagnosis was 63 (37-88). In this work we tried to correlate known biological factors (FISH, LDH,) with other innovative ones (NOTCH1). Despite the small numbers and the retrospective characteristics of data collection we are able to make some interesting observations. In particular, also in our series the tris12 is significantly correlated with the increase of LDH values (diagnosis 612 U/L range 193-1188 pre-treatment 850 U/L range 220-1759), distinguishing itself from the other FISH anomalies. NOTCH1 alleles were investigated in 146 patients, with NOTCH1 mutation in 22 patients (15%). Furthermore, approximately 50% of patients with tris12 are also characterized by a NOTCH1 mutation. A further field of investigation was to verify the incidence of NOTCH1 mut in the different FISH sub-categories identified, in order to verify the known correlation between NOTCH1 and tris12 mutations and any other possible associations 46% of patients with tris12 also had a NOTCH1 mutation, in line with what was reported in the most recently published literature. In the other sub-categories the percentage association was the following:FISH neg: 25.7%, TP53 +: 25%, Del11: 20%, Complex mutations: 12.5%,Del13: 10.5%, Del17: absent. Then we confirm the prognostically unfavorable value of the NOTCH1 mutation, with worsening of the overall survival outcome. This unfavorable prognostic impact is further evident in the IGVH unmutated / NOTCH1 mut subgroup. In conclusion the desirable future is represented by the introduction and validation of new prognostic markers - among these NOTCH1 and others currently under study - which will constitute a valid instrument of evaluation and prognostic stratification together with what has already been used in clinical practice. The recent implementation of new biological diagnostic investigations and their evolution in the field of onco-hematological pathologies are able to ensure increasingly innovative investigation tools. For this reason we are sure that the integrated clinical and biological evaluation can constitute an essential synergy with a common goal, that is the offer of the best diagnostic-therapeutic strategy in a field in continuous evolution.

P012

## CONVALIDA DELLE STRUMENTAZIONI DI LABORATORIO NEI CENTRI DI QUALIFICAZIONE BIOLOGICA: USO DELLE PREPARAZIONI DI RIFERIMENTO DELL' ISS PER LA RICERCA DEL WNV

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Premessa: La convalida delle strumentazioni analitiche di Laboratorio è una procedura che,attraverso l'evidenza documentata, dimostra che un'apparecchiatura fornisce una prestazione conforme alle specifiche dichiarate dalle Ditte produttrici.Per i Servizi Trasfusionali tutto ciò è regolato dal Decreto 300 del 28.12.2015 il quale dà disposizioni sui requisiti di qualità e sicurezza del sangue. Il protocollo redatto ed approvato per ogni strumento utilizzato prevede l'individuazione delle modalità di esecuzione della qualificazione del processo.Scopo del lavoro è illustrare la Convalida del metodo analitico per la ricerca del WNV,un Arbovirus ad RNA costituito da 2 ceppi(Lineaggio 1 e Lineaggio 2) che si trasmette con un ciclo enzootico tra insetti ematofagi (zanzara) e ospiti Vertebrati (uccelli); l'uomo è un ospite occasionale.
Materiali e Metodi: Sono state utilizzate N° 10 preparazioni di Riferimento per ogni Lineaggio fornite dall'ISS positive per il marcatore analitico in esame a concentrazione nota per definire il Detection Limit della metodica,per allestire studi di Proficiency e per convalidare gli operatori. E' stato convalidato lo Strumento Cobas 6800 System Roche che impiega una tecnologia Real Time PCR,totalmente automatizzato per il dosaggio dell'RNA del WNV. Sono stati valutati,secondo le linee guida del CNS, i seguenti parametri: Ripetibilità(esecuzione di N°3 sedute analitiche,ciascuna costituita da 5 repliche delle preparazioni e in 3 giornate diverse),Accuratezza (analisi di N°10 campioni positivi e N° 10 campioni negativi per WNV),Contaminazione(analisi di N° 10 campioni positivi e N°10 campioni negativi distribuiti in maniera alternata),Sensibilità Analitica(analisi delle preparazioni diluite 6x95% LOD,3x95%LOD,1x95%LOD,0.5x95%LOD per ogni Lineaggio).
Risultati e Conclusioni: L'analisi dell'accuratezza diagnostica per la strumentazione in esame ha mostrato Sensibilità e Specificità del 100% e non è stata riscontrata contaminazione nel corso delle sedute analitiche; il LOD dichiarato dalla Ditta è stato rispettato con qualifica dello Strumento.

P013

## PROTEOMIC AND METABOLOMIC STUDY FOR BIOMARKERS DISCOVERY IN UROLITHIASIS

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Introduction Urolithiasis, the process of stone formation in the urinary tract, is a common disease with an increasing prevalence and up to 50% of patients may have a relapse over the years. The etiology is only partly known and the main problem is the lack of efficient therapeutic pharmacological approaches. Proteomics and Metabolomics are powerful tools to identify specific biomarkers that could represent new drug targets.The aim of this pilot study is to propose a novel approach, based on “omics” methods, to improve the quality of urinary calculus analysis and to provide insights on the etiology of urolithiasis.

Material and Methods Samples of 8 renal calculi with different composition (calcium oxalate, uric acid, brushite and struvite) were characterized with morpho-constitutional analysis. Bottom-up analysis were performed on an ACQUITY MClass System coupled to High Definition Synapt G2-Si Mass spectrometer. Data were analyzed using software PANTHER Classification System and STRING (Search Tool for the Retrieval of Interacting Genes/Proteins). For metabolomics analysis a panel of 36 amino acids and derivatives was assessed in the urine of 15 patients compared to 15 healthy subjects by UPLC-MS. Univariate (Mann Whitney U test) and multivariate (PLS-DA) statistical analysis were performed to identify significant results.

Result and Discussion Results from proteomics analysis revealed a peculiar protein profile for each type of stone and only a fraction of matrix proteins were common to all types. Most of the matrix proteins involved in inflammatory process and oxidative stress are present in oxalate calcium and acid uric kidney stones. Proteins related to coagulation process are prevalent in the matrix of brushite stone. Finally, proteins involved in immune response signaling pathway were identified in struvite stone. The results of metabolomics study show a statistically significant difference between patients and healthy subjects. Compared with healthy subjects patients with urolithiasis have a significant decrease in urine concentration of amino acids (alanine; alpha amino butyric acid; arginine; asparagine; cystine; ethanolamine; glycine; histidine; isoleucine; leucine; lysine; methionine; phenylalanine; serine; taurine; threonine; tryptophan; tyrosine ; valine). Furthermore, PLS-DA model allows a correct classification of 76±5.1% of the patients with urolithiasis and.75.3±4.1% of healthy subjects. This preliminary proteomic and metabolomic data, in addition to clinical information, allow the clinicians to better understand the molecular signature of the patient and, in perspective, this approach may help to monitor the disease progression.

P014
## NEXT GENERATION SEQUENCING IN PATIENTS AFFECTED BY EARLY ONSET PARKINSON'S DISEASE

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Parkinson's disease (PD) is a neurodegenerative disease affecting the basal ganglia with progressive neuron death in the substantia nigra with reduction of the dopamine neurotransmitter production. The average age of onset is commonly between 60 and 70 years, but about 10% of the patients show early onset in the fourth decade of life. To date, 28 distinct chromosomal regions related to PD are discovered, but only few genes are known to associate conclusively with the pathology. In this study we designed and validated a new targeted-NGS method using the Ion Torrent PGM™ platform to analyze 36 patients enrolled by the Parkinson center of University of Brescia/Spedali Civili of Brescia. The genes studied in the panel were: SNCA, PARK2, PINK1, PARK7, LRRK2, FBXO7, GBA, and HFE gene that we previously studied in PD population to verify the correlation with brain iron overload. The panel included 268 amplicons and the target region was 48.370 Kb with "in silico" coverage of 95.5%. 1,749 bases in 7 of the 8 genes included in the panel were not covered and were analyzed with Sanger sequencing. The regions covered with less than 20X reads were also analyzed by Sanger. Comparison between NGS and Sanger sequencing showed that all SNPs and variants were correctly called with specificity and sensitivity equal to 100% in the validation phase; for this purpose we tested 3 controls and 1 PD patient carrying the pathogenic mutation c.4321C> T (p.R1441C) in the LRRK2 gene. In patients' cohort we found: a new heterozygous variant in SNCA gene (c.50C>A, p.A17D) predicted as deleterious by PolyPhen software, 1 rare heterozygous VUS in PARK2 gene c.310C>T p.R104W, 1 heterozygous causative mutation in LRRK2 (c.6055G>A G2019S), 3 heterozygous variants in GBA gene (c.1093G>A E365K, c.1226A>G N409S, c.1448T>C L483P) known as risk factors. Two variants were called as false positive respectively in LRRK2 and FBXO7, and one as false negative in GBA. All these anomalies were verified and solved using IGV software and Sanger sequencing.In conclusion, a new NGS panel to verify genetic predisposition in early onset PD was developed. This method shows very good metrics, high specificity and sensitivity and excellent reproducibility.

P015
## URINARY ALBUMIN TO CREATININE RATIO IN DIAGNOSIS AND RISK STRATIFICATION OF RENAL AL AMYLOIDOSIS

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The measurement of 24 hour proteinuria (24hUP) is fundamental for the assessment of renal involvement in patients with AL amyloidosis. However, it is cumbersome and prone to errors. The urinary albumin to creatinine ratio (UACR) has been proposed as an alternative to measure urine protein loss. We evaluated the performance of UACR in diagnosing renal involvement and predicting renal outcome in 436 newly diagnosed (2013-2017) patients with AL amyloidosis. Pearson's r test was performed to evaluate correlation between UACR and 24hUP. UACR cutoffs were identified by ROC analysis. Sixty-two percent of patients had kidney involvement [24hUP >0.5 g/24h (predominantly albumin)]. Renal stage based on 24hUP (cutoff 5 g/24h) and estimated glomerular filtration rate (eGFR, cutoff 50 mL/min per 1.73 $m^2$) was I in 49% of cases, II in 38%, and III in 13%. Median (interquartile range) 24hUP and UACR were 1.4 g/24h (0.23-6 g/24h) and 1921 mg/g (66-5655 mg/g), respectively. There was a strong correlation between 24hUP and UACR (Pearson's r = 0.90, 95%CI: 0.87-0.92). The best UACR cutoff for the diagnosis of renal involvement (defined as 24hUP >0.5 g/24h) was 600 mg/g (area under the ROC curve 0.96, 95%CI: 0.94-0.98; sensitivity 90%; specificity 98%). The definition of renal involvement with 24hUP and UACR was concordant in 93% of cases. After a median follow-up of living patients of 24 months, 46 (11%) required dialysis. The UACR cutoff best discriminating patients who required dialysis at 24 months was 3500 mg/g. This was used to substitute the 24hUP cutoff in the renal staging system. There was a 83% concordance in renal staging with the 24hUP and the UACR based staging systems. Both staging systems discriminated 3 groups with significantly different rate of progression to dialysis. A >30% decrease in UACR best predicted dialysis at 12 months, which is used to define renal response in the absence of >25% decrease in eGFR. Patients who achieved a renal response, defined either per standard criteria (based on a >30% decrease in 24hUP) or using the novel UACR criteria, had a significant lower risk of progressions to dialysis. These data indicate that UACR can be used to identify renal involvement, predict renal outcome and assess renal response in AL amyloidosis.

P016

## DIAGNOSTIC POTENTIAL OF CIRCULATING miRNAS IN OSTEOPOROSIS AND SKELETAL MUSCLE WASTING DISORDERS

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Introduction: Aging is a process characterized by several pathophysiological changes, including skeletal muscle wasting and loss of bone mineral density, that affect overall health status and quality of life. Early diagnosis, prevention and treatment of these often coexisting ageing-associated diseases should be necessary to improve the quality of life. Circulating miRNAs are emerging as a new class of noninvasive biomarkers that allow to discriminate between these two pathological conditions. Aim of this study was to identify circulating miRNAs associated with reduced muscle mass in a population of postmenopausal osteoporotic women.

Methods: Total free circulating miRNAs were extracted from plasma of 13 osteoporotic (O group) and 8 osteoporotic women with reduced skeletal muscle mass and appendicular skeletal muscle mass index (ASMMI) <5.45, (O-MW group). A panel of 179 miRNAs was assayed by RT-qPCR. The relative expression of each miRNAs was calculated by the $2^{-\#\#CT}$ method using, as normalizer, the average value of all expressed miRNAs. The results were analyzed by Exiqon GenEx software ver6. Only miRNAs with a statistically significant fold change $\geq \pm 2.5$ were considered for the diagnostic accuracy analysis. The ROC (receiver operating characteristic) curves were calculated for each single miRNAs and their combination. Spearman's rank correlation analysis was performed to correlate miRNAs, total skeletal muscle mass and ASMMI.

Results: Among the 179 circulating miRNAs, hsa-let-7c-5p, hsa-miR-127-3p and hsa-miR-339-5p were significantly lower in O-MW group compared to O group. hsa-let-7c-5p, hsa-miR-127-3p and hsa-miR-339-5p, singularly, revealed an AUC of 0.931 (p= 0.004), 0,826 (p=0.022), and 0.776 (p=0.038), respectively. Only the combination of hsa-let-7c-5p+hsa-miR-339-5p displayed a significant AUC of 0,786 (p=0.037). Furthermore in O-MW group, hsa-miR-885-5p negatively correlated with both total skeletal muscle mass and ASMMI (r= -0.805, p=0.017 and r= -0.756, p= 0.031, respectively).

Conclusions: From these preliminary results, hsa-let-7c-5p emerges as the miRNA with the best diagnostic potential to discriminate between O and O-MW women. Further analyses are required to validate the diagnostic applicability of circulating miRNAs in osteoporosis and skeletal muscle wasting.

P017

## DIFFERENCES IN CLINICAL PRESENTATION BETWEEN LIGHT CHAIN DEPOSITION DISEASE (LCDD) AND RENAL LIGHT CHAIN (AL) AMYLOIDOSIS: THE ROLE OF CLONAL AND ORGAN BIOMARKERS

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Monoclonal light chains can form fibrillar or non-fibrillar renal deposits in the two most common monoclonal gammopathies of renal significance (MGRS): light chain (AL) amyloidosis and light chain deposition disease (LCDD), respectively. LCDD is a rare condition and few small case series have been reported so far, while renal outcome in AL amyloidosis is better known and it is well evaluated at baseline with biomarkers [24h proteinuria and estimated glomerular filtration rate (eGFR)]. We compared a consecutive cohort of LCDD patients from three different centers [N=74: 61 diagnosed in Pavia, 8 in Padova (Italy) and 5 in Calgary (Canada)] and a series of 207 consecutive patients affected by renal AL amyloidosis followed at the Pavia Amyloid Center. Only stage I non-cardiac AL amyloidosis patients were included in order to avoid the confounding effect of heart involvement. Patients with LCDD had lower eGFR (32 vs. 70 mL/min, P<0.001) and lower proteinuria (2.6 vs. 6.2 g/24h, P<0.001) at baseline. The k free light chain (FLC) istotype was more frequent (85 vs. 23%, P<0.001) and the difference between involved and uninvolved FLCs (dFLC) was significantly higher in the LCDD-group (90 vs. 75 mg/L, P<0.001). Time to dialysis was longer in AL amyloidosis (median not-reached vs. 9 years, P<0.001) and progression to end stage renal disease was more common in the LCDD-group than in the AL-cohort (43% vs. 20%; P<0.001). In AL amyloidosis, renal outcome was predicted by the proteinuria (cutoff 5 g/24h)/eGFR (cutoff 50 mL/min) staging system. In LCDD patients eGFR (best cutoff 30 mL/min [HR 5.3, P<0.001]) but not proteinuria predicted dialysis. A profound reduction of dFLC after 6 months of treatment (cutoff 40 mg/L, which is the definition of very good partial response according to the International Society of Amyloidosis criteria) was associated with prolonged renal survival in both groups. LCDD is characterized by the presence of a FLC $\kappa$ clone in most of cases. Renal dysfunction is often severe at diagnosis and predicts renal outcome, but proteinuria is less prominent than in AL amyloidosis. Quantification of FLC has a prominent role in response assessment and a profound reduction of dFLC is associated with better prognosis.

P018

## DUODENAL TRANSCRIPTOME HIGHLIGHTS THAT MITOCHONDRIAL DYSFUNCTION IS ASSOCIATED WITH INFLAMMATION AND ENERGY IMBALANCE IN OBESITY

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Background: Over the past decades, obesity has emerged as a leading health problem worldwide being also a risk factor for several other diseases. Obesity causes several metabolic changes in the body, such as inflammation, oxidative stress, mitochondrial dysfunction and apoptosis. However, most of these observations are based on studies focusing on adipose tissue, while little is known on duodenal involvement. The duodenum is a tract of intestine involved in harvesting energy from nutrients and is emerging as a key player in metabolic diseases.

Aim: Our aim was to perform a transcriptomic analysis of duodenum samples obtained from obese patients and normal weight subjects in order to identify alterations in metabolic pathways and networks associated with obesity.

Materials and methods: We consecutively enrolled: A) 24 obese patients (12 females) (BMI=35.0-40.0 kg/m$^2$), and B) 23 normal weight age-matched control subjects (10 females) (BMI 20.0-24.9 kg/m$^2$), among those undergoing gastro-duodenal endoscopy. Total RNA was isolated from duodenal biopsies and sequenced by Next Generation Sequencing (Illumina, HiSeq1500-2500). Bioinformatic tools were applied to highlight differences in gene expression levels between obese and control groups.

Results: We found several differentially expressed genes, including NDUFA4, ATP5J2, COX7A2, NDUFB6, COX7C, NDUFAB1, NDUFC2, COX4I1, ATP5G1, COX7A2L, PPA2, PPA1, NDUFB2, UQCR11, NDUFS4, SDHD, and several genes encoding for ribosomal proteins in obese subjects. Metabolic prediction showed several metabolic impairments, in particular in relation to mitochondrial activity, oxidative phosphorylation and translational processes.

Conclusions: Globally, duodenal transcriptome obtained in our obese and normal weight subjects highlights that mitochondrial alterations are significantly associated with energy imbalance and inflammation observed in obesity.

P019

## ROLE OF A2AR DEFECTIVE SIGNALING IN EXACERBATING XENOBIOTIC-INDUCED INFLAMMATORY RESPONSE IN PATIENTS WITH MULTIPLE CHEMICAL SENSITIVITY

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Multiple chemical sensitivity (MCS) is a syndrome involving an aberrant susceptibility response to a broad range of physic-chemical and biological factors[1]. MCS presents with systemic manifestations, i.e. cognitive, musculoskeletal, skin-related, airway or mucous membrane symptoms, triggered by high-dose or chronic low-dose exposure to xenobiotics, heavy metals, radiations, iatrogenic factors, microbial and food allergens[2]. Systemic inflammation and immune activation are striking features of MCS[3]. It is known that the release of pro-inflammatory cytokines, as well as immune activation, can be inhibited by adenosine receptor 2A (A2AR) activation[4]. ADORA2A rs2298383 polymorphism affects the transcription rate of ADORA2A gene, coding for A2AR, and has been involved in aberrant immune activation[5]. Here we aimed to assess the distribution of ADORA2A rs2298383 genotypes in 156 MCS patients and 135 healthy subjects (CTR), and the transcript levels of ADORA2A, IL-8 and IFN-γ in peripheral blood mononuclear cells of randomly selected individuals with different genotypes (48 MCS, 39 CTR). Genotyping and transcriptomic analyses were made by Real-time PCR-based allelic discrimination and Sybr Green-based quantitative RT-Real-time PCR, respectively. Serum cytokine levels were assessed by ELISA.The ADORA2A rs2298383 TT mutated genotype was significantly more frequent in MCS than CTR subjects ($p<0.00001$) and proved to be a genetic risk factor for MCS (O.R.=3.44, C.I. 95% 2.111 - 5.623). Notably, ADORA2A gene transcript levels were reduced in subjects having the ADORA2A T mutated allele, and the lowest amount was found in MCS patients with TT genotype. Concomitantly, IL-8 and IFN-γ transcript and protein levels were significantly increased in TT subjects, and the highest amounts were found in MCS patients.

These findings confirm that A2AR defective signaling increases the individual susceptibility to inflammatory stimuli, and may play a relevant role in exacerbated inflammatory response observed in MCS patients upon excess environmental exposure to toxic agents.

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P020

## FRAILTY AND AGING THROUGH GENE EXPRESSION ANALYSIS

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Background: Aging is accompanied by multiple biological disarrangements and a higher clinical complexity, mining the sustainability of the healthcare services. Frailty, condition characterized by increased vulnerability to stressors due to a reduction of homeostatic reserves, has been indicated as a promising way for capturing the biological aging of the individual. The Frailty Index (FI) may optimally serve for this purpose estimating the biological aging through a quantitative approach to the age-related accumulation of health deficits. Use of the FI has been proven a valuable method for identifying older people at risk for increased vulnerability, disease, injury, and mortality. Given that frailty is age-related, we propose to generate a biological FI by a panel of biomarkers associated with the “hallmarks of aging” pathways. The biological FI might help to depict the relationship between frailty and the mechanisms of intrinsic aging and to predict adverse outcomes together with clinical FI.

Study design: We have designed an array (QuantStudio 12K Flex) to evaluate, at the same time, the gene expression of 50 genes underlying aging.We have analysed the gene expression of peripheral blood mononuclear cells (PBMCs) from 350 older persons (> 65 years old) characterized by different degree of “fragilization” evaluated by a multidimensional geriatric assessment.

Results: First, we have tested the ability of our array to grade the degree of cognitive impairment assessed by Mini Mental State Examination (MMSE) test.Interestingly, the MMSE correlates with expression of genes belonging to different pathways: collagen 6a2 ($\beta$=0.181 p=0.04) involved in muscle remodelling, ceramide synthase 2 ($\beta$=0.199 p=0.02) and HDL binding protein ($\beta$=0.170 p=0.05) in lipid metabolism, SYK ($\beta$=-0.193 p=0.02) in neuroinflammation, PIN1 ($\beta$=0.219 p=0.01) and insulin like growth factor 1 receptor ($\beta$=-0.208 p=0.02) in cellular homeostasis and serpin1 ($\beta$=0.197 p=0.01) in synaptic plasticity.

Conclusions: These preliminary data are able to suggest which biomarkers are pathognomonic of cognitive impairment and not to the individual’s age.The biological FI could provide a signature of frailty patients and could suggest clusters of patients characterized by specific pathogenetic mechanisms.

P021

## A WIDE NEXT-GENERATION-SEQUENCING PANEL IMPROVES THE MOLECULAR DIAGNOSIS OF DYSLIPIDEMIAS

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Introduction: Hyperlipidemias are common clinical conditions and important determinants of cardiovascular diseases. Among these, the Familial Hypercholesterolemia (FH) is the most frequent genetic disease with an autosomal dominant inheritance; Hypertriglyceridemia (HTG) has two forms, a rare monogenic form with autosomal recessive inheritance and a very severe phenotype, whereas polygenic form is more common and associated with a mild phenotype. The aim of this study was to evaluated the usefulness of a wide next-generation-sequencing panel of 25 genes involved in lipid metabolism for molecular diagnosis of dyslipidemias.Materials and Methods: We reported preliminary data about reanalysis of 13 patients (9 FH,4 HTG) previously analyzed only for causative genes. We selected patients carriers of different type of variants in different genes (single nucleotide variants (SNV) and copy number variations (CNV)), previously analyzed with Sanger sequencing and MLPA. DNA libraries were prepared using Agilent SureSelect target enrichment, and the sequencing was performed using Illumina MiSeq Reagent Micro Kit V2. Sequencing results were analyzed using Agilent SureCall and Agilent Alissa Align&Call and Intrepret. Results: All previously identified SNV and CNV variants were confirmed by NGS. Additional rare variants, in some cases never associated with dyslipidemias, were found in 12/13 patients, although the pathogenicity evaluation of these variants is still in progress. In particular, in a patient with severe HTG that carried only 1 pathogenic variant in APOA5 gene (causative of HTG), we found another pathogenic variant in APOB gene, described as associated with mild hypercholesterolemia. Conclusions: Our results confirm that this NGS-based method is able to detect different type of variants, including CNV. The identification of additional rare variants could be useful to better understand the genetic basis of dyslipidemias and could be useful for diagnosis and management of patients.

P022

## ANALISI MOLECOLARE DI PML/RARα NEI PAZIENTI CON LEUCEMIA PROMIELOCITICA ACUTA

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Introduzione: la leucemia promielocitica acuta (APL) è una rara forma di leucemia mieloide acuta, considerata un'emergenza medica, il cui marker molecolare distintivo è la traslocazione reciproca bilanciata t(15;17) con fusione del gene PML/RARα, target della terapia con acido all-trans retinoico (ATRA) con tasso di remissione completa del 90-95%.

Scopo del lavoro: valutare l'analisi dei PML/RARα risultati positivi nei campioni di sangue midollare e/o periferico nei pazienti con APL giunti al Laboratorio di Biologia Molecolare della U.O. Ematologia di Pisa nel 2018, con l'utilizzo di una nuova metodica.

Materiali e metodi: il protocollo prevede un isolamento cellulare con gradiente di densità specifico per linfomonociti, estrazione di RNA e sua retrotrascrizone in cDNA e PCR Real Time. Il primo metodo, utilizzato fino al 2018, ci da un'indicazione qualitativa della risposta (presenza/assenza della traslocazione), quello nuovo fornisce una risposta quantitativa con il numero di copie della traslocazione.

Risultati e conclusioni: lo studio di correlazione metodologica è da poco iniziato, al momento abbiamo analizzato i campioni di 15 pazienti (10 maschi e 5 femmine, età media 43 anni). Con il metodo quantitativo si valutano tutte e 3 le isoforme di PML/RARα a differenza del primo metodo che permette l'analisi delle 2 isoforme principali bcr1 e bcr3; tre pazienti infatti sono risultati positivi anche nell'isoforma bcr2 (la più rara). Per quanto riguarda il monitoraggio dell'efficacia del trattamento in pazienti sottoposti a terapia con ATRA, il metodo quantitativo ci permette di definire la stratificazione del rischio di recidive in base al numero di copie normalizzate di PML-RARα durante il follow up della malattia minima residua (MRD), cosa che non è possibile fare con il solo metodo qualitativo. Questi dati preliminari ci hanno permesso, dal 2019, di inserire nell'uso quotidiano il metodo quantitativo.

P023

## BIOPSIA LIQUIDA NEL CARCINOMA POLMONARE NON A PICCOLE CELLULE, MUTAZIONI A CARICO DI EGFR: ESPERIENZA DEL LABORATORIO DI GENOMICA DELLA AORN DEI COLLI, NAPOLI

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Il tumore del polmone rappresenta una delle prime cause di morte nei Paesi industrializzati, ed in particolare nel nostro Paese questa neoplasia è la prima causa di morte per tumore negli uomini e la terza nelle donne causando quasi 34.000 morti in un anno. La biopsia tissutale pone diversi problemi che ne limitano l'utilità come la difficoltà di acquisire quantità adeguate di tessuto, la limitata ripetibilità, gli errori di campionamento dovuti al prelievo di una piccola area di un singolo tumore e l'eterogeneità del tessuto. Uno dei metodi alternativi alle ripetute biopsie è rappresentato dalla biopsia liquida. La biopsia liquida, essendo un metodo a ridotta invasività, può essere ripetuta più volte richiedendo un semplice prelievo ematico ed è destinata ad integrare le tecniche di biopsia tradizionali. La rilevazione del DNA tumorale circolante (cfDNA) si rivela la metodica più completa. Il cfDNA può essere isolato sia nel siero che nel plasma dei pazienti affetti da neoplasie, in quanto in questi soggetti i livelli risultano essere più alti rispetto ai livelli rilevati nei soggetti sani. Lo studio è stato effettuato su 29 casi, 5 dei quali hanno evidenziato presenza di mutazioni. In tre pazienti si è evidenziata la presenza di mutazioni del gruppo delle delezioni dell'esone 19: è la più frequente tra le mutazioni EGFR riscontrate e dà la possibilità al paziente di essere idoneo alla terapia con inbitori della tirosin-chinasi. L'altra la L858R nell'esone 21 è stata riscontrata in due pazienti, uno dei quali aveva sviluppato anche la mutazione da farmaco resistenza T790M. Se venisse sempre più utilizzata la tecnica della biopsia liquida, i campioni di sangue potrebbero essere prelevati a intervalli più frequenti per valutare la risposta terapeutica del paziente; consentire di monitorare lo sviluppo di resistenze al trattamento; decidere tempestivamente eventuali modifiche dell'iter terapeutico; accelerare la calendarizzazione degli esami di diagnostica per immagini. Il cfDNA ha un'emivita breve (circa 2 ore) ed è quindi potenzialmente in grado di fornire un'istantanea precisa della carica tumorale nel momento del prelievo ematico. L'analisi del cfDNA potrebbe fornire una valutazione dinamica dei cambiamenti nella carica e nella genetica del tumore.

P024

## BIRT-HOGG-DUBÉ, FROM NON-INVASIVE DERMATOLOGIC ASSESSMENT TO GENE TESTING AND ULTRASTRUCTURAL HISTOLOGIC ANALYSIS, DEFINING THE ROLE OF FLCN IN RENAL AND SKIN CLINICAL FINDINGS

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The Birt-Hogg-Dubé syndrome (BHDS), firstly described in seventies, is a rare disease characterised by cutaneous manifestations like fibrofolliculomas, trichodiscomas, and others. The severity of the disease vary significantly, even within individuals of the same family, and due to this heterogeneity, the syndrome is often under diagnosed. The patients develop also bilateral lung cysts and pneumothorax with an high incidence. These type of lesions are characterized by cystic alveolar dilatations, located predominantly in basal or medial segments. A further important complication of BHD, is the association with renal cell cancer (RCC). These patients, differently to the sporadic RCC, shows an high association with multifocal or bilateral manifestation. The presence of pre-cancer cysts leads to kidney functional modification detected by clinical chemistry indicators. FCLN gene has been recently associated to this conditions, it encodes for a 64 kDa protein mainly localized in the nucleus and highly conserved across species. Its function is not completely elucidated, but it seems to act as tumour suppressor. In our report, we describe a case of a fibrofolliculoma affected patient, in which the genetic evaluation reported the presence of the p.Leu460Glnfs mutation in FLCN, leading to the diagnosis of BHD. Further Diagnostic imaging showed also the presence of lung cysts, clinical laboratory analysis showed kidney function abnormalities, and radiographic imaging confirmed the presence of renal cysts, thus completing the triade of BHD phenotypes. To investigate the role of the FCLN mutation in the pathogenesis of the skin phenotype we performed a deep pathological analysis using confocal immunofluorescence, leading to hypothesize a link between FCLN, mTOR and p63, has responsible of hyperplasia of follicular epidermal keratynocytes.

P025
**CARATTERIZZAZIONE MOLECOLARE DELLE MALATTIE LINFOPROLIFERATIVE DI TIPO B**

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Introduzione: i disordini linfoproliferativi di tipo B originano da cellule del sistema immunitario in diversi stadi di differenziazione, con il risultato di un'ampia gamma di aspetti morfologici, immunologici e clinici.
Scopo del lavoro: valutare l'espressione del "marker" IgH nei pazienti con malattia linfoproliferativa B, in particolare leucemia linfatica cronica, leucemia linfatica acuta B, mieloma multiplo, linfoma follicolare, linfoma mantellare, linfoma non-Hodgkin e linfoma diffuso a grandi cellule B. Sono stati presi in esame i campioni di sangue midollare e/o periferico giunti al Laboratorio di Biologia Molecolare della U.O. Ematologia di Pisa nel 2017 e successivi controlli nel 2018 al fine di analizzare l'espressione dei marker molecolari alla diagnosi e nel follow up.
Materiali e metodi: il protocollo prevede un isolamento cellulare con gradiente di densità specifico per linfomonociti, estrazione del DNA, amplificazione tramite singola PCR e analisi della clonalità con corsa elettroforetica capillare su sequenziatore ABI PRISM® 3500 e software GeneMapper v3.5.
Risultati e conclusioni: i campioni sono stati 403, età media 63aa, 226 maschi e 177 femmine. Sono risultati 131 casi clonali (32.5%), 235 policlonali (58.3%) e 37 non amplificabili (9.2%), rendendo valida l'utilità della ricerca della clonalità con indagini di biologia molecolare nel paziente alla diagnosi. Lo studio necessita di una continuità per permettere l'analisi con casistica di dimensioni maggiori in modo da valutare la % nelle singole patologie e soprattutto di seguire i dati nel follow up. Al momento vengono esaminati i casi più critici, che risultano dubbi all'amplificazione con analisi frammenti o di difficile interpretazione con tecniche innovative quali Next Generation Sequencing (NGS). La tecnologia NGS permette la caratterizzazione della sequenza del clone e di tutti gli altri frammenti della curva che otteniamo rendendo disponibile un dato che aiuta nel follow up a confermare il clone nella sua interezza di sequenza, se ancora presente o se è presente una diversa clonalità. La perfetta caratterizzazione molecolare, se fatta alla diagnosi, da un notevole contributo per predire la qualità di risposta e la prognosi.

P026
**CIRC_100219 EXPRESSION IN COLORECTAL CANCER MUCOSAE**

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Aim: Circular RNAs (circRNAs) have been recently identified as endogenous non-coding RNAs, with covalently linked ends, harbouring diverse physiological and pathological functions. Circ_100219 was found to be expressed in breast cancer and has an important role in carcinogenesis. Whether it participates in colorectal cancer (CRC) remains unclear, however. This study is hence aimed at investigating potential regulation of colorectal cancer by circ_100219 and its putative role as biomarker.
Methods: Total RNA was isolated from 37 CRC tissues and paired normal tissues by using Trizol Reagent (Thermo Scientific, Wilmington, Delaware, USA). The expression level of circ_100219 was analyzed with quantitative Real-Time Polymerase Chain Reaction (qRT-PCR). GAPDH was chosen as reference for normalizing expression levels. Significance of differences was assessed with Mann-Whitney test or Wilcoxon signed-ranks test (when appropriate), whilst correlation analyses were performed with Spearman's test. Diagnostic performance was calculated with receiver operating characteristics (ROC) curve analysis. The level of statistical significance was set at $p < 0.05$.
Results: The expression levels of circ_100219 were significantly lower in CRC tissue compared to normal tissue ($p<0.0001$). A statistically significant difference was noted in circ_100219 expression between tumoral stages 1-2 and 4 ($p=0.0371$). The area under the ROC curve (AUC) of circ_0002138 was 0.725 (95% confidence interval, 0.607-0.843; $p=0.001$).
Conclusions: Circ_100219 may be seen as potential biomarker in CRC diagnostics. Additional studies are needed to confirm the diagnostic performance in patients' samples. Reference: Lü L, Sun J, Shi P, et al. Identification of circular RNAs as a promising new class of diagnostic biomarkers for human breast cancer. Oncotarget 2017;8:44096-107

P027

## DIAGNOSIS OF PML-RARA GENE REARRANGEMENTS IN ACUTE LEUKEMIA: MOLECULAR CHARACTERIZATION BETWEEN QUALITATIVE PCR, REAL TIME MONITORING AND Q-LAMP ASSAYS

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Acute promyelocytic leukaemia is a rare variant of acute myeloid leukaemia characterised by a riarrangiaments between 15 an 17 cromosome with typical presentation, morphology, molecular pathogenesis and prognosis. Patients often present as a medical emergency, with disseminated intravascular coagulopathy (DIC) occurring frequently and resulting in associated haemorrhagic and thrombotic complications. Early therapy with all trans retinoic acid (ATRA) and arsenic demonstrated a significant reduction in early haemorrhagic death. Management of complications as thrombocytopenia, hypofibrinogenaemia and coagulopathy is need in the early stages to minimize this risk. Therefore, prompt initiation of therapy is indicated when APL is suspected until the diagnosis is confirmed or refuted Role of molecular laboratory is crucial in early response of 15, 17 rearrangemets chromosomes.

The comparison of 3 different diagnostic methodologies is the aim of our study as to verify the compatibility between the accuracy of the result, the times of execution of the diagnostic tests and above all the non-complexity in the execution of the same.

During the last year, 6 suspected cases of acute promyelocytic leukemia afferent to our clinical unit were studied with 3 methods. In all cases, bcr1, bcr2 and bcr3 isoforms were studied, as qualitative PCR, real time PCR and Q-lamp technology were performed. All positive cases for t( 15;17) translocation were confirmed by karyotype analysis with FISH assay. At diagnosis 6 patients in the last year with suspected APL have been analyzed.Qualitative PCR amplification, real time Q-PCR and Q Lamp technology were performed, and only in positive riarrangements FISH have been investigated. For Q Lamp technology assay, GUS beta as internal contros was used, while in qualitative PCR and real time Q-PCR Abelson gene was used. Not treated patients were enrolled for to investigate in t( 15;17) translocation and Bcr1, Bcr2, and Bcr3 isoforms.The morphological and cytofluorimetric analyzes had been performed. On 6 patients the 33,3% were positive for t( 15;17) respectly one bcr1 isoform and another bcr3 isoform. All diagnostic assay qualitative PCR, real time PCR and Q lamp pcr agree with the positivity and the related isoforms.

Conclusions :In t( 15;17) study for APL leukemia at diagnosis, the Q-lamp with isothermal amplification method in unique set to provide improvements for the molecular diagnosis of APL leukemias.The Q-lamp with real time monitoring is reliable, rapid ,the final result are availables in 40 minutes or generally after 15, 20 minutes is possible to see as preliminary indications of positive samples.The simplicity of the method, its accuracy plays a role crucial in the molecular laboratory for one early response of t(15, 17) rearrangemets at diagnosis.

P028

## EFFECTS OF COMMON PRE-ANALYTICAL VARIABLES ON DETECTABILITY AND STABILITY OF MICROVESICLE-ASSOCIATED AND FREE CIRCULATING miRNAs

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Introduction: Pre-analytical phase standardization is a main requirement for the clinical implementation of biomarkers and, specifically, of circulating miRNAs. miRNAs are present in blood as free (e.g., associated with argonaut protein, low-density lipoproteins) and microvesicle-associated forms. Based on their release, free-circulating miRNAs are considered biomarkers while microvesicle-associated miRNAs mostly carry out the role of hormone-like compounds. This study was aimed at evaluating the effects of common pre-analytical variables on detectability and stability of a panel of microvesicle-associated and free circulating miRNAs.

Methods: Venous blood from 10 male volunteers was collected into standard K2EDTA tubes and plasma preparation tubes (PPT) with a separator gel. Plasma was obtained by centrifugation according to the manufacturer's indications (1-K2E, PPT), while an aliquot of K2EDTA plasma was further centrifuged (2500g, 15min, room temperature) to deplete the platelets (2-K2E). Samples were immediately frozen or stored for 24h at either room temperature or 4°C. The microvesicle-associated and free circulating fractions of 179 miRNAs was assayed by RT-qPCR.Results. Detectability of free miRNAs was greater in PPT samples than in 1- and 2-K2E samples; particularly, 2-K2E samples displayed the lowest detectability over all the conditions. Detectability in 1-K2E samples was mostly affected during storage, regardless the condition, while in PPT sample miRNA expression levels remained stable. Also for the exosome-associated miRNA fraction, samples collected in 2-K2E displayed the lowest detectability and stability but, in this case, the storage conditions had comparably no effects on miRNAs stability in PPT and 1-K2E samples, although it resulted slightly improved in 1-K2E samples.

Conclusion: Results indicate that blood collection in PPT, with gel separator, guarantee a greater stability for free-circulating miRNAs (i.e., the biomarker-like fraction), compared to K2E samples, and also for microvesicle-associated miRNAs (i.e., the hormone-like fraction) the performance of this tube are good. The stepwise centrifugation strongly affected miRNA detectability and stability of miRNAs and, therefore, it may not be applied in miRNA testing.

P029

## ISOCITRATE DEHYDROGENASE 2 (IDH2) GENE MUTATIONS DETECTION IN ACUTE MYELOID LEUKEMIA (AML): SCREENING AND MINIMAL RESIDUAL DISEASE (MRD) MONITORING BY "DROP-OFF" DIGITAL DROPLET PCR (ddPCR)

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Background: the screening of IDH2 mutations plays an important role in categorization of AML and can drive ab initio the treatment strategy due to the introduction of IDH inhibitors. Mutations are found in 20% of AML; they are single-nucleotide variants involving the R140 or R172. Prognostic assessment of IDH2 is controversial, but enadisenib leads the possibility to use IDH2 as a marker of MRD. (Petrova et al, 2018). Sanger sequencing is the gold-standard method for detection, but it is not quantitative and it has limited sensitivity. qPCR methods have been developed, based on the amplification refractory mutation system PCR techniques. Aims: we set a new multiplex ddPCR method, then used at diagnosis and as tool for MRD detection.
Methods: a drop-off ddPCR FAM/HEX Assay (Biorad), used for setting the technique, requires a pair of probes to detect and quantify different mutations in a single reaction: the FAM-probe binds a reference sequence distant from the target but still within the same amplicon; the HEX-probe binds the wild-type sequence in the target site (sensitivity $1x10^{-3,}$ as qPCR assay, qBiomarker Somatic Mutation, Qiagen).
Results: at diagnosis we identified 11/60 (18.3%) IDH2-mutated patients, 9 carrying the R140Q and 2 R172K. The median mutational burden was 13.7%; it did not correlate with blasts percentage or histotype. Sanger sequencinq was not able to identify mutations in 5/11 cases, because of their low mutational burden (0.4-12%). In our series of patients, the IDH2 mutational status did not significantly impact on survival, neither on the achievement of the clinical response. We also performed ddPCR in follow-up samples after induction and consolidation therapy in 4 IDH2 mutated patients (2 $CR_{MRD-}$, 1 $CR_{MRD+}$ and 1 resistant). In the $CR_{MRD-}$ patients the IDH2 mutational status became negative; in the resistant one IDH2 correlated with disease persistence; in the $CR_{MRD+}$ patient IDH2 mutation was 0% while the MRD positivity was still detected by NPM1.
Conclusion: we demonstrated that the ddIDH2 PCR could represent a valid tool for detecting and quantifying IDH2 mutations. Our results about MRD suggest that the application of ddPCR to IDH2 mutations is today a hot matter of debate. It is relevant that the sensitivity of our method is promising.

P030

## VALUTAZIONE DELLA PRESENZA DELLA VARIANTE ARV7 DEL RECETTORE ANDROGENICO NELLE CELLULE TUMORALI CIRCOLANTI DI PAZIENTI AFFETTI DA CARCINOMA PROSTATICO

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Il carcinoma prostatico è la neoplasia più frequente nel sesso maschile. La terapia di deprivazione degli androgeni è il trattamento di elezione, ma l'insorgenza di resistenza ha portato allo sviluppo di farmaci (abiraterone/enzalutamide) che inibiscono il recettore androgenico (AR). Dopo un lasso di tempo variabile si sviluppa resistenza a questi farmaci, in seguito a mutazioni a livello di AR e della sua cascata segnalatoria, fra le quali è clinicamente significativa la variante di splicing ARV7. ARV7 è stata identificata anche nelle cellule tumorali circolanti (CTC) di pazienti con carcinoma prostatico metastatico castrazione-resistente aprendo la possibilità di una determinazione non-invasiva della sua presenza attraverso un prelievo di sangue (biopsia liquida). L'obiettivo dello studio è la valutazione di un protocollo per l'arricchimento e la caratterizzazione molecolare delle CTC per quanto riguarda la presenza/assenza di ARV7. L'arricchimento delle CTC è stato effettuato con un metodo immunomagnetico e l'identificazione della loro presenza è stata realizzata mediante una multiplex RT-PCR avente come bersagli trascritti associati alla neoplasia o al tessuto prostatico (PSA, PSMA, EGFR), mentre l'individuazione di ARV7 e della forma normale di AR nelle CTC è stata effettuata mediante RT-qPCR.Sono stati analizzati 12 campioni di sangue prelevati da pazienti con carcinoma prostatico castrazione-resistente prima dell'inizio della terapia con enzalutamide/abiraterone: 4 (33,3%) sono risultati positivi per la presenza delle CTC, e 3 (25%) presentavano la forma normale di AR, mentre 1 (8,3%) la variante ARV7. La prevalenza di ARV7 è simile a quanto riportato in letteratura. Non sono state riscontrate correlazioni fra la presenza/assenza di CTC né di ARV7 e l'andamento dei valori ematici del PSA nel tempo (marcatore utilizzato per valutare l'andamento della terapia), ma il follow up è ancora troppo breve per poter valutare l'outcome clinico.Complessivamente il metodo considerato risulta efficace per l'individuazione della variante AR-V7 e per la sua quantificazione nelle CTC. Un follow-up più lungo ed un ampliamento della casistica consentirà di verificare il ruolo prognostico e predittivo delle CTC e della variante AR-V7 nella nostra coorte di pazienti.

P031
## FLC: INDEPENDENT BIOMARKER FOR THE MANAGEMENT OF MONOCLONAL GAMMOPHATIES

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Aim of the study: Free Light Chains assay (sFLC) is an immunological test well established in the Laboratory/Clinical practice, recommended by Guidelines for the management of Monoclonal Gammophaties. The calculation of the ratio between k/λ provides diagnostic and prognostic indications and in the monitoring of plasma cell dyscrasias.
In fact sFLCs are independent biomarkers of the disease process. This is of potential clinical use particularly when the tumour produces large amounts of FLCs and small amounts of intact monoclonal Igs.
We report a clinical case in which the sFLC determination ("Freelite®" The Binding Site, N-Latex Siemens), among the other conventional routine tests, provided a crucial contribute from the Diagnosis, Risk Stratification, Monitoring and Relapse Detection of Multiple Myeloma.
Patient and Methods: We report a case of 59 years old man whom arrived the first time at our attention in April 2017. At first investigation (Hematological suspicion) were found MC IgG-K 3g/L; λ free (not quantifiable with ETF); rFLC k/λ 0,09. MGUS Risk Stratification: Low-intermediate (rFLC < 0,26). Due the stratification risk monitor within 3-6 months. After 5 months (Diagnosis): MC IgG-K 9g/L; λ free 6g/L; IgD-λ 2g/L. rFLC k/λ 0,002. BOM confirms Multiple Myeloma; start of therapy. Despite the low concentration of MCs the rFLC indicates severe linforproliferative disorder (SLiM Biomarker) and leads the diagnostic deepening. After 2 months (Monitoring): no evidence of CM. rFLC k/λ 0,29. The normalization of the FLC ratio confirms therapy's efficacy. After 16 months: MC IgD-λ 3g/L: λ free (not quantifiable with ETF). rFLC k/λ 0,00. Disease relapse. Despite the low concentration of MCs the rFLC indicates severe disease relapse.
Conclusion: Confirming what is already established in literature, this case emphasizes the importance to consider sFLC test as an independent biomarker. In fact, especially in absence of correlation with CM concentrations obtained in ETF methods, rFLC may provide crucial information in order to assess the pathology and lead to the most appropriate clinical evaluations. Based on these evidences FLC test should be performed every time there is a suspicion of Monoclonal Gammophaty and during the Monitoring as suggested by the Guidelines.

P032
## IgE TYPE MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE: A RARE CONDITION DIFFICULT TO RECOGNIZE

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Clinical Case: We report a case of a double monoclonal gammopathy of undetermined significance (MGUS) with IgE-kappa and IgG-kappa paraprotein types. An 85-year-old woman with IgG-kappa MGUS diagnosed two years earlier, performed blood tests for a periodic routine check-up. We found a normal blood count with Hb 12.6 g/dL, creatinine 0.76 mg/dL, eGFR 72 mL/min/1.73 m2, normal sodium and potassium levels. Serum protein electrophoresis showed two peaks in the gamma zone. Immunofixation clearly showed an IgG-kappa paraprotein, but the presence of two bands in Kappa induces a further deepening with anti-IgD, IgE, and Kappa-free antisera, thus highlighting also an IgE-kappa paraprotein. Considering the good clinic conditions of the patient, the attending physician did not consider necessary to proceed to further investigations, thus referring the patient to an annual MGUS follow-up.
Discussion: The frequency of the monoclonal gammopathies (MG) isotype roughly reflects their serum concentrations; accordingly, IgE MG are less frequently observed. According to data reported in the literature, multiple myeloma with IgE paraprotein is more aggressive, with a reduced survival time compared to other Ig-type myeloma, also showing a higher incidence of plasma cell leukemia. The lack of use of anti-IgE antiserum in the workup for monoclonal component (MC) typing makes IgE paraprotein problematic to recognize. The picture becomes even more complicated if it is accompanied by another MC linked to the same light chain, as in this case IgG-kappa. By re-evaluating the immunofixation performed two years earlier, it was possible to notice a further, less marked band reactive to the anti-kappa antiserum, which however was not sufficiently investigated since the attention of the operator was captured by the more evident IgG-kappa. If few cases of IgE multiple myeloma have been described so far, even rarer are the cases of IgE MGUS, which are very difficult to identify as patients have no symptoms leading to the diagnosis. Considering that IgE myeloma are often aggressive disease, it would be important to describe all cases of IgE-MGUS in order to expand the knowledge of this rare pathology. The experience and practice of the laboratory become decisive for a correct diagnosis of a rare gammopathy difficult to detect.

P033
**BIOMARCATORI DEL LIQUIDO CEFALORACHIDIANO NELLA SCLEROSI MULTIPLA: IL RUOLO DEL LABORATORIO**

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Background: Molte patologie neurologiche sono dovute all'instaurarsi di processi infiammatori a carico del Sistema Nervoso Centrale (SNC), con relativa sintesi intratecale di anticorpi. Questo processo infiammatorio può essere rilevato dalla presenza di bande oligoclonali di sintesi intratecale di IgG nel liquor, ma non nel siero, mediante isoelettrofocalizzazione e immunoblotting. Questa metodica è considerata il gold standard data la sua elevata sensibilità, nonostante la scarsa riproducibilità. Pertanto il dosaggio delle catene leggere libere Kappa e Lambda delle immunoglobuline nel liquido cefalorachidiano, potrebbe essere considerato un valido test di supporto diagnostico.
Pazienti e Metodi: Abbiamo valutato la validità della determinazione delle catene leggere libere, espressa come kFLC index e λFLC index, nella diagnosi della Sclerosi Multipla. Oggetto del nostro studio è il caso di una donna di 65 anni con ipostenia dell'arto inferiore destro associata a parestesia dell'arto superiore destro. È stata eseguita una TC cranio che ha messo in evidenza 2 aree sfumate ipodense a livello dei centri semiovali e in mesencefalica destra. Sono stati effettuati esami ematochimici di routine, anticorpi organo e non organo specifici, determinazione quantitativa e ricerca delle IgG con profilo oligoclonale mediante isoelettrofocusing del liquor.
Risultati: L'esame liquorale ha dato esito positivo per la presenza di tre bande oligoclonali IgG. Sono stati calcolati IgG index, κFLC index e λFLC index a conferma della diagnosi.
Conclusioni: L'uso combinato degli indici κ e λ e dell'IgG index aumenta la sensibilità diagnostica e consente il monitoraggio della malattia in maniera precisa, semplice e dai costi relativamente contenuti.

P034
**L'ADRENOMEDULLINA COME POTENZIALE INDICATORE DI OUTCOME DEL PAZIENTE CON MENINGITE BATTERICA: CASO CLINICO**

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Background: L'Adrenomedullina (ADM) è un peptide regolatore espresso e secreto in diversi tessuti e organi come cuore, vasi sanguigni, reni, polmoni, cervello e pancreas. Questa vasta distribuzione suggerisce un suo ruolo nella regolazione di numerose funzioni corporee e fisiologiche. Inoltre l'ADM presenta proprietà antimicrobiche sia contro batteri gram-positivi che gram-negativi. Grazie alla sua struttura anfipatica, costituita da domini idrofobici carichi e separati spazialmente, può intercalarsi nelle membrane batteriche. La concentrazione di ADM necessaria per uccidere o inibire la crescita microbica è superiore alla concentrazione fisiologica dell'ormone circolante; tuttavia, in determinate circostanze, come la sepsi, la sua concentrazione plasmatica aumenta significativamente.
Pazienti e Metodi: Abbiamo descritto il caso di un paziente afferente al Pronto Soccorso dell'Ospedale Infettivologico Cotugno di Napoli, con sospetta diagnosi di meningite batterica, e dosato i valori di Procalcitonina (PCT) e Adrenomedullina (MR-proADM) sia nel siero/plasma che nei campioni di liquido cefalorachidiano. Abbiamo poi calcolato il rapporto $ADM_{liquor}$/ $ADM_{plasma}$ per stabilire se tale indice possa essere di aiuto nella valutazione, in tempi brevi, del danno cerebrale.
Risultati: I risultati degli esami biochimici condotti, hanno confermato la diagnosi di meningite purulenta franca. La determinazione della Procalcitonina sierica è risultata essere di 0,38 ng/mL e di 3,48 ng/mL su liquor; mentre il valore di MRproADM plasmatica era di 1,22 nmol/L e su liquor di 14,21 nmol/L. Il rapporto $ADM_{liquor}$/ $ADM_{plasma}$ >10.
Conclusioni: MRproADM è un marcatore di danno d'organo. Valori > 6 nmol/L su plasma sono associati a esito infausto, rappresentando probabilmente un cut-off di non ritorno. Su liquor l'ADM potrebbe, invece, indicare danno cerebrale, soprattutto per quanto concerne la valutazione del rapporto $ADM_{liquor}$/ $ADM_{plasma}$.

P035
## UN CASO CLINICO PARTICOLARE: AGGLUTINAZIONE ERITROCITARIA DA CRIOAGGLUTININE NON REVERSIBILE CON TERMOSTATAZIONE

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La presenza di crioagglutinine è causa di errori nel conteggio dei globuli rossi (RBC) e degli indici eritrocitari (MCV, HCT, MCH ed MCHC). In particolare, risultano sottostimati RBC ed HCT, e sovrastimati MCV, MCH ed MCHC. Normalmente l'agglutinazione eritrocitaria è reversibile dopo termostatazione del campione a 37°C per 30 minuti. Presentiamo il caso di un paziente di 80 anni inviato al P.S. dal medico curante per acrocianosi dopo esposizione al freddo e livedo reticularis. Agli esami ematochimici si evidenzia marcata agglutinazione eritrocitaria (RBC: 0.45 10^12/L, MCV: 120 fL, HCT: 5.4 %, MCH: 188.9 pg, MCHC: 157,4 g/dL) non risolvibile dalla termostatazione del campione a 37°C anche per oltre 30 minuti. L'unico parametro strumentale attendibile risulta essere la concentrazione emoglobinica (8,5 mg/dL), in quanto determinata fotometricamente dopo lisi eritrocitaria. Gli approfondimenti diagnostici rivelano un quadro di severa anemia emolitica autoimmune: LDH: 1061 UI/L, bilirubina indiretta: 3.8 mg/dL, aptoglobina < 10 mg/dL, con positività del test di Coombs Diretto in presenza di autoanticorpi freddi ad altissimo titolo (> 1/4096). Nei giorni seguenti, nonostante il trattamento con plasmaferesi, steroide e anticorpo monoclonale antiCD20 (Rituximab), persiste grave agglutinazione anche dopo termostatazione prolungata. Per refertare correttamente i parametri eritrocitari abbiamo utilizzato la lettura dei globuli rossi con metodo ottico (RBC-O) sul canale termostatato a 41°C dell'analizzatore automatico Sysmex XN 9000. L'HCT è stato ricavato da lettura di microcapillare dopo centrifugazione, gli altri indici eritrocitari sono stati calcolati mediante applicativo gestionale Dasit EPU. Abbiamo ritenuto opportuno condividere la nostra esperienza essendo, questo, il primo caso di tale complessità di gestione laboratoristica giunto alla nostra osservazione.

P036
## DUPLICATION OF SETBP1 GENE IN A BOY WITH NEUROLOGICAL DISORDERS

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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 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 growth and psychomotor delay, movement and language disorder. 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 duplication of around 42.996 Kb on the 18 chromosome at q12.3 region that includes SET binding protein 1 (SETBP1) gene. The protein encoded by this gene has been shown to bind the SET nuclear oncogene, which is involved in DNA replication. It is well described that mutations in this gene are associated to Schinzel-Giedion midface retraction syndrome (SGMFS), a disorder characterized by severe mental retardation, distinctive facial features, and multiple congenital malformations including skeletal abnormalities, genitourinary and renal malformations. Moreover, SETBP1gene rearrangements have been found in subjects with cognitive disability-expressive aphasia-facial dysmorphism syndrome. De novo mutations of the SETBP1 gene probably result in a gain of function or a dominant negative that suggest a possible role of the duplication of the SETBP1gene in the phenotype of the proband.

P037
**MIELOMA MULTIPLO (MM): DA PLASMOCITOMA A COINVOLGIMENTO MULTIORGANO**

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Paziente (pz) maschio di 71 a, iperteso, affetto da diabete tipo II, pregresso IMA. Effettua PET/TC (2/16) per dolore all'emitorace sx che evidenzia accumulo di tracciante al sigma e più moderatamente alla prostata, costa sx e ala iliaca sx. Esegue asportazione polipo al sigma e FNAC costale che non mostra alterazioni patologiche; il 7/16, dopo seconda biopsia della II costa sx, si evidenzia un quadro morfologico ed immunoistochimico riferibile a plasmocitoma di basso grado. Nel 9/16, ricoverato in Ematologia esegue: elettroforesi sieroproteica (SPE) e immunofissazione (CM IgG k, 8.4 g/L); immunofissazione urinaria (lieve presenza PBJ k); mielobiopsia (plasmacellule 8.7%). Ad una SPE mostrata, eseguita 8 mesi prima presso altra struttura, si evidenzia un picco anomalo in zona gamma di modesta entità, non segnalato né tipizzato. Viene confermata diagnosi di plasmocitoma costale; effettua radioterapia con acceleratore lineare. Il pz si ripresenta dopo 6 mesi riferendo episodi febbrili e lombalgia ed esegue controlli ematochimici: CM=19,1 g/L; misura nefelometrica catene leggere libere sieriche (FLC) k=342 mg/L (6.7-22.4), lambda=28,2 mg/L (8.3-27), rFLC=12,1 (0.31-1.56). All'RMN si evidenziano lesioni multiple D6-D9; diagnosi MM IgG k, III A DS. Sottoposto a diversi schemi chemioterapici di induzione, si ottiene graduale riduzione della CM e della iFLC (involved FLC), fino a 4,6 g/L e 61,2 mg/L rispettivamente; i parametri rimangono stabili ed il pz sospende la terapia (2/18). Nei mesi successivi si osserva incremento della CM=23,7 g/L, marcata positività PBJ, iFLC=494 mg/L. Sottoposto a terapia, non si ottiene risposta ma: netto peggioramento delle condizioni cliniche; coinvolgimento epatico: AST=67U/L (<34); ALT=102 U/L (<55); ALP=762 U/L (40-150) GGT=1315 U/L (12-64), ALB=19 g/L (32-46), confermato da agobiopsia epatica su lesioni nodulari; insufficienza renale acuta, CREA=5,7 mg/dL (0.72-1.25). Il pz è sottoposto a trattamenti emodialitici, va incontro ad edema polmonare, nei giorni seguenti decede. Il MM può risultare particolarmente aggressivo. Il caso presentato, esordito come plasmocitoma, è evoluto, in un breve lasso di tempo, in MM ad interessamento multiorgano. È da segnalare il mancato approfondimento dell'SPE e della PET/TC nelle fasi antecedenti la diagnosi.

P038
**QUANDO LA CONOSCENZA SUPERA LA CASUALITÀ; LA DIAGNOSI DI MALARIA NEL LABORATORIO DI BASE: CASE REPORT**

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Alcuni emocitometri di ultima generazione sono dotati di allarme morfologico per le emazie parassitate, il quale pone il sospetto di infezione malarica anche per quei casi privi di quesito specifico.
Presentiamo il caso di un paziente giunto al Pronto Soccorso del nostro ospedale con vomito, febbricola e diarrea.
L'emocromo è stato eseguito utilizzando l'analizzatore Sysmex XN-9000 il quale ha evidenziato, in presenza di uno scattergram del canale WDF senza alterazioni, gli allarmi morfologici "RET Abn Scattergram?" e "Thrombocytopenia" che hanno portato alla revisione microscopia dello striscio con rilevazione di parassita malarico (trofozoita); è stata erroneamente segnalata una reticolocitosi, rivelatasi una pseudoreticolocitosi, in quanto le emazie parassitate sono state riconosciute come reticolociti ad alto grado di maturazione. La diagnosi è stata poi confermata dal microbiologo con il riscontro di una positività agli antigeni comuni per P. Falciparum, Vivax e Ovale e presenza di parassiti con livello maturativo allo stadio di trofozoiti. Il paziente, trattato con artesunato, ha avuto un miglioramento senza complicanze. In regime di follow up, un campione dello stesso paziente è giunto in laboratorio per un controllo ematologico di routine. In questo caso lo strumento ha segnalato l'allarme "pRBC?" il cui WDF scattergram di riferimento è stato un valido supporto per la diagnosi di una recidiva di malaria; l'analisi ha infatti mostrato un cluster cellulare atipico apparentemente riferibile alla popolazione degli eosinofili ma caratterizzato da una minor intensità di fluorescenza e da una minor complessità. L'esame microscopico dello striscio ha confermato la presenza di emazie parassitate e l'esame microbiologico è risultato positivo per Plasmodium Ovale con una parassitemia a diversi stadi maturativi (trofozoita e gametocita), confermando la recidiva di malattia. Trattato con clorochina e con primachina il paziente è stato dimesso in buone condizioni.
Questo caso sottolinea come la formulazione di allarmi strumentali dedicati e la conoscenza della morfologia degli scattergrams suggestivi di patologia malarica sia fondamentale per indurre lo specialista di laboratorio alla revisione microscopica, al fine di confermare o escludere la presenza del parassita.

P039

## IDENTIFICATION OF THE HB VARIANT CITY OF HOPE IN A PATIENT OF ARGENTINA-ITALIAN DESCENT WITH NON-TRANSFUSION-DEPENDENT β-THALASSEMIA (NTDT)

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Non-transfusion-dependent β-thalassemia (NTDT) is characterized by high clinical and molecular heterogeneity.
Ineffective erythropoiesis, chronic anemia and increased intestinal iron uptake contribute to worsening clinical complications of the disease, therefore early identification and molecular characterization of these patients play a key role to define most appropriate therapeutic protocols.Here we report the case of a 40-year-old woman of Argentina-italian descent with microcytic hypochromic anemia (Hb 8.0 g/dL, MCV 61.2 fL, MCH 19.4 pg, MCHC 31.7 g/dL), HbA2 5.5% and HbF 7.2%, reticulocytosis, hemosiderosis, sub-jaundice, altered hemolysis indexes, splenomegaly and cholelithiasis at the third decade of life.Molecular screening by reverse dot blot (commercial kit) had only shown heterozygosity for the HBB:c.[118C>T] ($\beta^0$ codon 39) mutation. Sequence analysis of the entire β-globin gene, beside confirming the HBB:c.[118C>T] mutation, showed the presence of another mutation, HBB:c.[208 G>A], responsible for the synthesis of Hb City of Hope [beta 69 (E13) Gly>Ser], a variant that is undetactable at cation-exchange HPLC. By MLPA analysis of the α-globin gene cluster we also found the α-anti 3.7 triplication.
The same genotype, except for Hb City of Hope, was detected in the propositus's sister presenting with a milder form of NTDT.It is known that the association of the HBB:c.[118C>T] mutation with the α-anti 3.7 triplication is responsible for NTDT conditions. Therefore, the presence of Hb City of Hope, although reported as asymptomatic so far, contributes to worsening the clinical phenotype. This case highlights a novel pathogenetic role for this variant and emphasizes the importance of a wide range of molecular diagnostic tests that are required to avoid pitfalls in molecular diagnosis of hemoglobinopathies.

P040

## HEMOGLOBIN HYDE PARK: THE FRAGILITY OF THE SISMEX XN1000 ANALYZER AS A TOOL TO IDENTIFY UNSTABLE HEMOGLOBIN VARIANTS

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The Department of Laboratory Medicine (DAiMeLab) of AOU FEDERICO II offers well-established diagnostic services for screening and molecular characterization of thalassemia and other hemoglobinopathies. For blood count testing, we use either the Siemens ADVIA 2120i analyzer or the Sysmex XN3000 analyzer. Some hemoglobin variants may interfere with the DIFF analytical channel of Sysmex blood analyzers. Here we report the case of a young woman with severe hemolytic anemia, reticulocytosis, indirect hyperbilirubinemia. Blood count testing had shown abnormal eosinophilia, lymphopenia and immature granulocytes (Immature GIs). The ADVIA 2120i hemocytometer did not confirm eosinophilia and other leukocyte abnormalities, eosinophilia was also excluded by blood smear examination. The absence of any abnormalities on a buffy coat sample on Sysmex allowed us to hypothesize an erythrocyte pathology. To confirm our hypothesis, molecular investigation was carried out, revealing the presence of an abnormal hemoglobin variant, Hb Hyde Park. This variant belongs to the group of hemoglobins M, a group of abnormal hemoglobins with altered oxygen transport, generally characterized by a replacement of a single amino acid that promotes the formation of methemoglobin even with normal methemoglobin reductase activity. Hb Hyde Park is generated by a point mutation on the b globin gene causing the His99Tyr replacement. This substitution is able to oxidize the heme iron that becomes resistant to the hemoglobin reductase, thus causing cyanosis, but it is also responsible for Hb protein instability. On one hand this report sheds light on the operational "fragility" of the Sysmex analyzer. On the other hand, this "fragility" could help identify cases of rare hemoglobinopathies that is of main interest in prevention programs for hemoglobinopathies. Moreover, in this case molecular characterization, beside contributing to clarify the pathogenetic mechanisms of this type of variant, provided an explanation to the blood count abnormalities detected by the Sysmex analyzer and suggests further hypothesis to study the Diff anomalies on the Sismex XN3000 analyzers.

P041
**MOLECULAR ANALYSIS TO PREVENT THE RISK OF SUDDEN CARDIAC DEATH IN ATHLETE UNKNOWINGLY SUFFERING FROM CARDIAC DISEASE**

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Purpose: The prevention of sudden cardiac death (SCD), in asymptomatic athletes, unknowingly suffering from cardiac disease, is an important objective that involves many areas of the medical profession and of the healthcare systems in general. In these years, the sports authorities have focused on pre-participation screening that may predict the risk of SCD and hence initiate preventive measures in at-risk athletes.
Case presentation: Here we report the case of an asymptomatic amateur cyclist, who, in the setting of pre-participation screening, to obtain clearance to take part in competitive sporting event, underwent cardiac clinical and instrumental examinations. Screening consisted of family and personal history, physical examination and 12-lead electrocardiography (ECG) as a first-line evaluation. He had no symptoms at medical examination. There was no personal history of shortness of breath during physical exertion or of dyspnea or syncope; however, he reported two episodes of lipothymia unrelated to physical exercise.
Results: ECG revealed mild repolarization abnormalities and echocardiography showed borderline septal wall thickness. Molecular analysis for mutations in eight sarcomeric genes, revealed double heterozygosity for mutations in the TNNT2 (c.832C>T; p.Arg278Cys) and MYBPC3 (c.2689_2690insCCTGGCTGTGGCTACAGCA; p.Gly897Alafsx159) genes. The p.Arg278Cys is a previously described mutation, found in Hypertrophic Cardiomyopathy (HCM) patients with relatively mild and sometimes subclinical hypertrophy, but with a high incidence of sudden death. The p.Gly897Alafsx159 mutation, is not previously described, which produces a frame-shift reading error and the consequent generation of a premature termination codon.
Conclusions: This case report highlights that molecular analysis can reveal DNA alterations in asymptomatic athletes, which in many cases could cause SCD. This and previous cases show that Clinical Molecular Biology is now an essential addition to the clinical and instrumental approach of the pre-participation screening, to the evaluation of cardiac wellness, which could otherwise remain obscure.

P042
**RUOLO DELL'ESAME EMOCROMOCITOMETRICO E DEI NUOVI ANALIZZATORI DI MICROSCOPIA DIGITALE NELLA DIAGNOSI DI MALARIA**

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Introduzione: La malaria è una delle malattie infettive più diffuse nel mondo e la più frequente malattia tropicale importata in Italia. Cinque specie di parassita sono in grado di causare manifestazioni cliniche nell'uomo: Plasmodium (falciparum, vivax, ovale, malariae, knowlesi). Nonostante l'esame microscopico dello striscio periferico rappresenti il gold standard per la diagnosi della malaria, l'esame emocromocitometrico e in particolare i nuovi analizzatori di microscopia digitale si dimostrano un valido aiuto. Caso clinico: N. H. 10 anni, originario di Burkina Faso, in Italia da pochi mesi, si presenta al P.S per piccolo ematoma del glande. Esame obiettivo ed esame urine sono negativi. Viene rinviato al medico curante che dopo una settimana chiede: Emocromo (Hb 76g/L, Hct 0,250 L/L, MCV 60,0 fL, MCH 18,2 pg, PLT 74 x 10^9/L, WBC 3,48 x 10^9/L, Neutrofili 1,05 x 10^9/L), VES 42 mm/h, PCR 13.08 mg/dL. Lo striscio periferico conferma la piastrinopenia e presenza di anisopoichilocitosi. Il bambino torna al nostro laboratorio dopo 10 giorni: Emocromo (Hb 72g/L, Hct 0,261 L/L, MCV 65,6 fL, MCH 18,1 pg, PLT 314 x 10^9/L, WBC 3,41 x 10^9/L, Neutrofili 1,19 x 10^9/L), VES 28 mm/h, Ferritina 22 ng/mL. L'assetto emoglobinico presenta una variante HbC eterozigote. Il citometro a flusso XN-9000 (Sysmex) presenta lievi alterazioni dei citogrammi del conteggio leucocitario "WNR" e della conta differnziale "WDF", suggestive di infezione malarica, in più segnala l'allarme pRBC (emazie parassitate). Lo striscio periferico viene analizzato su lettore digitale Cella Vision DM9600 che visualizza la presenza di rari gametociti e trofozoiti compatibili con Plasmodium falciparum. Il test molecolare Alethia malaria su Illumipro (Meridian) risulta positivo. L'emoscopia diretta su goccia spessa dà conferma definitiva della presenza del Plasmodium e l'analisi morfologica su striscio sottile consente l'identificazione della specie e la valutazione della parassitemia (0,3%). Il bambino viene ricoverato in pediatria. Conclusioni: L'esame emocromocitometrico su citometri a flusso di ultima generazione e l'applicazione di nuovi analizzatori di microscopia digitale si dimostrano di valido aiuto nello screening e la diagnosi della malaria, soprattutto quando l'infezione non è attesa.

P043

## GENETIC AND EPIGENETIC ANALYSIS OF A PREGNANCY-RELATED ORAL SQUAMOUS CELL CARCINOMA EXHIBITING AN UNEXPECTED LOW AGGRESSIVE BEHAVIOR

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Oral squamous cell carcinoma (OSCC) is the most frequent neoplastic disease in head and neck region and it is usually diagnosed in an advanced stage, which is associated with worse prognosis (5-years survival rate after diagnosis: 50%). The development of OSCC during pregnancy is a very rare event. During gestation immunological suppression together with high metabolic state, increased circulating growth factors, and amplified hormonal responses may predisposes the subject to malignancies (1). Purpose of the present report is to describe the genetic and epigenetic profile of an OSCC affecting a 41 years old pregnant woman with an oral tumor mass located at the hard and soft palate with bone involvement and lymph-node metastases (T4N1M0). She has been treated with a conventional multimodal radio and chemotherapy. The follow-up at eight years did not show evidence of disease. Genetic and epigenetic analysis were performed in multiple samples from different area of the tumor and from non-neoplastic mucosa to verify tumor heterogeneity. DNA mutation analysis was investigated by NGS in a set of 11 OSCC driver genes (2). DNA methylation analysis was evaluated by bisulfite-NGS in a set of 13 genes known to be altered in OSCC (3). Three genetic mutations only were detected: the TP53 p.R282W shared in all three different tumor area, while TP53 p.R249K and NOTCH1 p.D1609N were detected in only one collected tumor sample. Additionally, the same aberrant DNA methylation pattern was detected in all tumor specimens. Non-neoplastic mucosa adjacent to OSCC showed a methylation profile typical of normal mucosa in healthy donors. The presence of a shared TP53 driver mutation and the same DNA methylation profile in different tumor cell populations suggest a simple clonal homogeneous carcinoma which usually has been associated to low aggressive behavior (4). Additionally, no genetic and epigenetic alterations in non-neoplastic oral mucosa were detected, demonstrating the absence of field cancerization. These data suggest that pregnancy related OSCC has a different molecular transformation pathway, offering new perspectives for further investigations.

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P044
**NON DIMENTICHIAMO LE IgD: RARO CASO DI MIELOMA MULTIPLO IgDK**

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Il mieloma multiplo (MM) IgD è una patologia rara che rappresenta circa il 2% di tutti i mielomi ed ancora meno frequenti sono quelli con componenti monoclonali (CM) di tipo IgDK (0,2%); la breve emivita di questa classe di immunoglobuline (2,8 gg) ne rende difficile l'individuazione. Dal punto di vista clinico, il MM IgD è caratterizzato da un comportamento più aggressivo rispetto agli altri mielomi ed è associato più frequentemente ad insufficienza renale, ipercalcemia, amiloidos ie proteinuria di Bence Jones (BJ).
Nel dicembre 2018 il paziente (65 aa) accede al pronto soccorso con trauma cranico per caduta accidentale in seguito ad episodio vertiginoso ed i principali esami di laboratorio (fra cui creatinina ed eGFR) risultano nella norma. Dopo circa 3 mesi si presenta nuovamente al pronto soccorso con insufficienza renale (creatinina 2,53 mg/dL (0,64–1,20) ed eGFR secondo CKD-EPI25 (>60)). In questa occasione, dato il sospetto clinico di mieloma, viene richiesta anche l'elettroforesi sieroproteica (SPE), l'immunofissazione sierica ed urinaria (s-IFE e u-IFE) ed il dosaggio delle catene leggere libere su siero (sFLC).
La SPE eseguita ad aprile 2018 (normale nel marzo 2016) mostra 2 sospette CM (19%, 2%) in zona $\gamma$ confermate in s-IFE rispettivamente come IgDK e K libere. L'u-IFE risulta positiva per la presenza di BJ Kappa ed il rapporto k/$\lambda$ delle sFLC è fortemente alterato: 211 (0,31-1,56). L'aspirato midollare, eseguito successivamente, evidenzia un infiltrato plasmacellulare del 50%. Viene quindi posta diagnosi di MM IgDK. Il paziente inizia un trattamento con Bortezomib, Lenalidomide, Desametasone. Al termine del primo ciclo la SPE mostra la scomparsa delle CM ed una marcata ipogammaglobulinemia. Dopo il quarto ciclo di trattamento il paziente mostra un quadro in netto miglioramento con recupero della funzionalità renale.
Poiché la prognosi di MM IgD è fortemente dipendente dall'interessamento renale, in caso di insufficienza renale di dubbia eziologia è importante che venga sospettata una discrasia con relativa investigazione del quadro proteico. Per un corretto inquadramento diagnostico è altresì determinante l'esecuzione della s-IFE che, in caso di positività delle sole catene leggere, deve prevedere l'utilizzo di specific iantisieri anti IgD e IgE.

P045
**16p11.2-p12.2 MICRODELETION IN A GIRL WITH SCHIZOPHRENIA AND SEVERE MENTAL DELAY**

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Several new microdeletion syndromes have been identified by using the array-Comparative Genomic Hybridization (a-CGH) analysis. Their identification and clinical description is most relevant for the clinical approach, counseling, and the management of patients with severe mental retardation and neurological disorders. Among these, a newly identified syndrome is the recurring microdeletion 16p12.2-p11.2.In this study, we describe a female child with schizophrenia and serious mental delay. 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 8.168 Mb on the 16 chromosome at p12.2-11.2 region that includes several OMIM genes such as SCNN1G, SCNN1B, PALB2,COG7, IL4R, IL21R, CLN3, ATP2A1, CD19 and TUFM.Minor facial anomalies, feeding difficulties, significant delay in speech development, and recurrent ear infections are common symptoms observed in the microdeletion syndrome 16p11.2-p12.2. Moreover, behavioral problems have been also identified in patients with 16p11.2-p12.2 microdeletion, such as anxiety, irritability, hyperactivity, and short attention span.The deletion described in this study could be correlated to the complex clinical phenotype of our patient.

P046
## A PATIENT WITH DIFFUSED BONE PAIN: THE ROLE OF THE LABORATORY IN DIAGNOSTICATING ITS CAUSE

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Introduction: Multiple Myeloma (MM) represents 10% of all hematological malignancies, in 15% of thoughts with MM the monoclonal component is considered by light chains only, kappa or lambda. This particular form of MM is known as Light Chain Multiple Myeloma (LCMM).Clinical Case: A 53-year-old patient at the Corelab Laboratory (AOU-AUSL of Modena) blood tests by a general practitioner for bone pain. The full blood count does not show significant alterations, whereas a slight hypogammaglobulinemia (5.5 mg / L) can be seen at the electrophoresis of serum proteins (ELF) in capillary technique (Capillarys, SEBIA, Florence). A serum immunofixation (IFE) on agarose gel (IF 2/4 SEBIA, Florence) which highlighted the presence of free kappa light chains not attributable to any heavy chain and the dosage of serum free light chains (FLC) with Turbidimeter Optilite and Antisera Freelite, (The Binding Site, Birmingham, United Kingdom) with the following results: FLC-k 2677.17 17 mg / L; FLC-λ 6,15 mg / L; FLC ratio (rFLC), 435.31. The value of rFLC detected, diagnostic for MM, induced a GP who sent the doctor at the Oncohematology of the Policlinico of Modena who contacted the laboratory for the timely sending of the results of the examinations performed. The patient was then admitted for suspected LCMM then confirmed by the bone marrow biopsy.
Discussion: The LCMM represents the third most common type of MM and difficult to recognize because it almost does not produce changes in the electrophoretic pattern.Although not very evident, these alterations do not go unnoticed by an expert eye and, together with the indication of the MMG of bone pain, were decisive for orienting the work to autonomous examination.Crucial and very useful for the control of MM words is the d-FLC dosage, diagnostic according to international guidelines. The laboratory professional's own initiative therefore translates into "personalized medicine" that is related to the patient's needs. The competence of the laboratory, in the light of the guidelines and scientific knowledge, proves important in the success.

P047
## AN UNEXPECTED PRENATAL DIAGNOSIS

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Maturity Onset Diabetes of the Young (MODY) designates a group of heterogeneous disorders characterized by diabetes onset in young (generally before 25 years of age), no signs of autoimmunity, autosomal dominant inheritance and dysfunction of the pancreatic β cells due to mutations of at least 14 different genes. MODY1, MODY2, MODY3, and MODY5 are the most frequent forms. N.R.G., a pregnant woman, was addressed to geneticists from a dermatological center to perform a prenatal investigation because she was affected by Incontinentia Pigmenti, a rare, X-linked dominant, multisystem ectodermal dysplasia. At the 16th week of pregnancy she performed amniocentesis, and molecular analysis on fetal DNA was negative for the maternal disease. Because the ultrasound had shown fetal severe abnormalities of the kidneys, an NGS analysis was performed, aimed at the studying of genes associated with renal abnormalities. The analysis revealed a mutation in the HNF1b gene (c.344+2T>C), that was confirmed after birth. Mutations in this gene are associated with the development of MODY5 diabetes generally in puberty. Immediately after the birth of a Small for Gestational Age (SGA) infant, pediatricians monitored the blood glucose of the infant by Continuous Glucose Monitoring (CGM) which showed an increase in glycemia after feeding. These data, although not confirming the presence of neonatal diabetes, indicate the presence of a glycemic defect due to the fetal insulin deficiency and suggest the need to closely monitor the patient over time in order to early diagnose diabetes and treat the patient appropriately so preventing or avoiding the development of complications related to the disease. In addition, the use of corticosteroids, when the child will receive a kidney transplant due to severe renal failure, could contribute to determining the early onset of diabetes. This case shows how the NGS analysis has played a crucial role in the correct fetal diagnosis, allowing to highlight an unexpected genetic defect useful for the correct clinical management of the young patient

P048

## ANTIDOTE - YES OR NO? ASSESSMENT OF DABIGATRAN PLASMA CONCENTRATION CAN AID IN MANAGING TREATMENT OPTIONS IN CASE OF DABIGATRAN OVERDOSE

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Introduction: Dabigatran (DBG) is a direct thrombin inhibitor mainly used for the stroke and systemic venous tromboembolism prevention. Since it has a dose predictable anticoagulation activity, routine laboratory monitoring of its anticoagulant effect is usually not necessary. Nevertheless, when overdose with DBG is suspected, an urgent laboratory assessment of its plasma concentration is required. We are describing a case of a DBG overdose in an elderly female due to an acute renal insufficiency.

Methods: A case report.

Results: An 87-year-old female patient on DBG, with a history of atrial fibrillation and chronic heart failure, presented with the complaints of generalized weakness and dehydration. Although instantly rehydrated, she developed oliguria and laboratory tests revealed high serum creatinine (2.1 mg/dL). Coagulation tests showed significantly prolonged prothrombin time (PT) and activated partial thromboplastin time (aPTT). DBG accumulation was suspected and confirmed by detecting high concentration in plasma (763 ng/mL). Significant drop of DBG plasma concentration was observed over the next 4 days (145 ng/mL), which was followed by normalization of the coagulation tests (PT-ratio: 1.31, aPTT-ratio: 1.41). Since our patients' overall bleeding risk was regarded low, specific treatment intervention, including the administration of an antidote, was not necessary. Considering her low eGFR (36 ml/min/1.73m$^2$), long-term diuretic treatment was prescribed, while DBG was replaced by Apixaban (APX), an anti X-a direct oral inhibitor. Regular monitoring of blood pressure, creatinine level and hydration status was advised to prevent future episodes of acute renal failure and APX overdose.

S Management of patients with DBG overdose remains challenging. DBG is the only drug for which a reversal agent - Idarucizumab was Conclusions: licensed for use in a case of an overdose associated with the risk of severe bleeding. Laboratory monitoring of aPTT can be a predictor of DBG activity, but its value may not accurately correlate with DBG concentration. Therefore, measuring DBG concentration in plasma can be of great aid in assessing the bleeding risk, overall outcome and managing treatment options in patients who accumulate DBG either due to overdose or renal insufficiency.

P049

## LA QUANTIFICAZIONE DELLE CATENE LEGGERE LIBERE CIRCOLANTI INDIVIDUA UN PICCOLO CLONE AMILOIDOGENICO IN UN PAZIENTE CON AMILOIDOSI CARDIACA E PERMETTE DI CORREGGERE LA DIAGNOSI

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Un uomo di 68 anni con storia di sindrome del tunnel carpale nel giugno del 2018 esegue una visita cardiologica per comparsa di dispnea. L'ecocardiogramma evidenzia un quadro di sospetta amiloidosi cardiaca e la concentrazione del peptide natriuretico di tipo B (BNP) risulta 360 ng/L (v.r. <99 pg/mL). L'immunofissazione del siero e delle urine non mostra componenti monoclonali. La scintigrafia con $^{99m}$Tc-HDP ha mostrato un'elevata captazione del tracciante a livello cardiaco, ritenuto compatibile con un'amiloidosi da transtiretina (visual score 3). L'aspirato del grasso periombelicale non ha mostrato depositi di amiloide e l'indagine genetica non ha mostrato la presenza di mutazioni a livello del gene per transtiretina. È stata posta diagnosi di amiloidosi cardiaca ATTRwt (senile) ed è iniziata una terapia con doxiciclina. Dopo un anno di trattamento, il paziente è giunto alla nostra osservazione per peggioramento dello scompenso cardiaco nel giugno 2019. In questa occasione la concentrazione del frammento N terminale del peptide natriuretico di tipo B (NT-proBNP) era 1300 ng/L (v.r.: <334 pg/mL) e la troponina I 0,05 ng/mL (v.r. <0,04 ng/mL come 99°percentile della distribuzione). L'immunofissazione del siero e delle urine non ha rilevato componenti monoclonali, mentre la quantificazione delle catene leggere libere circolanti (FLC) ha mostrato catene leggere libere $\kappa$ 250 mg/L (v.r. 3.3-19.4), con un rapporto $\kappa/\lambda$ 15 (v.r. 0.26-1.65). Secondo le linee guida attuali, anche in presenza di una captazione cardiaca di bisfosfonati alla scintigrafia, nei pazienti con componenti monoclonali, è necessaria una diagnosi istologica per definire il tipo di amiloidosi. Pertanto, è stata eseguita una biopsia endomiocardica, nella quale sono stati individuati depositi di amiloide alla colorazione con rosso Congo. L'analisi immunoistochimica ultrastrutturale è risultata positiva per catene legge $\kappa$ e negativa per transtiretina. È stata posta diagnosi amiloidosi AL cardiaca, la terapia per amiloidosi da transtiretina è stata sospesa ed è iniziata una chemioterapia contro il clone plasmacellulare. La quantificazione delle FLC è uno strumento utile per l'identificazione dei piccoli cloni plasmacellulari ed è un esame indispensabile per la diagnosi differenziale dell'amiloidosi cardiaca.

P050
## MONITORAGGIO MOLECOLARE DI ISOCITRATO DEIDROGENASI 2 (IDH2) IN PAZIENTE CON LEUCEMIA MIELOIDE ACUTA (LMA) RECIDIVATA

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Introduzione: La caratterizzazione molecolare della LMA è essenziale per diagnosi, terapia e prognosi. Tra i marcatori emergenti, l'individuazione di mutazioni di IDH2 permette l'impiego nella pratica clinica di inibitori anti-IDH2. La mutazione puntiforme, presente nel 20% dei casi, coinvolge i codoni R140 e R172 alterando il differenziamento cellulare. Il monitoraggio del target permette di valutare la malattia minima residua (MRD) e l'efficacia della terapia.
Presentazione: Nel novembre 2016 un uomo di 38 anni viene ricoverato nell'U.O di Ematologia con diagnosi di LMA negativa per i marcatori molecolari convenzionali. Effettua un ciclo di induzione senza risposta (20% di blasti) seguito da terapia di salvataggio, anch'essa senza risposta (13% di blasti) per cui viene sottoposto a protocollo terapeutico sperimentale con il quale ottiene remissione ematologica completa (CR). Il paziente viene avviato a procedura trapiantologica a giugno 2017 ma dopo 7 mesi presenta recidiva di malattia. Non avendo a disposizione il test per la valutazione della mutazione di IDH2 al momento della diagnosi, si è valutato l'andamento del marcatore retrospettivamente. All'esordio viene riscontrata la mutazione R172K (29%) presente anche dopo le due linee di terapia. Alla remissione ematologica, in assenza di blasti circolanti, il paziente presentava ancora un 2% di MRD con cui quindi è andato al trapianto. Il primo time-point post-trapianto, in CR, presentava chimerismo 100% donor e WT1 4 (copie WT1/copie ABL*1000). Alla recidiva ematologica i marcatori sono entrambi positivi: WT1 3401 e IDH2 7,8%. Il time-point precedente risultava 100% donor e i due marcatori discordanti: negativo WT1 e positivo IDH2 (0,1%).
Conclusioni: Nel nostro caso il monitoraggio di IDH2, benché non raccomandato come marcatore di MRD dalle linee guida ELN, avrebbe inquadrato la remissione pre-trapianto come "CR MRD+" indicando la persistenza del clone leucemico. Per quanto riguarda la CR post-trapianto, IDH2 avrebbe identificato la recidiva molecolare 2 mesi prima di quella ematologica permettendo di anticipare il trattamento. Inoltre, la sua caratterizzazione alla diagnosi avrebbe indirizzato la terapia verso l'impiego di inibitori anti-IDH2 migliorando le possibilità di remissione in prima linea.

P051
## RUOLO DEL LABORATORIO NELLA VALUTAZIONE DI UNA PAZIENTE CON CRISTALLURIA DI NATURA DA DETERMINARE

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Il caso riguarda una paziente di 30 anni alla 24[a] settimana di gravidanza, in accesso al Pronto Soccorso con febbre e dolore al fianco destro. Il Laboratorio Generale riceve il campione di urine della paziente per l'esecuzione dell'ECMU e risultano alterati i seguenti valori: WBC 174 cell/µL (IR < 18 cell/µL), esterasi leucocitaria 500 e pH 7 (IR 5-6.5) (IRICELL 2000 Beckman Coulter, Inc). La paziente viene ricoverata presso il reparto di Ginecologia. Dopo 16 ore dalla prima raccolta, viene inviato un ulteriore campione di urine, i cui esiti risultano: WBC 150 cell/µL, esterasi leucocitaria 250 e pH 6.5. Inoltre, il campione presenta numerosi cristalli aghiformi di colore nero e con elementi intrecciati a forma di "freccia", non ulteriormente classificabili. Il reparto viene prontamente informato dal Dirigente di turno, riportando nel referto: "Si segnala la presenza di cristalli aghiformi di natura da determinare. Comunicato telefonicamente al medico richiedente".La determinazione della natura dei cristalli ritrovati nelle urine della paziente viene effettuata mediante lo spettrometro FT-IR IRAffinity (Shimadzu, Co) in uso presso il Laboratorio della Medicina del Lavoro. Gli spettri prodotti ($\lambda$ 4000-400 $cm^{-1}$, risoluzione 4 $cm^{-1}$) analizzando il sedimento urinario sono confrontati con la biblioteca di riferimento per i calcoli renali NICODOM, 1994-2007 (NICODOM, Ldt). Nel sedimento delle urine della paziente vengono identificati cristalli di amoxicillina (score: 87.7%), in accordo con quanto solo successivamente verificato (trattamento con amoxicillina endovena). Dopo ulteriori 12 ore, viene inviato un terzo campione di urina, che risulta senza alterazioni dell'esame chimico-fisico e senza cristalli.Nei giorni successivi, l'analisi con lo spettrometro FT-IR sia di cristalli urinari che di calcoli renali di altri pazienti conferma in tempi molto brevi i risultati inizialmente ottenuti rispettivamente con lo strumento IRICELL per le urine e la metodica colorimetrica per i calcoli in uso presso il Laboratorio Generale.Questo approccio di approfondimento rappresenta l'integrazione di metodiche analitiche di routine con tecniche avanzate a livello molecolare e strutturale, ai fini di una più rapida, esatta ed appropriata diagnostica di routine.

P052
**TERAPIA ANTICOAGULANTE ORALE E FATTORI GENETICI LEGATI AL TEV - CASO CLINICO**

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L°incidenza stimata di tromboembolismo venoso (TEV) è di l-3 eventi ogni 1.000 individui/anno circa metà dei pazienti che presentano un primo episodio di TEV idiopatico, è portatore di uno stato trombofilico congenito. Il TEV è una patologia multifattoriale dovuta all'interazione di fattori genetici, ambientali e comportamentali. E" nota da tempo l°associazione tra TEV e stati fisiologici (età, gravidanza, puerperio), patologie intercorrenti (traumi, interventi di chirurgia maggiore, allettamento, neoplasie, presenza di cateteri venosi), terapie farmacologiche (estroprogestinici, terapia ormonale sostitutiva, chemioterapici), obesità; più recente è l'associazione con i viaggi aerei di lunga durata. Spesso la combinazione di uno di questi fattori di rischio con un fattore genetico, aumenta il rischio di TEV in maniera moltiplicativa. Elemento essenziale per la diagnosi di disordine ereditario trombofilico è la diagnosi di portatore di deficit di antitrombina, proteina C, proteina S, di omozigosi per il fattore V Leiden, omozigosi per la protrombina G202l0A o anomalie combinate, tramite i metodi di laboratorio. Non è invece di alcuna utilità clinica la ricerca di mutazioni a carico del gene che codi¿ca per Penzima metilen-tetra-idrofolato-reduttasi (MTHFR), della via metabolica metionina cisteinaIl riscontro di famiglie con elevata frequenza di eventi trombotici correlati a fattori genetici è riportato in letteratura già da molti decenni.Il caso clinico in questione e' riferibile ad episodi di TEV recidivante in appartenenti ad un nucleo familiare (padre , 1 figlia,1 nipote)L'aspetto piu' " originale" di questa storia familiare e' l'interesse rivolto allo studio genetico di questa patologia, solo al momento della gravidanza della nipote (26 anni) con storia clinica di poliabortivita' (5 aborti).Esami genetici condotti sul nucleo familiare hanno rivelato il seguente aspetto geneticoGenitori; padre con episodi recidivanti di TEV; in trattamento con Warfarina. Omozigote mutato per il fattore V Leiden, proteina C anticoagulante Funzionale inf.al 30% , madre omozigote mutato per MTHFR C677 T,valori di omocisteina sup.a 40 micromoli/L.Figlia: omozigote mutato per MTHFR C677 T (1 gravidanza a termine con parto eutocico)Nipote: poliabortivita'; proteina C anticoagulante funzionale 25%; omocisteina sup.a 50 micromoli /LLa paziente opportunamente indirizzata presso centro specialistico, con la collaborazione di varie figure professionali e le cure del caso, partorisce con taglio cesareo Francesco.In accordo ai dati riportati in letteratura,si conferma l'importanza dello studio genetico multifattoriale in pazienti con episodi di TEV associato a stati fisiologici, nonche' l'inutilita' clinica della ricerca di mutazioni a carico del gene MTHFR.

P053
**UN CASO DI DISCRASIA PLASMACELLULARE CLONALE NON MIELOMATOSA CON DISCREPANTI DOSAGGI DI CATENE LEGGERE LIBERE IN PLASMA E URINE**

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Ad una donna di 56 anni nel 2010 fu diagnosticata leucemia linfatica cronica per la quale intraprese chemioterapia. Nel 2014 la paziente manifestò riacutizzazione di malattia con linfocitosi e piastrinopenia, splenomegalia e linfoadenopatie sopra e sottodiaframmatiche; contemporaneamente comparve una componente monoclonale di classe IgM tipo kappa. Le indagini midollari esclusero M.di Waldenstrom ed evidenziarono massiva infiltrazione di elementi linfoidi con parziale differenziazione linfoplasmocitoide secernenti IgM kappa.
Dal gennaio del 2018 il tracciato elettroforetico delle sieroproteine mostra un aumento del picco già presente in zona beta2-gamma, con massiccio incremento della componente monoclonale IgM (5760 mg/dl) e la paziente manifesta sintomi da iperviscosità con comparsa di epistassi e alterazione del campo visivo. Inizia quindi un trattamento bisettimanale di plasma exchange.
In occasione di una delle sedute di plasma exchange (8 Aprile 2019) vengono raccolti campioni sui quali sono eseguite ulteriori analisi. Sul campione prelevato prima della seduta di plasma exchange, le IgM sono 9320 mg/dl, e l'immunofissazione sierica evidenzia la componente monoclonale IgM kappa. Dopo plasma exchange le IgM sono ridotte a 5950 mg/dl. Le catene leggere libere (FLC) kappa plasmatiche, misurate con il reattivo Freelite della TheBindingSite e con il reattivo N Latex FLC della Siemens su nefelometro BNII, prima del plasma exchange sono rispettivamente 6,3 mg/dl e 64 mg/dl, mentre dopo plasma exchange sono 5,65 mg/dl e 52,6 mg/dl, evidenziando una discrepanza di risultati tra i due reattivi. Il plasma, sottoposto a Western Blot (WB) sviluppato con anti-kappa, evidenzia le bande corrispondenti alle IgM e alle FLC presenti prevalentemente come monomero.
Sul campione di urine raccolto nella stessa data l'immunofissazione evidenzia abbondanti catene leggere libere di tipo kappa. Le FLC misurate con Freelite sono risultate essere 200 mg/dl, e con N Latex FLC 811 mg/dl confermando la discrepanza osservata nel plasma. Anche il campione di urina sottoposto a SDS-PAGE e WB evidenzia FLC in forma prevalentemente monomerica. Il campione di urina, sottoposto a LC-MS, ha permesso di determinare con esattezza il peso molecolare del monomero, che è risultato di 24605 Da.

P054
## UN CASO DI IgD LAMBDA CON CATENE LEGGERE "NON REATTIVE" IN MIELOMA MULTIPLO

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Introduzione: Il mieloma multiplo (MM) a componente monoclonale (CM) IgD è una rara neoplasia plasmacellulare che rappresenta ~1-2% dei casi di MM. In letteratura sono stati riportati casi in cui cambiamenti conformazionali nella struttura delle catene leggere (LC) possono interferire con reazioni di precipitazione con specifici antisieri, rendendole "non reattive" alle convenzionali tecniche di immunofissazione sierica (s-IFE). Il caso clinico qui presentato riguarda una CM IgD con catene leggere lambda non reattive.
Caso clinico: Un paziente maschio di 74 anni è stato ricoverato a gennaio 2014 nel reparto di medicina per astenia, perdita di peso e dolori ossei diffusi. All'indagine si evidenzia anemia e ipogammaglobuninemia (elettroforesi capillare, Sebia). Agli esami di approfondimento diagnostico si evidenziano lesioni osteolitiche diffuse, la s-IFE evidenzia la sola presenza di catene leggere libere lambda (FLC λ) monoclonali in β-2, l'immunofissazione urinaria positiva per PBJ λ. La s-IFE con anti-D ed E rileva la presenza di catene pesanti IgD migranti in zona γ e FLC λ monoclonali in zona β-2, non associate. Abbiamo ritenuto potesse trattarsi di un caso di LC non reattive associate alla CM IgD e quindi trattato il campione eseguendo una immunosottrazione (IS) a prolungato tempo di incubazione. Nello specifico abbiamo preincubato a 4°C, per 48 ore, 2 aliquote di siero con aggiunta di anti-k e anti-λ in eccesso. Successivamente eseguite due s-IFE a lenta migrazione con anti-D, anti-k, anti-λ. Si ipotizzava che la preincubazione con eccesso di antisiero verso le catene leggere potesse precipitare anche la catena pesante monoclonale legata ad esse, e quindi renderla non rilevabile alla successiva immunofissazione eseguita sul surnatante. Nel nostro specifico caso la CM IgD è scomparsa a seguito del trattamento con eccesso di antisiero anti-λ e ciò ha permesso di caratterizzarla come CM IgD lambda.
Conclusioni: La IS è risultata una tecnica semplice che potrebbe essere utilizzata per tipizzare le CM con catene leggere non reattive alla s-IFE.

P055
## ACQUIRED HEMOPHILIA IN PATIENT WITH WALDENSTROM'S MACROGLOBULINEMIA

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Background: The WHO defines Waldenström Macroglobulinemia (WM) as a B-cell lymphoplasmacytic lymphoma (LPL), associated with a monoclonal immunoglobulin M (IgM) protein in serum, and infiltration of bone marrow with lymphoplasmacyte cells. Some monoclonal proteins from patients with WM possess antigen-binding activity directed at autogenous or foreign antigens, so that a number of autoimmune syndromes have been identified as being mediated by monoclonal macroglobulin. Monoclonal IgM may also interact with protein expressed on cell membranes or with plasma coagulation proteins including fibrinogen, and Factors V, VII e VIII, and may be responsible for abnormal bleeding and clotting times.
Method: A 61-year-old male patient was referred to our hospital with serious muscle and skin bleeding. The abdomen-pelvis CT scan showed a 70 x 67 mm hematoma of the iliopsoas muscle. Successively, due to a low platelet count (61x10^3/μL), the patient was subjected to a bone marrow biopsy, with a diagnosis of B-cell lymphoplasmacytic lymphoma. The laboratory diagnostic workup was oriented both to the evaluation coagulation tests and to the research of the monoclonal component, with serum protein electrophoresis (CZE), serum Immunofixation (s-IFE), urine Immunofixation (u-IFE) (Sebia) and sFLC (The Binding Site).
Results: The coagulation studies showed a normal prothrombin time (PT) and prolonged activated partial thromboplastin time (aPTT) not corrected by incubating the patient's plasma with equal volumes of normal plasma (mixing study), reduced Factor VIII activity and a high anti-FVIII inhibitor titer: diagnosis of acquired hemophilia A. CZE showed a monoclonal spike of 1.01gr/dL, s-IFE confirmed IgM-kappa, u-IFE showed monoclonal k chains, serum free light chain assay showed a ratio of 4.21. Conclusions: Hemostatic disorders are common in dysprotidemias and are characteristic of WM. Clinical and laboratory study indicate that paraproteins may act as specific inhibitors of one or more coagulation Factors; in this case, it is likely that it acted as an inhibitor of Factor VIII.
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P056

## CLINICAL SIGNIFICANCE OF ANTIBODIES DIRECTED AGAINST DOMAIN 1 OF β2-GLICOPROTEIN 1 IN ANTIPHOSPHOLIPID SYNDROME

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Background: Antiphospholipid syndrome (APS) is a systemic autoimmune disorder characterized by the presence of persistent antiphospholipid antibodies (aPL) in patients with thrombosis and/or pregnancy complications. Laboratory diagnosis depends upon the detection of a Lupus Anticoagulant (LA), and/or anticardiolipin (aCL) and anti-β2-glicoprotein 1 (aβ2GP1) antibodies. In APS anti-domain 1 β2-glicoprotein 1 antibodies (aβ2GP1-D1) have been involved in the immunopathogenesis of APS. However, the clinical significance of anti-D1 β2GPI in APS thrombotic risk assessment is unknow. The aim of study was to assess the diagnostic value of aβ2GPI-D1 antibodies in APS.
Methods: Sixty-four patients with suspected APS were included. Levels of aCL,aβ2GP1 (IgG and IgM class) antibodies, together with aβ2GPI-D1 antibodies were measured by ACL ACUSTAR chemiluminescent immunoassay and LA with coagulometric methods.
Results: The level of IgG aβ2GP1-D1 antibodies were significantly increased in patient with APS, compared with patients with no APS ($p<0.001$). Moreover the levels of IgG aβ2GP1-D1 antibodies were significantly correlated with levels of IgG aβ2GP1in all patients ($p<0.0001$). In addition, significat correlation were also observed between the levels of IgG aβ2GP1-D1 antibodies and the levels of IgG aCL anibodies and LA ($p<0.0001$and $p<0.001$).The levels of IgG aβ2GP1-D1 antibodies were also evaluated in APS patients with the triple, double and single aPL positivity. The triple a PL posititivity has been considered as a risk factor for aPL -mediated clinical manifestations. Importantly, significantly higher levels of IgG aβ2GP1-D1 antibodies were found in patients with triple a PL positivity ($p< 0,05$), compared with patients with double and single aPL positivity (respectively $p<0.01$ and $p<0.01$).
Conclusions: Our results suggest that testing for anti-D1 β2GPI antibodies may contribute to APS risk assessment, specially with more aggressive aPL profiles, such as triple positivity and lupus anticoagulant activity.
Ref: Iwaniec T et al. Clinical significance of anti-domain 1 β2-glycoprotein I antibodies in antiphospholipid syndrome.Thromb Res. 2017;153:90-94.

P057

## TERAPIA ANTICOAGULANTE CON ANTAGONISTI DELLA VITAMINA K: LA SCELTA DEL FARMACO PUO' CONDIZIONARE LA QUALITA' DEL TRATTAMENTO NEL SINGOLO PAZIENTE?

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Introduzione: Il trattamento anticoagulante con anti-vitamina K è caratterizzato da una stretta finestra terapeutica per cui la stabilità della risposta terapeutica è un parametro fondamentale. Da alcuni anni sono stati introdotti nella pratica clinica parametri quali il Time in Therapeutic Range (TTR) ed il Variance Growth Factor (V-score) che permettono di monitorare la 'stabilità' della risposta al trattamento con l'obiettivo di ridurre il rischio di eventi tromboembolici ed emorragici. Svariati studi hanno cercato di identificare quale tra gli antivitamina K fosse in grado di dare la migliore stabilità della risposta anticoagulante senza che tuttavia sia stata ottenuta una risposta univoca e definitiva.
Obiettivi: L'obiettivo dello studio è stato di valutare se in pazienti in terapia anticoagulante con Acenocumarolo e con scarsa stabilità del trattamento, la conversione al Warfarin fosse in grado di migliorare la stabilità della risposta terapeutica indicata dal TTR e del V-score.
Pazienti e metodi: È stata condotta un'analisi retrospettiva su un gruppo di 58 pazienti in terapia con acenocumarolo da più di sei mesi nei quali è stata eseguita una conversione dell'anticoagulante con passaggio a trattamento con Warfarin. Abbiamo analizzato il valore del TTR e del V-score prima e dopo la modifica della terapia. I dati sono stati elaborati mediante il test statistico di Wilcoxon e l'analisi della varianza per misure ripetute di Friedman.
Risultati: In seguito alla conversione da acenocumarolo a Warfarin si è ottenuto un miglioramento statisticamente significativo della mediana del TTR che è passata dal 45,7% del periodo in Acenocumarolo ad un valore di 59,3% del periodo in Warfarin e il V-score da 1,475 a 0,51. Valutando l'andamento dei singoli pazienti abbiamo osservato, inoltre, che in 36 pazienti su 58 (62%) il valore mediano del TTR è migliorato di oltre il 10% in 14 su 58 pazienti (24%) ha avuto una variazione inferiore a ±10% e in 8 pazienti su 58 (14%) il valore del TTR è peggiorato di oltre il 10%.
Conclusioni: I risultati del nostro studio indicano che nel singolo paziente la stabilità della risposta anticoagulante può essere influenzata dal tipo di farmaco antivitamina K utilizzato. Nei pazienti in trattamento con acenocumarolo con scarsa stabilità della terapia, la conversione a Warfarin è risultata vantaggiosa nella maggioranza dei casi tuttavia, in una minoranza di pazienti, si può avere un ulteriore peggioramento della risposta terapeutica.

P058
**INTERAZIONE TRA FARMACI ANTIEPILETTICI E FARMACI ANTICOAGULANTI DIRETTI: ESPERIENZA IN 15 PAZIENTI**

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Introduzione: le caratteristiche farmacocinetiche dei farmaci antiepilettici (AEDs) di vecchia e nuova generazione e alcuni studi in vitro suggeriscono la possibilità di interazione con i farmaci anticoagulanti diretti (DOACs). Tale interazione non è stata studiata in vivo e non esistono dati sulla concentrazione plasmatica dei DOACs in corso di AEDs. E' stato condotto uno studio retrospettivo per valutare l'interazione fra DOACs e AEDs attraverso la determinazione plasmatica (DP) dei DOACs ed il monitoraggio di eventi trombotici o emorragici su 15 pazienti in terapia con almeno 1 AED.
Materiali e metodi: La concentrazione plasmatica del DOAC è stata ottenuta in 14 pazienti utilizzando metodi cromogenici basati sull'attività anti-Xa per Apixaban, Edoxaban e Rivaroxaban e con test di Ecarina per Dabigatran (Diagnostica STAGO).
Risultati: Nessun paziente ha presentato eventi clinici (follow up medio 17 mesi). 7 pazienti su 14 (6 in Rivaroxaban e Levetiracetam, Carbamazepina, Fenobarbital, Zonisamide, Valproato, Topiramato, Primidone e 1 in Dabigatran e Levetiracetam) presentavano una DP al di sotto dei 30 ng/mL, che è al di sotto della concentrazione plasmatica media attesa per i DOACs, anche se i valori possono rientrare nell'intervallo di confidenza a causa di una ampia variabilità biologica. Alcuni studi (RELY, ENGAGE) hanno dimostrato una correlazione tra bassa concentrazione plasmatica dei DOACs e aumentato rischio di eventi ischemici.
Conclusioni: La numerosità dello studio è ancora troppo bassa per conclusioni certe, ma possono esistere in vivo interazioni fra DOAC e AED e nella pratica clinica la misura della concentrazione plasmatica dei DOACs potrebbe essere appropriata in questa tipologia di pazienti.

P059
**POST ANALYTICAL VARIABLES IN COAGULATION TESTING: COGNITIVE INVESTIGATION IN PIEMONTE AND VALLE D'AOSTA**

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Introduction: The use of modern laboratory instrumentation with high levels of test reliability and appropriate quality assurance measures will lead to very few analytical errors within hemostasis testing. Nevertheless, incorrect or inappropriate test results are still reported, often due to events outside the control of the laboratories performing the tests. This is due primarily to pre-analytical events related to sample collection and processing but also can include post analytical events related to the reporting and interpretation of test results.
Aim: Aim of Piedmont and Aosta Valley Laboratory Coagulation Group (GEPAL) was to introduce regional consensus in preanalytical errors management and in reporting of laboratory results of first level coagulation assays: Prothrombin Time (PT), Activated Partial Thrombplastin Time (APTT), Fibrinogen (Fib), Antithrombin (AT), Ddimer (DD), Direct Oral Anticoagulants (DOACs).
Methods: We sent a questionnaire to all laboratories of our two regions asking about: assays performed in routine/emergency laboratory; units of measure utilized on the laboratory report; reference ranges (calculated, datasheets, literature); critical values (identification, communication, management) pre-analytical coagulation variables management.
Results: We received 13 questionnaires, only a few part of laboratories of two regions area. In GEPAL group after discussion of questionnaires data and analysis of updated guidelines we provide a consensus on coagulation first level assays report of test results in adherence with Italian and International Guidelines. Moreover, because clinicians are less aware of the issue of pre-analytical variables, and would base their clinical response on the test result that they received, we provide

recommendation for specimen pre-analytical coagulation variables management.
Conclusion: Laboratory scientists are ultimately responsible of coagulation test results and there is a duty to provide accurate and precise results expressed in correct units of measures with specific reagent/instrumentation calculated reference ranges in strictly adherence of pre-analytical coagulation variables recommendation management in order to enable clinicians to manage patients appropriately. Laboratory workers collaboration gave us the possibility to improve Western Italy laboratory reporting of first level haemostasis assays.

P060
**HEMOLYSIS, ICTERUS AND LIPEMIA INDICES PERFORMED ON COBAS t511 COAGULATION NEW ANALYZER**

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In the coagulation laboratory, spurious hemolysis, icterus and lipemia (HIL) in test samples represent by far the leading diagnostic prenalytical challenges. The aim of this study was to assess the performance of the preanalytical module on the new hemostasis analyser Cobas Roche t511. We assessed the influence of HIL on prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (Fib), antithrombin (AT) and DDimer (DD) on plasma pools aliquots with different interference degrees. Moreover, we evaluated spontaneous haemolysis by comparing results on 50 paired samples (hemolysed versus non hemolysed). Spurious hemolysis interference studies highlight the absence of a clinical significant impact on PT, APTT and AT test results at all haemoglobin concentration investigated. For Fib and DD assays a clinically significant difference was observed in the most hemolysed aliquot for Fib and in the two most hemolysed aliquots for DD. Spontaneous hemolysis interference studies showed no clinical significant differences for PT and AT assays, instead for APTT, Fib and DD we found significant statistical and clinical differences between hemolysed and non hemolysed specimens. Bilirubin interference studies and lipemic samples interference studies enable us to confirm that the differences in the results obtained between the different aliquots and reference pool is not clinically significant for all assays. HIL check preanalytical module of Cobas Roche t511 analyzer displaied excellent performance for routine use in clinical laboratories. Regardless of analytical considerations, the type of interference encountered with spurious hemolysis, icterus and lipemia is substantially different and requires different approaches.

P061

## PREPARAZIONE DI POOL DI PLASMI NORMALI (NPP - NORMAL POOL PLASMA) SU BASE REGIONALE PER IL CALCOLO DELLA RATIO DEI TEST DI SCREENING DELLA COAGULAZIONE

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Premessa: I test di screening della coagulazione, il tempo di protrombina (PT) e il tempo di tromboplastina parziale attivato (APTT), sono i test più frequentemente utilizzati per indagare le anomalie della coagulazione. I risultati del PT sono stati espressi in secondi, percentuale di attività, ratio (rapporto tempo paziente/tempo plasma di riferimento) e INR (International Normalized Ratio); i risultati dell'APTT vengono invece espressi in secondi ed in ratio. Come suggerisce CLSI (Clinical and Laboratory Standards Institute) è auspicabile che i risultati di entrambi i test vengano espressi solo in ratio allo scopo di minimizzare la variabilità intra-laboratorio; nel caso di pazienti in trattamento con anticoagulanti anti-vitamina K, il PT deve essere espresso in INR ($Ratio^{ISI}$). Per il calcolo della ratio vengono solitamente utilizzati plasmi commerciali, ma sarebbe preferibile la preparazione di NPP, in quanto questi hanno caratteristiche simili ai plasmi dei pazienti da testare.
Materiali e metodi: Sono stati preparati due pool presso il Laboratorio di Siena (AOUS) e il Laboratorio di Firenze (Torregalli). In entrambi i laboratori sono stati utilizzati i plasmi di 40 donatori di sangue reclutati secondo i criteri di CLSI. Gli strumenti ed i reagenti utilizzati (dello stesso tipo e dello stesso lotto) sono stati i medesimi sia per il PT che per l'APTT in entrambi i centri (coagulometri ACL TOP 750, ReadiPlasTin per il PT, SynthASil per l'APTT).
Risultati: PT (media geometrica dei dati): 11,5 (Siena), 11,6 (Torregalli); APTT (media geometrica dei dati): 30,4 (Siena), 30,6 (Torregalli).
Conclusioni: La recente gara per l'assegnazione di un sistema unico per l'esecuzione di esami nel settore di Emostasi per l'intera Regione Toscana offre interessanti opportunità. L'utilizzo degli stessi sistemi (strumenti e reagenti) permette di fornire ai centri che non hanno la possibilità di preparare per vari motivi NPP al loro interno, i valori dell'NPP preparati nei due centri di riferimento. Questo permette di armonizzare le risposte dei test di screening in piùlaboratori; inoltre, offre ulteriori opportunità quali lo studio delle miscele, l'utilizzo come CQI,l'utilizzo come plasma calibrante per il dosaggio dei fattori e degli inibitori fisiologici della coagulazione.

P062

## ANALYTICAL PERFORMANCE EVALUATION OF THE NEW ROCHE T411 AND T511 COAGULATION ANALYSERS

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Background: The increasing demand for coagulation tests as well as the economic pressure for reducing laboratory costs and decreasing reagents budgets have raised interest in haemostasis laboratory automation. In the present study, the Roche T411 and T511 analysers have been compared to the performance of the top ACL coagulation analyser for the determination of routine coagulation parameters [4]. These fully automated coagulation analysers use multiple wavelength technology to measure clotting (e.g., activated partial thromboplastin time – aPTT, prothrombin time – PT, fibrinogen – FBG), chromogenic (e.g., antithrombin – AT) and immunological (e.g., D-dimers – DDi) assays.
Materials and methods: We evaluate the performance of the T411 and T511 coagulation analysers versus the coagulation analyser ACLTop 500. Patient samples, human pooled plasma and quality control samples were used. The patient samples were obtained from the routine laboratory after the requested patient care analyses were performed. The samples were tested anonymously. The citrated plasma (0.109 M sodium citrate) was prepared within 4 hours after blood drawing. Plasma was obtained by centrifugation at 2500 g for 10 minutes at room temperature. A total of 915 samples, up to 220 patient samples for comparative instruments were assayed by compare the cobas T411 and T511 coagulation analyzers. The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).
Results: We verified the repeatability and precision of the test methods using a quick protocol to check brand's product declaration. The protocol used was a 3x5 (3 replicates for 5 days) over a relatively long time interval (at least 2 weeks). The verification was carried out using 2 control quality materials used by the company in the phase of validation and reported in the technical sheet. The within-day and between-day imprecision, accuracy and total error were all acceptable.Passing-Bablok, Bland-Altman and Mountain plot were used to compare the data with those obtained with ACL 500 TOP automated coagulation analyser.
Conclusion: The operational performance of the T411 and T511 compared with the coagulation analyser ACL TOP, indicated a good results and performances for the T511, on the other hand the T411 exhibits less satisfactory results within a lower quality standards respect to T511.

P063
**PLASMA PHOSPHOLIPID DYSREGULATION IN PATIENTS WITH CYSTATHIONINE-β SYNTHASE DEFICIENCY**

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Rationale: Patients with cystathionine β-synthase deficiency (CBSD) exhibit high circulating levels of the amino acid homocysteine (Hcy). Enhanced lipid peroxidation has been documented in CBSD patients. No information is available about lipidomic profile in CBSD.
Objective: To characterize lipid signature in CBSD patients and controls (CTRL).
Methods and Results: Using an untargeted lipidomic approach, changes in plasma lipid metabolism were determined in 11 CBSD patients. Higher than CTRL content of medium and long-chain polyunsaturated fatty acids (PUFA) in phosphatidylethanolamine (PE) and lyso-phosphatidylethanolamine (LPE) species (p always <0.02), and depletion of lyso-phosphatidylcholine (LPC p=0.0033) and of phosphatidylcholine (PC, p=0.0248) species containing docosahexaenoic acid (DHA) were found in CBSD patients, suggesting impaired phosphatidylethanolamine-N-methyltransferase (PEMT) function. As PEMT needs methyl groups to convert PE into PC, the key plasma source of methyl groups S-adenosylmethionine (SAM) and the major transmethylation inhibitor S-adenosylhomocysteine (SAH) were measured by a liquid chromatography tandem mass spectrometry method. Mean SAH and SAM concentrations in CBSD patients were 2.8-fold ($p = 0.009$) and 1.3-fold ($p = 0.005$) higher than in CTRL, respectively. Ultrasound evaluations ruled out an association between liver steatosis/fibrosis and phospholipid dysregulation.
Conclusions: An untargeted lipidomic approach reveals depletion of PC species containing DHA and accumulation of PUFA in PE/LPE species in plasma from CBSD patients. Metabolic perturbations -including slight changes in plasma SAM and SAH concentrations – are associated with such phospholipid dysregulation. An impaired PEMT-mediated PE→PC conversion -as evaluated by the related metabolites- provides a highly sensitive functional marker of the multiple biochemical abnormalities occurring in CBSD patients.

P064
**LE NON CONFORMITÀ PRE-ANALITICHE NEL LABORATORIO ANALISI DEL DIPARTIMENTO MILITARE DI MEDICINA LEGALE DI PADOVA: UNO STUDIO QUANTITATIVO E QUALITATIVO**

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La fase preanalitica rappresenta, secondo la letteratura scientifica, l'anello debole nel processo di produzione del dato laboratoristico: in particolare gli errori si concentrano in attività esterne al laboratorio, quali identificazione del paziente, modalità di prelievo dei campioni, tempi prolungati di trasporto e temperature di conservazione non adeguate. Le predette problematiche, pur non essendo dipendenti dall'operato del laboratorio, devono essere prontamente rilevate al fine di evitare la produzione di dati analitici non attendibili.
Allo scopo di valutare numero e tipologia delle non conformità preanalitiche dei campioni ematici, nel nostro Laboratorio è stata intrapresa una registrazione sistematica delle stesse elaborando delle procedure standardizzate per il trattamento dei campioni non conformi, tra cui dei commenti precodificati da inserire nei referti. Sono stati quindi implementati indicatori di qualità utili per valutare il processo preanalitico.
Nel corso del periodo oggetto di studio (12 mesi) sono pervenuti al laboratorio 41602 campioni ematici. In totale sono state registrate 323 non conformità relative alla fase preanalitica (0.78% dei campioni).
In numero assoluto le non conformità erano così distribuite: 173 campioni di siero per chimica clinica e immunologia, 134 campioni per coagulazione, 16 campioni per emocromo.
In percentuale il tipo di campione più frequentemente affetto da non conformità è quello dedicato agli esami di coagulazione (1.69%) e la problematica più frequente l'emolisi nei campioni di siero (0.90%), seguita dal non adeguato riempimento delle provette di coagulazione (0.59%), provetta non idonea (0.23%), campione coagulato (0.06%).
La standardizzazione delle procedure interne al nostro Laboratorio per la gestione delle non conformità preanalitiche dei campioni ci ha permesso di rilevare le principali criticità, ponendo le basi per l'introduzione di apposite misure preventive (stesura di istruzioni operative per i prelevatori, standardizzazione delle procedure di trasporto, programmazione di corsi di formazione) il cui esito sarà valutato monitorando nel tempo gli indicatori di qualità introdotti.

P065

## MALDI-TOF/MS PROFILING OF BD BARRICOR TUBE REVEALS A DIFFERENT PEPTIDOMIC PATTERN WITH RESPECT TO PST II TUBE

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Backgrounds: Mass spectrometry (MS) analysis of plasma represents a relevant tool for the identification of new biomarkers. However, both suboptimal centrifugation conditions of blood collection tubes and the presence of gel separator may reduce the overall quality MS analyses. The aim of this study was to compare the peptidomic profiling obtained by MALDI-TOF/MS analyses of BD Vacutainer® Barricor™ Plasma collection tube (Barricor) centrifuged at different conditions, with respect to BD PST II plasma tube (PSTII).
Materials and methods: One PSTII, centrifuged at 1300gx10min, two Barricor tubes centrifuged at 4000gx3min and 4000gx10min and one plasma sample ultracentrifuged at 12000gx5 min (Purified plasma) were collected from 29 subjects at fasting conditions. High abundant proteins were precipitated by adding to plasma acetonitrile (1:1, v/v). After evaporation and desalting, peptides were evaluated by MALDI-TOF/MS, in a mass to charge (m/z) range from 1000 to 4000. After signals median normalization, differences in features intensities were evaluated by random effect analyses.
Results: Out of 77 total MALDI-TOF/MS features identified in all plasma types, 35 features behaved differently among the studied conditions. 11/35 features intensities varied in the different plasma types with respect to the Purified Plasma condition ($p<0.05$). Independently from centrifugation protocol, in Barricor's plasma features intensities at m/z 1626, 1638, 1739, 1740, 1896 varied with respect to PSTII ($p<0.05$). Differences in Barricor's plasma associated with the centrifugation protocol were found for features at m/z 2037, 2054, 2109, 2544, 2842, 2858 ($p<0.05$). With respect to Purified plasma, the features intensities at m/z 1598, 1767, 2166 and 2228 increased progressively in Barricor centrifuged 4000gx10min, 4000gx3min and PSTII ($p<0.05$).
Conclusions: Barricor peptidomic profiles were more comparable with ultracentrifuged plasma than PSTII. This improvement could potentially benefit the proteomic study for the discovery of new disease-associated biomarkers.

P066

## CONTROLLI DI QUALITA' DEGLI EMOCOMPONENTI COME INDICATORI DEL PROCESSO DI SCOMPOSIZIONE DEL SANGUE INTERO

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Introduzione: I controlli di qualità devono essere eseguiti sugli emocomponenti per garantire uno standard di prodotto e per standardizzare le procedure. In ottemperanza al DM 2 novembre 2015, i controlli a campione devono prevedere un numero appropriato, statisticamente rappresentativo dei volumi di produzione (Allegato V/B) e deve essere previsto un controllo statistico di processo SPC (Allegato I). Presso il Servizio Trasfusionale Asl Caserta è stato implementato, come metodo per il SPC, l' uso della carta di controllo.
Materiali e metodi: La procedura del Servizio Trasfusionale prevede l'esecuzione dei controlli di qualità (CQ) sul 5% delle unità di emazie concentrate filtrate e su tutti i concentrati piastrinici da pool di buffycoat sono sottoposti al CQ. Tutti i parametri sono registrati sul sistema informatico (Eliot 3.0 by Engineering). Con cadenza semestrale, tutti i dati prospettici vengono tracciati su carte di controllo appositamente costruite in base ai dati storici relativi all' anno 2017.
Risultati:Nell'anno 2018 sono stati prodotti 23.298 concentrati eritrocitari leucodepleti di cui 1.389 (5.5%) sottoposte a CQ. Di questi, 97(0.07%) sono risultati non conformi per un valore di emoglobina inferiore a 40g/U mentre 125 (0.09%) sono risultati non conformi per un numero di globuli bianchi residui (r-WBC) superiore a $1x10^6$/U. Sono stati prodotti 900 concentrati piastrinici (CP) di cui il 99% sottoposti a controlli di qualità. Di questi, 18 (2%) CPsono risultati non conformi per un numero di piastrine inferiore a $2x10^{11}$/U; 5 (0.5%) sono risultati non conformi per r-WBC superiore a $1x10^6$/U; 4 (0.4%) sono risultati non conformi per un valore del pH a fine conservazione inferiore a 6.4.
Conclusioni: Dall' interpretazione delle carte di controllo: i dati prospettici dei CQ seguono il pattern dei dati storici e nessun dato è risultato consecutivamente al di sotto oppure al di sopra dei limiti di controllo predefiniti. Di conseguenza, il processo di scomposizione del sangue intero risultato essere "in controllo" e non è stato necessario intraprendere alcuna azione correttiva. Tale strumento risulta essere fondamentale nel monitoraggio costante dei prodotti finiti e, pertanto, come garanzia della sicurezza degli emocomponenti.

P067
**UN PROBLEMA STRUMENTALE RISOLTO: L'UTILITÀ DELL'INTEGRAZIONE DELLE INFORMAZIONI DEL CQI E DELLA VEQ**

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Il Laboratorio riceve il report dell'esercizio numero VEQ 2 chimica clinica (provider CRRVEQ AOU Careggi Firenze) in
cui la misura dell'LDH, eseguita su AU 480 Olympus Beckman, presenta errore totale (ET) +23.70%. I limiti di accettabilità (LA) stabiliti dal provider sono 11%. La misura dei CQI in quella seduta, non aveva originato violazioni delle regole impostate. Non viene attuata nessuna azione correttiva.
Il successivo report, campione VEQ 3, riporta AST con ET +20.69% (LA 10%) e creatinina ET -14.06 (LA 12%). Il CQI ha
evidenziato un analita, su 27 parametri monitorati, con uno scostamento superiore a 3 DS ma ripetuto è rientrato nelle specifiche. Si decide di attuare il protocollo di verifica precisione strumentale CLSI EP5A3E e di allertare l'assistenza tecnica della Ditta.
Un pool di sieri residui da pazienti (n 65), viene misurato 41 volte in successione per 27 analiti. L'assistenza tecnica esegue prove di precisione con un materiale specifico denominato orange.
Il protocollo CLSI fornisce 10 dati aberranti su 1107 risultati ovvero un outlier(metodo di Huber) ogni 110.7 misurazioni, (0.91%). Gli aberranti presentano tutti una sovrastima media di circa il 20%. La prova con materiale " orange" evidenzia dati anomali.
Il QCI non ha evidenziato il problema perchè per 27 analiti su 4 livelli la probabilità di avere un outlier era di 0.98% per seduta. La presenza di un valore out per seduta viene interpretato come valore aberrante casuale dal momento che ripetendo il QC il valore rientrava nei limiti di accettabilità.
L'errore così a bassa frequenza rende estremamente complicata l'azione dei tecnici. Dopo numerosi interventi in cui
vengono verificati e sostituiti vari componenti del sistema viene individuato il problema: 4 cuvette leggermente difformi. La difformità è talmente lieve che i dati di calibrazione fotometrica eseguita settimanalmente non l'hanno
rilevata. Sostituite le cuvette il problema è stato risolto.
I risultati dei pazienti sono stati rilasciati fino a quando non è stata conclusa la prima prova di precisione.
Solo il confronto con i dati VEQ ha innescato lo studio delle cause e l'azione correttiva, poiché la bassa frequenza e l'imprevedibilità dell'errore non ha permesso la rilevazione mediante QCI.

P068
**COMPARISON BETWEEN QUALITATIVE METHODS ACCORDING TO CLSI EP12-A2:2008 "USER PROTOCOL FOR EVALUATION OF QUALITATIVE TEST PERFORMANCE": THE EXAMPLE OF CHEMICAL DIPSTICK ANALYSIS OF URINE**

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Background: Method comparison is an essential part of the evaluation of measurement procedures. Qualitative methods pose additional challenges in comparison to quantitative ones, regarding data analysis and, more importantly, given the lack of clinically sound acceptance criteria for the differences. Unfortunately, urine dipstick testing suffers from substantial analytical variability among manufacturers. Our aim was to evaluate the level of agreement between 2 commonly used dipsticks for total protein (TP), hemoglobin (HB), leukocyte esterase (EST) and nitrites (NIT) according to CLSI document EP12-A2:2008.
Methods: A comparison was conducted between "candidate" method iChem Velocity (IRIS; Beckman Coulter) and "comparative" method Uriflet (Menarini Diagnostics). Routine samples were collected over 15 days, gathering at least 50 positive and 50 negative results for each test; the level of agreement was then estimated after conversion of all results to only 2 outcomes (positive/ negative).
Results: We collected 297 random samples from in- and outpatients, analysed within 2 hours of receipt. Overall percent agreement (95%CI) was 93,9 (90,6-96,1) for TP, 95,6 (92,7-97,4) for HB, 92,2 (88,7-94,8) for EST and 97,6 (95,2-98,9) for NIT. Positive percent agreement (95%CI) was 94,9 (91,0-97,8) for TP, 100 (96,9-100,0) for HB, 84,4 (77,1-89,7) for EST and 91,7 (78,2-97,1) for NIT. Negative percent agreement (95%CI) was 92,2 (86,6-95,6) for TP, 92,7 (87,9-95,7) for HB, 98,2 (94,9-99,4) for EST and 94,5 (96,1-99,4) for NIT.
Conclusions: Despite agreement rates >90% for almost all parameters, acceptance of the "candidate" method required careful evaluation of results (e.g. if discordancy was due to trace levels of measurand) and expected effects on the workflow (i.e. correlation with urinary sediment findings). In fact, straightforward acceptance criteria are currently needed for agreement rates. This implies that differences between methods should be carefully evaluated in respect to possible consequences on the analytical phase and, more importantly, clinical classification of patients.
References: CLSI document EP12-A2:2008. User Protocol for Evaluation of Qualitative Test Performance, 2nd Edition. Clinical and Laboratory Standards Institute, Wayne PA, 2008.

P069

## DETERMINATION OF MAGNESIUM EXCRETION IN 24-HOUR URINE SAMPLES: COMPARISON BETWEEN SPECIMEN COLLECTION WITH AND WITHOUT ACID PRESERVATIVE

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Background: The measurement of urinary Magnesium (Mg) excretion is usually performed in a 24-hour sample. As such, it is generally deemed necessary to collect urine with the addition of an acid preservative to allow for a pH<3, which should inhibit the precipitation of Mg salts. However, the evidence supporting this pre-analytic procedure is scarce and outdated, having been challenged by recent findings. We compared urinary Mg measurement in samples collected with and without the acid preservative, to test for any significant difference between the two modalities of collection.
Methods: The comparison was performed on consecutive requests submitted to our laboratory for routine testing that included both an acidified (HCl 6M) and non acidified 24-hour urine sample. Urinary Mg was measured on the Beckman Coulter AU680 with a colorimetric method. Data analysis was performed with SiBioc Excel worksheet MetComp ver 1.0.
Results: 97 coupled urine samples were included. Median Mg concentration in non acidified urines was 4.9 mg/dL (interquartile range: 3.2-6.7 mg/dL), whereas in acidified samples was 4.5 mg/dL (interquartile range: 3.3-6.4 mg/dL). Passing-Bablok regression analysis did not reveal any significant difference between acidified and non acidified samples (intercept: -0.1 mg/dL, 95%CI -0.4;0.2; slope: 1.0, 95%CI 0.9; 1.1), a finding confirmed by Bland Altman plot, that did not show a systematic difference between the two collection methods.
Conclusions: Our results show that the addition of an acid preservative is probably of limited or no added value for the measurement of urinary Mg excretion, depite its widespread use in clinical Laboratories. This finding should stimulate Laboratory professionals to critically evaluate pre-analytical procedures: in this case, the results of our comparison ultimately led to a simplification of the 24-hour urine collection for patients.
References: CLSI document EP09-A3:2013. Measurement Procedure Comparison and Bias Estimation Using Patient Samples, 3rd Edition. Clinical and Laboratory Standards Institute, Wayne PA, 2013. CLSI document GP16-A3:2009. Urinalysis, 3rd Edition. Clinical and Laboratory Standards Institute, Wayne PA, 2009.

P070

## POST-ANALITIC CRYOGLOBULIN ASSESSEMENT. THE GOAL OF FACING AND MANAGING: THE LABORATORY VARIABILITY IN EMILIA ROMAGNA

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Introduction: The research, quantification and characterization of cryoglobulins (CRG) are fundamental for the diagnosis and subsequent monitoring of patients.Comparing ourselves with colleagues from the Emilia Romagna Region and observing the results of the VEQ (UK NEQAS) we found considerable differences between the participating laboratories. Probably due to the intrinsic heterogeneity of CRGs as well as the lack of standardization in all phases of the process.
Materials and Methods: We proposed a comparison on the post-analytical phase to the laboratories of the Emilia Romagna Region, taking inspiration from the VEQ.All participants, 8 laboratories for a total of 13 operators, were sent 30 immunofixations on agarose gel (IFE) scanned as JPEG images. Each participant had to give his own interpretation without comparison with colleagues so as to be able to verify the actual inter and intra-laboratory variability.
Results: Out of 30 IFE read by each operator only 3 did not present any variability in the response. The remaining 27 IFEs presented inter and intra-laboratory variability, therefore also between operators of the same center.The descriptive statistics analysis revealed an inter-operator variability of 38% on the 27 non-concordant IFEs.
Conclusions: What emerges from this preliminary comparison is that regardless of the pre-analytical and analytical phases there remains a considerable criticality on the post-analytical phase. Inter-operator variability denounces a shared difficulty in reading IFEs. The national documents are not exhaustive on this phase of the process and there are no indications even for the use of a univocal classification among all those who report the CRG. It is an exam performed manually and IFE inspection is visual with significant subjectivity in reading. This makes the preparation and experience of the laboratory technician central. The participation of more laboratories in the VEQ program (UK NEQAS) can be an important solution to the problem.

P071
**A CHE PUNTO E' LA NOSTRA ARMONIZZAZIONE? RISULTATI DI UNA SURVEY NATA DALLA COLLABORAZIONE FRA ISTITUZIONE E SOCIETA' SCIENTIFICA**

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Introduzione: Da sempre SIBioC è in prima linea nel promuovere iniziative di armonizzazione volte a ridurre i rischi derivanti da differenze nei risultati degli esami, nella loro terminologia, nelle unità di misura utilizzate, negli intervalli di riferimento, ecc (Biochim clin 2015;39:48-55). Per questo motivo, nell'ambito della collaborazione con il Centro di Riferimento Regionale per la Qualità dei Servizi di Medicina di Laboratorio (organo istituzionale di Regione Lombardia), il gruppo di studio SIBioC "Qualità analitica" ha contribuito alla preparazione e all'analisi di una survey su alcuni aspetti inerenti lo stato di armonizzazione dei 34 misurandi inclusi nel programma VEQ "Ormoni e marcatori tumorali", gestito dal Centro.
Metodi: Un questionario per la raccolta di informazioni sugli strumenti, sugli standard di riferimento, le unità di misura, i limiti inferiore (LOQ) e superiore dei range di misura e gli intervalli di riferimento utilizzati dai partecipanti al programma VEQ è stato preparato e somministrato a 180 Laboratori lombardi e toscani.
Risultati: La survey ha messo in evidenza un'estrema eterogeneità nelle informazioni riportate per i diversi misurandi, anche fra gli utilizzatori dello stesso sistema analitico e anche nel caso in cui la fonte dichiarata di origine dei dati era la stessa (datasheet della metodica). Ad esempio, nel caso del TSH sono stati indicati 27 strumenti diversi, 2 standard primari (62 utilizzatori su 158 non hanno indicato alcuno standard), 7 diverse unità di misura espresse sui referti, 19 LOQ e 16 limiti superiori del range di linearità, 39 intervalli di riferimento. Analoghi risultati sono stati rilevati per tutti gli altri misurandi. Di particolare rilievo è il fatto che solo nel 4,8% dei casi l'unità di misura riportata sui referti appartiene al Sistema Internazionale, o che solo nell'8,7% dei casi la concentrazione è rapportata al litro.
Conclusioni: Il quadro che sembra emergere dalla survey mette in evidenza che gli sforzi delle società scientifiche per promuovere il processo di armonizzazione rischiano di essere vanificati se non incontrano la disponibilità dei singoli laboratori ad applicare a livello locale le indicazioni, le raccomandazioni e le linee guida proposte.

P072
**ANALISI RETROSPETTIVA SUL TEST Ab-ANTI HLA: LA NOSTRA ESPERIENZA**

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Introduzione: La produzione di anticorpi diretti contro i geni HLA del donatore, dopo il trapianto di rene, si associa ad un peggiore outcome del graft, in quanto sono responsabili di rigetti umorali sia di tipo acuto che cronico. Per questo motivo è necessario determinare precocemente la presenza degli stessi.
Metodi: In questo studio abbiamo selezionato le richieste di Ab anti-HLA ricevute da Gennaio 2017 a Luglio 2019. I campioni sono stati analizzati mediante Luminex 200 (Luminex Co-Texas USA) e quantificando l'intensità media di fluorescenza (MFI). E' possibile rilevare la presenza o assenza di IgG anti-HLA identificando il tipo di classe anticorpale (Screening - LMX), gli specifici anticorpi (test ID I e ID II) ed il PRA% (Panel Reactive Antibody). Nei casi con PRA elevato si studiano gli antigeni a livello allelico (LSA I e LSA II). Infine, C3d I e II vengono eseguiti nei pazienti in cui il test LSA risulta DSA positivo (Donor Specific Antibody).
Risultatii: Nei 200 pazienti, analizzati in 2 anni, abbiamo effettuato 1323 test, di cui 29,5% erano test di Screening (LMX); 22,3% test ID I e 22,6% test ID II; il 10,2% e il 15% erano i test LSA I e II. Il test C3d costituiva solo lo 0,4% dei test. Il controllo di qualità positivo ha mostrato valori medi, per la probe 77 (biglia di controllo positivo), di 21000 MFI e CV% compresi tra 8,9-10,1 per i test LMX, ID I e ID II; per i test LSA I e LSA II la media del controllo positivo era 18100 MFI (CV%: 9,8-14,5); i test C3d I e II hanno mostrato un valore medio di 18500 MFI (CV%: 5,1-12,7). Il PRA% nel controllo positivo variava dal 95-100%. Il controllo di qualità negativo ha mostrato variabilità, per la probe 77, sovrapponibili al controllo positivo per tutti i test eseguiti (LMX, ID I, ID II, LSA I, LSA II, C3d I, C3d II) con CV% compresi tra 8,3-13,5. Il PRA% era inferiore al 2%.
Conclusioni: La metodica è risultata riproducibile dal punto di vista analitico e dimostra una buona attendibilità per il monitoraggio dei pazienti, utile nella modulazione della terapia immunosoppressiva.

P073
**L'IMPORTANZA DELL'UTILIZZO COMBINATO DEI PROGRAMMI DI CQI E VEQ PER LA VALUTAZIONE DELLE PRESTAZIONI ANALITICHE**

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Scopo: Il monitoraggio della fase analitica prevede due specifici macro processi: il Controllo di Qualità Interno (CQI) e la Valutazione Esterna di Qualità (VEQ), ampiamente descritti nel percorso di Accreditamento Istituzionale e nella norma ISO 15189, che attualmente rappresenta lo standard internazionale di accreditamento per i Laboratori. Lo scopo di questo lavoro è mettere in evidenza l'utilità della partecipazione ai programmi di VEQ per la rivelazione degli errori di inaccuratezza non identificabili dalla sola esecuzione del CQI.
Materiali e metodi: Le prestazioni analitiche del settore Emocitometria del Laboratorio Analisi dell'Ospedale "Papa Giovanni XXIII" di Bergamo vengono assicurate dall'esecuzione di un CQI (XN CHECK in 3 Livelli Alto, Medio, Basso)e dalla partecipazione a programmi di monitoraggio esterni della Regione Lombardia (VEQ RL) e UK NEQAS.Nel mese di febbraio e marzo 2019 sono stati eseguiti, sui 5 moduli XN9000 (Sysmex, Kobe, Japan) in dotazione presso il laboratorio, due campioni di controllo del programma UK NEQAS e VEQ RL inviati come da calendario.
Risultati: L'elaborazione dei dati del NEQAS riporta un coefficiente di variazione (CV) interstrumentale massimo del 3.7% per i parametri di uso comune dell'esame emocromocitometrico, con una Deviazione Standard massima di 3 g/L per l'Emoglobina (HB). In base al numero di eritroblasti i campioni della VEQ RL mostrano una differenza sul conteggio di Globuli bianchi (WBC) di circa 1,5 $10^9$/L in difetto o in eccesso. L'anomalia quantitativa è a sua volta visibile dallo spostamento del cluster dei Leucociti all'interno dello scattergram del canale WNR (white-cell nucleated). Le carte di controllo interno Levey-Jennings non mostravano nessuno shift o trend sui parametri indicati.
Conclusioni: L'analisi dei dati combinata tra CQI e VEQ ha richiesto in un caso la necessità di un intervento tecnico di calibrazione dell' HB, mentre nell'altro ha messo in evidenza un problema di conteggio dei WBC legato alla matrice della VEQ RL, ma che ci ha permesso di approfondire tutti i dati del CQI relativi ai parametri che interessano il canale WNR.Il presente lavoro mostra come l'utilizzo combinato di CQI e VEQ sia fondamentale per valutare la qualità totale delle prestazioni analitiche.

P074
**VERIFICA DELL'ALLINEAMENTO E VALUTAZIONE DELLE PERFORMANCE: L'ESPERIENZA DEI LABORATORI HUB E SPOKE NELLA PROVINCIA DI AREZZO.**

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Introduzione: L'aumento dell'età della popolazione ha prodotto un aumento significativo della domanda di servizi diagnostici. In aggiunta, le esigenze di budget, hanno favorito la nascita di laboratori centralizzati che eseguono servizi diagnostici su larga scala e laboratori satelliti che eseguono soltanto alcuni test di 1° livello. Il modello organizzativo, nella provincia aretina, è costituito da una rete con un laboratorio centrale (HUB) ad Arezzo che esegue i test di I e II livello e quattro laboratori "SPOKE" (Bibbiena, Cortona, Sansepolcro, Montevarchi) che eseguono test di I livello.
Scopo: Scopo del presente studio è quello di verificare un allineamento accettabile delle prestazioni tra HUB e SPOKE. Ciò è essenziale ai fini del monitoraggio dei pazienti e assicura che qualsiasi variazione dei risultati dei test rifletta un cambiamento reale delle condizioni del paziente e/o della risposta alla terapia.
Materiale e metodi: Per lo studio sono stati considerati 5 analiti eseguiti in tutti i laboratori: ALT, Glucosio, Creatinina, Calcio, LDH. Sono stati estratti i risultati di 48 giorni (4 gg per ogni mese, 1°, 10°, 20° e 28° ) nel corso dei 12 mesi del 2018 di cui sono state calcolati le mediane, il CV% e successivamente confrontati tra di loro. Inoltre è stata eseguito un confronto con le imprecisioni del relativo esercizio della VEQ regionale, il suddetto confronto è stato eseguito con i corrispondenti CV% medi mensili calcolati dalle determinazioni globali dei 4 gg.
Risultati e discussione: Il confronto delle medie delle mediane degli analiti, ha mostrato un ottimale allineamento tra i 5 laboratori, in particolare per quanto riguarda ALT, Glucosio e Creatinina. Per il Calcio è stata riscontrata una situazione critica, con i laboratori non perfettamente allineati tra loro. L'LDH ha mostrato una situazione mista, con 3 dei 4 laboratori satelliti ben allineati con quello centrale e una tendente diversità tra i laboratori spoke. Il laboratorio HUB, ha mostrato valori più bassi del CV% delle determinazioni rispetto l'imprecisionedel report dell'esercizio delle VEQ per ALT, Calcio e Creatinina e valori comparabili per Glucosio e LDH. Tale situazione è stata riscontrata, negli altri laboratori, solo per il Calcio. Il confronto tra i dati delle VEQ ha mostrato, al contrario, un perfetto allineamento tra tutti i laboratori, con ancora qualche criticità per il calcio.
Conclusioni: A tutt'oggi, valutare e mantenere le prestazioni dei dosaggi in modo continuo e adeguatamente allineato è una grande sfida, soprattutto in un territorio con analizzatori decentrati ma interdipendenti. Il nostro studio ha esplorato un metodo per studiare il controllo di analizzatori omogenei nell'area aretina, nel nostro caso abbiamo registrato un buon

allineamento con un CV% inferiore nel Laboratorio di Arezzo, molto probabilmente dovuto alla numerosità elevata del campione, ma nella variazione sicuramente ci sono anche ragioni di qualità analitica che emergono dalle VEQ regionali. Questo metodo dimostra di essere uno strumento valido per controllare e talvolta anticipare la qualità analitica registrata dalla VEQ, soprattutto in una logica organizzativa di Area Vasta.

P075

**USE OF GLYCATED ALBUMIN AS A NEW GLYCEMIC METRIC IN PATIENTS WITH DIABETIC KIDNEY DISEASES**

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Glycaemic control is usually evaluated with HbA1c in patients with diabetes. In the subpopulation of patients with diabetic kidney disease (DKD), HbA1c could be less accurate due to several factors that alter haemoglobin turnover and its glycation rate. Recently, the use of alternative glycaemic metrics, such as Glycated Albumin (GA), has been proposed for the evaluation of glycaemic control in such patients, but the relation between GA and other glycaemic metrics in relation to factors that could interfere with Hb glycation rate remains unexplored.

Inpatients with DKD referring to the University Hospital of Palermo were consecutively included from January to May 2019. Diabetes was defined according to the American Diabetes Association. Kidney disease was defined by eGFR<60 ml/min and/or ACR > 30. Patients with acute kidney injury were excluded. GA was measured on plasma-EDTA by quantILab® Glycated Albumin (IL, A Werfen Company). HbA1c was measured by ion exchange chromatography (D100, BioRad).

The study included 60 patients. When patients were stratified according to GA quartiles, patients with higher GA levels had higher HbA1c but no differences of FPG, albuminemia, eGFR, presence of albuminuria, were detected. To further evaluate the relationship between GA and HbA1c, patients were stratified according to the presence of conditions associated to altered Hb turnover (transfusions in the last 3 months, use of erythropoiesis stimulating agents, severe anemia according to WHO criteria). In the subgroup of patients with no altered Hb turnover (n=35), GA was significantly correlated to HbA1c (r= 0.55, P=0.0006). In patients with altered Hb turnover (n=25) this correlation was attenuated, although still statistically significant (r=0.43; P=0.03).

In patients with DKD, GA is independent by albuminemia and albuminuria and it correlated to HbA1c. Alterations of Hb turnover could alter this relationship, suggesting that GA could reflect more accurately glucose homeostasis in this setting.

(1) Glycated albumin as a glycaemic marker in patients with advanced chronic kidney disease and anaemia: a preliminary report. Bellia C, Cosma C, Lo Sasso B, et al. Scand J Clin Lab Invest. 2019 May 9:1-5.

P076
**EMOGLOBINA GLICATA, ALBUMINA GLICATA E FRUTTOSAMINA A CONFRONTO**

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Background: il valore dell'emoglobina glicata(HbA1C) è utilizzato per la diagnosi dello stato diabetico ed è il parametro con cui si monitora il controllo glicemico a lungo termine (2-3 mesi) nelle persone affette da diabete mellito. La fruttosamina e l'albumina glicata (AG) sono, invece, indicatori a breve termine (2-4 settimane) del controllo, che potrebbero coadiuvare HbA1C nella definizione dello stato patologico. Scopo del presente lavoro è quello di valutare quale tra i due test, AG e Fruttosamina, sia più adatto ad affiancare l'HbA1C.
Metodi: sono stati dosati: HbA1C (HPLC Variant II, Biorad), fruttosamina (metodo colorimetrico Roche Diagnostics), AG (metodo enzimatico-colorimetrico, Instrumentation Laboratory) su 68 pazienti senza distinzione di sesso ed età, afferenti al Centro Prelievi dell'Ospedale Pediatrico Bambino Gesù nel periodo Gennaio-Dicembre 2018 per il dosaggio della HbA1C.
Risultati: sono stati utilizzati i seguenti valori soglia per definire il sospetto di diabete: HbA1C 48mmol/L, AG 15,2% e fruttosamina 280 mmol/L. Utilizzando come riferimento l'HbA1C, il 30,9% dei pazienti superava il valore soglia, con l'AG il 23,5 % e con la fruttosamina il 2,9%. Sono stati analizzati i dati confrontando i test AG e fruttosamina, e si è evidenziato che presentano una concordanza del 33%, in particolare tra i valori sotto la soglia di riferimento. Inoltre, rispetto all'HbA1C, l'AG mostra il 67% di concordanza nella rilevazione del sospetto diabetico e la fruttosamina il 10%. In un paziente con carenza di Hb (9g), l'AG ha permesso di evidenziare un sospetto diabete rispetto alla fruttosamina (rispettivamente 15,3% e 187 mmol/L, con HbA1C 30 mmol/L).
Conclusioni: dai dati esaminati, l'AG sembrerebbe essere più accurata rispetto alla fruttosamina per affiancare l'HbA1C nella diagnosi e nel monitoraggio del diabete mellito e può essere in grado di coadiuvare e/o sostituire l'HbA1C nei casi di anemie, come già ipotizzato in letteratura.

P077
**GLYCATED ALBUMIN: A NEW POSSIBLE BIOMARKER FOR GLUCOSE MONITORING IN DIALYSED PATIENTS**

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Background: Glycated hemoglobin (HbA1c) is currently considered the gold standard test to estimate the average glycemia of the last 8-12 weeks. However, the concentration of HbA1c in the blood can be influenced also by clinical situations which may interfere with the metabolism of hemoglobin, , such as hemolytic, secondary or iron deficiency anemia, hemoglobinopathies, pregnancy and uremia. Glycated Albumin (GA) is a test that reflects, short-term (1-2 weeks) basal and post-prandial glycemia. The aim of our multicenter study was to verify the efficacy of the GA compared to HbA1c dosage in non-diabetic and diabetic dialysed patients.
Materials and methods: GA and HbA1C detection was performed every 30 days for three months (T0, T1, T2 and T3) and a final withdrawal was made after 6 months (T4). Basal dosage of HbA1c on whole blood and of GA on plasma-EDTA sample. HbA1c was obtained through the use of Capillarys Flex Piercing (SEBIA, France), the GA was performed using the quantILab Glycated Albumin kit (Instrumentation Laboratory) implemented on Roche C702 modular. Detections were all performed at the UOC Clinical Pathology S. Filippo Neri HUB of ASL Roma1.
Results: The data obtained in this way allowed us to highlight the possible improved glyco-metabolic monitoring of the GA compared to HbA1c in the diabetic nephropathic patient. During this study, preliminary data highlighted that in some cases GA reflected more early the glyco-metabolic alterations compared to HbA1c, both in diabetic patients (out of 203 total determinations, 46 GAs discordant with HbA1c, equal to 22.6%) and in non-diabetic patients (out of 370 total determinations, 77 GAs discordant with HbA1c, equal to 20.8%).

Conclusion: Data showed that GA is an earlier marker of HbA1c in hemodialysis, predisposed to developing anemia and often requiring EPO therapy, factors that can influence HbA1c monitoring.

P078

## ACIDIFIED MIXTURE BLOOD: A CANDIDATE QUALITY MATERIAL FOR GLUCOMETERS

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Introduction: Self-monitoring of blood glucose (SMBG) is considered an integral part of the current strategy of diabetes treatment. The control of analytical quality of SMBG is recommended as a routine procedure in diabetes management. Here we present a preliminary study on a possible new quality control (QC) material for glucometers.
Material and methods: The study was conducted at the Laboratory of Esine and at the Laboratory of St Bortolo Hospital in Vicenza. At Esine hospital 2 whole blood sample tubes containing citrated buffer, NaF and EDTA-2mL volume (FC-Mix Greiner BioOne) were collected from a subject at 60' during OGTT. One sample was maintained at room temperature (RT) (20-30°C) and another one at 2-8°C for 15 days (dd). Blood glucose was determined in duplicate using Abbott Free-Style Optium Neo glucometer, using reagent strip lot# #4500180543 for the next 15 dd. QC was determined daily according to manufacturer. At Vicenza hospital 3 different whole blood sample pools containing citrated buffer, NaF and EDTA were prepared and maintained at RT until analysis. Blood glucose was determined in duplicate for 14 consecutive dd using Roche Accu-Check and different strips lots. QC was determined daily. The criteria for assessing glucose sample stability were based on total error (TE) according to IFCC WG GMECC :<8.33% at glucose concentration ≥100 mg/dL and <6 mg/dL if <100 mg/dL (good analytical performance).
Results: Mean glucose concentration was 117 mg/dL at zero day in Esine. During the 15 dd evaluation the mean difference from the initial value was +2.99% (range: -2.99/+6.84%) and +4.13% (range: -2.14/+8.12%) in sample maintained at RT and at 2-8°C, respectively. CV of blood sample was 2.54% at RT and 2.69% at 2-8°C. Control1 (mean value: 43 mg/dL) CV was 8.79% and control2 (mean value: 319 mg/dL) CV was 10.53%. Whole blood glucose concentrations were: 81.0 mg/dL, 136.5 mg/dL and 161.5 mg/dL in pool 1, 2 and 3, respectively, in Vicenza. During the next 14 dd the mean difference from the initial value was -1.68 mg/dL (range: -4.00/+1.50 mg/dL) for pool1, and -5.21% (range: -1.83/-7.69%) and -6.41% (range: -3.72/-8.36%) for pool2 and 3, respectively. CV were 2.14%, 2.28% and 2.29% for pool 1, 2 and 3. Control1(mean: 44.6 mg/dL) CV was 3.37%, while control2 (mean: 288.9 mg/dL) CV was 1.76%. Conclusions. This study show that glucose in citrated buffer/NaF /EDTA whole blood is stable at 2-8°C and at RT for at least 2 weeks using glucometers, showing their good analytical performance. Citrated blood samples should be used as an EQAS material for SMBG for checking accuracy and imprecision of glucometers at home or in hospital setting. Other

studies on different glucometers could be performed to corroborate our findings.

P079

## THE QBC STAR HEMATOLOGICAL POCT: THE ANALYTICAL PERFORMANCE EVALUATION

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Purpose: Point-of-care testing (POCT) is becoming important in laboratory practice and recently in laboratory hematology also. QBC Star is a POCT that provides a blood count on venous or capillary blood and it is composed of the following parameters: Leukocytes, Hemoglobin, Hematocrit, Mean corpuscular hemoglobin concentration (MCHC), Platelets, Differential leukocyte count with three populations.
This study was carried out with the aim of verifying the analytical performance of the QBC Star system versus the Sysmex XN series hematology analyzer (Sysmex, Kobe, Japan).
Materials and methods: 98 peripheral blood samples collected in K3EDTA tubes (Becton Dickinson) were evaluated. 50 of them were from healthy adult donors and 48 from pediatric patients. In order to perform the comparative evaluation between the two analytical systems, specific statistical parameters were used. Passing and Bablok regression slope (SL) and intercept (IN) were estimated with their 95% confidence interval (IC), calculation of Bland Altman Bias with the Analyze-it software 3.90.1 were performed. The study involved the use of pre-existing samples, immediately made anonymous after the routine investigations. The study was conducted in accordance with the Helsinki Declaration and in compliance with current legislation.
Results: Data from 98 samples were included in the result evaluation. For 18 of all pediatric samples no evaluation was available due to recurrent errors on the POCT instrument.
Regarding the analysis regression, SL from a minimum of 0.60 for MCHC to a maximum of 1.08 for PLT was recorded. The relative Bias changed from -2.82% for MCHC to 17.29% for the PLT. The predetermined goal of desirable Bias HCT, MCHC, PLT, and polymorphonuclear granulocytes was not exceeded. The parameters Hb and WBC can be correlated (Hb Bias: 1.06% SL 0.98 / WBC Bias: 4.27% SL: 1.05).
Conclusions: The method of sampling from capillary puncture and the execution of blood counts in a pediatric microtube are the emerging critical issues on the POCT QBC Star. From statistical parameters analysis most of the results obtained on QBC STAR instrumentation cannot be correlated with the ones from the XN modul. QBC STAR cannot be recommended for execution of complete blood counts for diagnostic purposes, but just for the monitoring of the two parameters Hb and WBC.

P080
**RET-HE (RETICULOCYTE HEMOGLOBIN EQUIVALENT ): PARAMETRO UTILE E PRECOCE PER PREVENIRE L'ANEMIA SIDEROPENICA IN DONATORI PERIODICI DI SANGUE INTERO**

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Introduzione:La donazione di sangue intero determina la perdita di 450 ml di sangue, causando una netta diminuzione dei depositi infatti già dopo una o due donazioni la ferritina si riduce nettamente. Particolarmente utile per individuare precocemente la deplezione dei soggetti marziali è la possibilità di misurare direttamente la percentuale delle emazie microcitiche, ipocromiche e il Ret-He (reticulocyte hemoglobin equivalent).
Scopo: E' stato quello di valutare su donatori abituali e periodici (periodici sono tutti quei donatori che donano almeno una volta in 24 mesi(Art.1del D.M. 2/11/2015)) gli indici eritrocitari :MCV(volume corpuscolare medio)e MCH(concentrazione di emoglobina media) e reticolocitari Ret-He, correlato con l' assetto ferrico (sideremia, transferrina e ferritina).
Materiali e Metodi:Nell'anno 2018 a 1000 donatori periodici di sangue intero afferiti presso il Servizio Trasfusionale Asl Caserta sono stati eseguiti: esame emocromocitometrico(Sysmex X1000,Dasit), dosaggio della ferritina(Vitros3600), dosaggi di sideremia e transferrina(Modular,ROCHE).
Risultati:Dei 1000 donatori(65% maschi e il 35% femmine)103 donatrici (10.3%) e 75 donatori (7.5%) all' esame emocromocitometrico hanno presentato valori di emoglobina pari a 13,5±0,5g/dL,valori di MCV<80fL(65± 5fL),valori di MCH<26pg(20±3pg).
Tali donatori sono stati sospesi alla donazione ed è stato eseguito il Ret-He risultato<35pg(39±3pg);successivamente sono stati indirizzati allo studio dell'assetto ferrico.La ferritina è risultata<20 ng/mL (10±3ng/mL),la transferrina<200 mg/dL(120±20 mg/dL) e la sideremia <80µg/dL(65± 5µg/dL).
Discussioni e Conclusioni: Sebbene nei donatori sottoposti allo studio, l' Hb rientra nei parametri stabiliti dal D.M 2/11/15, essi sono stati indirizzati ad una terapia con ferro e un successivo controllo dopo 10 giorni. Il parametro Ret-He consente di valutare la qualità dell'eritropoiesi midollare in tempo reale, aumentando già dopo 1-3 giorni dall'inizio della terapia rispetto all' MCH il quale aumenta dopo 2-4 settimane.Tali donatori dopo 10 giorni , presentavano un Ret-He incrementato,per questo hanno potuto effettuare la donazione di sangue intero,rispetto le donne in età fertile che sono state rinviate a donazione dopo un mese.

P081
**LE ANEMIE EMOLITICHE AUTOIMMUNI DA ANTICORPI FREDDI: ESPERIENZA SIT ASL CASERTA**

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Introduzione: Le anemie emolitiche autoimmuni possono essere determinate da anticorpi caldi, freddi o a reazione mista. Gli autoanticorpi possono essere idiopatici o correlati a una condizione sottostante come infezione, neoplasia o malattia immunitaria. L' anemia emolitica autoimmune da anticorpi freddi è una patologia rara caratterizzata dalla presenza di crioagglutinine. Lo scopo del nostro studio è stato quello di esaminare le caratteristiche cliniche e patologiche dei casi di anemia emolitica autoimmune da anticorpi freddi riscontrati.
Materiali e metodi: Ai Pazienti che presentavano un test di coombs diretto positivo ed indiretto positivo panagglutinante, sono state determinate le agglutinine fredde ed effettuata l'anamnesi. I pazienti sono stati classificati in basi alla patologia, al valore di emoglobina e alla risposta alla terapia.
Risultati: Da gennaio 2018 su 6000 richieste di type e Screen 20 pazienti hanno presentato il test di Coombs diretto positivo ed indiretto positivo panagglutinate; sette sono risultati positivi al test per le agglutinine fredde. Quest'ultimi 4 avevano specificità anti I; 3 avevano specificità anti H. Dall'anamnesi effettuata è emerso che un paziente era affetto da Epstein Barr virus , due pazienti da Mycoplasma pneumoniae, i restanti 4 pazienti non presentavano alcuna patologia. Tutti i pazienti avevano un valore di emoglobina inferiore ad 8 g/dl quindi tutti sono stati trasfusi previa premedicazione con cortisonici.
Conclusioni: I sette pazienti risultati positivi erano tutti maschi contraddicendo così il dato presente in letteratura che vede una preponderanza della patologia nelle donne ( Berentsen et Al Primary cronic cold agglutinin disease: a population based clinical study of 86 patients. Haematologica. 2006). Nei pazienti dove la patologia ematologica è stata secondaria ad una problematica infettivologica la cura della patologia primaria è stata fondamentale per la risoluzione della problematica ematologica. I 4 pazienti, in cui l'anemia emolitica autoimmune era la patologia primaria, sono stati indirizzati al reparto di ematologia. Il gold standard per la cura della suddetta patologia è il Rituximab, trattamento efficace ed in grado di permettere una remissione completa.

P082

## PERFORMANCE OF BIOMARKER-BASED STAGING SYSTEMS IN ELDERLY PATIENTS WITH AL AMYLOIDOSIS

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Immunoglobulin light chain (AL) amyloidosis is a rare, aging-associated plasma cell dyscrasia in which monoclonal light chains misfold and deposit in form of amyloid fibrils, leading to progressive dysfunction of affected organs. Biomarker-based staging systems have been developed and validated in the general population of AL patients. Whether they retain their prognostic validity also in the subset of the frailer, elderly patients with AL remains unknown.

We analyzed data from 168 consecutive AL patients >75 years of age evaluated at the Pavia Amyloidosis Center between 2004 and 2015, who were prospectively followed for response to anti-plasma cell therapy and survival.

The overall survival was 11.6 months. The Mayo 2004 cardiac staging system, based on NT-proBNP (cutoff: >332 ng/L) and cTnI (cutoff: >100 ng/L), retained its prognostic significance among elderly AL patients, with a median survival of 54, 22.5 and 8 months in patients with stage I, II or III (neither, only one or both markers above cutoff), respectively ($p<0.001$). Alkaline phosphatase (ALP) levels above the upper reference limit (url) was a negative prognostic factor (median survival: 4.4 vs 20.2 months compared to patients with ALP<url, $p=0.0013$). Among patients with intermediate risk (Mayo2004 stage II), ALP>url identifies a subset of patients with poorer survival (7.3 vs 30 months compared to patients with ALP<url, $p=0.0016$). A low plasma cell clonal burden, as assessed by difference in concentration between involved and non-involved light chain isotype (dFLC) below 50 mg/L, bared prognostic effect (median survival: 27 vs 9.3 months compared to patients with dFLC>50 mg/L, $p=0.027$). The renal staging system, based on proteinuria (cutoff: >5 g/24h) and estimated glomerular filtration rate (cutoff: <50 mL/min per 1.73 $m^2$) efficiently predicted risk of dialysis at 2 years (median time to dialysis of 54 months vs not reached for patients in renal stage III, with both markers beyond their respective cutoff, compared to the other two risk classes, with none or only one marker beyond its cutoff, $p<0,04$). Reaching any type of hematologic response after anti-plasma cell chemotherapy (dFLC decrease at best response >50% compared to baseline) bared a positive prognostic effect (median survival of 45 vs 9.5 months compared to patients with no response, $p<0.001$).

Biomarker-based staging systems proved their validity also in the subset of AL patients older than 75 years. ALP levels can help to further stratify risk. Hematologic response to therapy improves survival also in these frail patients.

P083
**UTILIZZO DELL' EMOCITOMETRO "HORIBA YUMIZEN H2500" COME SUPPORTO NELLA DIAGNOSTICA DELLE SINDROMI MIELODISPLASTICHE**

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Introduzione: Le MDS sono un gruppo di patologie dovute alla perdita di capacità delle cellule staminali emopoietiche, di completare la loro maturazione, con conseguente anemia, piastrinopenia e granulocitopenia. Ciò è dovuto ad alterazioni molecolari che si riflettono in specifiche atipie morfologiche, per cui secondo WHO, la valutazione microscopica dello striscio di sangue periferico, rappresenta il gold standard per la diagnosi.
Scopo del lavoro: Numerosi studi hanno dimostrato la capacità dei moderni emocitometri di individuare la disgranulopoiesi, che è uno degli aspetti caratteristici delle MDS. Il nostro intento è stato valutare la capacità dell'emocitometro, HoribaYumizen H2500, nell'evidenziare tali alterazioni, fornendo un valido supporto nella diagnosi delle MDS.
Materiali e metodi: Esso utilizza, per la conta differenziale, una metodica citochimica basata sull'uso di Sudan B modificato (Clorazol Black E) correlata al contenuto di lipidi degli organelli citoplasmatici che unita al metodo impedenziometrico per la determinazione del volume, fornisce uno scattergramma bidimensionale dei WBC. La conta dei basofili avviene, previo stripping, citoplasmatico con reagente Baso-Lyse, nel canale BASO. Per lo studio sono stati valutati 193 campioni di 62 pazienti con disgranulopoiesi MDS, 40 di donatori sani, 39 di pazienti con MPN, 41 di pazienti con neutrofilie "reattive" e 40 di neutropenie non displastiche.
Risultati: La valutazione dello scattergramma LMNE, evidenzia nella maggior parte delle MDS con disgranulopoiesi, una riduzione della positività al Sudan B per i granulociti, con spostamento verso il basso del cluster dei neutrofili, che invade la regione di linfociti e monociti, con conseguente sovrastima. In un minor numero di casi, la popolazione granulocitaria è sdoppiata con solo una quota spostata verso la parte inferiore dello scattergramma, come da riduzione della sudanofilia.Valutando quantitativamente questa deviazione, la mediana dell'assorbanza (MLA) dei neutrofili nel canale LMNE è 58(range 54-66) nei pazienti MDS($p<0.001$) rispetto ai campioni normali 65 range 62-69), ai campioni MPN 62(range 60-68), alle neutrofilie reattive e alle neutropenie non MDS, 61(range 60-69).

P084
**THE TELE-HEMATOLOGY NETWORK OF THE SOUTH-EAST TUSCANY AREA**

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Introduction: The Tuscan South East Area Vasta (SEAV),which covers 11562 square kilometers,consists of the 3 provinces of Arezzo,Grosseto and Siena.For the distances and the characteristics of the territory,the laboratory medicine services are organized according to the model "Hub and Spoke".The samples from the territorial sampling centers and from the spoke centers (SC),where only urgent samples are analyzed,are transported to the Hub centers (HC) in the provincial headquarter.In this paper we present a project of a tele-hematology network (NW)of blood counts (BC) and digital images of peripheral blood smears (PBS) among the various centers of the NW.
Materials and methods: The territorial tele-hematology NW of the SEAV includes HC in Arezzo,Grosseto,Campostaggia and Nottola,which perform BC with an automated instrumental leukocyte formula (AILF) and PBS;and SC in Cortona,Bibbiena,Sansepolcro,Valdarno,Massa Marittima,Orbetello,Pitigliano,Abbadia San Salvatore and Castel del Piano,which perform BC with an AILF of urgent samples,while non-urgent samples are sent to the HC.All laboratories (labs) use XN ematology cell counters,which are stand alone in SC and organized in XN chains with SP-1000 slides and image analyzer DI-60 in HC.The instrumental data are shared by all the centers through the DMS WEB middleware (Sysmex).
Results: Every day in the SEAV of Tuscany about 2300 routine BC and about 700 urgent samples are performed.The DMS WEB allows all the labs to be connected to one another with numerous clinical and organizational advantages:the HC can advise and validate the results of urgent BC of their own SC at a distance;they can share digital images with each other and allow the "second opinion",in a short time,of the evaluation of PBS;in addition,clinical hematology,inserted in the NW,can evaluate PBS,analyzed in the HC of the whole vast area,with the possibility of clinical consultations a distance.
Conclusions: The tele-hematology NW allows better patient care,guaranteeing results achieved with uniform,advanced and standardized methods and technologies;the patient is placed in a NW that allows the collaboration of specialists with much faster diagnosis and treatment times;and the effectiveness of care is allowed by a less rigid and leaner organization.

P085
**DEVELOPMENT OF AN ALGORITHM WITH MORPHOLOGICAL-FUNCTIONAL LEUKOCYTE XN PARAMETERS FOR THE DIAGNOSIS OF CLI**

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Introduction: Modern hematology analyzers (HA), in addition to the canonical parameters recognized and reportable, also determines a series of search parameters. In particular, the XN HA (Sysmex) measure morphological-functional leukocyte (MFL) research parameters, studied so far for myelodysplastic syndrome and sepsis. In this work the reference values (RV) of the MFL parameters of the XN HA in the healthy population were calculated, in line with the literature [1] and the same parameters were evaluated in patients with Chronic Lymphatic Leukemia (CLL) for the development of a prediction algorithm for the diagnosis of CLL.
Materials and methods: For the determination of the RV of the MFL parameters of the XN HA, 200 healthy subjects aged between 20 and 90 years who had perfectly normal blood counts were enrolled. For the evaluation of MFL parameters in CLL, 30 patients with an established diagnosis of CLLs between 50 and 90 years old were enrolled. The blood count was performed with the XN HA and the statistical evaluations were performed with the SPSS version 11.0 software.
Results: The RV of the MFL research parameters, in line with the literature [1] are the following:NE-SSC(144.1-159.7), NE-FSC(82.0-90.5), NE-SFL(41-48) , NE-WX(283.2-243.8), NE-WY(550.2-655.0), NE-WZ(577.2-847.8), LY-X(79-83), LY-Y(61-70), LY-Z( 55-58.2), LY-WX(397-564), LY-WY(723.1-1003.8), LY-WZ(433.7-758.1), MO-X(116-121), MO-Y(94-111), MO-Z(63-68), MO-WX(229.2-318.3), MO-WY(567.2-862.1), MO-WZ(508.0-913.1). In patients with CLL compared to normal values, a constant trend was observed for the NE-SSC, NE-FSC, NE-SFL, NE-WZ, LY-Y, LY-WZ, MO-Y, MO-WX, MO-WY, MO-WZ parameters; an increase for the NE-WX, NE-WY, LY-X, LY-Z, MO-Z parameters; and a decrease for the parameters LY-WX, LY-WY, MO-X.
Conclusions: These evaluations emerge from the preliminary results which, if confirmed by a larger and statistically significant number of cases, will allow the development of multiparametric patterns based on MFL parameters characteristic of the pathology for the prediction of the diagnosis not only of CLL, but probably also of other conditions pathological.
[1] Evaluation of reference intervals for complete blood count on Sysmex XN 9000. S Buoro, C Ottomano, G Lippi et al. Biochimica Clinica 2015;39(4)256-263.

P086
**CONFRONTO TRA METODICA MODIFICATA ED ALTERNATIVA PER L'ESECUZIONE DELLA VES IN UNA POPOLAZIONE PEDIATRICA**

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Introduzione: Le recenti linee guida ICSH sull'esame della VES hanno riconosciuto che sul mercato IVD esistono metodologie diverse per eseguire il test: Westergren modificato e Westergren alternativo. Le metodiche Westergren modificato sono basate sulla lettura fisica del livello di sedimentazione delle emazie in sangue anticoagulato in citrato o EDTA entro un intervallo di tempo prefissato e sono una diretta filiazione della metodica di riferimento di Westergren. Le metodiche "alternative", invece, estrapolano il valore di VES dal grado di aggregazione delle emazie misurato su di un campione di sangue intero anticoagulato in EDTA prelevato dal tubo primario. Entrambi i metodi sono ritenuti accettabili dalla comunità scientifica, ma essendo basasti su principi analiticamente diversi, in taluni casi possono fornire risultati devianti da quelli ottenibili con la metodica di riferimento, ed in questa sede vorremmo descrivere la nostra particolare esperienza per quel che riguarda l'utilizzo delle due metodiche quando applicate a pazienti pediatrici.
Materiali e metodi:Sono stati analizzati campioni da 212 bambini suddivisi in 3 gruppi: soggetti sani, soggetti con patologie infiammatorie e soggetti con patologie neoplastiche ematologiche.Gli strumenti utilizzati sono stati per il Westergren modificato il Ves-Matic Cube 30 (DIESSE Diagnostica Senese SpA, Monteriggioni (SI)), e per la metodica "alternativa" il Test-1 (Alifax Polverara (PD)) seguendo le istruzioni dei produttori. I risultati forniti da entrambi i sistemi sono stati confrontati con quelli della metodica manuale di Westergren e sottoposti ad analisi statistica.
Risultati: I 3 metodi presentano una buona correlazione sia per i soggetti sani che per i pazienti con patologie infiammatorie. Per alcuni pazienti oncoematologici, il metodo Roller Test1 non mostra risultati sovrapponibili agli altri due metodi, in particolare in presenza di valori bassi dell'ematocrito, mostrando complessivamente una correlazione insoddisfacente (r= 0,26).
Discussione:la metodica modificata e quella alternativa per l'esecuzione del test della VES sono basate su principi analitici molto diversi. La prima, infatti, in accordo alla metodica originale di Westergren (che è poi la metodica di riferimento) misura la distanza fisica percorsa dalle emazie aggregate nel plasma autologo entro un lasso di tempo predeterminato, preservandone le tre fasi (aggregazione, sedimentazione ed impaccamento). La metodica alternativa, invece, è basata sulla misura del grado di aggregazione degli eritrociti, eseguita in pochi secondi, da cui viene estrapolato un valore riferito come VES. Vista la tipologia di pazienti che afferiscono al nostro ospedale, si evince che il metodo Vesmatic Cube 30, attualmente in uso nel nostro laboratorio risulta essere dotato della maggiore accuratezza diagnostica, mostrando una buona correlazione con il metodo di riferimento, senza essere influenzato dai bassi valori di ematocrito.

P087

## DIAGNOSTIC UTILITY AND PITFALLS OF ADVIA 2120i PATTERN IN ACUTE PROMYELOCYTIC LEUKAEMIA: FROM ONSET TO RETINOIC ACID INDUCED MATURATION

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Acute Promyelocytic Leukemia (APL) represents the most important haematologic emergency, requiring a prompt diagnosis in order to start specific therapy and to prevent life-threatening haemorrhagic complications.APL accounts for about 5-8% of all Acute Myeloid Leukemia (AML) adult cases. Morphologic diagnosis relies on the identification of abnormal promyelocytes, charachterized by large size, azurophilic granules and frequent multiple Auer rods. Confirmation of diagnosis requires detection of PML-RARalfa transcript. Nevertheless, timing of molecular test doesn't meet clinical needs. Therefore, the diagnostic practice is firstly based on Complete Blood Cell Count (CBC), coagulation tests and morphological blood smears review. Actual hematological analyzers provide specific patterns able to direct diagnosis in the case of APL. In particular Siemens ADVIA 2120i is known to offer a specific APL pattern in the perox channel. According to our experience, we present a case report with an interesting behaviour and a particular evolution of the instrumental pattern. A 60 years old male outpatient came to observation following his routine laboratory controls for HCV cirrhosis. At onset, he presented petechiae, lower limbs bruising, gingival bleedings and mild haematuria appeared in the previous days. CBC showed severe leukopenia, anaemia and thrombocytopenia with normal coagulation tests, except for D-dimer elevation. Despite graphic instrumental pattern was unrelevant, the diagnosis was suspected by the morphological observation of rare atypical promyelocytes. All-trans Retinoic Acid (ATRA) therapy was started on the morphologic suspicion, according to guidelines. ATRA determined the increase of leukocyte count (>2000/ul, day 7), leading to the appearance of the typical ADVIA pattern related to APL. This trend is consistent with the known mechanism of the drug, which favours leukaemic clone maturation, without directly causing cell death. According to this knowledge, first neutrophilic population shows the same peroxidase features of the leukemic cells. Only at time of complete remission (day 60), the normalization of the graphic patterns of ADVIA 2120i was observed.

P088

## LE CRIOGLOBULINEMIE E L'EMOCITOMETRIA: UN RISCONTRO "RAPIDO" PER UNA DIAGNOSTICA "FRAGILE"

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Nel Laboratorio di Ematologia del DAIMELAB della A.O.U Policlinico Federico II di Napoli si effettuano esami emocromocitometrici di pazienti ricoverati o afferenti ai vari Ambulatori. Per l'esecuzione di tali esami si utilizza l'analizzatore ADVIA 2120i (Siemens), 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 e l'analizzatore XN300 (Sismex), che utilizza la fluorescenza e la diffusione ottica. Nell'ultimo anno sono venuti alla nostra osservazione 11 casi di pazienti con crioglobulinemia diagnosticati attraverso l'approfondimento degli allarmi sul sistema ADVIA. I pazienti provenivano dall'ematologia (6), reumatologia (4), ed immunologia clinica (1) ed avevano ricevuto la diagnosi di mieloma multiplo (4), leucemia linfatica cronica (1), linfoma (1), LES (2), artrite reumatoide (2). Un ultimo caso afferiva dal reparto di immunologia clinica per vasculite. La possibilità di identificare la presenza di crioglobuline ci è data da alcune anomalie grafiche prodotte all'interno dello scatter piastrinico (grafico di distribuzione volumetrica delle piastrine) dell'analizzatore ADVIA 2120i. Su questi sistemi, grazie all'introduzione del citogramma piastrinico PLT scatter e PLT volume, si ha la possibilità di una rappresentazione citografica delle crioglobuline. In uno dei campioni analizzati, tuttavia, oltre alla falsa piastrinosi, che si normalizzava dopo incubazione a 37°C, abbiamo riscontrato un'anomalia sul grafico perox, mai segnalata in precedenza, nel box appartenente alle "grandi cellule non colorate" LUC che si estendeva fino alla coincidenza e che, allo stesso modo, si normalizzava dopo incubazione a $37^0$C. Tale riscontro rappresenta, quindi, un nuovo allarme citografico per la diagnostica delle crioglobuline. L'osservazione microscopica e la peculiarità delle indagini condotte sulla proteina crioprecipitata ci hanno condotto ad ulteriori approfondimenti. Tali osservazioni confermano la validità di sistemi di rapido riscontro per patologie "complesse" di difficile inquadramento diagnostico.

P089
**MISURA DELLE FREE LIGHT CHAINS PLASMATICHE NELLA VALUTAZIONE DELLA RISPOSTA AL TRATTAMENTO CON DARATUMUMAB IN SOGGETTO AFFETTO DA MIELOMA MULTIPLO A CATENE LEGGERE KAPPA (LCMM) ASSOCIATO A MALATTIA DA DEPOSITO DI CATENE LEGGERE (LCDD)**

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Background: La malattia da deposito di catene leggere (LCDD), caratterizzata dalla deposizione a livello renale di frammenti di immunoglobuline monoclonali prodotte da linfociti-B e/o plasmacellule, nel 10-30% dei casi si associa a Mieloma Multiplo. L'outcome di queste malattie è migliorato grazie all'introduzione del test diagnostico Free Light Chain (sFLC) e dell'utilizzo di nuove terapie come l'anticorpo monoclonale Daratumumab. E' risaputo che l'immunoterapia, interferendo sui test elettroforesi (CZE) ed immunofissazione sierica (sIFE), non consente una corretta valutazione della risposta clinica. Un appropriato workup diagnostico di laboratorio ed ematologico è utile sia per la diagnosi che per il trattamento precoce, migliorando l'outcome clinico del paziente. La biopsia renale è essenziale per determinare l'esatta natura della lesione e la severità del danno d'organo.
Metodi: Donna di 64aa, con Insufficienza Renale Cronica ed anemia si ricovera presso l'U.O.Ematologia. Il workup diagnostico all'ingresso è stato orientato sia alla valutazione del danno renale che alla ricerca della componente monoclonale con CZE, s-IFE, u-IFE su gel di agarosio HR (Hight Resolution) (Sebia) e dosaggio delle sFLC (The Binding Site). Per una corretta valutazione della risposta al trattamento è stato utilizzato il test Hydrashift (Sebia). Risultati All'esordio la CZE non presentava alterazione morfologica; s-IFE: negativa, u-IFE: catene monoclonali kappa; sFLCk 1487 mg/L, sFLCλ 40,24 mg/l, ratio k/l 36,95.
Gli esami ematochimici: eGFR 27mL/min, Hb:10g/dL, proteinuria 1231 mg/24h. La biopsia osteomidollare evidenziava una plasmocitosi del 15% con clonalità kappa; la biopsia renale rilevava una LCDD kappa; diagnosi: LCMM associata a LCDD. Dopo "Minimal Response" a terapia di I linea: Velcade, Ciclofosfamide e Desametasone (VCD) si intraprese terapia con: Daratumumab, Velcade e Desametasone ottenendo Risposta Completa (RC): ratio k/l 3,87.
Conclusioni: L'elevata sensibilità diagnostica della ratio k/l alterata non solo si è rivelata un importante indicatore di clonalità per il managment della LCDD ma ha evidenziato anche la mancata RC stringente al trattamento. Il caso clinico suggerisce la necessità di ulteriori indagini diagnostiche per definire l'esatta natura del clone.

P090
**NEW SCREENING TEST FOR HEMOGLOBINOPATHIES: TGA/CHEMOMETRIC ANALYSIS**

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Thermogravimetry coupled with chemometrics proved to be a rapid and cost effective diagnostic tool for β-thalassemia screening [1]. This model, consisting of chemometric PLS-DA, permitted the discrimination of thalassemic patients and healthy individuals, using the TG curves of blood samples. In addition, the TGA screening test permitted to differentiate thalassemia according to clinical severity and was not influenced by drug therapies (aspirin) commonly used by thalassemia patients who undergo splenectomy [2-3]. The TGA/ Chemometric analysis is able to provide the screening of thalassemia in blood samples stored at 4°C until 15 days. Healthy donors were considered as reference subjects and a typical thermal behaviour as a function of aging was estimated and compared to thermal behavior of thalassemia subjects. Despite blood changes with aging, thealthy and thalassemic subjects may be significantly differentiated by TGA/Chemometrics after 15 days from blood collection with a 100 % of correct classification rate. This new method applied to aged samples was able to discriminate thalassemia in transfused patients that is generally not possible by the common first level protocol, and in dβ-thalassemias, and β-thalassemia combined with Hb Lepore, usually requiring the molecular analysis for diagnosis. This study, for the first time, describe a screening method for thalassemia able to detect thalassemia on whole blood samples stored for 15 days [4].In conclusion the application of TGA/chemometric analysis has proved to be a particularly useful diagnostic tool for the screening of the hemoglobin defects, in a short time and at low cost, of this case of congenital hemolytic anemia of difficult diagnosis. This method results particularly suitable in pediatric patients as it requires small sample volumes and is able to characterize patients subjected to transfusion. [1] Risoluti, R., Materazzi, S., Sorrentino, F., Bozzi, C., Caprari, P. Talanta183 (2018) 216-222 doi 10.1016/j.talanta.2018.02.071[2] Risoluti, R., Materazzi, S., Sorrentino, F., Maffei, L., Caprari, P. Talanta 159 (2016) 425-432 doi 10.1016/j.talanta.2016.06.037[3] Risoluti, R., Gullifa, G., Fabiano, M.A., Sorrentino, F., Caprari, P., Materazzi, S. J of Ther. Anal and Cal. 134(2) (2018) 1299-1306 doi 10.1007/s10973-018-7262-3.[4] Risoluti, R., Caprari, P., Gullifa, G., Massimi, S., Sorrentino, F., Buiarelli, F., Materazzi, S. Microchemical J 146 (2019) 374-380 doi 10.1016/j.microc.2019.01.008

P091

## 1,25(OH) VITAMIN D AND FIBROBLAST GROWTH FACTOR (FGF-23) IN PATIENTS WITH KLINEFELTER SYNDROME

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Background: Recently, we demonstrated a normal response to human chorionic gonadotropin (hCG) stimulation in Klinefelter Syndrome (KS); We speculated that the impaired testosterone (T) production, despite the first steps of steroidogenesis are functional, could be due to a reduced hydroxyl-steroid dehydrogenase activity. Scientific evidences describe a central role of testes in vitamin D homeostasis, indeed, lower levels of 25-hydroxy vitamin D (25(OH)D) were significantly associated with lower levels of T. In this setting, human fibroblast growth factor 23 (FGF23) is involved in bone metabolism and no studies had evaluated FGF23 and 25(OH)D correlation with testicular production in the KS setting. Objective: To analyze FGF23 production in KS, and its relationship with hormonal production after hCG stimulation. Methods: 13 KS patients (36±9 years) not receiving T replacement therapy and 12 eugonadal controls (32±8 years) were evaluated at baseline and for 5 consecutive days after intramuscular injection of 5000 IU hCG, measuring 1,25(OH)D and FGF23 (LIAISON®XL, DiaSorin S.p.A, Saluggia (VC), Italy). Results: 1,25(OH)D was not normally distributed (p=0.811), the mean value at baseline was 42.19+9.94 pg/ml. All subjects had 1,25(OH)D serum levels within the reference range (RR), without significantly change after hCG stimulation (p=0.401). No correlations among 1,25(OH)D and sexual steroids (R=0.024, p=0.846) were found. FGF23 was not normally distributed (p=0.866), with a mean value at baseline of 31.09+9.66 pg/ml. Two patients (18.2%) showed baseline levels below the lower limit of RR. FGF23 did not significantly change after hCG stimulation (p=0.320), even when only patients with detectable FGF23 basal levels were assessed (p=0.589). FGF23 did not correlate neither to 1,25(OH)D (R=-0.106, p=0.475) nor to T (R=-0.160, p=0.276). Conclusion: 1,25(OH)D levels did not change after hCG stimulation, suggesting that testicular vitamin D activation is only slightly sensitive, in contrast with recent studies depicting a strong correlation between vitamin D and testicular function. Similarly, FGF23 was not influenced by hCG stimulation, without any difference between KS and eugonadal men, suggesting a secretion pattern independent to steroidogenesis stimulation.

P092

## PREVALENCE OF SUBCLINICAL HYPOTHYROIDISM IN A PEDIATRIC POPULATION OF THE MOLISE REGION: THE IMPORTANCE OF THE LABORATORY IN THE DIAGNOSIS

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Subclinical hypothyroidism (SCH) is a clinical condition defined by the presence of thyroid-stimulating hormone (TSH) values above the age normal range, associated with normal free thyroxine levels (FT4). Its prevalence in pediatric age is 2% and includes both autoimmune and non-autoimmune forms. The most frequent cause of the former is Hashimoto's thyroiditis (HT), but also transplacental passage of maternal antibodies. Among the SCH non-autoimmune causes, we can consider iodine deficiency/excess, genetic forms and thyroid dysgenesis. Individuals with autoimmune diseases, genetic syndromes (Turner, Down) or obesity should be considered at risk of SCH. Most patients show few clinical manifestations and the diagnosis is made only with the support of laboratory.The aim of the present study was to identify the prevalence of SCH in a pediatric population of the Molise region (Italy), as well as evaluating the distribution of obesity, celiac disease (CD), Hashimoto's thyroiditis (HT) and Down syndrome (DS) in this population. Possible correlations between anthropometric parameters, such as body mass index (BMI) and waist-hip ratio (WHR), ultrasound findings, familiarity and TSH values were also investigated. The medical records of 1052 subjects have been evaluated, 609 females and 443 males. The children underwent anamnesis, auxological examination, anthropometric evaluation and laboratory analysis such as TSH, FT4, free triiodothyronine (FT3), antithyroperoxidase antibodies (TPOAbs), antithyroglobulin antibodies (TgAbs), immunoglobulin A anti-tissue transglutaminase antibodies (IgA-tTG), antibodies against native gliadin (AGA), karyotype.The study confirmed that also in the Molise region there is an association between obesity and SCH, both in children and adolescents, in particular a significant correlation was demonstrated between TSH values and BMI and, although of a lesser entity, between TSH and WHR. The study also allowed to analyze and classify the various etiological frameworks of subclinical hypothyroidism, largely confirming the expected evidence for cases associated with CD and DS. A high prevalence of obese or overweight subjects was found among the SCH group, higher than the general population and in line with the high prevalence of obesity in Molise.

P093

## ANALYSIS OF FAT SOLUBLE VITAMINS IN SERUM USING UPLC-MS/MS FOR CLINICAL RESEARCH

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Background: Extraction and analysis of vitamin A and E by LC-MS/MS has historically involved laborious sample preparation and lengthy analysis time with high solvent consumption, whilst vitamin $K_1$ is a challenging analyte due to its hydrophobicity (logP 9.70). Phospholipids, which are present in high levels in serum samples, elute in the same chromatographic region as vitamin $K_1$, causing ion suppression when performing reversed-phase UPLC-MS/MS. Clinical research methods have been developed using a reverse-phase Solid Phase Extraction (SPE) sorbent in 96-well plate format that minimizes interference from phospholipids. This sample preparation research method in combination with an analysis time of only 3 minutes, reduces solvent consumption and allows for high throughput sample analysis.
Methods: Certified reference materials were used to create in-house calibrators and QC materials in stripped serum. Samples were pre-treated with internal standards, centrifuged, and for vitamin A and E analysis a fixed volume of supernatant was diluted prior to SPE using Waters™ Oasis™ PRiME HLB µElution plates. All samples were quantified using a Waters ACQUITY UPLC™ I-Class FTN/Xevo™ TQD System with a 2.1x50 mm HSS PFP column and a water/methanol/ammonium acetate/formic acid gradient.
Results: For vitamin A and E the method was shown to be linear over the measuring ranges (100-2000 ng/mL) and (1.1-21.1 µg/mL) respectively. Coefficients of variation (CV) for total precision and repeatability of low, mid and high QC samples (n=25) were all ≤6.9%. No significant carryover was observed from high concentrations serum samples into serum blanks and over-range samples were successfully diluted (1:4) with accuracies ranging from 86% to102%. Analytical sensitivity (bias ≤15% and CV ≤20%, over 5 occasions) was shown to be 50 ng/mL for vitamin A and less than 1.1 µg/mL for vitamin E. EQA samples demonstrated good agreement between this analytical method and the EQA ALTM mean values, with mean bias of -7.0% for vitamin A and -10.9% for vitamin E. For vitamin $K_1$ the method was shown to be linear over the range 0.1 – 20 ng/mL, with precision performance ≤10%. Structurally related compounds were chromatographically separated, and endogenous interference studies gave recoveries within 85-115% for all vitamins.
Conclusions: We have successfully quantified vitamin A, vitamin E and vitamin $K_1$ in serum using SPE with UPLC-MS/MS for clinical research purposes. This method demonstrates good linearity, precision and accuracy, whilst removing phospholipids and providing high throughput capabilities.

P094

## UPLC-MS/MS Analysis of DHT, DHEA, Testosterone, Androstenedione, 17-OHP and Progesterone in Serum for Clinical Research

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Background: Here we evaluate an offline automated method for the measurement of serum dihydrotestesterone (DHT), dehydroepiandrosterone (DHEA), testosterone, androstenedione, 17-hydroxyprogesterone (17-OHP) and progesterone, enabling steroid profiling for the investigation of metabolic dysfunction biomarkers for clinical research. A UPLC-MS/MS method was developed using a mixed-mode Solid Phase Extraction (SPE) sorbent in 96-well plate format, improving workflow and reducing sample preparation time. Chromatographic resolution between structurally related steroid species was achieved to help obtain highly precise and accurate data, particularly at low physiological concentrations.
Methods: Certified steroid hormone reference material purchased from Sigma Aldrich (Poole, UK) were used to create calibrators and QC materials in 1% Bovine Serum Albumin (BSA) in Phosphate Buffered Saline (PBS). Serum samples purchased from NEQAS (Birmingham, UK) for testosterone, androstenedione and 17-OHP were analyzed and concentrations were compared to the EQA MS mean for each steroid hormone. Lyophilized serum samples purchased from RCPA (NSW, Australia) for DHT were compared to the target values. Additionally, samples previously analysed by an independent LC-MS/MS clinical research method were evaluated for comparison. Sample preparation was automated offline using a Tecan® Freedom Evo 100. 100 µL serum samples were pre-treated with internal standard, methanol and water. Waters Oasis MAX µElution SPE was performed, allowing direct injection of the SPE eluate. Using an ACQUITY UPLC I-Class system, samples were injected onto a 2.1 x 50 mm Waters CORTECS $C_{18}$ column with an in-line 0.2 µm filter using a water/methanol/ammonium fluoride gradient and quantified with a Waters Xevo TQ-S micro mass spectrometer.
Results: The developed method was shown to be linear over the measuring range for the serum steroid hormones.
Coefficients of variation (CV) for total precision and repeatability on five separate days for low, mid and high QC samples were ≤6.5% (n=25) for all analytes.
Analytical sensitivity investigations performed over five occasions demonstrated a CV < 20% (S:N>10) at 0.025 ng/mL for DHT, 0.1 ng/mL for DHEA, 0.005 ng/mL for testosterone, 0.01 ng/mL for androstenedione, 0.01 ng/mL for 17-OHP and 0.005 ng/mL for progesterone. Matrix Factor experiments demonstrated the internal standard compensated for ion suppression observed in the method, with accuracies of 95–101% and CVs ≤3.4% for the serum steroids. The method has shown to be analytically selective through separation of isobaric steroid species and matrix specific interferences such as albumin, intralipid, cholesterol, triglycerides and bilirubin.

Agreement between this analytical method and the EQA LC-MS mean values have been demonstrated with mean method bias of -1.4%, +0.2% and -5.6% for testosterone, androstenedione and 17-OHP, respectively. Agreement between this analytical method and RCPA target values for DHT was +4.9% mean bias and method comparison to an independent LC-MS/MS clinical research method for DHT demonstrated a mean bias of -6.6% for the samples analysed.
Conclusions: This offline automated clinical research method demonstrates excellent linearity, analytical sensitivity, selectivity, precision and accuracy. The method quantifies serum samples for DHT, DHEA, testosterone, androstenedione, 17-OHP and progesterone using UPLC-MS/MS, providing sample tracking capabilities and high sample throughput capabilities whilst minimizing operator error. For Research Use Only. Not for use in diagnostic procedures.

P095
**RAPID UPLC-MS/MS DRIED BLOOD SPOT ANALYSIS OF STEROID HORMONES FOR CLINICAL RESEARCH**

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Background: Dried Blood Spots (DBS) are an established microsampling technique providing a low-cost approach of collecting, shipping and analyzing samples for clinical research. Ligand-binding assays (LBAs) are often used as the frontline testing methodologies for DBS samples in steroid hormone analysis. Although rapid, the relatively low analytical specificity of the LBAs may necessitate follow-up, using liquid chromatography – tandem mass spectrometry (LC-MS/MS). The challenge is to create a fast, analytically sensitive and selective LC-MS/MS methodology.
Methods: Certified androstenedione, 17-OHP, cortisol, 11-deoxycortisol and 21-deoxycortisol reference material purchased from Sigma Aldrich (Poole, UK) were used to create calibrators and QC materials in whole blood; prepared by mixing 50/50 (v/v) red blood cells from BioIVT (West Sussex, UK) and MSG4000 stripped serum from Golden West Biologicals (CA, USA). Blood spots were prepared by adding 50µL whole blood calibrators and QCs to Whatman 903 Protein Saver Blood Spot cards from Sigma Aldrich (Poole, UK) and then left to dry. Two 3mm DBS samples were pre-treated with an internal solution and mixed for 5 minutes. SPE was carried out with a Waters Oasis™ MAX µElution 96-well plate, allowing direct injection of the SPE eluate. Offline automated extraction was performed using a Tecan® Freedom Evo 100. Using an ACQUITY UPLC I-Class system, samples were injected onto a 2.1mm x 50mm CORTECS $C_{18}$ 2.7µm column with pre-column CORTECS $C_{18}$ 2.7µm VanGuard using a water/methanol/ammonium fluoride gradient and quantified with a Xevo™ TQ-S micro mass spectrometer.
Results: The method enabled rapid separation in 1.4 minutes (2.3 minutes injection to injection) for 17-OHP, androstenedione, cortisol, 11-deoxycortisol and 21-deoxycortisol with baseline resolution of steroid isobars.
Calibration lines were linear from 0.5 – 500 ng/mL for androstenedione and 11-deoxycortisol; and 1.0 – 500 ng/mL for cortisol, 17-OHP and 21-deoxycortisol with correlation coefficients ($r^2$) >0.99 over five occasions.
Coefficients of variation (CV) for total precision and repeatability over five occasions at four concentrations; 2, 5, 50 and 400ng/mL, were ≤ 9.3% (n = 25) with accuracies ranging from 94 – 110%.
Conclusions: The challenge was met by using Ultra Performance Liquid Chromatography (UPLC™) combined with CORTECS™ 2.7µm particle columns to provide UPLC separations at high linear velocities with minimal loss in column performance. This offline automated method demonstrates excellent linearity, analytical sensitivity, precision and accuracy, while providing high sample throughput capabilities for the

analysis of androstenedione, 17-OHP, cortisol, 11-deoxycortisol and 21-deoxycortisol in dried blood spots for clinical research purposes.For Research Use Only, Not for use in diagnostic procedures.

P096

## TESTOSTERONE AND CORTISOL MODIFICATIONS IN THE PROFESSIONAL MALE CYCLIST ACROSS THE AGONISTIC SEASON

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Background and objective: The testosterone to cortisol (T/C) ratio has been proposed as an indicator for the balance between anabolic and catabolic metabolism. A 30% decrease of the ratio during training is suggestive of a condition of overreaching. While there is agreement that a bout of exercise induces an acute increase of cortisol (C), the interpretation of testosterone (T) changes is challenging because of contradictory results reported in published studies; nevertheless, when read together, they suggest that the response of sex steroid hormones to exercise might be influenced by the duration and the intensity of exercise itself. Here, we evaluated a long-lasting physical exercise such as cycling race with the aim to explore the variations of serum total T and C in a group of professional male cyclists before and after a single race, during an agonistic season.

Methods: Prospective longitudinal observational study, including 17 professional adult male cyclists (mean age 45.7+11.9 years) belonging to the same team. For each subject, blood samples were collected at the beginning, in the middle and at the end of the agonistic season. The pre-race blood collection was obtained few days before the competition and the post-race upon the arrival (usually around 16:00). Serum total T and C were assessed by immunoenzymatic assays. Delta T and delta C were calculated to assess the hormonal change after race, considering the difference between post- and pre-race serum levels. T/C ratio was calculated as index of overreaching when increased >30% of baseline values.

Results: Total T serum levels significantly decreased after the race ($p<0.001$), whereas serum C significantly increased ($p<0.001$). Accordingly, T/C ratio significantly decreased post-race ($p<0.001$). However, neither T, C nor T/C ratio significantly differed among the 3 time points.

Conclusions: High-intensity physical activity induces rapid changes in hormonal and biochemical parameters. In particular, the cycling race leads to an acute T reduction together with a C increase after the competition. However, this effect is acute and rapid and hormones returns within the baseline levels along the agonistic season, without impairing the chronic anabolic/catabolic balance, as confirmed by the unchanged T/C ratio

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## QUANTITATIVE SERUM AMH DETERMINATION: EVALUATION OF A FULLY AUTOMATED CLIA ASSAY AND COMPARISON WITH THE CURRENT MANUAL ELISA ASSAY.

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Anti-Müllerian hormone (AMH) is a glycoprotein hormone belonging to TGF-β family, mainly studied for its regulatory role in male sex differentiation. AMH produced by foetal testis arrests the development of female reproductive tract, while after birth it is produced by ovary as well. In both sexes AMH is secreted in serum and represents a specific marker of Sertoli cell function and spermatogenesis in men, and a clinical marker of ovarian reserve in women. Aim of research is to compare a manual AMH assay with respect to an automated one (AMH Gen II ELISA assay versus Access AMH assay) both produced and marketed by Beckman Coulter. The AMH Gen II ELISA assay is an enzymatically amplified two-site manual immunoassay, performed in our lab on Grifols Triturus ELISA instrument, with AMH concentration directly proportional to absorbance. Access AMH assay is a highly automated paramagnetic particle CLIA, performed on Beckman Coulter UniCel DxI 600 platform, with light production directly proportional to AMH concentration. To compare the two methods, a total of 154 serum samples covering the whole measurable interval and the range of values clinically observable, were used and data statistical analysis was performed. The Access AMH assay was also partially validated by linearity dilution analysis, matrix effect analysis and LoQ determination.From linear regression analysis compared methods showed a good relationship but non-parametric analysis highlighted a slight constant and proportional systematic error. The Bland-Altman graphical analysis, confirmed a slight systematic but not significant error with a general tendency to overestimate results for the in use method.The new method showed good linearity over a wide range of dilutions, matrix effect was insignificant and the LoQ was set to 0.01 ng/mL, in line with what stated by assay manufacturer.In conclusion, this study conducted on 154 samples with AMH concentrations covering the entire reference range demonstrate that automated ACCESS AMH assay correlate well with AMH Gen II ELISA assay and, for characteristics of its performances also documented by the manufacturer, it is suitable to replace the method used in our laboratory.

P098

## CARDIOVASCULAR DISEASE RISK EVALUATION IN A COHORT OF HIV-AFFECTED PATIENTS TREATED WITH RITONAVIR: THE ROLE OF PHARMACOGENETIC BIOMARKERS

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Ritonavir (RTV) is an anti-HIV drug related to increased lipodistrofy and cardiovascular disease (CVD) risk. HIV-infected patients are more predisposed to have accelerated atherogenesis, which could depend on genetics. Polymorphisms in metabolizing or transporting proteins encoding genes are able to affect RTV drug concentrations, thus probably its related toxicities, but no data are available in literature.For these reasons, aim of this study was to analyze the role of variants in genes (ABCB1, ABCC2, ABCG2, VEGF and HNF4α) involved in RTV transport and metabolism in predicting metabolic and cardiovascular risk biomarkers. Levels of total cholesterol (TC), low density lipoproteins (LDL), high density lipoproteins (HDL), triglycerides, pancreatic amylase, glycemia, aspartate amine transferase, alanine aminotransferase, gamma glutamyl transferase, haemoglobin were analyzed at baseline (BL) and weeks 12, 24 and 48. CVD risk was evaluated calculating the ratio of total cholesterol/high density lipoproteins, considering values>5 as higher risks. Allelic discrimination was assessed through real-time PCR.99 patients were analyzed. ABCB1 3435 TT was associated with TC (BLp=0.044, W12p=0.008, W24p=0.039) and triglycerides (BLp=0.048, W12p=0.045, W48p=0.049), ABCB1 2677 TT with viral load (BLp=0.030, W12p=0.013, W24p=0.023, W48p=0.046), ABCC2-1249 AA with glycemia (BLp=0.025, W12p=0.031, W24p=0.012), ABCC2-24 GA/AA with TC (BLp=0.009, W24p=0.003, W48p=0.009), LDL (BLp=0.008, W24p=0.004, W48p=0.010) and triglycerides (BLp=0.041, W12p=0.043, W24p=0.045, W48p=0.025), ABCG2 1194+928 CC with pancreatic amylase (BLp=0.015, W12p=0.033, W24p=0.039, W48p=0.013), HNFα 975 CG/GG with TC (BLp=0.006, W12p=0.001, W24p=0.009) and LDL (BLp=0.001, W12p=0.001, W24p=0.011) and VEGFα with LDH (W12p=0.012, W24p=0.031, W48p=0.030). In regression analyses, ABCB1 2677GG/GT and aspartate aminotransferase>40 U/L, whereas age>50 years, ABCC2-24GG and gamma-glutamyl transferase<71 U/L predicted CVD risk>5 at baseline and at 48 weeks, respectively. This is the first study reporting the SNPs' influence on RTV-associated haematological markers and CVD risk in HIV-affected patients. This work could suggest the potential for personalized, pharmacogenetic-based, antiretrovirals' selection.

P099
**ESPERIENZA DI APPLICANZIONE DI UN PROTOCOLLO PER COMMISSIONI MEDICHE LOCALI PER IDONEITA' ALLA GUIDA NEI CONDUCENTI SANZIONATI PER l'art.186 C.d.s.**

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Scopi ed obiettivi: Lo stato derivante dalla dipendenza da alcool compromette i requisiti fisici e psichici richiesti nella valutazione d'idoneità alla guida."La Commissione Medica Locale deve accertare l'idoneità alla guida e a tal fine: può avvalersi di singoli consulenti oppure di istituti medici specialistici appartenenti a strutture pubbliche, con onere a carico del soggetto esaminato (D.P.R. 495/92, art. 330, c.6)". Per tali accertamenti è stato predisposto da tempo nel nostro Laboratorio, un Protocollo che vede oltre alla tradizionale quantificazione della CDT su siero, quella dell'Etilglucuronide (ETG) su matrice cheratinica, con l'esclusione di tutti i marcatori indiretti tradizionali (transaminasi, MCV ecc.). I risultati di questo Protocollo hanno generato in Liguria, un Documento Regionale, deliberato alla fine del 2018 , che riporta precise indicazioni sulle indagini utili alle Commissioni Mediche Locali della stessa regione (Delibera 321 del 19.12.2018).Tramite uno studio retrospettivo andremo a dimostrare come l'utilizzo del nostro Protocollo, sia uno strumento efficace nel monitoraggio per l'idoneità alla guida dei soggetti sanzionati per l'art.186 del C.d.s. e come questo ci abbia permesso di semplificare la procedura rendendola al contempo più efficace.
Materiali e metodo: Le indagini su matrice cheratinica (ETG) 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 relativi alla Commissione Medico Locale della Spezia, emerge che su 631 utenti monitorati nel 2018 per guida in stato di ebrezza a cui è stato applicato il nostro Protocollo, circa il 11% ha presentato valori di ETG ( Etilglucuronide) superiori a 30 pg/mg, soglia che indica un consumo elevato di alcool. Confrontando la positività al marker ematico tradizionale di abuso alcolico CDT (1.8%) con quella dell'ETG (11%) su matrice cheratinica, abbiamo riscontrato un aumento di casi di inidoneità alla guida del 9.2 %. Inoltre, dividendo i campioni con ETG > 30 pg/mg in sottocategorie quantitative, si evidenzia che gli unici casi di CDT positivi sono stati riscontrati per valori di ETG superiori a 50 pg/mg. Possiamo concludere che l'introduzione del nostro Protocollo, esteso a tutta la Regione Liguria, può sicuramente essere di ausilio alle Commissioni Medico Locali fornendo nuovi e più efficaci elementi valutativi per l'idoneità alla guida.

P100
**La Tossicologia Penitenziaria napoletana**

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La L.419/98 afferma l'esigibilità del diritto alla salute nei luoghi di detenzione.L'art.2,c.2,D.Lvo 230/99 recita:"L'assistenza sanitaria ai
detenuti...è organizzata secondo principi di globalità dell'intervento sulle cause di pregiudizio della salute,di unitarietà dei servizi e
delle prestazioni...".Il 63% dei detenuti nasce in Italia,il 32% al Sud,in testa Campania,per maschi e femmine.Gli stranieri sono Africani
(50%),specie Marocco e Tunisia,ed Europei (38%).Tale aumento pone nuove sfide alla sanità penitenziaria (il 50% dei detenuti campani
è di oltre 50 paesi diversi).Nel mondo i detenuti stranieri sono circa il 14%,meno del 3% in molti paesi.In Europa sono il 22-38%
del totale sino al 70% in Svizzera.In Italia gli stranieri in carcere sono il 37%.In Campania sono attive 15 carceri per 6131 posti, al
31/5/19 n.7841 detenuti,395 donne e 1024 stranieri (12,59% della nazione e 12,79% di sovraffollamento). Lo stato di salute:1su 5 usa
sostanze d'abuso,con doppia diagnosi probabile in almeno 5-6 su 10;epatopatie nei tossicodipendenti 1 su 4, 6 su 100 HIV positivi;il 60%
fuma.Carceri di Napoli: Centro di Secondigliano è del 1992, ha 4 reparti:3 di Alta Sicurezza e 1 per detenuti comuni.Dipendenze:190
soggetti in carico nel 2018.In trattamento con farmaci agonisti (eroinomani):30.Cocainomani puri e/o consumatori di cocaina:43.Alcolisti
puri/consumatori di alcol:9. Poliabusatori -cocaina(+ +),eroina,alcol,THC-:108. Casa Circondariale di Poggioreale è del 1918:capienza
1611, presenti 2299 (surplus 41,71%).Dipendenze:soggetti in carico 300,59 di loro (20%) con trattamento sostitutivo all'eroina (55 MTD
e 4 BUP).Circa il 90% è trattato dall'ingresso con MTD.Istituto per Minorenni di Nisida è del 1930,capienza 42 ospiti.Al 15/4/19 ospita 63
detenuti, maschi e femmine.Al 15/7/18 51 italiani e 17 stranieri. Presenza media/die 64,9,ragazzi e ragazze.I minorenni sono 18:di questi,1
nella fascia d'età 14-15 anni e 20 nella fascia 16-17 anni. 43 i giovani adulti, divisi nelle fasce d'età 18-20 (n.28) e 21-24 anni (n.15).Dal
2016 i delitti più numerosi,dopo quelli contro il patrimonio,sono stati quelli delle violazioni della normativa sugli stupefacenti. Dal 1/1/05
al 31/12/18 abbiamo esaminato 18021 campioni (13793 sono risultati positivi a stupefacenti e 4228 negativi).5991 i detenuti in terapia.

P101
## LA TOSSICOLOGIA E LA SICUREZZA STRADALE NELLA CITTÀ METROPOLITANA DI NAPOLI

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L'ACI collabora con l'Istat alla raccolta delle informazioni statistiche sull'incidentalità in congiunta con Ministero dell'interno, Polizia Stradale, Carabinieri, Polizia Municipale, Polizia Provinciale, gli Uffici di statistica dei comuni capoluogo di provincia e gli Uffici di statistica di alcune province.Sono monitorati gli incidenti stradali che si verificano in una strada aperta alla circolazione pubblica, in seguito ai quali una o più persone sono rimaste ferite o uccise e nei quali almeno un veicolo è rimasto implicato.Nel 2017 sono stati 174.933 gli incidenti stradali con lesioni a persone in Italia, in leggero calo rispetto al 2016, con 3.378 vittime e 246.750 feriti.Il numero dei morti torna a crescere rispetto al 2016 (+95 unità, pari a +2,9%) dopo la riduzione registrata due anni fa.Ancora non sono disponibili i dati relativi al 2018. Tra il 2010 e il 2017 la riduzione media annua del numero di vittime della strada è stata del 3,1% nella UE e del 2,8% in Italia, variazioni comunque inferiori a quelle stimate per raggiungere l'obiettivo europeo di dimezzare il numero di morti in incidenti stradali entro il 2020.Per rispettare il target fissato, nel periodo 2018- 2020 il numero di vittime nella UE e in Italia dovrebbe ridursi, in media annua fino al 2020, di circa il 15%.In Campania dal 2013 al 2017 gli incidenti ed il numero di feriti sono lievemente aumentati mentre sono cresciuti dal 2016 al 2017 gli incidenti mortali.Nel 2017 Polizia Stradale, Carabinieri e Polizie Locali dei Comuni capoluogo hanno elevato le seguenti contestazioni:art.186 e 186 bis n.41476 (+2,5%), art.187 n. 5289 (+11,7%).Anno 2017 le contravvenzioni elevate per guida in stato di ebbrezza e sotto l'effetto di stupefacenti in occasione di incidente stradale (dati Comando Generale dell'Arma dei Carabinieri e Servizio della Polizia Stradale del Ministero dell'interno) sono: art.186 e 186bis n.4575 (7,8%), art. 187 n.1690 (2,9%).Anno 2017 sanzioni elevate per guida in stato di ebbrezza e sotto l'effetto di stupefacenti in occasione di incidente stradale art 186 e 186bis n. 2126, art.187 n.462. Dai 5 P.S. cittadini dal 2005 al-2018 sono giunti alla ns. osservazione n.2425 campioni di cui n.1329 negativi e n. 1096 positivi (n.531 una sola sostanza d'abuso tra cui 128 cannabinoidi, 127 etanolo, 60 cocaina- n.565 poliassuntori).

P102
## ISAVUCONAZOLE IN PEDIATRIC PATIENTS WITH ONCO-HAEMATOLOGIC DISEASES AND HAEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)

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Background: Isavuconazole (ISA) is a new triazole approved for IFI treatment in adults with activity against both moulds and yeasts spp, excellent oral bioavailability without relevant food or gastric pH effect, poor drug-drug interactions. Due to the good-predictability of the dose-exposure relationship over a wide range of doses in healthy adult, the dose adjustment TDM-based seems not necessary. However, in children efficacy, safety, dosage and schedule have not yet been tested and established
Method: A retrospective analysis in paediatric patients under chemotherapy or HSCT who received ISA as off-label treatment for both IFI treatment or prophylaxis. Due to the lack of recommended dosing and of PK/PD target value, plasma conc. were measurement at $Ctrough_{ss}$ and C3h after drug intake by a validated LC-MS/MS assay. In some patients, additional sampling times were carried out Results: 29 children (M/F 20/9), median age and weight 14,5 yrs and 47 Kg were included. Ten patients received chemotherapy and 19 HSCT. ISA was used as prophylaxis in 5 patients and as IFD treatment in 24: 20 for previous therapeutic failure with other antifungal drugs, 4 as first line therapy. Patients under 30 Kg received half ISA dose, the others received adult recommended schedule. 10 patients received oral therapy, in 19 IV route was switched to oral during treatment. ISA was administered for a median of 75,5 days in combination with other antifungals. Overall response rate was 70.8%. We obtained 12 complete remission

and 5 partial; 5 treatment failure and 3 stable fungal lesions. No breakthrough infections in prophylaxis group. PK monitoring was applied to 17 patients: median ISA $Ctrough_{ss}$ was 4.91 mg/L; Conc/Dose(kg) ratio was 1.13. A statistical significant relationship between Ctrough/dose ratio and age was obtained[Ctrough(mg/L)/Dose(mg/kg)] = ln (age,yrs)–1,2 (r=0,81,p<0.001)In 6 cases a 12h-PK profile was carried out and a median $AUC_{0\text{-}12h}$ of 153,2 mgxh/L was obtained
Conclusions: ISA may be useful and safe in children, even in the HSCT setting. The obtained Ctrough/dose ratio suggests that the same treatment strategy cannot fit all patients, confirming that ISA clearance is faster in younger patients and a higher dose may be necessary. PK monitoring could help to identify optimal treatment individualization strategies especially in younger children

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## A NEW UHPLC-MS/MS METHOD FOR THE SIMULTANEOUS QUANTIFICATION OF BICTEGRAVIR AND FOURTEEN OTHER ANTIRETROVIRAL DRUGS IN PATIENT PLASMA

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Patients affected by HIV often report several comorbidities, resulting in burdened polytherapy with high risk of side effects. In this context, Therapeutic Drug Monitoring (TDM) could be a good strategy for a better management of drug-drug interactions in fragile patients. Recently, a new unboosted integrase strand transfer inhibitor (INSTI), named bictegravir (BIC), has been developed. BIC reveals improved resistance and safety profile compared to other INSTIs. Up to now, no analytical method is reported in literature for the quantification of BIC together with other antiretroviral drugs (ARVs) currently in use.The aim of this work has been the development and validation of a new sensitive, robust and cheap Ultra High Performance Liquid Chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) method for the simultaneous quantification of BIC and other ARVs (efavirenz, lopinavir, darunavir, amprenavir, nevirapine, dolutegravir, atazanavir, elvitegravir, raltegravir, etravirine, rilpivirine, ritonavir, maraviroc and cobicistat) for TDM purpose.This method has been validated according to FDA and EMA guidelines. Only 50μL of sample are mixed with 100μL of internal standard, which included [$^{13}C$, $^{2}H_2$, $^{15}N$]-Bictegravir, 6,7-dimethyl-2,3-di(2-pyridyl)quinoxaline, and other stable isotope-labeled compounds. Then, 600μL of acetonitrile and methanol (50:50 v:v) are added for protein precipitation. After centrifuge, 300μL of supernatant are diluted in 600μL of water. Chromatographic separation has been performed on an Acquity® UPLC HSS T3 1.8μm 2.1×150mm column (Waters), with a gradient of water and acetonitrile, both added with 0.05% formic acid; 5μL of sample are then injected with a flow rate of 0.4 ml/min for 15 min at 50°C in the LX-50 UHPLC system, coupled with a Q-Sight 220 detector (Perkin-Elmer). Regression coefficients of calibration curves are all >0.998, with a linear calibration model with a weighting factor 1/X. Preliminary tests have showed that accuracy, intra- and inter-day precision fitted FDA and EMA guidelines for all analytes, whereas recovery and matrix effects resulted stable and reproducible among different plasma lots. When the validation will be complete, this method will be applied to plasma samples from patients affected by HIV.

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## UN NUOVO METODO UHPLC-MS/MS PER IL THERAPEUTIC DRUG MONITORING (TDM) IN ROUTINE DI QUINDICI ANTIBIOTICI IN PLASMA E SUA APPLICAZIONE A PAZIENTI PEDIATRICI CRITICI

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L'individualizzazione delle terapie antimicrobiche è necessaria nelle aree critiche per la gestione ottimale dei pazienti e per il contenimento delle resistenze antimicrobiche. L'ottimizzazione dei dosaggi dovrebbe essere basata sulla relazione tra l'esposizione al farmaco e la suscettibilità antimicrobica al sito di infezione (MIC). Il raggiungimento dei target PK/PD ottimali nei pazienti critici è reso difficoltoso da diversi fattori patofisiologici e/o iatrogeni che possono condizionare la PK degli antimicrobici.La disponibilità di metodi analitici affidabili per il TDM degli antibiotici nei laboratori clinici è ancora limitata. L'uso di tecniche cromatografiche per la misurazione dei farmaci è preferibile agli immunoassay perché esse consentono una quantificazione specifica in un'ampia gamma di concentrazioni, la possibilità di rilevare simultaneamente diversi farmaci nella stessa corsa (multiplexing) e un buon rapporto costo-efficacia.
In questo lavoro mostriamo un nuovo metodo analitico basato sulla cromatografia liquida ad altissime prestazioni accoppiata alla spettrometria di massa tandem (UHPLC-MS / MS), per la quantificazione di 15 antibiotici appartenenti a diverse classi che sono utilizzati nelle nostre unità di terapia intensiva (ICU)pediatriche . Il metodo si basa su semplici protocolli di preparazione del campione basati sulla precipitazione proteica con l'aggiunta di standard interni deuterati. Lo scopo del lavoro è quello di combinare nella stessa piattaforma un ampio spettro di farmaci con proprietà chimiche diverse: da amicoglicosidi idrofili (amikacina, gentamicina e tobramicina) a glico / lipopeptidi (vancomicina, teicoplain, daptomicina e colistina), penicilline (piperacillina) , inibitore della beta-lattamasi (tazobactam), carbapenemici (meropenem), fluorochinoloni (ciprofloxacina), tetracicline (tigeciclina), cefalosporine (ceftazidime) e ossazolidononi (linezolid) per avere un'unica procedura indipendentemente da quale sia l'antibiotico determinato.
Il metodo è stato validato utilizzando le linee guida per la validazione dei metodi analitici. In particolare, poiché la stabilità è critica per alcuni antibiotici, in particolare per i beta-lattamici, è stata ampiamente studiata per tutti i 15 farmaci in diverse condizioni operative al fine di istituire protocolli operativi affidabili da applicare nella pratica clinica.
L'applicabilità del nostro metodo UHPLC-MS / MS alla routine è stata verificata su diversi campioni clinici di pazienti pediatrici in condizioni critiche durante il trattamento con uno o più antibiotici inclusi nel nostro protocollo di antimicrobial stewardship.

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## TDM DEGLI ANTI-TNF (INFLIXIMAB E ADALIMUMAB) IN PAZIENTI PEDIATRICI CON MALATTIA CRONICA INTESTINALE: ESPERIENZA DELL'ISTITUTO GIANNINA GASLINI

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Nel presente lavoro descriviamo la nostra esperienza nell'uso del TDM degli anti-TNF nella gestione dei pazienti (pz) pediatrici con malattia infiammatoria cronica intestinale (MICI).I livelli sierici di infliximab (IFX) e adalimumab (ADA) e degli anticorpi anti-farmaco (Ab) sono stati misurati rispettivamente con il kit "Quantum blue" (Buhlman) e IDKmonitor total ADA ELISA (Immundiagnostik, Technogenetics).Nel periodo dicembre 2018-giugno 2019 sono state effettuate 50 determinazioni (12 per ADA e 38 per IFX) in 19 pz, di cui 10 in terapia con IFX e 9 con ADA. Tra i 10 pz in IFX: 4 affetti da M. di Crohn (età media 12 anni e 10 mesi), di questi 2 con primary failure in presenza di livelli di IFX adeguati e in assenza di Ab, con modifica terapeutica con altre classi di farmaci; 2 con remissione clinica di cui nel follow-up 1 ha richiesto dopo 1 anno intensificazione terapeutica per bassi livelli con sviluppo progressivo di Ab fino a passaggio ad ADA. I restanti 6 pz in IFX affetti da colite ulcerosa (CU) (età media 12 anni e 3 mesi): 1 con primary failure; 2 in persistente remissione clinica con progressivo sviluppo di Ab dopo 20 mesi e 15 giorni (min 15 mesi-max 26mesi) e consensuali bassi livelli di farmaco fino a sospensione di IFX; 3 in remissione clinica con necessità di intensificazione terapeutica dopo una media di 8 mesi e 19 giorni con beneficio in 2 pz e perdita di risposta e conseguente cambio di terapia in un pz (per adeguati livelli di IFX in assenza di Ab). Tra i 9 pz in ADA: 2 affetti da CU (età media 15 anni e 8 mesi), 1 primary failure e 1 in remissione clinica con comparsa di Ab dopo 2 anni e conseguente associazione di Azatioprina. I restanti 7 pz affetti da M. Crohn (età media 13 anni e 4 mesi) tutti in remissione clinica, 3 con formazione di Ab dopo una media di 78 mesi (min 12-max 60) e associazione di immunosoppressore, 4 con adeguati livelli senza Ab dopo una media di 15 mesi (min 6- max 24).Il TDM degli antiTNF ci ha permesso di migliorare la gestione della terapia guidandoci nella modulazione della dose per garantire livelli ottimali, nell'associazione di immunomodulatori all'inizio della formazione degli Ab e nella sospensione/modifica terapeutica.

P106
**LC-MS/MS METHOD FOR SIMULTANEOUS DETERMINATION OF LINEZOLID, MEROPENEM, PIPERACILLIN AND TEICOPLANIN IN HUMAN PLASMA SAMPLES.**

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Antibiotic therapy is a crucial aspect of the management of hospitalized patients, however, current standard dosing protocols have been shown to often attain inadequate plasmatic concentrations which may impair the clinical outcome and promote the selection of multidrug-resistant bacteria.
The aim of this study is to establish and validate a robust and fast liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the simultaneous analysis of four commonly used antibiotics (Meropenem, Piperacillin, Linezolid and Teicoplanin) in human plasma according to the European Medicines Agency (EMA) guidelines.
Samples preparation was performed using a commercially available extraction kit which needs a very small amount of sample (50 μl). Antibiotics were detected, following a 7 min gradient separation, in multiple reactions monitoring (MRM) mode using a Qtrap 5500 triple quadrupole instrument equipped with an electrospray source operating in positive ion mode. The method, covering the antibiotics' clinically relevant concentration ranges, is also able to quantify, individually, the major teicoplanin components. The high reproducibility and the need of a small amount of sample, associated with the use of a commercial kit, together with a short chromatographic time, makes the method particularly suited for high-throughput routine analysis.
Monitoring of plasma antibiotic levels, as part of the clinical routine, would result in a quick therapy adjustment leading to a higher probability of eradicating the infection as well as a potential reduction of multidrug-resistance prevalence. The method was successfully applied to monitor the antibiotic concentration of 90 patients under therapy.

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**ON-SITE DETECTION OF CANNABINOIDS IN HEMP FOOD PRODUCTS BY MicroNIR/CHEMOMETRICS**

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In the field of forensic toxicology, the use of non destructive and easy to use techniques deserves remarkable attention, especially in such situation involving the public health and security [1]. In addition, with respect to forensic applications, the miniaturization and portability of one-touch devices for the detection of specific threats is required more and more [2].In this work, the capabilities of a novel miniaturized and portable MicroNIR spectrometer were investigated in order to propose a practical and intelligible test allowing the rapid and easy screening of cannabinoids in hemp food products. Multivariate statistical analysis was used to process spectra and chemometric rules were involved to correlate results and to develop models of prediction for cannabidiol (CBD), $\Delta^9$-Tetrahydrocannabinol (THC) and cannabigerol (CBG).In order to develop predictive models able to identify and simultaneously to quantify the residual amount of cannabinoids, specimens from hemp flours and oils commercially available on the markets were considered and spiked with increasing amount of Cannabidiol (CBD), $\Delta^9$-Tetrahydrocannabinol (THC) and Cannabigerol (CBG). Results demonstrated that MicroNIR/Chemometric platform is statistically able to identify the presence of CBD, THC and CBG in simulated samples containing cannabinoids, with the accuracy and sensitivity of the reference official methods actually proposed. In addition, all the samples were simultaneously analyzed by GC-MS and a good correlation of the reference versus predicted samples was observed for both flours and oils speciments (correlation coefficient of 0.9989 and 0.9972, respectively). The method was checked against false positive and true positive response, permitting to propose the MicroNIR/ Chemometric platform as a fast and accurate method for the monitoring of cannabinoids in hemp based products. [1] Risoluti, R., Gregori, A., Schiavone, S., Materazzi, S. "Click and Screen" Technology for the Detection of Explosives on Human Hands by a Portable MicroNIR-Chemometrics Platform 90 (7) (2018) 4288-4292.[2] Risoluti, R., Pichini, S., Pacifici, R., Materazzi, S. Miniaturized analytical platform for cocaine detection in oral fluids by MicroNIR/Chemometrics. Talanta 202 (2019) 546-553.

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## VALIDATION AND APPLICATION OF A UHPLC-MS/MS METHOD FOR THE SIMULTANEOUS QUANTIFICATION OF TWELVE CURRENTLY USED ANTIRETROVIRAL DRUGS WITHIN PERIPHERAL BLOOD MONONUCLEAR CELLS FROM PATIENTS WITH HIV INFECTION

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Recently, Highly Active Antiretroviral Therapy (HAART) improved the management of HIV infection, avoiding the onset of AIDS. Nevertheless, the pharmacokinetic properties in terms of intracellular penetration of the last antiretroviral drugs (ARV) are poorly explored, partly due to methodological challenges. In this work, a validated UHPLC-MS/MS method is described for the simultaneous quantification of twelve ARVs within the Peripheral Blood Mononuclear Cells (PBMCs): Nevirapine, Efavirenz, Ritonavir, Cobicistat, Maraviroc, Rilpivirin, Dolutegravir, Raltegravir, Atazanavir, Darunavir, Elvitegravir and Etravirine. PBMCs were isolated through Cell Preparation Tubes (CPT®), while cell counts and mean cell volumes were determined through an automated counter. PBMCs pellets were lysed with $H_2O$:$CH_3OH$ (30:70), divided in two aliquots of 500µL and stored at -80°C. Sample preparation consisted in the addition of an internal standard (IS - 40µL for each sample) working solution, containing stable-isotope-linked drugs, sonication (10 min at room temperature), centrifugation (21000xg for 10 min at 4°C) and drying (vacuum centrifuge at 50°C 1.5 h). Dry extracts were dissolved with 100µL of $H_2O$:$CH_3CN$ (70:30) and 10µL were injected in the LX-50 UHPLC (Perkin Elmer) chromatographic system.The chromatographic separation was performed on an Acquity® UPLC HSS T3 column, 2.1×150mm, 1.8µm (Waters) maintained at 50°C. Flow rate was maintained at 0.4 mL/min, with a gradient of two mobile phases (MP): MP-A (HPLC grade $H_2O$ + 0.05% formic acid) and MP-B (HPLC grade $CH_3CN$ + 0.05% formic acid). Briefly, chromatographic gradient consisted in an increase of MP-B percentage from 30% to 95% in 11 min, then the column was washed with 95% MP-B 1.5 min and reconditioned for 1.8 min (total runtime 15 min). The method was then applied to 50 samples from patients undergoing HIV treatment, allowing the description of the intracellular disposition of antiretroviral drugs.The method underwent full validation following FDA and EMA guidelines, showing accuracy, precision, recovery and IS-normalized matrix effect data that fitted the requirements; furthermore, the validation was performed considering a wide range of cell numbers, in order to mimic the wide variability expected in HIV-positive patients.

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## THE AUTOMATION AND THE TRACEABILITY OF THE ANALYTICAL PROCESS IN GENETICS: THE NOVARA EXPERIENCE

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Background: Genetic investigations for suspected inherited thrombophilia and hemochromatosis represent a high percentage of routine tests carried out in a genetic laboratory.These analyses can be performed by Real Time PCR and involves different steps: DNA extraction from whole blood samples, PCR setting-up, amplification and analysis of results.The manual procedures do not guarantee the traceability of the sample during the entire analytical process and require the employment of dedicated and highly specialized full-time technical personnel. To date, several analyzers are available to automate the extraction phase, while few are able to guarantee the automation of the entire workflow, including the genetic test report on LIS (Laboratory Information System). We here report our experience of the first 6 months of automation in our laboratory.
Methods: From January 2019, in the genetic laboratory, OMNIA LH 75 (Masmec Biomed) has been utilized in combination with Real Time PCR kits for the analysis of Factor V Leiden R506Q_Factor II G20210A and C282Y_H63D_S65C (HFE gene) mutations (AA831, AA832, AA1493; Nuclear Laser Medicine srl) and the "Real Gene" interpretative software (DO022, NLM).
Results: In the first 6 months of 2019, 945 genetic tests were carried out: 135 Factor V, 570 Factor II and 80*3 Hemochromatosis (C282Y, H63D and S65C). It was not necessary to repeat any assay.The time for the preparation of each analytical session on OMNIA LH 75 (24 samples/run) has been estimated equal to 30 minutes while the time required for the semiautomatic extraction phase plus the manually PCR setting-up or for entire workflow manually, had been previously estimated equal to 1 and 2 hours, respectively.
Conclusion: The employ of OMNIA LH 75 (Masmec Biomed) together with the use of hereditary thrombophilia and hemochromatosis kits (Nuclear Laser Medicine srl) has automated the entire workflow, reducing the operator time. The use of OMNIA LH 75 does not require particular technical preparation (user friendly, guided interface) and allows performing different tests in a single analytical session. Finally, the presence of the barcode reader and the connectivity with the management LIS, guarantee the traceability of the samples for the entire diagnostic path and the minimization of human error.

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**CALCOLO URINARIO IN EXCEL**

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Il nostro Laboratorio da tempo ha abbandonato la determinazione delle componenti di un calcolo urinario effettuata con metodo semiquantitativo ed attualmente esegue sette dosaggi fotometrici (calcio, fosforo, magnesio, acido urico, ammonio, cistina e ossalato), effettua la visualizzazione dei carbonati e fa uso di un opportuno software in Excel estrapolante le più comuni componenti di un calcolo urinario.L'acquisto dello spettrofotometro all'infrarosso (FTIR) della ditta Shimadzu, tecnica di riferimento per la determinazione di un calcolo, ha fatto comprendere meglio i limiti di alcune tecniche fotometriche usate, il quantitativo di calcolo da pesare e dissolvere in acido solforico, la non facile valutazione della presenza dei carbonati e le possibili componenti chimiche presenti, specie se trattasi di un calcolo misto.La preparazione del campione è la seguente: 10 mg di calcolo frantumato sono trattati con 125 μL di acido solforico concentrato; dopo completa dissoluzione al vortex, si aggiungono 10 mL di acqua distillata. Dopo miscelazione e successiva centrifugazione, il sopra natante ottenuto viene testato sulle strumentazioni biochimiche Cobas 6000 Roche (calcio, fosforo, magnesio, acido urico ed ammonio) e Viva E Siemens (cistina ed ossalato). A parte si identifica la presenza dei carbonati.Il programma in Excel evidenzia principalmente la presenza di struvite, calcio ossalato, cistina, acido urico e urato di ammonio, carbonato apatite e calcio fosfato, caratterizzando particolarmente brushite ed apatite. Le concentrazioni rilevate dei 7 analiti sono tutte trasformate in mmol/dL: quote equi-molecolari di magnesio, fosfato ed ammonio evidenziano presenza di struvite, così come identiche quantità di calcio ed ossalato indicano costituzione di calcio ossalato. Gli eventuali eccessi di calcio e presenza di fosfato possono identificare calcio fosfato (CaP, brushite ed apatite a seconda del rapporto molecolare Ca/P). Le componenti ottenute, a seconda del loro peso molecolare, si ritrasformano in mg/dL al fine di ottenere la relativa percentualizzazione. Cangiano G, Di Maina E , Buccino G et Al. Determinazione delle componenti di un calcolo urinario: nuovi accorgimenti metodologici ed informatici. Biochimica clinica, 2016, vol. 40, SS: S151

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**INTEGRATION AND CONSOLIDATION: THE NEW "CORELAB" AREA OF ASST "POPE JOHN XXIII" OF BERGAMO**

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Purpose: The purpose of this work is the impact evaluation of the new technical-organizational of the "Corelab" Area of the ASST Analysis Laboratory Pope John XXIII of Bergamo.
Materials and methods: The area named "Corelab" is one of the three Diagnostic Areas of the Complex Operating Unit "Laboratory Medicine Service - Clinical Chemical Analysis". The organization of the total Area starting From 2018 , on the occasion of the renewal of the in service tender notice. The work was focused not only on the instrument automation but above all on the organization of the whole process that was supposed to integrate the Pre-Analytical, Analytical and Post-Analytical Phases.
Results: The new "Corelab", active since June 2019, is a highly automated facility for check-in, pre-treatment, sorting and distribution of all Laboratory Analysis samples. They can be performed Biochemistry, Immunochemistry, Pharmacology, Toxicology, Hematology and Coagulation exams on whole blood, plasma, serum, urine and biological fluids both in routine and in urgency for inpatients and external patients. The Pre-analytical and Post-analytical phases are managed by the integrated "Aptio Automation" (Siemens) system; The Analytical Phase, regarding the Biochemistry and Immunometry tests, is managed by Atellica (Siemens) systems; The Coagulation phase is managed by the CS-5100 ( Siemens) and for blood tests the XN system (Sysmex) is used.
Conclusions: The project, created for the automated management of the whole diagnostic process, proved to be an analytical tool for product and process control. The integration of the Preanalytical Phase allowed to handle a total of 5,000 test tubes a day and over 5 million tests per year working on any and recurrent laboratory errors. The Analytical Phase has involved an important rationalization and optimization of the staff, while the integration of the Post-analytical Phase has allowed a modern management of the analyzed and / or of "to be analyzed" samples. Finally, the whole process is a "closed sample" with a significant increase in operator safety and the elimination of biological accidents.

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## THE DRONES IN THE NEW VISION OF HEALTH CARE AND IN THE EVOLUTION OF LABORATORY MEDICINE. FIRST EXPERIENCE IN ITALY

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Medicine at this particular current moment is experiencing a major transformation due to the exponential development of digital and emerging new technologies. Ultramodern technological devices are present in most Italian and worldwide hospitals, now even drones can contribute to the further evolution of healthcare, both hospital and territorial. The drones today, thanks to the evolution of information technology applied to innovative technologies, can be used in the connection between hospitals and other health facilities for the transport, even in urgency, of defibrillators, drugs, blood bags and / or blood products and other biological material (test tubes of blood, urine, tissue samples, etc.) even over long distances (max. 100 km). The drone, equipped with a safety container that meets all national and international standards for the transport of biological substances, can be loaded manually or through a fully automated station that allows dedicated and specially trained operators to load the container by inserting it directly in the appropriate adapter of the drone itself without any manual contact with the APR, to then make it take off also through a specific application (App) on a smartphone. The Study Group on Management and Organizational Innovation in Laboratory Medicine proposed to SiBioC an experimental project on process innovation in Laboratory Medicine and Health Services entitled "infrastructure and mobility with Drones in a digital ecosystem" that has achieved the patronage of Company on 25 October 2018. The project was called "Philotea" and the SIBioC Delegate of the Campania Region was identified for the coordination of all related activities. The experience that took place for the first time in Italy on 7 March 2019, with the authorization of the National Civil Aviation Authority (ENAC) and of the other authorities in charge, established the connection of two hospital hubs of a Company Health of the metropolitan city of Naples (also known for the great difficulties of connections linked to constant vehicular traffic) by testing thirty parameters (hematology, coagulation and clinical chemistry tests) before and after the flight to verify the perfect correspondence to the indispensable precision and accuracy laboratory tests to guarantee first and foremost patients and subsequently also health professionals. The evaluations carried out allow us to positively evaluate this first experience, followed by others, even in different weather conditions.

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## DEVELOPMENT AND VALIDATION OF AN IT-BASED ALGORITHM FOR APPROPRIATE TUMOR MARKER RETESTING

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Background: The use of tumor markers (TM) is appropriate mainly for monitoring. The minimal retesting intervals (MRI) vary and depend on TM, tumor type and monitoring time (early/late follow-up). Aim. To improve appropriate TM retesting, our aim was to develop and validate an informatic algorithm (IT-TM) supporting TM retesting.
Methods: IT-TM was developed for CEA, CA 15-3 and PSA and applied to the University-Hospital of Padova (UH-Pd) IT system for the requiring clinicians. Different MRIs were identified (guidelines and sharing with clinicians): 1, 3 and 6 months for post-surgery, early and late follow-up respectively. When MRI is less than 1 year, the IT-TM requires the choice (drop-down menu) of the clinical reason for retesting including the possibility to overcome any limitation by selecting “suspect of new tumor”. TM requests from external hospitals (Ext-H) were not IT-TM guided. TM requests trend before (2 years) and after (2 years) IT-TM application (October 2016) was evaluated. For comparison AFP, CA 19-9 and CA 125 were included.Results. In UH-Pd, PSA requests declined ($\chi^2$:p<0.001, p for trend <0.001), while they increased in Ext-H ($\chi^2$:p<0.0001, p for trend<0.0001). CEA did not vary in UH-Pd nor in Ext-H (p:ns). CA 15-3 was reduced in UH-Pd only ($\chi^2$:p=0.2167, p for trend=0.0433). AFP, CA 19-9 and CA 125 did not vary over the 4-years study in UH-Pd, while in Ext-H AFP ($\chi^2$:p=0,016, p for trend=0.042) and CA 19-9 ($\chi^2$:p=0.0028, p for trend=0.0003) increased. 1425 retesting were registered in IT-TM: 63% CEA, 19% PSA and 18% CA 15-3. Retesting was higher in medical area (67%), surgery (12%) and gastroenterology (9%), and lower in oncology (3%) and urology (0.4%). For CEA, 57% retesting reason was “suspected new tumor”.
Conclusions: An IT-TM guided tool for appropriate TM retesting was developed and demonstrated to reduce the overall number of PSA and CA 15-3, not of CEA requests. The success of the IT-TM was mainly observed in specialized areas, its failure in general medical area. Our data indicates that TM retesting, CEA in particular, is often inappropriate.

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## FRAILTY IN IDENTIFICATION OF UNSUITABLE SAMPLES: THE CASE OF HAEMOLYSED SAMPLES

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Background: Quality of samples is prerogative of reliable laboratory information, however unsuitable samples are the most common source of total testing process errors. Haemolysis is the most frequent pre-analytical artefact interfering with accurate measurement of analytes inducing a positive or negative bias depending on the analyser/method. A prompt haemolysis identification and reporting is, therefore, fundamental to avoid the release of incorrect results preventing negative patient outcome. Aim of this work is to identify the weaknesses in identification of haemolysed samples (HS) and its impact on patient safety.
Results: Analysis of literature and measurement of the relevant quality indicator (QI) in the IFCC WG-LEPS benchmark program demonstrate that HS are the most common type of errors. However, the correct identification of HS strongly depends on the procedure used, automated or manual. In fact, QIs data (2017 and 2018) highlight that the percentage of HS analysed by automated haemolytic index (HI) is higher than those detected by visual inspection (VI) (HI=2.0 and 1.81 vs VI=0.30 and 0.29). Moreover, wide variability was observed in both groups of labs results. It can be explained by operator subjectivity in case of VI, and by different thresholds defined in the different diagnostic platform to detect HI.QI data concerning the samples rejected due to haemolysis (2017: 0.32% and 2018: 0.43%) are difficult to understand if compared with the percentage of HS, resulting lower than expected. Applying risk management principles, it emerges that the failure to detect or notify haemolysis can negatively impact on laboratory information (diagnostic errors, delay in appropriate diagnosis/treatment due to the necessity to repeat blood collection). In the notification process, it is very important to correctly identify analytes that can be affected by haemolysis and, for each of them, at what concentration release the result with comment or did not release it.
Conclusions: Nevertheless, national and international guidelines on the correct management of HS are available, the road is still long to achieve the harmonization of procedures among labs. A close collaboration is, furthermore, needed, to improve the harmonization of HI measurement.

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## THE IMPACT OF PREANALYTICAL AUTOMATION ON RISK ASSESSED WITH FMECA

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Since up to 70% of errors in laboratory testing occur in the preanalytical phase, to improve patient safety it is necessary to carefully manage the related risk, as required by accreditation: the assessment discloses criticalities and helps in decisions. We aim to provide methodological elements for an effective management, by objectively measuring the risk connected to the phases handled by man compared to those automated. Our clinical laboratory belongs to the University Hospital of Siena and offers diagnostics both to inpatients and to outpatients. Since the management of these two flows has different degree of automation, it allows to evaluate the risk associated with different kind of preanalytics. To this aim we applied the proactive methodology FMECA (Failure Mode Effects and Criticality Analysis). Our multidisciplinary team divided the 3 phases (pre-preanalytical, preanalytical outside and within the lab) in 11 subphases (formulation of clinical question, test selection, prescription; acceptance, preparation, identification, labelling, collection, conservation, transportation; preparation for analysis) and defined 18 main activities, recognising the ones already automated from those still human handled. For each we identified failure modes and effects on clinical outcome. According to literature data and our quality indicators, we assigned scores to severity, probability and detectability using ten-point scales. We calculated the risk indexes (RI) that varied from 32 to 243. The sum of RI obtained from human activities resulted much higher than the one produced by automation. The most critical steps (RI>150) were: manual acceptance of test orders, patient identification, tube labelling and sample collection. Our results suggest to introduce automated phlebotomy tray preparation systems, already available for outpatients, in wards. Although automation has a fundamental role, each organization is different about workloads and competencies, so the most suitable management must be tailor-made. Our methodology represents a useful tool to predict the risk related to scenarios with more or less automation and to choose the best balance according to the needs. The cyclic repetition of this analysis allows to measure the effectiveness of the action adopted.

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## APPLICATION OF THE BIG DATA METHOD FOR STUDYING A RARE PATHOLOGY: CRYOGLOBULINEMIA

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Introduction: Big data method refers to large amount of information created by automation and digitization. By using advanced technologies, it is easy to convert data into information, useful for improving diagnosis of patients affected by rare conditions such as cryoglobulinemia (CRG). CRG is caused by immunoglobulins (Ig) precipitating at low temperatures. There are 3 types of CRG: I, monoclonal Ig; II, monoclonal Ig + polyclonal Ig; III, 2 polyclonal Ig. The aim of the study was to analyse available data obtained in the Department of Laboratory Medicine, OCSAE (Modena, Italy) to describe a cohort of patients with CRG.
Materials and methods: Data from the Modena Labs network were extracted by means of the software "Pagoda", directly connected to the Laboratories Information System (LIS). The analysed time covered 19 years ranging from 2000 and 2018.
Results: The samples analysed were 28,847; 4,901 (17%) were positive for cryoglobulin (CR). The positive samples were obtained from 2,528 patients. The typing positive CR were 4,190 (85%); those of type I were 327 (8%), those of type II 2,031 (48%) and of type III 1,832 (44%). As gender, 62% of patients were women, with average age of 66 years (y) ±16, whereas 964 (38%) were men, average age62±16 y. Overall 50% of patients had a clinical suspect supporting the search for cryoglobulins; those with hepatitis C infection were 604 (48%), with chronic hepatitis, 177 (14%), with rheumatologic disorders, 107 (9%) with other autoimmune diseases, 83 (7%), with unknown nephropathies, 80 (7%). The positive HCV patients were 290 (48%). In respect of genotype, 47% were 1b, 22% were 2a/2c, 9% were 3a, 22% showed other genotypes.
Conclusions: Our study shows that CRG is a disorder affecting mostly elderly and women as it happens in several autoimmune and rheumatological diseases. In our cohort the predominant CRG type was the II. The clinical conditions most frequently encountered were HCV positivity and chronic hepatitis. The most common HCV genotypes were 1b and 2a/2c.
We wish to highlight the possibility of obtaining prompt diagnosis and treatment of CRG related conditions. Big Data method represents an important goal for public institutions for improving such recognition.

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## IL CONTRIBUTO DELLA DECISION-ORIENTED HEALTH TECHNOLOGY ASSESSMENT NELLA VALUTAZIONE DELLE SOLUZIONI TECNOLOGICHE DEL CORELAB DELL'OSPEDALE PEDIATRICO BAMBINO GESU'

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Il progetto di riorganizzazione del Laboratorio Analisi dell'Ospedale Pediatrico Bambino Gesù è iniziato a seguito della opportunità di riallocare le attività in altra sede, e di centralizzare la maggior parte delle indagini diagnostiche in un sistema ad elevata complessità, automazione e performance, definito "Corelab". In tale contesto, si è deciso di utilizzare il metodo Health Technology Assessment (HTA) per la valutazione delle tecnologie e delle soluzioni organizzative più idonee, che prevedessero la ridefinizione delle attività ed i flussi specifici, garantendo sempre standard elevati di sicurezza, efficacia ed efficienza, con l'obiettivo di un miglioramento continuo della qualità delle prestazioni erogate. Lo studio di HTA è stato condotto utilizzando la metodologia Decision-oriented HTA (DoHTA) ed ha coinvolto un Gruppo di Lavoro multidisciplinare (28 professionisti afferenti a diverse aree dell'ospedale). L'attività è stata condotta nel seguente modo:
• Analisi del contesto e delle esigenze clinico-tecnologiche
• Elaborazione e condivisione dello schema di valutazione secondo il metodo Do-HTA, prendendo in considerazione le dimensioni identificate per la valutazione di una tecnologia sanitaria: Sicurezza, Efficacia Clinica, Aspetti economici, Caratteristiche tecniche, Aspetti organizzativi
• Definizione del Sistema di pesi
• Stesura della Scheda di valutazione utilizzando 17 indicatori di I livello e 30 di II livello, specifici per ogni area di interesse, riconducibili agli indicatori del metodo DoHTA
• Valutazione delle soluzioni proposte dalle Ditte del diagnostico, in coerenza con quanto emerso dal metodo Do-HTA.
La valutazione comparativa tra le due aziende partecipanti ha evidenziato una sostanziale equivalenza qualitativa delle due soluzioni tecnologiche ed organizzative proposte, confermando l'alto livello tecnologico di entrambe le alternative considerate.
L'adozione del processo HTA ed in particolare della metodologia DoHTA, ha permesso di valutare in maniera approfondita e dettagliata le diverse soluzioni proposte, consentendo di supportare efficacemente e consapevolmente, attraverso i dati e le informazioni prodotte, le Direzioni/Funzioni preposte alla valutazione. Ha consentito inoltre la condivisione del percorso valutativo con tutti i diversi professionisti, in un'ottica realmente multidisciplinare, determinando una valutazione globale e condivisa del valore della tecnologia in esame, in modo strutturato e trasparente.

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**THE TELE-URINALYSIS NETWORK IN THE TUSCAN SOUTH-EAST AREA VASTA**

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Introduction: The development of automation in the field of urinary exam (UE) has allowed the integration of chemical-physical (chem-phy) and sediment data through a middleware (mdw).Nowadays,the latest mdw systems installed are on the network (nw),allowing data and images to be transmitted from one laboratory to another,when connected.The connection and transmission of data and diagnostic images is strategic in a territory such as the Tuscan South East Area Vasta(TSEAV),where some centers are organized in hub and spoke.In this work,the construction of a telemedicine nw applied to UE,consisting of all the laboratories (labs) of the Area Vasta,is proposed.
Materials and methods: The tele-urinalysis nw of the TSEAV includes the territorial labs of the Azienda Usl South East of Tuscany,such as the hub centers of Arezzo and Grosseto with the spoke centers of Cortona,Bibbiena,Sansepolcro,Valdarno,Massa Marittima,Orbetello and Pitigliano;the labs of Montepulciano,Campostaggia,Abbadia San Salvatore,Castel del Piano and the laboratory of the University Hospital of Siena.The equipment used by the labs is Aution Max 4030 and Pochet Chem for the chem-phy exam,Sedimax Contrust and the mdw software DirectorWeb (Menarini).The rules and the filters of inconsistencies,the characteristics of the report and the microscopic revision of the urinary sediment,must be necessarily shared among all the labs of the nw,according to their own catchment area.
Results: The tele-urinalysis nw consists of all the labs in the TSEAV,which are interconnected through the DirectorWeb mdw,which allows the sharing of chem-phy analysis data and urinary sediment images.Through the Director Web mdw software it is possible,in fact, for the hub labs not only to consult and validate the urine exam carried out by the spoke centers of the territory,but to receive and give advice in "second opinion" among the nw labs.
Conclusions: The use of the same analytical systems and the common computer system throughout the TSEAV allows all the labs that take part in the nw,to obtain harmonized and standardized results,for diagnosis and treatment.The nw-urynalysis,in fact,allows the collaboration of specialists with much faster diagnosis time and a less rigid and leaner organization of work,uniform throughout the territory.

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**RUOLO DELL'AUTOMAZIONE NELL'ORGANIZZAZIONE DELL'ATTIVITÀ DI LABORATORIO DEL CORELAB DEL P.O. BOLOGNINI**

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La variazione dell'assetto organizzativo aziendale in poliblocco dovuta al contenimento dei costi, il conseguente aumento del carico di lavoro e dei test, la richiesta di maggiore tracciabilità dei campioni e standardizzazione dei processi dettate dalle direttive regionali, hanno fatto sì che il nostro laboratorio, circa un anno fa, abbia intrapreso un percorso di adeguamento tecnologico dell'attività svolta che ha coinvolto tutto il personale del laboratorio e afferente ad esso.In particolare l'installazione della nuova strumentazione di chimica clinica e PEX ha realizzato, in linea ai principi manageriali Lean, obiettivi di snellimento e miglioramento dell'attività lavorativa nel Corelab in termini di standardizzazione, efficienza, efficacia e sicurezza, quali:
- a livello analitico, gestione automatica e semplificata di operazioni prima manuali, quindi operatore dipendenti (carico e scarico dei campioni sugli analizzatori disponibili con omogenea distribuzione, passaggio di uno stesso campione su più analizzatori in base ai test richiesti, stoccaggio tracciato e recupero dei campioni dall'area di stoccaggio);
- massimizzazione dei re-run e gestione automatica di test reflex da middleware;
- migliore gestione e impiego delle risorse umane disponibili;
- maggiore sicurezza biologica grazie alla minore manipolazione dei campioni, soprattutto stappati, e allo smaltimento automatico degli stessi dopo 5 giorni dalla processazione.

Tutto ciò ha comportato il superamento della vecchia organizzazione a favore di un maggiore adeguamento dei flussi di lavoro, in termini di volumi di lavoro e tempi di risposta, più omogeneo per fasce orarie e analizzatori disponibili, una conseguente riduzione del TAT di validazione e infine una pianificazione ottimale del processo di presa in carico dei campioni afferenti da sedi decentrate.
Il software gestionale Labitup ha permesso di monitorare le varie fasi del processo, i picchi di lavoro e valutare l'impatto dell'implementazione effettuata sia sull'attività svolta che sul personale impiegato.

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**PREECLAMPSIA: AN EXAMPLE OF CASE MANAGEMENT AND EVALUATION OF ECONOMICAL IMPLICATIONS**

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Objectives: The objective of this work was evaluate the economical implication of Preeclampsia (PE) management. This condition, potentially life-threatening for the fetus and mother, affect around 2-8% of pregnancies in Italy. The typical signs and symptoms are not always exhaustive for a clinical diagnosis. The ratio of sFlt-1/PlGF is a reliable short-term predictor of PE. The evaluation of economical implication on a single case could help the clinical decision maker to optimize PE cases management.
Methods: The economic indicators related to PE there were analyzed also including expenditure by Local Health Unit for the case management. The case included the diagnostic and therapeutic pathway started in Emergency Care Unit and concluded with the childbirth. Using the Lombardy regional tariffs, the SfIt-1/PlGF ratio was simulated as test for detecting PE in two different setting, the first one in the early stage of typical symptoms in the second one as screening.
Results: the analysis performed in this work have measured an economic savings of € 14,000 in the in SfIt-1/PlGF ratio early scenario and € 18,000 for the screening.
In conclusion the results described qualitatively as an evaluation of a case can give multiple information not only for future clinical management but also at economical level, in order to give an additional tool to the clinical decision maker in the management of patients with suspected diagnosis of PE syndrome.

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**PROCALCITONIN IN CHILDREN: A 3-YEAR EXPERIENCE IN A MEDIUM-SIZED LOCAL HOSPITAL**

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Diagnosing sepsis in children, especially in newborns, is difficult because both clinical and laboratory data can vary widely. Procalcitonin (PCT) is considered an early-onset and reliable marker for bacterial sepsis. Its role is well defined in adults, while less studies have investigated it in the paediatric population. The purpose of this work is to analyze PCT results in paediatric population admitted to S. Andrea Hospital in Vercelli since the test introduction in 2016. Our laboratory experience consists of 113 PCT tests in paediatric patients, including 13 newborns under one month-old and 31 under twelve months.We compared PCT data with other laboratory markers generally associated with sepsis such as CRP, WBC, neutrophil percentage and serum lactate. We also associated laboratory data to microbiological results, with particular attention to blood cultures. Among 113 PCT determinations, 25 resulted over 2 µg/L, which is commonly considered the cut-off for bacterial sepsis. Not all patients had a contemporary assessment of CBC, CRP or lactate. 67/111 had an elevated CRP (>0.80 mg/dl). While only 27/108 had an elevated leukocyte count, 7 had decreased WBC. 62/107 cases had elevated neutrophils. Lactate assessments were only 47 and 29 of them resulted over 1.6mmol/L. We observed poor correlation when comparing quantitative data, while there was significant ($p<0.05$) categorical agreement considering PCT elevation (>2µg/L) and high neutrophils, CRP >0.8mg/dl and lactate >1.6mmol/L.Interesting considerations can be achieved when comparing PCT and microbiological results. Blood cultures were assessed in 43 cases, belonging to 41 patients. Only for one 17 year-old patient two sets of aerobic and anaerobic bottles were collected, while for all other patients clinicians opted for a single paediatric bottle. Among 20 patients with blood cultures and PCT levels >2 µg/L, only 3 (15%) had a positive blood culture. This finding is significantly lower when compared with adult population, among which, in our experience, 50% of blood cultures are positive when PCT is elevated. Microbiological findings other than blood cultures, like urine or respiratory tract cultures, resulted independent from PCT levels.

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**STUDIO PRELIMINARE DEI LIVELLI DI VITAMINA D IN UNA POPOLAZIONE PEDIATRICA DELLA PROVINCIA DI ANCONA**

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Introduzione: La vitamina D svolge un ruolo importante nella regolazione dei processi di mineralizzazione ossea promuovendo l'assorbimento di calcio e fosforo a livello intestinale e favorisce il deposito di calcio nelle ossa per conferire loro la solidità e la resistenza che le caratterizzano. L'ipovitaminosi D caratterizzata da bassi livelli di 25(OH)D è una condizione estremamente diffusa nel mondo, sia nei paesi sviluppati che nei paesi in via di sviluppo, come dimostrato da numerosi studi epidemiologici che evidenziano un'elevata prevalenza di ipovitaminosi D (superiore al 50%) anche sul territorio italiano durante le varie fasce dell'età pediatrica.(1,2) Scopo del presente lavoro è stato quello di verificare i livelli di vitamina D su una popolazione pediatrica sottoposta a screening per celiachia in età scolare (6 -10 anni) nella provincia di Ancona.
Materiali e metodi: sono stati analizzati retrospettivamente 200 campioni di sangue, mantenuti congelati presso la Clinica Pediatrica dell'Azienda Ospedaliera, di pazienti pediatrici HLA- DQ2/DQ8 positivi. La determinazione della Vitamina D totale è stata eseguita con metodo automatizzato in chemiluminescenza (Liaison XL DiaSorin). Sensibilità 4 ng/ml. Cut-off utilizzati ≥30 ng/ml sufficienza, 20-29 ng/ml insufficienza, < 20 ng/ml deficit, < 10 ng/ml deficit grave.
Risultati e conclusioni: 77 pazienti (38.5%) avevano livelli sufficienti di Vitamina D (media 45,2 ng/ml), 69 (34.5%) avevano una lieve insufficienza (media 24,8 ng/ml), 43 (21.5%) avevano un deficit di vitamina D (media15,0 ng/ml) e 11 (5.5%) presentavano un deficit grave (media 6,8 ng/ml). Di questi ultimi 7 avevano valori di PTH al di sotto del cut-off (media 15,4 pg/ml). La prevalenza di ipovitaminosi D riscontrata nella nostra casistica (61.5%) è sovrapponibile ad altri studi italiani. Molte Società scientifiche suggeriscono la supplementazione con vitamina D in particolari situazioni a rischio pertanto il ruolo del laboratorio nel controllo dei livelli ematici per identificare eventuali carenze in ambito pediatrico diventa fondamentale.
Bibliografia: Lippi G, Montagnana M, Meschi T, et al.Vitamin D concentration and deficiency across different ages and genders. Aging Clin Exp Res 2012; 24(5): 548-51 Vierucci F, Del Pistoia M, Fanos M, et al. Prevalence of hypovitaminosis D and predictors of vitamin D status in Italian healthy adolescents. Ital J Pediatr 2014; 40: 54.

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**RAPPORTO sFLT1/PLGF NEL PRIMO TRIMESTRE DI GRAVIDANZA: PRIME VALUTAZIONI SUL VALORE PREDITTIVO DI PRE-ECLAMPSIA**

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Introduzione: La preeclampsia è una patologia che colpisce circa 1 gravidanza su 20. È una delle principali cause di nascita prematura e dei conseguenti problemi di salute o decesso dei neonati. Se non diagnosticata e adeguatamente gestita, la preeclampsia può provocare perfino il decesso.Il test immunologico sFlt-1/PlGF valuta il rapporto di due proteine sFlt-1 (soluble fms-like tyrosine kinase-1) e PlGF (placental growth factor) presenti nel sangue materno. Un rapporto sFlt-1/PlGF inferiore o pari a 38 può escludere lo sviluppo di preeclampsia nella settimana successiva al test, con un valore predittivo negativo del 99,3% (studio Prognosis). Gli studi condotti fino ad ora sono stati svolti per valutare la predittività a breve termine della preeclampsia, scopo del presente lavoro è stato quello di verificare la concentrazione delle due proteine nelle donne in gravidanza tra l'undicesima e la quattordicesima settimana di gestazione. Materiali e metodi: nel periodo 01/03 – 01/05/2019 sono stati analizzati 80 campioni di sangue di donne in gravidanza afferenti agli ambulatori ginecologici dell'Azienda Ospedali Riuniti di Ancona. Il metodo utilizzato per la determinazione quantitativa di sFlt-1 e PlGF è un immunodosaggio automatizzato (Cobas e601 Roche Diagnostics) in elettrochemiluminescenza. Sensibilità di 10 pg/ml per sFlt-1e 3 pg/ml per PlGF. Cut-off utilizzati rispettivamente di 2501 pg/ml e 122 pg/ml, rapporto sFIT-1/PlGF 55.
Risultati e conclusioni: Delle 80 donne in gravidanza studiate (età media 31,7 anni) 21 erano all' 11° settimana di gravidanza, 45 alla 12° e 14 alla 13°. I valori medi ottenuti sono 1535,5 pg/ml per sFIT-1, 52,8 pg/ml per PlGF e 32,6 per il rapporto. 2 donne avevano valori di poco superiori al cut-off solo per PlGF (132,7 pg/ml), entrambe non avevano fattori di rischio particolari. 4 avevano valori superiori al cut-off per sFIT-1 (media 3258, pg/ml). 6 donne avevano il rapporto considerato patologico (media 70,06). Di queste 3 avevano anche valori elevati di sFIT-1, 4 avevano fattori di rischio per preeclampsia. Il dosaggio delle due proteine nel primo trimestre potrebbe valutare il rischio di insorgenza di questa grave patologia durante la gravidanza, consentendo di individuare precocemente le donne a rischio e programmandone un attento monitoraggio.
Bibliografia: Zeisler et al. Predictive value of the sFlt-1:PlGF ratio in women with suspected preeclampsia. NEJM 2016; 374: 13-22

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## EVALUATION OF SERUM URIC ACID AS A PREDICTIVE TEST FOR MATERNAL COMPLICATIONS IN PRE-ECLAMPSIA. META-ANALYSIS AND GRADE APPROACH RATING DIAGNOSTIC ACCURACY

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Background: Hyperuricemia is often associated with pre-eclampsia. We evaluated the accuracy and clinical value of serum uric acid (SUA) in predicting maternal complications.
Methods: We performed systematic electronic searches in electronic databases as well as a list of reference literature for papers published until June 2019. Studies were included if they evaluated the diagnostic accuracy of high SUA levels. The expected utility of SUA is related to the probability of occurrence of severe maternal complications (i.e. HELLP syndrome or eclampsia). The assessment of risk of bias was done using the QUADAS-2 tool. For each included study, we collected data about study characteristics and diagnostic test accuracy to construct 2 x 2 tables for maternal complications. Pooled sensitivity (Se), specificity (Sp), likelihood ratios for positive (LR+) and negative (LR–) test results, diagnostic odds ratio (DOR) and Receiver Operating Characteristic (sROC) curve were estimated using a bivariate model. Grading the quality of the evidence was carry out using the GRADE method relating to directness of the evidence, consistency and precision of the results. Evidence for test accuracy were used to assess the value of SUA in the management of women with gestational hypertension.
Results: Ten studies, testing 3002 women with gestational hypertension, met the inclusion criteria. The majority of studies were judged at low risk of bias. The pooled Se of SUA was 0.67 (95% CI 0.65-0.70), Sp was 0.53 (95% CI 0.50-0.55), LR+ 1.63 (95%CI 1.25-2.12), LR- 0.58 (95%CI 0.41-0.81). The AUC was 0.70 ±SE 0.07, the DOR was 3.17 (95%CI 1.62 – 6.19). Out of 1000 women with gestational hypertension, 14% had a complications. There are no limitations in terms of risk of bias, imprecision and indirectness of results. The GRADE rating was downgrade for inconsistency due to the presence of substantial heterogeneity among studies. The overall quality of evidences was high.
Conclusions: Applying the GRADE approach, the laboratory professionals have a valid tool to support clinical decision based on the certainty of evidence for test accuracy. Based on the GRADE evaluation, SUA seems to be a useful test in the management of women with gestational hypertension, but its accuracy remains still uncertain.

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## THE CLINICAL UTILITY OF D-DIMER/PLATELET COUNT RATIO IN PREGNANT WOMEN AFFECTED BY PREECLAMPSIA

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Objectives: Several markers have been considered to predict preeclampsia. We performed a retrospective study to assess the clinical utility of a new index, D-dimer/platelet count (DD/PLT) ratio, in discriminating preeclampsia from normal pregnancy and gestational hypertension during third trimester and time of delivery, compared to the biomarkers currently used, such as D-dimer (DD), platelet (PLT), lymphocyte (LIN) and neutrophil (NEU) counts, fibrinogen (FIB), PLT/NEU, NEU/LIN and PLT/LIN ratios.
Design & Methods: We retrospectively recruited 213 subjects. Of them, 163 and 50 were singleton pregnant and healthy non-pregnant women, respectively. Among pregnant women, 105 had normal pregnancy, 33 had gestational hypertension, and 25 had preeclampsia.
Results: Using Receiver Operating Curve (ROC) analysis, DD/PLT ratio showed significant higher area under the curve (AUC) (0.90; 95% confidence interval (CI) 0.84 to 0.95) in discriminating preeclampsia from normal pregnancy compared to those of DD, NEU, FIB, LIN, PLT/NEU, NEU/LIN and PLT/LIN ratios ($p < 0.03$), and close to significance to that of PLT ($p = 0.09$). In discriminating preeclampsia from gestational hypertension, the DD/PLT AUC (0.90; 95% CI 0.79 to 0.96) was significantly higher than those of DD, NEU, FIB, LIN, NEU/LIN and PLT/LIN ratios ($p < 0.03$), and not statistically different from those of PLT ($p = 0.22$) and PLT/NEU ratio ($p = 0.46$).
Conclusions: This study shows that DD/PLT ratio could be a promising index to evaluate the risk of preeclampsia. Large-scale studies are needed to verify its clinical usefulness, and to suggest more appropriate cut-off values for a widespread use.

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## SENTINEL BIOMARKERS IN HCV POSITIVE PATIENTS WITH MIXED CRYOGLOBULINEMIA

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Background: Infections, autoimmunity and cancer have been implicated as determinants of etiology in Hepatitis C virus (HCV) related mixed cryoglobulinemia (MC). Due to the complex virus-host interactions, MC represents an excellent model to investigate the pathogenetic mechanisms of HCV infection. Several risk factors have been suggested as markers of pathogenesis and progression of HCV-related MC into B cell Non-Hodgkin's Lymphoma (B-NHL). Here we aimed to evaluate the possible use of IgG subclass distribution, free light chains (FLCs) and vascular endothelial growth factor (VEGF) as a new combination of biomarkers.
Methods: We assessed IgG subclasses, FLCs and VEGF levels in sera from 53 HCV-MC, in comparison with 40 sera from HCV negative patients with rheumatoid arthritis (RA) and 30 from healthy blood donors (HBD).
Results: We observed that IgG3 levels were significantly higher in HCV-MC patients with a decrement of IgG2 and IgG4; a significant increase of FLC levels was observed in both MC and RA patients' groups serological levels of VEGF were higher in HCV-MC patients than in HBD.
Conclusion: Different profile of IgG subclasses distribution that we found in HCV-MC patients could reflect a different immune response and FLCs may play a pathogenetic role with VEGF. Therefore, our results suggest that a specific IgG subset together with raised levels of FLCs and VEGF could represent the biomarker "signature" of a worsening progression for HCV-MC patients into overt B-NHL. The biomarkers presence could be fundamental in precision medicine to evaluate the progression of HCV-related MC into lymphoproliferative disorders.

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## THE NEW RESEARCH HEMATOLOGIC PARAMETER NEUT-RI IN THE SYSTEMIC BACTERIAL INFECTION

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Background: Many cell subset data, that reflect the detailed status of specific cells, have become available in automatic blood cell analyzers. In particular, Sysmex XN-2000 can analyze two granulocyte items, i.e. the structural neutrophil parameters NEUT-RI (a measure of the fluorescence intensity of the neutrophil population) and NEUT-GI (a measure of the cytoplasmic granularity of the neutrophil population).The aim of our study was to investigate the correlation between NEUT-RI or NEUT-GI and the procalcitonin (PCT) plasma levels in the laboratory management of systemic bacterial infection.
Methods: This is a retrospective study based on 175 patients hospitalized from January to June 2019 at University Hospital "Maggiore della Carità" (Novara, Italy), with complete blood count (CBC) and PCT request laboratory tests.
Results: PCT plasma levels were significantly correlated with white blood cell (WBC), neutrophil (NEUT) and platelet (PLT) counts ($r = 0.317$, $p < 0.001$; $r = 0.326$, $p < 0.001$; $r = -0.249$, $p = 0.003$, respectively: Spearman test). Furthermore, PCT plasma levels were significantly correlated with NEUT-RI ($r = 0.441$, $p < 0.001$), but not with NEUT-GI. Then, the 175 patients were divided into three groups, based on PCT plasma levels (<0.5 ng/mL: group 1; 0.5-2 ng/mL: group 2; > 2 ng/mL: group 3); the WBC, NEUT and PLT values were significantly different in the groups ($p < 0.001$; $p = 0.002$; $p = 0.002$, respectively; Kruskal Wallis test). Comparison of NEUT_RI values in the groups showed that group 3 displayed significantly higher values than group 1 and 2 ($p < 0.001$ and $p = 0.003$, respectively; Mann Whitney test, Bonferroni's correction).
Conclusion: The new structural neutrophil parameter NEUT-RI, that represents the neutrophil metabolic activity, is correlated with PCT and significantly increased in patients PCT > 2 ng/mL. Therefore, NEUT-RI may be used in monitoring systemic infections.

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## ANALISI RETROSPETTIVA SULL'APPROPRIATEZZA DELLA RICHIESTA DI PROCALCITONINA: LA NOSTRA ESPERIENZA

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Introduzione: La sepsi rappresenta un'importante causa di mortalità ospedaliera, per cui è presente un crescente interesse verso la diagnosi precoce. La Procalcitonina (PCT) costituisce, ad oggi, un sensibile marcatore diagnostico per individuare i pazienti con sepsi, con un tempo di retesting minimo di 24 ore.
Metodi: In questo studio abbiamo selezionato i pazienti appartenenti ai reparti che, nell'anno 2018, hanno richiesto il maggior numero di PCT. Sono stati selezionati e analizzati campioni provenienti da 3 reparti clinici, 1 reparto chirurgico e 3 reparti di terapia intensiva, con metodica immunometrica (ELFA; VIDAS BRAHMS PCT, BioMérieux) su siero. I controlli di qualità interni misurati per il livello 1 sono risultati: 17,26 ng/ml +/-1,62; CV 9,43% (valori attesi: 18 ng/ml +/-1,5; CV<10%); per il livello 2 sono risultati: 1,85 ng/ml +/-0,15; CV 8,3% (valori attesi: 1,8 ng/ml +/-0,14; CV<10%). RISULTATI : I reparti studiati hanno richiesto un totale di 11.532 PCT/ anno con una richiesta media di 3,4 PCT/paziente. In particolare, i reparti intensivi hanno richiesto il 75% delle PCT totali con una richiesta media di 3,6 PCT/paziente. Inoltre, il conteggio delle richieste di PCT ripetute per lo stesso paziente nell'arco delle 24 ore, ha rilevato che i reparti intensivi hanno richiesto complessivamente l'89% di tutte le ripetizioni intra-day effettuate. Dunque, è facilmente deducibile ed osservabile che, i costi, corrispettivi a queste ripetizioni, siano maggiormente attribuibili ai reparti di tipo intensivo, con l'89% della spesa relativa alle ripetizioni inappropriate.
Conclusioni: L'inappropriatezza della richiesta analitica ed i relativi costi maggiormente attribuibili ai reparti intensivi, ci portano a ritenere che una migliore collaborazione tra clinici e laboratoristi possa migliorare la corretta appropriatezza prescrittiva, l'interpretazione e l'utilizzazione dell'informazione di laboratorio.

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## BIOMARKERS OF MINIMAL RESIDUAL DISEASE IN RITUXIMAB TREATED PATIENTS WITH MIXED CRYOGLOBULINEMIA

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Background: Hepatitis C virus (HCV) is the major risk factors for mixed cryoglobulinemia (MC), a small-vessel vasculitis considered as a B-cell benign lymphoproliferative disorder which may further evolve into an overt B-cell non-Hodgkin's lymphoma. B-cell clones persist in MC patients long after HCV infection has been eradicated. AIM: In this study we aimed to determine in patients with HCV-related MC if the measurements of serological free light chains (FLCs), IgM k and λ pair chains and VEGF may provide valuable information on the evolution of disease in response to rituximab (RTX) and may support for the early diagnosis of a minimal residual disease (MRD), that is widely desired to improve patients management.
Methods: We assessed serological levels of FLCs, heavy-light chain (HLC) pairs IgM k and λ and vascular endothelial growth factor (VEGF) in 34 HCV-MC patients, treated with low-dose RTX. Clinical and laboratory responses, defined on the basis of vasculitis activity and decrease of cryocrit, were evaluated in correlation to candidate biomarkers.
Results: We observed a clinical response (complete + partial) in 29 out of 34 patients, and a laboratory response (complete + partial) in 17 out of 17 patients; in contrast the mean levels of FLCs, HLCs and VEGF were substantially unaffected by RTX and remained above the normal range.
Conclusions: Our candidate biomarkers could represent the signature of "dormant" B cell clones activity and their employment in the laboratory routine of MC patients could be very useful to identify minimal residual disease or relapse or worsening outcome, as already demonstrated for FLCs and HLC in multiple myeloma and for VEGF in systemic autoimmune diseases.

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**DISTRIBUZIONE DEL GENOTIPO HCV NELL'USL TOSCANA SUD-EST: ANALISI EPIDEMIOLOGICA ALL'INIZIO DELLA STRATEGIA DI ERADICAZIONE**

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Introduzione: L'infezione attiva da HCV è un problema di salute pubblica perché si associa ad un aumento della morbilità e mortalità non solo epato-correlate. La regione Toscana, ha varato il piano di eradicazione dell'infezione da HCV da attuarsi nel triennio 2018-2020. Poiché i dati epidemiologici sono la base per lo sviluppo delle terapie con DAAs, sono stati valutati l'incidenza dell'HCV e la distribuzione del genotipo nella popolazione sottoposta a screening nei laboratori di Grosseto e Arezzo dal 2013 al 2018.
Materiali e metodi: La determinazione degli anticorpi HCV è stata eseguita con ADVIA Centaur System (Siemens). Campioni anti-HCV positivi, sono stati sottoposti a quantificazione della carica virale con il test Cobas Ampliprep/Cobas Taqman HCV v.2.0 (Roche Diagnostic). I campioni con carica virale rilevabile sono stati poi genotipizzati (NLM, s.r.l).
Risultati e discussioni: Tra i 1485 pazienti reclutati, il 53,8% era di genotipo 1, il 16,4% di genotipo 2, il 21,8% di genotipo 3 e il 7,5% di genotipo 4. La prevalenza dei sottotipi 1a e 1b è stata rispettivamente del 15,1% e del 27,5%. Per il sottotipo 2a/2c è stata riscontrata una prevalenza del 14,5%, mentre per il sottotipo 3a del 21,3%, secondo genotipo più rappresentato in contrasto con i dati epidemiologici italiani. L'incidenza dei genotipi 1b e 2a/2c risultata aumentata con l'aumentare dell'età. I genotipi 1a e 3a sono stati ritrovati maggiormente nei pazienti fino a 50 anni. Il genotipo 4 è stato rilevato principalmente in pazienti di età compresa tra 40 e 60 anni. I genotipi 1a e 2a/2c raggiungono il picco di incidenza nell'anno 2018. Il genotipo 3a ha raggiunto il picco minimo di incidenza nel 2018. Si è osservata una diminuzione del genotipo 1b nel 2018.
Conclusioni: Nel nostro studio è stata evidenziata una variazione nell'epidemiologia dei genotipi HCV. Secondo la strategia di eradicazione della Toscana, i genotipi 1b e 3a sono diminuiti della metà nel 2018. Il genotipo 1b è stato diagnosticato negli individui oltre 60 anni nonostante sia presente principalmente nei soggetti giovani. Ciò può essere dovuto all'età della diagnosi e non dell'infezione. Come atteso, il genotipo 1a è stato ritrovato principalmente nei soggetti fino a 50 anni, ed è mantenuto soprattutto da comportamenti sessuali o dall'utilizzo di droghe e può essere sottostimato, il che ne rende difficile l'eradicazione.

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**EXPRESSION OF A SECRETED LDLR/Tf CHIMERIC PROTEIN IN THE MUSCLE OF LDLR-DEFICIENT MICE AMELIORATES THEIR LIPID PROFILE**

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Familial hypercholesterolemia (FH) is an inherited disorder of lipid metabolism mainly due to mutations in the LDL receptor (LDLR) gene and is characterized by premature onset of cardiovascular disease due to high plasma LDL cholesterol concentrations. FH patients homozygous for LDLR mutations do not always respond to conventional therapies and have often a poor prognosis. Therefore, more effective therapeutic strategies, such as gene therapy, are still of main interest. We have recently developed a safe and effective gene therapy strategy based on liver expression of a secreted chimeric protein composed of the extracellular portion of the LDLR linked to a transferrin dimer using a helper-dependent adenoviral (HD-Ad) vector. This chimeric protein binds LDL and removes them from the bloodstream by receptor-mediated endocytosis through the transferrin receptor (TfR). As previously demonstrated, intravenous administration of this HD-Ad vector in LDLR-deficient mice resulted in an amelioration of the lipid profile and a reduction of aortic atherosclerosis. In order to increase safety of this strategy for a possible clinical application, we have recently generated a HD-Ad vector for a muscle-restricted expression of the mLDLR/mTF chimeric protein using a muscle-specific promoter and intramuscular administration of the vector. We observed expression of the chimeric protein after infection of C2C12 cell lines with our HD-Ad vector, expression of the chimeric protein after intramuscular administration in LDLR-deficient mice ameliorated the lipid profile compared to controls. In summary, we developed an innovative strategy for FH therapy based on the expression of a secreted chimeric protein after intramuscular administration of an HD-Ad vector. This approach reduces risks associated to systemic administration of viral vectors and, in principle, is applicable to other genetic diseases; collection of additional efficacy and safety data will further define its applicability in clinical settings.

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**PLATELET FUNCTION AND AUTONOMIC NERVOUS SYSTEM DYSREGULATION IN NEWLY DIAGNOSED HYPERTENSIVE CONDITION**

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Background: Platelets can contribute to the onset of hypertension not only through the induction of a pro-thrombotic condition but also by interfering with endothelial function. The autonomic system, which is widely known to be involved in the onset of hypertension too, can modulate platelets function. In spite of this, the relationship between platelet activity and sympathetic/parasympathetic drive in subjects at firstly diagnosed hypertension has not been clearly described, yet.
Methods: In a total of 99 normal subjects, arterial blood pressure was monitored by 24h holter monitoring. Lipidic and glicidic profile and body mass index (BMI) were quantified. In addition, platelet activity was measured by means of various stimulation tests. A 6-min electrocardiogram registration was taken for further analysis of heart rate variability (HRV), which was performed in the time domain (HRV, the heart rate beat-to beat variance) and in the frequency domain (Very Low Frequency, VLF, Low Frequency, LF, and High Frequency, HF, components). Sympathetic, parasympathetic and stress index were calculated, as well.
Results: Among the 99 subjects (M:31; F: 68, mean age 54), 59 were hypertensives. Hypertensives and non-hypertensives did not differ as regarding age, sex, smoke. BMI and HRV variables related to sympathetic activation were higher in hypertensives. In addition, the ristocetin-induced platelet aggregation and the thrombin receptor activating peptide-6 platelet aggregation tests shown higher responses in hypertensives versus non-hypertensives.
Conclusion: The dysregulation of autonomic nervous system and an increased trend of platelet aggregation could play a physio-pathological role in the onset of newly diagnosed hypertensive condition. Relation to endothelial dysfunction could be hypothesized.

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**ACCURATEZZA DIAGNOSTICA DEL PROTOCOLLO 0-3h DELLA hs-cTnT IN USO PRESSO L'AOU SENESE PER LA DIAGNOSI DI INFARTO MIOCARDICO ACUTO**

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Le troponine sono i biomarcatori di elezione per la diagnosi di infarto miocardico acuto (IMA), sensibili per il danno miocardico ma non IMA-specifiche. La valutazione della variazione della troponina (delta change) in determinazioni consecutive è utile per evidenziare il danno acuto. Da alcuni anni nell'Azienda Ospedaliera Universitaria Senese (AOUS) è stata adottata la troponina T ad alta sensibilità (hs-cTnT) con protocollo 0-3h e sono stati concordati con i clinici i seguenti limiti decisionali: per il rule-in di IMA è richiesto un aumento o diminuzione della hs-cTnT pari o superiore al 30% (delta) con almeno uno dei valori superiore a 14 ng/L (cutoff in uso per il danno miocardico, corrispondente al 99° percentile fornito dalla ditta). Si utilizza un cutoff di 50 ng/L per l'immediato rule-in di IMA al tempo zero. Poiché tali cutoff e il delta non sono derivati da uno studio sulla popolazione che afferisce all'AOUS, si è deciso di valutarne retrospettivamente l'accuratezza diagnostica nei pazienti afferiti al Pronto Soccorso. Sono state estratte dal sistema informatico tutte le richieste (5177) di determinazione seriale (curva) della hs-cTnT nel periodo di un anno, insieme alla diagnosi finale (ICD-9). Dalle tabella di contingenza (Gold Standard: diagnosi finali IMA vs NON-IMA; test: rule-in/out sulla base del protocollo 0-3h) sono stati ottenuti i seguenti valori predittivi: nel gruppo dei pazienti che hanno eseguito almeno due determinazioni (2741), considerando positivi per IMA i casi che avevano almeno un valore di troponina superiore a 14 ng/L e il delta≥30%, abbiamo riscontrato una sensibilità del 53%, una specificità del 90%, un PPV del 24% e un NPV pari al 97%. Tramite la valutazione delle curve ROC, solo per pazienti che avevano completato la curva (T0h-T3h), abbiamo identificato i cutoff desiderabili per la diagnosi di IMA: T0h=46,2 ng/L, T3h=49,52 ng/L e un delta change compreso tra 31,55% e 31,75%. I risultati ottenuti confermano che i cutoff in uso hanno la migliore accuratezza possibile in relazione alla metodica della troponina utilizzata e al protocollo in uso per la diagnosi di IMA.

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**LA FOSFOLIPASI A2 ASSOCIATA ALLE LIPOPROTEINE PREDICE L'ISCHEMIA CRITICA DEGLI ARTI INFERIORI NEI PAZIENTI EMODIALIZZATI**

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Introduzione: L'ischemia degli arti inferiori (LLI) è frequente tra i pazienti emodializzati (HDP). La Fosfolipasi $A_2$ associata alle Lipoproteine (Lp-$PLA_2$) è un enzima infiammatorio che destabilizza la placca aterosclerotica, predice gli eventi e la mortalità cardiovascolare. La Lp-$PLA_2$ è associata a LLI nella popolazione generale e nei pazienti cardiopatici, ma pochi dati sono disponibili nei pazienti nefropatici. Scopo dello studio è stato quello di valutare l'associazione tra i livelli di Lp-$PLA_2$ e lo sviluppo di LLI nei pazienti emodializzati.
Metodi: Sono stati arruolati 102 HDP nel giugno 2013, 64 (62%) maschi, età mediana 71anni; età dialitica 29 mesi, 35% diabete, 54% ipertensione, 40% coronaropatia e 31% arteriopatia periferica. Sono stati valutati Lp-$PLA_2$ (attività), profilo lipidico ed eventi vascolari periferici (ischemia critica, ulcera) nei successivi 5 anni.
Risultati: I valori mediani (IQR) di Lp-$PLA_2$, PCR, colesterolo totale, LDL, trigliceridi (TG) e apoB/apoA-I ratio erano rispettivamente 184 nmol/min/ml (156-214), 0,4 mg/L (0,1-0,9), 158 mg/dL (127-191), 79 mg/dL (63-102), 139 mg/dL (92-205) e 0,72 (0,58-0,89). I 43 HDP con Lp-$PLA_2$>194 nmol/min/ml (cut-off indicato dal Produttore) presentavano livelli significativamente più elevati di colesterolemia (171 vs 142 mg/dl, p=0,018), LDL (92 vs 67 mg/dl, p<0,0005), ApoB/A1 (0,83 vs 0,61, p<0,005) e incidenza di neo-ulcere (44% vs 17%, p=0,003). Alla regressione di Cox univariata, età (p=0,0027), Lp-$PLA_2$ (p=0,001), colesterolo-LDL (p=0,044), apoB/apoA-I ratio (p<0,0005), diabete (p<0,0005) e coronaropatia (p=0,012) erano significativamente associati a LLI. Tuttavia, solo Lp-$PLA_2$ (p=0,018) e diabete (p<0,0005) rimanevano indipendentemente associati a LLI all'analisi multivariata.
Conclusioni: Lo studio dimostra per la prima volta in HDP che la Lp-$PLA_2$ è un predittore indipendente di LLI.

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**DEVELOPMENT OF SENSITIVE AND RELIABLE UPLC-MS/MS METHOD FOR TRIMETHYLAMINE-N-OXIDE ANALYSIS IN PLASMA**

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Introduction: Trimethylamine-N-oxide (TMAO) is a small amine oxide whose plasma levels could be associated to future risk for major adverse cardiac events including myocardial infarction, stroke and death levels. Thus, the quantification of TMAO is clinically relevant.The aim of our study was to develop and validate an LC-MS/MS method to measure human TMAO plasma levels. To verify the utility in a pathological condition, plasma TMAO levels have been measured in patients with a first diagnosis of Acute Coronary Syndromes and in patients with Stable Angina.
Patients and methods: Patients with a first diagnosis of Acute Coronary Syndromes were divided in two groups: Non-ST elevation myocardial infarction (NSTEMI) and ST-elevation myocardial infarction (STEMI) confirmed at coronary angiography. The diagnosis of NSTEMI and STEMI was done according current guidelines.Patients with Stable Angina (SA), with symptoms of stable effort angina lasting more than 12 months, angiographically confirmed coronary artery disease, no previous acute coronary events, and no overt ischemic episodes during the previous 48 hours were also tested.UPLC separation was performed by hydrophilic interaction chromatography using acetonitrile/water containing 5 mmol/L ammonium acetate. TMAO was quantified using a triple quadrupole tandem mass spectrometry operating with an electrospray ionization (ESI) source in the positive mode. Selected reaction monitoring was performed following the transitions m/z 75.9>58.4 for TMAO and 85.9>66.0 for the internal standard TMAO-d9.
Results and conclusions: The method was linear up to 100 µg/mL, limit of detection and limit of quantification were 0.05 µg/L. Recovery was higher than 93%. Intra- and inter-assay imprecision were lower than 10%. The accuracy, expressed as bias %, was <6.5. TMAO levels were significantly different in STEMI group respect to both NSTEMI and SA groups (p value = 0.025 and 0.015 respectively) and there is not significantly differences between SA group and STEMI group (p value = 0.9)The validation parameters indicates the LC-MS/MS assay is rapid, sensitive and accurate. The results obtained show the utility in a pathological condition.

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## STUDIO MULTICENTRICO PER LA VALUTAZIONE DEI VALORI DI RIFERIMENTO NELLA POPOLAZIONE ITALIANA DEI METODI PER LA MISURA DELLA CTNI CON METODI AD ALTA SENSIBILITÀ

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Introduzione: È stato effettuato uno studio multicentrico con lo scopo di confrontare i valori di cTnI misurati con tre metodi ad alta sensibilità in soggetti volontari apparentemente sani e pazienti arrivati al pronto soccorso con sindrome coronarica acuta. Inoltre è stata determinata la distribuzione dei valori della cTnI e calcolato il 99° percentile nella popolazione italiana, divisa per sesso ed età.

Materiali e metodi: Sono stati raccolti campioni di plasma eparinato di 1511 soggetti sani da 8 istituzioni cliniche italiane (età media: 51,5 anni; SD: 14,1 anni; intervallo: 18-65; rapporto F/M: 0,95). tutti i volontari non avevano malattie acute o croniche e avevano valori normali per test di laboratorio routinari. Inoltre sono stati raccolti 1322 campioni di plasma eparinato di pazienti ammessi al pronto soccorso con sintomi clinici tipici di sindrome coronarica acuta. Nel laboratorio di riferimento sono stati misurati tutti i campioni raccolti con i tre metodi ad alta sensibilità: Architect hs-cTnI, Access hs-cTnI e ADVIA Centaur XPT. Le differenze tra metodi sono state analizzate anche con l'analisi delle componenti principali (PCA).

Risultati: È stata trovata una differenza media tra metodi del 31,2% CV. Il metodo ADVIA Centaur XPT misura valori di cTnI più elevati di circa due volte rispetto ai metodi Architect hs-cTnI e Access hs-cTnI. I risultati dello studio dimostrano che il valore del 99° percentile non dipende solo da sesso e età della popolazione di riferimento, ma anche dall'approccio statistico usato per il calcolo (metodo non parametrico robusto vs bootstrap). I valori del 99° percentile calcolati con il metodo non-parametrico robusto nella popolazione italiana sono risultati simili a quelli suggeriti dalle aziende produttrici.

Conclusioni: Considerate le differenze sistematiche tra i metodi hs-cTnI, che si riflettono anche su valori di riferimento, è necessario che i clinici e i pazienti siano informati sui limiti di riferimento dei metodi hs-cTnI e sulla necessità di eseguire la misura della cTnI sempre nello stesso laboratorio.

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## STUDIO MULTICENTRICO PER LA VALUTAZIONE DELLE PERFORMANCE ANALITICHE E DEGLI INTERVALLI DI RIFERIMENTO NELLA POPOLAZIONE ITALIANA DEL METODO hs-cTnI ACCESS CHE UTILIZZA LA PIATTAFORMA DxI

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Introduzione: Lo scopo di questo studio è stato quello di valutare le prestazioni analitiche in accordo con protocolli sperimentali standardizzati e i valori di riferimento nella popolazione italiana del metodo hs-cTnI Access, che utilizza la piattaforma DxI, recentemente distribuito in Italia.

Materiali e Metodi: Campioni di plasma eparinato sono stati raccolti da 1459 soggetti adulti sani in 8 centri clinici italiani allo scopo di determinare la distribuzione dei valori di cTnI nella popolazione italiana (età media 51,5±14,2 anni, intervallo 18-86; F/M=703/756). Ogni centro ha raccolto dai 50 ai 150 campioni di plasma. Tutti i volontari hanno negato la presenza di malattie acute o croniche e hanno valori normali negli esami di laboratorio (inclusi creatinina, elettroliti, glucosio ed emocromo). Sono stati anche raccolti 1322 campioni di plasma eparinato di pazienti ammessi al pronto soccorso di 9 differenti ospedali italiani con sospetto di sindrome coronarica acuta (età media 66.7±16.5 anni, intervallo 18 a 101 anni, F/M=570/752).

Risultati: I dati del profilo di imprecisione dimostrano che il valore del 99° percentile suggerito dalla azienda produttrice (17,5 ng/L) è misurato con una imprecisione di circa il 5%, cioè la metà del CV% richiesto dalle linee guida per i metodi ad alta sensibilità. I risultati del metodo hs-cTnI Access sono risultati essere in buon accordo con quelli misurati con il metodo hs-cTnI Architect in pazienti ammessi al pronto soccorso con sospetto di sindrome coronarica acuta (logAccess= 0,0859 + 0,9348 logArchitect; R=0,9829). La mediana della distribuzione della hs-cTnI nella popolazione italiana nelle 703 donne apparentemente sane è risultata 2,3 ng/L, cioè circa il doppio del valore di LoD del metodo (1,13 ng/L).

Conclusioni: I risultati di questo studio confermano che il metodo Access hs-cTnI soddisfa i due criteri che definiscono un metodo ad alta sensibilità, come richiesto dalle linee guida internazionali. I valori di riferimento calcolati risultano in accordo con quelli suggeriti sia dalla azienda produttrice, che quelli riportati in altre popolazioni. Inoltre, i risultati dimostrano che il calcolo dei valori del 99° percentile URL sono influenzati dall'età e dal sesso della popolazione di riferimento.

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**STUDIO MULTICENTRICO PER LA VALUTAZIONE DELLE PERFORMANCE ANALITICHE E DEGLI INTERVALLI DI RIFERIMENTO NELLA POPOLAZIONE ITALIANA DEL METODO ADVIA HS-CTNI CHE UTILIZZA LA PIATTAFORMA CENTAUR XPT**

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Introduzione: La valutazione del valore del 99° percentile, secondo le specifiche di qualità richieste dalle linee giuda internazionali, è una difficile sfida e spesso va oltre delle capacità del singolo laboratorio. Nel lavoro sono riportati e discussi i risultati di uno studio multicentrico incentrato sulla valutazione del 99° percentile e del reference change value (RCV) del metodo ADVIA hs-cTnI (TNIH), che utilizza la piattaforma XPT.
Materiali e metodi: La popolazione di riferimento in cui è stato valutato la misura di cTnI è di 1325 soggetti sani adulti (range di età: 18-86 anni); di cui 653 donne (età media: 50,7 anni; SD: 14,5 anni) e 672 uomini (età media: 50,9 anni; SD: 13,8 anni).
Risultati: Nello studio sono stati ottenuti i seguenti valori di LoB, LoD e LoQ 210% e 10% per il metodo hs-cTnI ADVIA Centaur XPT: 1,0 ng/L, 2,2 ng/L, 3,5 ng/L e 8,4 ng/L. Essendo la distribuzione dei valori di cTnI nella popolazione di riferimento altamente asimmetrica, sono stati usati i valori di cTnI log-trasformati per avere una distribuzione approssimativamente log-normale. Gli uomini hanno livelli di cTnI più elevati delle donne in tutti gli intervalli di età. I soggetti con età ≤55 anni hanno valori significativamente più bassi di quelli con età >55 anni ($p < 0,0001$). Il 62% delle donne e il 77% degli uomini hanno valori di cTnI ≥ del valore di LOD del metodo (2,2 ng/L).
Conclusioni: I risultati dimostrano che il metodo ADVIA Centaur hs-cTnI soddisfa entrambi i criteri e le specifiche di qualità richieste dalle linee guida internazionali per la definizione di metodo di misura di cTnI ad alta sensibilità.

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**SERUM FREE LIGHT CHAINS PREDICT LEFT VENTRICULAR DYSFUNCTION IN PATIENTS WITH ACUTE HEART FAILURE AFTER MYOCARDIAL INFARCTION**

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Background: Recent studies have shown a possible role of serum free light chains (sFLC) as a marker of inflammation and predictor of mortality in patients with chronic heart failure (HF). The potential causes of inflammation in heart failure patients are numerous, including the activation of innate immune responses following tissue injury, neurohormonal activation, oxidative stress, systemic hypoperfusion. However, patients with chronic heart failure often have comorbidities that could increase the concentration of sFLC, such as chronic kidney failure. Therefore, it is unclear whether the increase in sFLC is due to cardiac or kidney disease. The aim of our study is to evaluate the concentration of sFLC in patients with acute heart failure after myocardial infarction, in the absence of kidney failure, and left ventricular dysfunction.
Materials and Methods: We evaluated the sFLC in 226 patients with acute heart failure, after myocardial infarction who were treated with primary angioplasty in the Cardiology Department of the University Hospital Tor Vergata. Inclusion criteria: patient with acute heart failure after myocardial infarction in the absence of previous cardiovascular diseases. Exclusion criteria: diabetes, haematological diseases, kidney failure. For each patient during hospitalization we have determined blood concentration of sFLC and we also performed an echocardiogram to evaluate cardiac function. Left ventricular ejection fraction (LVEF) was measured by 2-dimensional echocardiography. Reduced systolic function was defined as LVEF <50%. The sFLC measurement was performed using N Latex FLC kit based on a mixture of monoclonal antibodies for use on the BN ProSpec® System analyzer (Siemens Healthcare Diagnostics).
Results: We observed that patients with LVEF> 50% had normal sFLC levels. Patients with LVEF <50% had an increase in sFLC. Therefore, depending on the ejection fraction greater or less than 50%, in almost all cases we have verified a correlation with the concentration of sFLC.
Conclusions: We have shown, for the first time, that sFLC correlates with left ventricular dysfunction in patients acute heart failure and without kidney failure. It can be hypothesized that a reduction of LVEF increases the systemic inflammation and activates the neurohormonal system, such as to increase the FLC. More studies are needed to better understand the role of sFLC in cardiovascular disease.

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## LIPOPROTEIN(A) AND CARDIOVASCULAR RISK FACTORS IN OBESE CHILDREN AND ADOLESCENTS

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Childhood obesity is a global phenomenon affecting all socio-economic groups, irrespective of age, sex or ethnicity. Many of these children have risk factors for later cardiovascular disease (CVD) and early signs of atherosclerosis. Novel biochemical markers such as lipoprotein (a) [Lp(a)] being used to determine CVD related morbidity and mortality. Elevated concentrations of Lp(a) have been shown to be an independent risk factor for atherosclerotic disease. We aimed to elucidate the role of the Lp(a) screening in obese children and adolescents.We prospectively screened for Lp(a) 86 consecutive patients (age 4-17 years old) admitted to the division of Center for Obesity and Related Endocrine Disorders, A.O.R.N. Santobono-Pausilipon.Samples for Lp(a) measurement with ELISA were collected during follow-up, in stable clinical conditions. Lp(a) concentration ≥30 mg/dL was considered elevated. In our obese pediatric population, Lp(a) resulted raised in the 25,6 % (n=22) of all subjects. Moreover, only the 3,5% (n=3) of patients had a Lp(a) value ≥70 mg/dL, identified as a threshold value for adult Lp(a) specific apheresis. We found Lp(a) levels >30 mg/dl in 12 girls and 10 boys, without significant differences in sex (27% vs 24 %, p= 0.69). Dividing our sample into 2 groups based on Lp(a) values (>30 and < 30 mg/dl), we observed that children with high Lp(a) levels had higher family history of premature CVD compared to children with normal levels (73% vs 40 %, p= 0,0094; respectively). Childhood obesity is an important cardiovascular risk factor because is often associated with dyslipidemia, endothelial dysfunction and early onset of diabetes, with the development of early atherosclerosis. In our very preliminary study on obese children, we found elevated levels of Lp(a), particularly in subjects with a family history of premature CVD, showing that these patients are exposed to an even higher risk. Systematic screening for Lp(a) might intensify the risk stratification and a further control of traditional risk factors in young obese population, already at higher cardiovascular risk, encouraging an action to bring the subject to a healthy lifestyle as early as possible.

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## SALIVARY AND SERUM ADMA LEVELS AS BIOMARKERS OF ENDOTHELIAL DYSFUNCTION IN PATIENTS WITH PERIODONTAL AND CARDIOVASCULAR DISEASE

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During the last few decades, observational studies have shown an association between chronic periodontitis (CP) and coronary heart disease (CHD), including myocardial infarction, stroke and peripheral vascular disease [1,2]. The aethiology of CP comprises immunological and inflammatory processes that cause dysregulation in the host response due to the superinfection of periodontal bacteria [3]. Few reports have associated CP and CHD with endothelial dysfunction [4,5]. Previous studies have demonstrated an association between high serum asymmetric dimethylarginine (ADMA), C-reactive protein (CRP) levels and endothelial damage [6]. In CP subjects, a cross-sectional study demonstrated that CP is associated, in a dose-dependent manner, with serum ADMA levels in untreated hypertensive patients [7]. We here aimed to evaluate the influence of either CP, CHD, or their combination (CP+CHD) on saliva and serum ADMA levels. Moreover, the association between both saliva and serum ADMA levels in either CP or CHD patients as well as the correlation between either salivary or serum ADMA levels and high sensitivity CRP (hsCRP) serum levels have been assessed. Increased values of hsCRP were observed among patients with CP, CHD and CP+CHD in comparison with healthy subjects ($p<0.001$). A higher prevalence of previous CVD events (atrial fibrillation, angina pectoris, stroke, heart failure) was observed in patients with CHD and CP+CHD, that regularly had more CVD drugs (antihypertensive, statins, low-dose aspirin, beta blockers) compared with CP and healthy subjects.The median concentrations of serum and salivary ADMA were higher in CHD ($p<0.01$) and CP+CHD ($p<0.001$) groups compared with controls. Serum and salivary ADMA concentrations were also significantly increased in CP+CHD patients in comparison with CP ones ($p<0.01$). Patients with CHD and CP+CHD presented higher levels of salivary and serum ADMA compared to healthy subjects and CP patients. hs-CRP is a significant predictor of increased salivary and serum ADMA levels.

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**EXPRESSION OF mRNA GALECTIN-3 IN HUMAN CAROTID ATHEROSCLEROTIC PLAQUES**

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Introduction: Atherosclerosis consists in the development of plaque in the intima-media layers of arteries due to lipid accumulation and oxidation together with a massive inflammation. Galectin-3 (Gal-3) is a pleiotropic protein involved in several biological processes such as macrophage chemotaxis, oxidative stress and cell proliferation. We aim to study Gal-3 mRNA expression in human carotid plaques.
Materials and Methods: Levels of the Gal-3 mRNA were measured in human carotid plaques and in their respective adjacent regions with lower grade lesions withdrawn from 35 patients undergoing carotid endarterectomy. Total RNA was extracted and cDNA was reverse-transcribed. Real Time PCR was performed in duplicate using TaqMan technology. Data analysis was performed by relative quantification and results are reported as expression units (eu) relative to the endogenous control (beta-Glucuronidase) and to a calibrator sample. Primary cells from human aortic endothelial (EC) and smooth muscle cells (SMC) were also used.
Results: Gal-3 resulted similarly expressed in atherosclerotic plaques and adjacent regions (1.58±0.69 vs 1.55±0.67 eu respectively, p=0.869 at paired T-test). No correlations have been observed between Gal-3 expression levels in adjacent regions and atherosclerotic plaques. Similar Gal-3 mRNA levels were observed among the different plaques types, or between complicated and uncomplicated plaques. No differences were found between Gal-3 expression levels among plaques with different plaque features such as presence of fibrosis, flogosis and calcification. To verify if different vessel cells express different Gal-3 levels, the mRNA quantification was performed in EC and SMC showing that the latter cells had higher levels of Gal-3 than EC (8.81 ± 2.36 vs 1.25 ± 0.13 eu respectively, p=0.033 at T-test).
Conclusion: Gal-3 is expressed by cells of atherosclerotic plaque. In particular SMC resulted to be the cells that most express Gal-3. However, its expression is independent from the different plaque features.

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**PREVALENZA DI COMPONENTI MONOCLONALI RISCONTRATE NEI DONATORI DI SANGUE AFFERENTI AL SIMT DI ASTI**

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Introduzione: La MGUS rappresenta oltre il 60% delle gammopatie monoclonali, con una prevalenza stimata di 1-3% nella popolazione di età superiore ai cinquanta anni. Il paziente solitamente è asintomatico ed il riscontro, all'elettroforesi delle siero-proteine (EF), è generalmente casuale ed avviene durante l'esecuzione di esami ematochimici di controllo.
La prevalenza di MGUS è in aumento a causa dell'invecchiamento e per la maggiore sorveglianza sanitaria.
I donatori di sangue rappresentano una risorsa chiave per l'autosufficienza nazionale di emocomponenti e pertanto sono sottoposti a esami di controllo annuali, a norma di legge, che possono comprendere anche l'esecuzione dell'EF.
Il nostro lavoro definisce la prevalenza di MGUS nei donatori afferenti al SIMT di Asti.
MaterialI e metodi: Estrazione dati: database AVIS (Apsi) su AS/400 (IBM)
Conferma dati: Eliot (Engineering) e Concerto (Dedalus)
Periodo: dal 01-05-2016 al 30-04-2019.
Proteine totali metodo biureto: Advia Chem XPT (Siemens);
EF: elettroforesi capillare Capillarys (Sebia);
Tipizzazione CM: eseguita sia su Hydrasys (Sebia) [IFE] che su Capillarys (Sebia)[ISE].
Analisi Statistica: MedCalc (versione 7.3.01)
Risultati: Donatori attivi nel periodo: 7768 (34,76% F, 65.24% M) di età compresa fra 18 e 65 anni.
Prevalenza complessiva CM: 0.73% (0.56% F; 0.83% M)
$\chi^2$ per differenza tra prevalenza CM in base al sesso: 1.449 (P = 0.2287: non significativo).
Mediana PT primo riscontro = 71g/L (uguale anche per sex).
Tipizzazione CM: 38IgG di cui 22κ e 16λ; 9IgA di cui 3κ e 6λ; 5IgM di cui 4κ e 1λ; 4biclonale (GκGκ; GκGλ; AκAκ; GλMκ); Non tipizzata:1.
Classe Ig monoclonale: G(70.49%), A(18.03%), M(9.84%), non tipizzata (1.64%).
Catena leggera monoclonale: 35κ (57.38%); 25λ (40.98%), non tipizzata (1.64%).
32 CM per donatori > 50aa (7F e 25M)
Discussione: Il nostro studio rileva una prevalenza di CM leggermente superiore (0.73 Vs 0.59). Tale dato risulta sovrapponibile ad altri studi riportati in letteratura.
Suddividendo per genere i dati si evidenzia un incremento della prevalenza nelle donne con indicazione ad eventuali approfondimenti diagnostici.
Bibiografia: Frezzotti A. et al. Prevalenza di componenti monoclonali in una popolazione di donatori di sangue. Biochimica Clinica 2014, vol. 38, n. 4.

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## VITAMIN D LEVELS IN WOMEN AFTER BREAST CANCER SURGERY: 12-MONTHS FOLLOW UP DEDiCA STUDY

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Background: Vitamin D deficiency is implicated in a variety of cancers. Several epidemiological studies and meta-analyses have investigated the association of blood 25(OH)D levels with breast cancer risk and showed that low 25(OH)D concentrations were associated with advanced tumor stage and relapses (1). We analyzed blood levels of 25(OH)D at baseline and at 12-months in a subgroup of women previously diagnosed with breast cancer participating in the DEDiCa study, a multicentre randomized controlled trial conducted in Italy of the effects of diet and lifestyle treatment with supplemental vitamin D in women after surgery for primary breast cancer. Our preliminary data show that, at baseline, 25(OH)D levels in women previously treated for breast cancer, 94% had low levels even in a country located in Southern Europe. Moreover, when supplemented with oral vitamin D there was still a large percentage (more than 50%) who did not reach blood sufficiency levels.We evaluated vitamin D levels in in 152 women after12-month treatment.

Methods: Eligible women (n=329) who had undergone surgery for primary histologically confirmed breast cancer (stages I-III) within the previous 12-months, were randomized to follow, for a maximum of 33 months, either one of the two treatments: group A (low glycemic index traditional Mediterranean diet + exercise + vitamin D to reach blood levels of 60ng/ml); Group B (standard care, general advice to follow a traditional Mediterranean diet and exercise + vitamin D to avoid insufficiency). Clinic visits every three months included the evaluation of 25(OH)D levels in blood samples which were analysed on the Liaison XL (Diasorin) according to the manufacturing instruction.

Results: At baseline the updated average circulating 25(OH)D was 24.2±12.7 ng/mL in 329 women aged 52.1±9.4. In women not previously taking oral vitamin D supplements (n= 201), only 12.4% reached sufficiency levels. In women previously taking oral vitamin D supplements (n=128) 56.3% reached sufficiency levels. However 43.7% remained in insufficiency levels, despite the supplementations.Patients were divided into two treatment groups (A and B). At baseline in group A the average circulating 25(OH)D was 22.8±12.1 ng/mL (deficiency 12%, insufficiency 28%, mild insufficiency 37.3%, sufficiency 22.7%). In group B the average circulating 25(OH)D was 21.6±11.8 ng/mL (deficiency 15,6%, insufficiency 32.5%, mild insufficiency 28.6%, sufficiency 23.4%).At 12-month follow-up of 152 patients (46% of total) the average circulating 25(OH)D levels in group A (n=75) were 49.6±12.5 ng/mL (deficiency 0%, insufficiency 1.3%, mild insufficiency 5.3%, sufficiency 93.3%). The corresponding values for group B (n=77) were 31.1±8.3 ng/mL (deficiency 0%, insufficiency 3.9%, mild insufficiency 42.9%, sufficiency 53.2%).

Discussion and Conclusion: The analysis at 12-month follow-up, show that vitamin D levels in the higher intensity group reached higher levels of sufficiency (93.3%) compared to group B in which there was still a 46.8% insufficiency. Therefore, close monitoring of 25(OH) D dosages is still necessary in women diagnosed with breast cancer and undergoing osteoporosis-inducing anti-estrogenic therapy.

References: 1-Vitamin D supplementation and total cancer incidence and mortality: a meta-analysis of randomized controlled trials - Keum N et al - Ann Oncol. 2019 May 1;30 (5):733-743.

P145
**VITAMIN K DEFICIENCY IN NEWLY DIAGNOSED PROSTATE CANCER PATIENTS**

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Prostate cancer (PC) is the second leading cause of cancer-related death affecting men worldwide. PSA is currently the only biomarker approved for PC screening. Levels of total PSA (TPSA) between 4-10 ng/mL are considered in "grey zone" for PC risk onset. Experimental studies have shown that vitamin K has antitumor effect on diverse cancer cells (i.e. PC) by enhancing oxidative stress and altering expression of proto-oncogene proteins that leads to cell cycle arrest and apoptosis. Recent investigations have focused on the role of vitamin K in the pathogenesis of some tumors, including PC: in animal and cancer cell studies it has been shown that vitamin K has anticarcinogenic activities. This vitamin can be indirectly evaluated by measuring the levels of des-gamma-carboxy-prothrombin (PIVKA-II), a protein induced by the absence of vitamin K. Aim of this study was to investigate vitamin K deficiency by measurement of PIVKA-II serum levels in PC patients with TPSA>10ng/mL at time of diagnosis and in men with increased risk of PC according to their TPSA levels (4-10 ng/mL). In laboratory of Tumor Markers of Policlinico Umberto I (Sapienza University of Rome), from December 2016 and December 2018, we collected and analyzed 91 serum samples from: 21 newly diagnosed PC patients (age range: 61-80 years), 35 men with TPSA in "grey zone" (age range: 39-65 years), and 35 healthy subjects with TPSA < 4 ng/mL as a control group (age range: 34-67 years). Total PSA was analyzed with the Hybritech calibration system (Beckman Coulter Access) while PIVKA-II determination was performed with an automated chemiluminescence instrument (CLEIA, LUMIPULSE® G 1200). PIVKA-II normal cut-off (<48mAU/mL) was previously established in our laboratory in a reference population. The results showed PIVKA-II values above the cut-off respectively in 48% of PC patients, in 6% of men with TPSA "grey zone" and in 14 % of men with TPSA <4 ng/ml. A statistically significant difference was observed between PC patients vs high risk PC men and healthy subjects ($p < 0,05$).These data seem to suggest an association between vitamin K deficiency and the onset of PC. Further studies will confirm these results, investigating more closely the role played by vitamin k in the prostate carcinogenesis process.

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**MINICHROMOSOME MAINTENANCE PROTEINS (MCM5) IN UROTHELIAL BLADDER CANCER PATIENTS IN FOLLOW UP: PRELIMINARY DATA**

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Urothelial bladder cancer is an oncological disease with a high incidence rate; in Italy alone, 25.000 new cases are registered each year. The 5-year overall survival rate is around 80% with a high relapse rate which makes a close follow-up of patients necessary especially during the first 2 years from diagnosis. Currently, cystoscopy is the gold standard diagnostic; on the contrary, the urine cytology appears to be not accurate enough in terms of specificity and sensitivity. MCM5 is a helicase and it is part of a replication complex which intervenes at the beginning of the cell proliferation phases and is present in the cells of the deep layers of the urothelium; the cell surface appears differentiated, the cells lose their replicative capacity and they lack the MCM5 protein. In case of carcinoma, neoplastic proliferating cells (MCM5 positive) reach the urothelium surface and flake off into the bladder lumen. Therefore, the presence of this protein in urine represents a biological marker of neoplasia. The determination of MCM5 in urine is carried out with ELISA method using DSX Equipment Technology (Technogenetics). In our study we tested urine samples of 35 patients with suspected urothelial carcinoma and 27 patients in follow-up for previous diagnosis of bladder cancer. All patients were subjected to cystoscopy and biopsy. The analysis of the ROC curves with SPSS statistical software vers.23 has shown an overall low diagnostic capacity of the test with AUC = 0.679. On the contrary, the same analysis conducted in patients with recurrence showed a very high diagnostic capacity with AUC = 0.873, sensitivity 85.7%, specificity 83.3% and VPP 94.7%. These preliminary data show that this non-invasive test can help in early identifying patients with bladder cancer recurrence during the follow-up, so they can have endoscopic examination with priority.

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**RED BLOOD CELL ANISOCYTOSIS IN RELATION WITH THE ANATOMICAL SITE OF ORIGIN OF COLORECTAL CANCER**

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Background: The pathological, clinical, and therapeutic features of colorectal cancer (CRC), one of the most common malignancies worldwide, depend on its anatomical localization. In this study, we investigated possible associations between the kinetics of red blood cell distribution width (RDW) and CRC localizations.
Methods: A consecutive series of 288 patients undergoing surgical resection of the large intestine for CRC from 1st January 2013 through 31st December 2017, was identified from the clinical archives of the second surgical unit of the University of Sassari. Demographic, clinical, pathological and laboratory data, including complete blood count parameters, were retrieved from clinical records and reports. Patients were classified in accordance with the anatomical site of the tumor: right, transverse, left and sigmoid colon, rectum and multiple lesions involving more than one district of the large intestine. Fasting blood samples were obtained with standard procedures and methodologies in accordance with current international and national guidelines; the samples were processed and analyzed in a certified laboratory, where the normal range for RDW was 11–13.5%.
Results: There were no significant differences in sex, age, stage, and grade of the tumors, as well as in relevant comorbidities between patients with right-sided CRC and those with CRC in all the other locations. However, median RDW values were significantly higher in patients with right-sided CRC when compared to those with CRC in other localizations (16.2, IQR: 14.5-20.0 vs 13.8, IQR: 13.0-16.1, $p<0.0001$). Anisocytosis was statistically associated with hemoglobin (Hb), mean hemoglobin concentration (MHC), and mean corpuscular volume (MCV) values in all the patient groups examined. A cut-off value of 14.3% was associated with right-sided localization with sensitivity and specificity of 76.3% and 64.2%, respectively (AUC 0.71).
Conclusions: Median RDW values are significantly higher in right-sided CRC, when compared to other tumor locations, and may represent an additional marker for differential diagnosis. Larger, prospective, studies are warranted to confirm these findings.

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**LABORATORY MEDICINE AND EXTRACELLULAR VESICLES IN THE DIAGNOSTIC PROCESS OF HEMATOLOGIC MALIGNANCIES: FRAILTIES AND STRENGHTS**

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Cells exchange information by secreting a heterogeneous population of micro and nanosized extracellular vesicles (EVs), i.e. exosomes, ectosomes (or microvesicles) and apoptotic bodies. These soft nanoparticles, enclosed by a membrane that is tailored for targeting, are vehicles for proteins, nucleic acids and metabolites derived from the cell of origin. Upon delivery, the cargo is able to modulate biological functions in target cells and/or their microenvironment. In this way EVs participate not only in the regulation of normal physiological processes but also in the pathology underlying diseases, such as the case of hematological malignancies. Indeed it has been evidenced that EVs play a crucial role in information transfer in those tumors. Recent findings suggest that specific EV microRNAs are involved in pathogenesis and have a prognostic role in Multiple Myeloma (MM). New interesting protein candidate biomarkers have also been discovered. As a research group we actively took part in this field by highlighting that serum derived EVs of MM and Monoclonal Gammopathy of Undetermined Significance (MGUS) patients differ in concentration, biological activity, and biochemical content. These modifications seem to be related to the free light chains (FLCs) associated with EVs and their pro-pathogenic properties. Moreover, the cellular processing of FLC-decorated EVs and their ability to activate proinflammatory mechanisms are different between MM and MGUS patients. On the basis of these elements we can hypotesize to develop in the near future an innovative EV-based multiparameter panel, very specific and highly sensitive, to monitor MGUS to MM switching. In this presentation we will cover the role of EVs in the field of MM and we will highlight EV future possible translation in clinical diagnostics.
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## COMPARTMENTALIZATION OF BILE ACIDS IN PLASMA AND BILE SAMPLES FROM PATIENTS WITH BENIGN AND MALIGNANT BILIARY DISEASES

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Background and aim: Cholangiocarcinoma (CCA) is a rare but devastating cancer accounting for 15% of all primary liver malignancies. Its diagnosis is challenging since there are no sensitive and specific biomarkers to accurately differentiate benign and malignant biliary disease. Since some studies recently suggested that bile acids (BA) may have a pathogenic role in cholangiocarcinogenesis, this study was aimed to compare the plasma and bile profile of BA in patients with CCA and biliary stones using an in-house developed liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay.

Methods: A panel of 17 BA were quantified in bile and plasma samples from 27 patients with CCA and 26 patients with biliary stones (sex and aged matched). LC-MS/MS analysis was performed using a ACQUITY UPLC I-Class System (Waters) followed by detection on TQS-Micro Tandem Quadrupole MS detector. Seven deuterated internal standards (IS) were used for quantification. Mann Whitney test was used to compare BA concentrations in CCA and control group. Spearman test was used to assess the correlations between bile and plasma samples. Statistical significance was set at $p<0.05$.

Results: Conjugate BA were found to be differentially expressed in CCA with respect to biliary stones samples ($p<0.05$ for all taurocholic and glycocholic plasma BAs). The glycochenodeoxycholic acid (GCDCA) and the glycodeoxycholic acid (GDCA) showed to be inversely correlated in bile and plasma samples in the whole population ($r=-0.45$, $p=0.0007$ and $r=-0.41$, $p=0.0023$ respectively). GCDCA was significantly inversed correlated in bile and plasma samples even in the CCA group taken alone ($r=-0.44$, $p=0.0228$). Median and range values of plasma and bile GCDCA in CCA and benign group were as follow: 896 ng/mL (80-10662 ng/mL) vs 418 ng/mL (19-2615 ng/mL) ($p=0.0159$) and 4232 ug/mL (446-46919 ug/mL) vs 9451 ug/mL (113-83305 ug/mL) ($p=0.1422$). Median and range values of plasma and bile GDCA in CCA and benign group were as follow: 112 ng/mL (25-896 ng/mL) vs 70 ng/mL (13-576 ng/mL) ($p=0.0453$) and 1235 ug/mL (86-21911 ug/mL) vs 4878 ug/mL (0-94240 ug/mL) ($p=0.0425$).

Conclusion: Our preliminary analysis of data showed a trend toward an inverse association between biliary and plasma glycoconjugated BA. Accordingly, GCDCA and GDCA showed a significant inverse compartmentalization in bile and plasma samples from patients with CCA and patients with biliary stones being higher in bile vs plasma samples of benign patients and higher in plasma vs bile samples from CCA patients.

Reference: Current and future roles of mucins in cholangiocarcinoma-recent evidences for a possible interplay with bile acids. Danese E, Ruzzenente A, Montagnana M, Lievens PM. Ann Transl Med. 2018;6(17):333

P150
## CARATTERIZZAZIONE MOLECOLARE DI PAZIENTI CON CARCINOMA EREDITARIO DELL'ENDOMETRIO

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Il carcinoma dell'endometrio è il quarto tumore femminile dopo il carcinoma della mammella, del colon e del polmone. Nel 95% dei casi è sporadico, nel 5% ereditario.Il carcinoma ereditario dell'endometrio si riscontra in famiglie con Sindrome di Lynch II, in cui sono presenti tumori primari anche in altre sedi, quali stomaco, vie epato-biliari, cervello, reni, ureteri, mammella ed ovaio e in famiglie con HBOC (Hereditary Breast and Ovarian Cancer).La Sindrome di Lynch II è da ricondurre a mutazioni germinali in uno dei geni del "MisMatch Repair" (MMR), l'HBOC principalmente a mutazioni nei geni BRCA.In questo studio analizziamo i principali geni del MMR, MLH1 e MSH2 e i geni BRCA1 e BRCA2 in pazienti con carcinoma ereditario dell'endometrio e nei loro familiari.L'indagine è stata condotta su 15 pazienti con carcinoma dell'endometrio, 11 appartenenti a famiglie con Sindrome di Lynch II e 4 con HBOC ed estesa ai familiari delle pazienti risultate mutate.I pazienti, dopo consulenza, sono stati sottoposti a prelievo di sangue periferico.L'analisi mutazionale è stata condotta con NGS, mentre l'estensione di mutazione nei familiari è stata condotta con sequenziamento di Sanger.5/15 (33%) pazienti sono risultate avere una mutazione, 3/5 (60%) in MSH2, 1/5 (20%) in MLH1 e 1/5 (20%) in BRCA1.Inoltre è stata identificata una nuova variazione in MSH2, c.2006-23T>G (IVS12-23T>G). Dall'analisi in silico, condotta col software Alamut, tale variante non risulta avere effetto sullo splicing.L'analisi ha permesso di individuare mutazioni nel 33% delle pazienti. In particolare l'80% delle mutazioni ricade in MLH1 e MSH2, confermando l'insorgenza del carcinoma endometriale come evento sentinella della Sindrome di Lynch II, mentre l'individuazione di mutazione in BRCA1 suggerisce l'associazione con l'HBOC.L'indagine genetica è stata estesa alla figlia della paziente con mutazione in BRCA1, risultata negativa, al figlio della paziente con mutazione in MLH1, risultato negativo.Inoltre, di 5 familiari appartenenti a una delle pazienti mutate in MSH2, 4 hanno ereditato la mutazione. L'individuazione di mutazioni nei geni di suscettibilità consente di selezionare i familiari sani con mutazione, a rischio di sviluppare una neoplasia, da sottoporre a percorsi di prevenzione oncologica.

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## MARCATORI BIOCHIMICI DI RISPOSTA ALLA TERAPIA DI INDUZIONE IN PAZIENTI AFFETTI DA MIELOMA MULTIPLO

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E' stata valutata in una popolazione di pazienti affetti da Mieloma Multiplo (MM) la risposta ematologica alla terapia di induzione, attraverso l'impiego dei principali parametri raccomandati dall'IMWG (2016): CM sierica, in elettroforesi capillare; catene leggere libere sieriche (FLC), in nefelometria (BNP-Prospec, Siemens); proteina di Bence Jones (PBJ), mediante immunofissazione urinaria. In alternativa alla misura quantitativa della PBJ (lettura della banda all'elettroforesi urinaria) è stata eseguita la misura nefelometrica delle catene leggere totali urinarie (uTLC), test poco sensibile e specifico, ma di facile applicabilità, non molto costoso, utile nel follow-up dei pazienti affetti da MM. La coorte di pazienti (pz) arruolata (6/16 -10/18), afferente al reparto di Ematologia, era composta da 33 MM e 14 MM a catene leggere (LCMM). Sono stati valutati CM, iFLC (involved FLC), PBJ e uTLC involved (uiTLC), a diagnosi e in corso di terapia, per stabilire il marcatore più sensibile di malattia. Schema chemioterapico di induzione somministrato a 44 pz per 6 cicli: 23 pz VTD=bortezomib (bort), talidomide, desametasone (desam); 16 pz VMP=bort, melphalan, prednisone; 4 pz RD=lenalidomide (lenalid), desam; 1 pz VD=bort, desam. Altri 3 pz: 1 pz, 5 cicli VTD, + 2 di VRD=bort, lenalid, desam; 1 pz, 2 cicli VRD + 5 KD=carfilzomib, desam; 1 pz, 1 ciclo VD + 4 PAD=bort, antraciclina, desam e 3 RD. Alla diagnosi i pz MM presentavano: 100% (33/33) CM; 91% (30/33) iFLC alterata; 82% (27/33) uiTLC alterata e PBJ positiva. Dopo terapia di induzione: nell'85% permaneva la presenza della CM; nel 58% e 39% erano alterate iFLC e uiTLC rispettivamente, PBJ era positiva nel 39 %. Per tutti i parametri le riduzioni percentuali pre e post terapia risultavano statisticamente significative (P <0,05). Dei 14 pazienti LCMM il 100% presentava alla diagnosi iFLC e uiTLC alterate e PBJ positiva. La CM non è stata valutata perché presente in 3/14 pazienti. Dopo terapia di induzione rimanevano alterati: iFLC nel 93%; uiTLC nel 57% e nel 71% PBJ positiva. In conclusione, l'analisi effettuata nella popolazione in studio ha confermato, in accordo con i dati della letteratura, i ruoli 'leader' della CM nel MM e della iFLC nell'LCMM nel valutare la risposta ematologica dopo terapia di induzione.

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## USEFULNESS OF SERUM FREE LIGHT CHAIN MEASUREMENT FOR THE INTERPRETATION OF PARTICULAR SERUM PROTEIN ELECTROPHORESIS: CLINICAL CASE REPORTS

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Background: Serum Protein Electrophoresis (SPE) is usually performed for the diagnosis of plasma cells dyscrasias. However, there are some conditions that make the interpretation of a SPE very difficult: when the M-protein is very small or not evident at all (light chain disease), in case of hypogammaglobulinemia, or when monoclonal proteins are hidden under the peak of β fraction. In these cases SPE should be followed by Immunofixation (IFE) to point out the presence or the absence of a Monoclonal Gammopathy. However, IFE is time consuming, so it can't resolve immediately doubt about the interpretation of a SPE, while the dosage of serum Free Lights Chain (sFLC) can be useful for an immediate detection of MG. sFLC assay has proved to be a useful laboratory test for the evaluation and management of MM and related plasma cell disorders in association with SPE.
Method: We retrospectively analysed 5 particular clinical cases in which the quantification of sFLC was useful for the interpretation of SPE. sFLC measurement was performed on Optilite (The Binding Site) utilizing a commercially kit (Freelite, The Binding Site). SPE was performed using semiautomatic analyzer Capillarys 2 (Sebia).
Results: SPE showed hypogammaglobulinemia in all the cases described. In one case there weren't qualitative or quantitative alterations of serum proteins. In the other cases, SPE showed also a little increase of β fraction. To verify the presence of Monoclonal Component (MC) we performed sFLC assay and, in all cases, we found an increase of sFLC κ or λ. Serum IFE confirmed the presence of IgA or IgM components that aren't usually visualized by SPE because they are hidden under the β fraction.
Conclusions: The detection of the sFLC and the estimated κ/λ ratio are excellent indicator of clonality. The evaluation of these parameters is recommended by IMWG guidelines for screening, prognosis, therapy and patient monitoring as well as for diagnosis and monitoring of all the conditions in which MC is hardly detectable and measurable. Our experience confirm that sFLC assay is useful to identify a MC in particular clinical cases avoiding a delay of diagnosis. Thus, the association SPE and sFLC is relevant expecially for patients with an IgA component not detectable with SPE (21% of MM).

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## ANTINUCLEAR ANTIBODIES (ANA) AS FRAILTY MARKER IN A MULTICENTRE EUROPEAN STUDY

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Aim: The increase in average life is paralleled by an enhanced number of elderly people, displaying different frailty conditions.Frailty is a clinical state associated with ageing and chronic disease, which usually anticipates disability.The FRAILOMIC initiative is a multicenter European Project designed to use biomarkers for identifying factors that may elucidate the transition from frailty into disability.The main objective is to develop clinical and laboratory tools for diagnosing and predicting the frailty risk.The antinuclear antibody (ANA) assay is used as a primary test to investigate many autoimmune disorders affecting several tissues and organs.
Materials and methods: We examined ANA in 320 elderly patients with immunofluorescence test and we tested the correlation between ANA positivity/title with the degree of frailty in the elderly.
Results: A statistically significant correlation with ANA positivity was observed in patients with frailty and disability (ADL disability $p = 0.01$; IADL disability $p = 0.05$; Mobility disability $p = 0.007$), whilst no significant association was found with other pathological conditions of the elderly, including congestive heart failure, diabetes, depression and cognitive impairment.ANA positivity, especially at low-level, seems to have a protective effect in cancer patients (22% in cancer patients vs 10% healthy patients), although this trend was not statistically significant. When we assessed all frailty conditions and divided patients into robust, pre-frailty and frailty, ANA positivity was of borderline significance ($p = 0.059$).
Conclusions: ANA testing seems an interesting screening tool for assessing the risk of frailty.The distribution of ANA positivity was different across various frailty models.

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**EVALUATION OF SPECIFIC SERUM BIOMARKERS RELATED TO DIAGNOSIS OF ATROPHIC GASTRITIS**

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Atrophic Gastritis(GA) is an alteration of gastric mucosa cells caused by Helicobacter Pylori(HP) or an autoimmune reaction and predisposes to gastric carcinoma, hypo or achlorhydria and malabsorption of iron and VitB12. The gold standard for the diagnosis of gastric diseases is gastroscopy. The introduction of a panel of 4 serum markers(Gastropanel® ELISA BIOHIT Healthcare): HP antibodies(Ab anti-HP), Gastrin 17(G17) and Pepsinogen I and II(PGI- II) has allowed a better classification of the patients also in the pre and sub-clinical phases of gastric pathology.The purpose of this work was the evaluation of Gastropanel® diagnostic efficacy, in conjunction with other serum biomarkers: autoantibodies against Parietal Cells(PCA), total Gastrin(G34) and Chromogramin-A(CrA). We have been selected 400 outpatients, sent by the attending physician and by the gastroenterologist for recurrent dyspeptic disorders, for the following blood tests: Gastropanel®, CrA Eurospital Diagnostic IFI), PCA (BRAHMS KRYPTOR TRACE $^{TM}$) and G34 (Immulite2000).According to Gastropanel®, the patients were divided into 4 groups: 1) 180 Healthy patients(45%); 2) 100 patients with moderate atrophic gastritis(25%); 3) 70 patients(17.5%) affected by body atrophic gastritis; 4) 50 patients(12.5%) affected by HP antrum atrophic gastritis. Group 3 have high levels of G17, low levels of PGI and Ab anti-HP negative. Moreover, they are all positive PCA and HP direct negative tests. 35 patients(50%) had elevated G34 and CrA levels and for 20 patients(28%) the serum results were confirmed by biopsy. Anamnesis highlights that patients of Group 3 are suffering from VitB12 deficiency and anemia. The patients of Group 4 show low value of G17 and Ab anti-HP positive, moreover they are all negative PCA and in 25 patients(50%) the result was confirmed by the histological report.Our study showed that the Gastropanel®, in association with the PCA assay, is an excellent diagnostic tool for both autoimmune atrophic gastritis and generated by HP. The use of the Gastropanel® is useful not only in the diagnostic phase but also during patients follow-up and its strong reliability and accuracy allows it to be used as an alternative to invasive methods such as gastroscopy.

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**ANTI-NUCLEAR ANTIBODIES SCREENING WITH COMBINED IMMUNOFLUORESCENCE AND SOLID PHASE ASSAY: REPORT FROM SIBIOC AUTOIMMUNITY GROUP**

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Anti-Nuclear Antibodies (ANA) test is still the first step in the diagnostic algorithm of a suspected autoimmune disease. As the spectrum of autoimmunity is rapidly expanding, ANA requests in Laboratory Departments have substantially increased, therefore new needs of reorganization and improvement in appropriateness and quality have emerged. In the past few years considerable interest arose on extended solid-phase Connective Tissue Disease (CTD) screen, whether with fluoroenzymatic immunoassay (FEIA) or chemiluminescent immunoassay (CLIA), as a possible, more specific, alternative to consolidated indirect immunofluorescence (IIF) assay on HEp2 cells, being the latter the gold standard method because of its high sensitivity.Hereby we report the results of a shared effort from Autoimmunity Units of Padova, Modena and Parma, on behalf of the SIBioC Autoimmunity Group, to combine IIF and CTD screen in a real world setting. A total number of 878 unselected samples (502 in Padova, 188 in Modena, 188 in Parma) consecutively received for ANA testing was included; patients were referred both from general practitioners and hospital units.IIF test was performed on HEp-2 cells currently used by each laboratory (NOVA Lite HEp-2 by Inova and HEp-2000 by Immuno Concepts), starting from dilution 1:80, cut-off for positivity was set on 1:160. CTD screen was performed on FEIA method (EliA CTD Screen by Thermo Fisher), cut-off for positivity was set on 1.0 ratio (unit/ml), as suggested by manufacturer; range 0.7-1 was labelled as borderline. Discordant results between IIF and CTD screen were further tested for specific antibody positivity according to each laboratory diagnostic algorithm for ANA-testing.Overall IIF and CTD screen agreed upon ANA positivity in 130 patients (14.8%) and ANA negativity in 524 (59.7%). 148 samples (16.9%) were IIF positive/CTD negative and 42 (4.8%) IIF negative/CTD positive. Out of 34 CTD borderline results, 18 (2.1%) were negative for IIF and 16 (1.8%) were positive.Our joint work supports the recent statements of an increase in the diagnostic accuracy for ANA screening when solid-phase assay is associated to IIF; however careful strategical evaluation of the impact of a new routine test should be done in each laboratory setting.

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**CHEMILUMINESCENCE ANTI-TRANSGLUTAMINASE ASSAY AND PREDICTION OF CELIAC DISEASE**

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Introduction: Recently new chemiluminescence anti-transglutaminase (atTG) assays with outstanding performance in terms of sensitivity and specificity have been available. The identification of patients with positive serology without duodenal damage is becoming more common with these new assays: thus, the necessity to investigate if the atTG could be use also as a predictive and prognostic test for the diagnosis of CD.
Material and methods: Between June 2012-December 2013, 516 consecutive patients referred to our laboratory were atTG positive (Quanta-Flash h-tTG IgA, Inova Diagnostics, INOVA, San Diego, California) using the manufacturer's cut off (20 CU). For all the patients the clinical, serological, genetic and histological information available were collected in a five years retrospective follow up. The patients with a CD diagnosis or who received a CD diagnosis within 6 months from the positive atTG result were excluded.
Results: 516 patients (133 males, 383 females, average age 20.9y, median age 13y, range age 8months-85y) were included. atTG results range was 20.4 CU-4965.5 CU, average value 672.2 CU, median value 74.6 CU. 190/516 (36.8%) were excluded because they already received a CD diagnosis. 207/516 (40.1%) received a CD diagnosis within 6 months and, according to our selection criteria, were excluded. For 119/516 (23.0%) the clinical data and record were collected: 14/119 (11.8%) are still classified as potential CD; in 19/119 (16.0%) CD was excluded; 4 patients never undergone other exams; 60/119 (50.4%) received a CD diagnosis during the five years of follow up. For 22/119 patients the data are still under investigation.
Conclusion: although these are preliminary data, atTG seems to be able to predict the CD diagnosis in 50.4% of cases during five years follow up.

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**INVOLVEMENT OF OXIDATIVE STRESS AND VITAMINS D, C AND A IN SYSTEMIC SCLEROSIS: IN RELATION TO CLINICAL DISEASE ACTIVITY PARAMETERS**

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Systemic sclerosis (SSc) is an immune-mediated rheumatic disease characterized by fibrosis of the skin and internal organs, vasculopathies, Raynaud's phenomenon (RP) with digital vasculopathies, and leg ulcers. Early diagnosis and personalized treatment are limited by few studies on SSc's pathogenetic mechanisms. In the last years the role of oxidative stress (OxS) and deficiency of some micronutrients, including vitamins, have been highlighted.We evaluated the role of OxS and the oxidative burst capacities of polymorphonuclear leukocytes (PMN) by examining derivative-Reactive Oxygen Metabolites (d-ROMs), Thiobarbituric Acid Reactive Species (TBARS) and PhagoBurst in 42 patients with SSc, 14 with RP and 20 healthy controls (HC). In addition the blood level of vitamins A, C, D, were measured. Correlation analysis was performed between OxS markers and clinical disease activity parameters (DA), evaluated by European Scleroderma Study Group (ESSG) index and the Number of microhaemorrage (NEMO) score.The markers of oxidative stress in blood of SSc patients were aberrant indicating an imbalance between oxidants and antioxidants. Both SSC and RP patients had higher OxS level compared with HC [d-ROM test: 380 (SSC), 330.5 (RP) and 280 UCARR (HC), <0.0001; TBARS: 0.1 (SSC),0.12 (RP) and 0.07 nmolMDA/mg protein (HC), p=0.009]. The differential analysis based on DA parameters demonstrated that d-ROMs values were higher in patients with: a) NEMO score ≥8 (451 vs 354; p=0.01) and the two parameters were positively correlated (r =0.42, p=0.005); b) scleredema (451 vs 348; p=0.04); c) digital ulcers (DU) (374 vs 427, p<0.0001). Lower levels of TBARS was measured in SSc with DU (0.05 vs 0.11; p=0.02). Data of the oxidative burst activity experiments upon stimulation with unlabeled opsonized bacteria (E.coli) demonstrated a reduced activity of granulocytes, producing fewer ROMs in SSc subjects. Reduced level of vitamin D, C and A was measured in SSc and their values were correlated with OxS parameters. Our results evidence that OxS correlates with the DA parameters which define both early skin and vascular signs, supporting the importance of its involvement in

these stages, and also suggest a novel approach for SSc treatment considering micronutrients' supplementations.

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## COMBINED USE OF K FREE LIGHT CHAIN AND OCB DETECTION IN MULTIPLE SCLEROSIS DIAGNOSIS

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Background: The diagnosis of Multiple Sclerosis (MS) is based on the integration of medical history, clinical examination, magnetic resonance imaging findings and cerebrospinal fluid (CSF) examination. The detection of oligoclonal bands (OCBs) in CSF mirrors the intratechal IgG synthesis and represents the gold standard for MS diagnosis. However, it has some analytical issue. The measurement of IgG k free light chains (kFLCs) in the CSF and, in particular, the kFLC index (kFLCi) [(CSF kFLC / Serum kFLC)/( CSF Albumin/Serum Albumin)], has been proposed as alternative tool to evaluate the intratechal synthesis of IgG. However, since kFLCi has lower specificity than OCB, kFLCi and OCB could be used in combination to support MS diagnosis.

Aim: The aim of the current study was to assess the usefulness of a diagnostic algorithm based on the evaluation of kFLCi, as screening test, followed by OCB detection, as confirmatory test.

Methods: We enrolled unselected consecutive patients suspected of MS with an OCB request. CSF and serum kFLC as well as CSF and serum Albumin were measured on samples stored at -80°C after collection by turbidimetric assay (Freelite®, The Binding Site Group Ltd., Birmingham, UK) on Optilite analyser System, according to manufacturer.

Results: We included a total of 56 patients, 39 with MS and 17 with Other Neurological Diseases (OND). The diagnostic performance of kFLCi for MS diagnosis was tested by ROC analysis. We chose a kFLCi cut-off of 2.3 with a sensitivity of 97.4% and a specificity of 64.7% in order to have a high sensitivity and an acceptable specificity. We performed a post-hoc analysis testing the following algorithm: patients with kFLCi ≥2.9 were considered at high probability of having MS and should perform the OCB detection; patients with kFLCi <2.9 had low probability of MS and do not need OCB evaluation. Applying retrospectively such algorithm, we found that among all patients (56), 11 had kFLCi <2.9; all these patients were OND except one that received the diagnosis of MS. Noteworthy, this patient was also OCB-negative. Among the 45 patients with kFLCi ≥2.9, 40 were also OCB positive. 93% of patients with kFLCi ≥2.9 and OCB-positive were diagnosed as MS. Overall, performing such algorithm we would have executed 20 % less of OCB analysis.

Conclusion: Findings of our preliminary study support the sequential use of kFLCi and OCB detection for MS diagnosis.

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## NEW TESTS FOR DIAGNOSTIC IMPROVEMENT IN AUTOIMMUNE BULLOUS DISEASES

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Aim: Autoimmune blistering dermatoses (ABD) are a heterogeneous group of rare diseases clinically characterized by erosions and/or blisters on the skin and mucous membranes. In ABD the immune system produces autoantibodies (Abs) directed against cell-cell or cell-matrix adhesion molecules. More common ABD entities, pemphigus and bullous pemphigoid, are identified by circulating and tissue-bound Abs against the desmosomal cadherins (mainly desmoglein1 and 3– DSG1/3) and dermal-epidermal junction components (BP180 and BP230), respectively. The diagnosis of ABD is based on a combination of criteria: histopathology, direct immunofluorence of a biopsy skin, indirect immunofluorescence (IIF) on monkey esophagus (ASA), BIOCHIP technology and ELISA. The identification of the target antigen is crucial for the diagnosis and patient management. At present several serological assays have been developed, however no accepted gold standard assay exists.
Materials and methods: We tested 104 patients suspected or diagnosed for ABD to evaluate the diagnostic performance of three different assays: traditional IIF-ASA, innovative BIOCHIP mosaic (array of six different diagnostic substrates including monkey esophagus, primate salt-split skin, dots of tetrameric BP180-NC16A as well as DSG1/DSG3 extracellular/transmembrane domains, BP230 C-terminal domain expressed in HEK293 cells) and ELISA based on recombinant target antigens (DSG1/DSG3/BP180).
Results. The tests profiles required were different between Specialist Departments (Dermatology and Ophthalmology) and General Medicine Departments: ASA IIF 7% vs 66%, ELISA 53% vs 34% and BIOCHIP 40% vs 0%, respectively. We observed different positive percentages between IIF ASA, ELISA and BIOCHIP assay: 36%, 45% and 75% respectively. The agreement between ASA-IIF and Antigen specific test (Ag) ( ELISA and BIOCHIP) was 75% (33/44). 7/44 patients (16%) were ASA- / Ag + and mainly represented by BP180 + (5/7); instead 4/44 patients (9%) were ASA + / Ag- mainly represented by intercellular substance aspecific pattern (3/4).
Conclusions: Our results regarding ABD patients support the opportunity to perform very sensitive and specific tests, as Antigen specific assay, in order to achieve a definitive diagnosis.

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## SERUM IMMUNOGLOBULIN FREE LIGHT CHAINS (FLCs) LEVELS IN SYSTEMIC AUTOIMMUNE RHEUMATIC DISEASES (SARDs)

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Serological immunoglobulin free light chains (FLCs) levels are frequently elevated in different B-cell-mediated autoimmune disorders as a direct marker of B cell activation. HCV can lead to B-cell lymphoproliferative disorders. Different polymeric forms, physico-chemical properties and bioactivities characterize each clone of FLCs. A strong association has been reported between increased FLC levels and HCV-related mixed cryoglobulinaemia (MC). The objective of this study was to validate the clinical usefulness of serum FLCs in patients with systemic autoimmune rheumatic diseases (SARD). We assessed FLC levels in sera from 198 SARD patients (37 rheumatoid arthritis, RA; 47 Systemic Lupus Erythematosus, SLE; 52 Anti Phospholipid Syndrome, APS; 62 primary Sjogren's syndrome, pSS), from 62 HCVMC and from 50 healthy donors (HD).All patients (SARDs + HCVMC) showed increased k levels when compared to HD; in HCVMC, free k chains were higher than SLE and APS patients. Free $\lambda$ levels displayed a significant increase only for HCVMC and SLE patients compared to HD. The faster increase of k compared to $\lambda$ takes account in a k/$\lambda$ ratio of approximately 1.6 for all the groups of patients.We confirm the association between FLC patterns and HCVMC. Moreover, we suggest that the elevated levels of FLCs assessed in SARD could act as mini autoantibodies playing a key role in the ongoing of autoimmune diseases. Unfortunately, specific biochemical structural differences distinguishing between normal from pathological FLCs have not been identified. Production of different FLC isotypes by B-lymphocytes is probably connected to any still unknown pathways.

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## STUDIO DI BIOMARCATORI LEGATI ALLO STRESS OSSIDATIVO NELL'ARTRITE REUMATOIDE

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L'artrite reumatoide (RA) è una malattia autoimmune progressiva cronica associata ad infiammazione sistemica che colpisce principalmente le articolazioni sinoviali. È caratterizzata dall'infiltrazione di cellule infiammatorie nella sinovia e conseguente iperplasia, che portano alla distruzione dell'osso e della cartilagine articolare, e svariate complicanze sistemiche[1]. Sebbene la causa esatta sia ancora sconosciuta, si ritiene che la RA sia il risultato di meccanismi immuno-mediati innescati da fattori ambientali in soggetti caratterizzati da un substrato genetico favorevole. Diversi studi hanno dimostrato che le specie reattive dell'ossigeno (ROS) sono implicate nella fisiopatologia dell'AR. Si tratta di specie chimiche altamente reattive che hanno il potenziale di danneggiare lipidi, proteine e DNA nei tessuti. Il presente studio è stato progettato per valutare lo stress ossidativo nei pazienti RA rispetto ai controlli sani. Sono stati reclutati 164 pazienti con RA (62 M/102 F, età media 55,0 ± 6,8 anni) e 101 controlli sani (50 M/51 F, età media 54,9 ± 5,6 anni). I TBARs (MDA e aldeidi) e l'attività della paraoxonasi-1 sono stati determinati come indici di danno lipidico misurando l'assorbanza rispettivamente a 535 nm e a 412 nm usando paraoxon come substrato. La determinazione dei gruppi -SH plasmatici è stata eseguita con DTNB come agente di titolazione, misurando l'assorbanza del coniugato a 405 nm. La capacità antiossidante (TEAC) è stata determinata utilizzando il metodo descritto da Re et al. La chinurenina e il triptofano, indici di infiammazione, sono stati determinati mediante rivelazione UV di elettroforesi capillare, come descritto in Zinellu et al.Dai risultati emergono differenze significative nei valori medi di PSH tra controlli e pazienti (mediana 3,77 vs 3,15 μmol/gr prot; p <0,001). Allo stesso modo è stata registrata una significativa riduzione dell'attività della PON tra i controlli e i pazienti (mediana 109,73 vs 128,09 U/L). Lo studio dei biomarcatori ossidanti e antiossidanti nei pazienti con AR è da considerarsi quindi utile per migliorare la comprensione della patogenesi e valutare la progressione della malattia. [1] Smolen, JS, Aletaha, D, McInnes, IB. Lancet. 2016;388:2023–2038

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## ALPHA 1 ANTITRYPSIN DEFICIENCY: A RARE NULL ALLELE IN PATIENTS FROM SOUTH OF ITALY

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Alpha 1 antitrypsin (AAT) is a water-soluble and tissue-diffusible, circulating glycoprotein: it inhibit a series of serine proteases as Neutrophilic Elastase (NE), which is released at the pulmonary level after the neutrophil activation. Congenital α1-antitrypsin deficiency (AATD) is a hereditary genetic disorder transmitted through an AR character with various forms of penetration and expressiveness. This condition is characterized by reduced or absent levels of α1-antitrypsin that depend on a misfolding of the latter which leads to incorrect polymerization. In Italy it is estimated that 1/5000 individuals may suffer from severe AATD, with frequency peaks in some areas of 1/2000 inhabitants. This disease is a misdiagnosed condition: the AATD pathogenesis is directly related to the gene mutations, which is highly polymorphic: more than 120 genetic variants closely associated with specific plasma glycoprotein concentrations have been identified. Emphysema affects 54% of patients diagnosed with this deficit.This study focuses on the most common mutated variants Pi*S and Pi*Z and on the rarer $PiM_{Malton}$ and $PiM_{Procida}$ variants detection by Real Time PCR. Of the 86 patients we analyzed, 64 are wild type, 12 are heterozygous Pi*S (allele frequency 0.14, serum concentration between 1.20 and 1.24 g/L), 5 heterozygous Pi*Z (allelic frequency 0.06, serum concentration between 0.79 and 0.97g/L) and 5 heterozygous $PiM_{Procida}$ (allelic frequency 0.06, serum concentration 0.74 g/L); one patient turned out to be composite heterozygous Pi*Z/$PiM_{Procida}$ (serum concentration 0.24g/L). The Procida variant is a rare and unique variant of the SERPINA1's gene which originates from a medium-sized deletion that involves the removal of a coding part of SERPINA1's genes, namely the part that – under natural circumstances - enables the A1AT protein's synthesis. It can have an important impact on the phenotype: we have indeed found that it involves a strong lowering of serum levels of A1AT even when not accompanied by the more frequent variations S and Z. We could observe in fact, its presence in eterozygosity lowers serum levels by 50% compared to the wild type, whilst its contribution to the phenotype becomes more important when in association with the more common variant Z.

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## SERUM MARKERS OF MITOCHONDRIAL DYSFUNCTION IN MELAS SYNDROME CORRELATE WITH BRAIN METABOLIC, STRUCTURAL AND MICROSTRUCTURAL IN VIVO ALTERATIONS

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MELAS (mitochondrial encephalomyopathy lactic acidosis and stroke-like episodes) syndrome is a rare multi-system disorder associated with mithocondrial-TL1 gene mutations. It's pathogenesis is not yet fully elucidated. Several studies pointed to the nitric oxide (NO) pathway dysfunction. Serum L-arginine, a NO-precursor, is reduced in MELAS and its administration is proposed to decrease stroke-like episodes frequency and severity. We examined in vivo neuro-axonal degeneration with bio-molecular markers such as proton MR spectroscopy ($^1$H-MRS) N-acetyl aspartate (NAA) content or microstructural diffusion weighted (DWI) parameters, and explored correlations with serum markers.
We enrolled 23 patients with MELAS molecular diagnosis (42±12yrs, 13M) and matched healthy controls (HC). The MR protocol (1.5T) included single voxel $^1$H-MRS in normal-appearing regions (medial parieto-occipital gray matter, POGM; parieto-occipital white matter, POWM; cerebellar hemisphere). NAA content relative to Creatine (Cr) and its diagnostic accuracy were evaluated. Voxel-based morphometry was performed on high resolution T1-w images. DWI voxelwise analysis was performed for mean diffusivity (MD) and fractional anisotropy (FA). Patients' serum concentrations of lactate, alanine and arginine were measured.
MELAS patients had lower NAA/Cr in POGM ($p=0.006$), POWM ($p=0.007$) and cerebellum ($p<0.001$). POGM lower NAA/Cr negatively correlates with lactate acidemia ($r=-0.682$, $p=0.021$). Cerebellar NAA/Cr reduction was the best discriminator (acc. 94%, sens. 83%, spec. 92%). Cerebral and cerebellar widespread GM loss was detected, negatively correlating with lactate acidemia (cerebral: $r=-0.56$, $p=0.045$) and positively with decreased L-arginine (cerebral: $r=0.69$,$p=0.018$, cerebellar: $r=0.62$, $p=0.040$). The main brain white-matter tracts showed diffuse microstructural alterations. Higher MD values positively correlated with alanine ($r=0.63$, $p=0.039$).
In conclusion, cerebellar in vivo metabolism, NAA/Cr ratio, is a valuable supplemental tool to guide MELAS diagnosis in clinical practice. The correlations between brain alterations detected by advanced and non-invasive MRI and serum metabolic markers suggest an interplay between mitochondrial and NO pathways in the mechanism leading to neurodegeneration.

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## SEARCHING FOR BLOOD BIOMARKER OF ALZHEIMER'S DISEASE: A COMPARATIVE STUDY AMONG MECHANICAL PROPERTIES OF RED BLOOD CELLS, BIOCHEMICAL PARAMETERS AND NEUROLOGICAL TESTS

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In this work we provide a comparative study of the mechanical response of red blood cells (RBC) obtained from 30 patients diagnosed with Alzheimer's disease (Group I) and 30 age-matched healthy subjects (group II) with the aim to identify a blood-derived mechanical biomarker of the pathology. To this purpose the viscoelastic response of RBCs at the cell level was systematically compared with biochemical parameters and neurological examinations. The RBC viscoelastic response was investigated using AFM in the force spectroscopy mode through the acquisition of force distance curves and creep-relaxation curves, which allowed us to measure cell relaxation times, cell rigidity and cell viscosity [1]. Our measures demonstrated that RBCs obtained from the pathological group display a significantly altered biomechanical response with respect to the healthy group. The performance of mechanical parameters in distinguishing between the two groups was assessed using receiving operator characteristic (ROC) curves, showing very large areas under the curves for selected biomarkers (AUC > 0.9). A more in-depth analysis of the pathological group showed a large positive correlation between: (i) RBC viscosity and rigidity and the grade of the pathology as measured with the mini-mental state examination and (ii) fibrinogen serum levels and RBC creep relaxation times. A significant negative correlation was found between: (i) fibrinogen serum levels and RBC rigidity and (ii) iron serum level and cell viscosity.
Taken together, our results showed that the combined use of biomechanical and biochemical parameters can be of potential use in the diagnosis and monitoring of Alzheimer's disease. [1] Ciasca, G., et al., Mapping viscoelastic properties of healthy and pathological red blood cells at the nanoscale level. Nanoscale, 2015. 7(40): p. 17030-17037.

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**RELATIONSHIP BETWEEN AMYLOID BETA42/40 RATIO AND APOE GENOTYPE IN PATIENTS WITH MILD COGNITIVE IMPAIRMENT**

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Amyloidβ 42 (Aβ42), total Tau (T-tau) and Tau phosphorylated (P-tau) represent the core CSF biomarkers for Alzheimer Disease (AD) diagnosis, with a slight superiority of CSF Aβ42/40 ratio with respect to Amyloidβ 42 (Aβ42) alone. ApoE e4 is known to be the most common genetic risk factor for AD and it promotes beta-amyloid deposition in the brain. Nevertheless, its influence on diagnostic performance of Aβ42/40 ratio for AD has not been fully elucidated yet. The aim of the study is to assess the impact of CSF biomarkers to distinguish AD from non-AD in a routinely diagnostic path and determine the influence of ApoE genotype on the diagnostic performance of Aβ42/40 ratio.
We analyzed data from 98 consecutive patients (51% M, 68±8y) affected by cognitive impairment, who underwent a complete diagnostic assessment (i.e. neurological and cognitive evaluation, structural neuroimaging and 18F-FDG-PET), including CSF biomarkers determination. An independent neurologist, blind to biomarkers concentration, classified patients in AD and non-AD. ApoE genotype was assessed by Real-Time PCR. CSF Aβ42 and Aβ40 were measured by CLEIA and ELISA, respectively. Comparison between median Aβ42/40 ratio was performed by Mann-Whitney test. Diagnostic accuracy of Aβ42/40 ratio was tested by ROC analysis. Statistical analysis was carried out by MedCalc Software. Clinical diagnosis was available for 84 patients. The Area Under the Curve (AUC) of Amyloid beta 42/40 ratio, P-tau and T-tau for AD were 0.70 (95%CI: 0.59-0.79; P=0.0009); 0.66 (95%CI: 0.55-0.76; P=0.009) and 0.66 (95%CI: 0.55-0.76; P=0.008). The subjects carrying at least one ApoE e4 allele (n=32) had lower levels of Amyloid beta 42/40 ratio in comparison with no e4 carriers (n=66) (0.10 [0.06-0.13] vs 0.069 [0.05-0.08], P=0.0017) but no differences of Aβ42 or Aβ40 were detected. When only carriers of e4 allele were considered, Amyloid beta 42/40 ratio retains its ability to distinguish AD patients from non-AD patients.
ApoE genotype influences Amyloid beta 42/40 ratio levels, but not its diagnostic performance for AD. The introduction of ApoE genotyping in the diagnostic work-up of patients with MCI could be useful for the correct interpretation of CSF biomarkers.

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**PLASMA PROGRANULIN IN NEURODEGENERATIVE DISEASES: CAN HELP US?**

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Introduction: Many studies suggest that Progranulin (PGNR), a pleiotropic protein can act as a neuroprotective agent particularly as it may play different roles in the clearance of Aβ, in tau phoshorylation, in neuroinflammation as well as in neuron survival. PGNR levels are found to be decreased in patients with Alzheimer's disease (AD). Thus plasma PGNR's levels should be used as an accurate predictor of PGNR-related neurodegeneretion disorders.
Methods: Proganulin was measured in plasma samples of patients monitored for neurodegenerative diseases (MCI, FTLD and AD) with a request for CSF biomarkers assay. PGNR was measured using Mediagnost Progranulin ELISA kit, whereas CSF biomarkers (β-amyloid 1,40 and 1,42, total and phospho Tau protein) using Fujirebio ELISA assays. The results obtained were expressed as median (95%CI), Mann-Whitney (p) using Analyse.it program.
Result: 23 patients were recruited (11 males and 12 females, aging from 57 to 75 y). On the basis of the phospho-tau cut-off (normal < 60 pg/mL), the patients were classified as Normal Phospho-Tau Group (NPTG) and Pathologycal Phospho-Tau Group (PPTG). Progranulin values observed in PPGT are 28,8 (21,34 – 37,67) μg/L vs 24,27 (21,66-26,69) μg/L (p= 0,1179) in NPTG. In PPGT group CSF biomarkers demonstrate values lower than those observed in NPTG and in particular: β–amyloid 1,42: 487 ng/L (379-564) vs 590 ng/L (562-830) (p<0.0045); β-amyloid 1,40: 8354 ng/L (6160-12610) vs 7122 ng/L (p=0,3527); -phospho-Tau: 98 ng/L (78-121) vs 47 ng/L (44-51) (p< 0,0001); total-Tau 787 ng /L (537-983) vs 276 ng/L(201-383) (p<0,0001). Furthermore, we found an inversely proportional trend, between phosho-tau and progranulin values in PPGT group.
Discussion and conclusion: Our preliminary data confirmed the possible protective role of progranulin particularly in patients showing pathological values of phospho-tau protein. The reverse correlation between progranulin and phospho-tau protein values in PPGT seems to confirm the hypothesis that the progranulin reduction (probably induced by up-regulation of cyclin-depedent kinases) can lead to the hyperphosphorylation of tau and the formation of neurofibrillary tangles.

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## K-INDEX CORRELATES WITH OCB FOR IMPROVE CEREBROSPINAL FLUID ANALYSIS

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Synthesis of immunoglobulins by CNS plasma cells is central in Multiple Sclerosis (MS) pathogenesis. The quantification of free light chains (FLCs) and albumin in serum and cerebrospinal fluid (CSF) allows calculation of the FLC index. An elevated index can support a diagnosis of MS and identifies Clinical isolated syndrome (CIS) patients with risk of MS conversion. The diagnosis of MS is still based on clinical criteria, magnetic resonance (MRI) flags and the detection of oligoclonal bands (OCBs). Recently the kappa free light chains (kFLCs) have been proposed as a diagnostic biomarker. The aim of this work was to calculate kFLC index of all samples sent to our laboratory with request of OCBs for suspected MS. We analysed serum and CSF samples of 120 patients from neurological Day Hospital. Before, CSF and serum FLCs concentrations were measured by quantitative turbidimetric immunoassay Freelite® (The Binding Site Ltd, UK) and then, on selected CSF and serum samples with Kindex>10,8 (our previous work), OCBs were determined by immunofixation (Hydragel 9 CSF kit isofocusing, Sebia Italia). Afterwards, OCBs analysis was compared to all samples against kFLC index with ROC analysis for diagnostic tests sensitivity and specificity. The statistical computing and graphics were performed with R-Project Statistical software.The final diagnosis of these patients , based on clinical criteria, were: 70 affected by MS and 50 with Other Neurological Diseases (ONDs).We obtained a kFLC index cut-off of 10,8 for MS diagnosis with sensitivity and specificity of 93% and 95% respectively , with ROC analysis of all calculated kFLC index. The diagnostic sensitivity and specificity of the OCBs were 94% and 90%, as shown by the ROC curve. The statistical computing of kFLC index in MS patients (70± 44,5), in ONDs ( 5,5± 3).Our results suggest to use of FLCs quantification assay for select initially samples that need OCBs analysis.This automated laboratory method is rapid and reliable, reducing long manual procedures and variability in result interpretation. FLCs assay may be easily performed as initial screening upon samples request submission.

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## HEMATOLOGICAL ALTERATIONS IN PATIENTS WITH RELAPSING REMITTING MULTIPLE SCLEROSIS SUBJECTED TO NATALIZUMAB THERAPY

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Introduction: Natalizumab is a drug for the treatment of patient with Relapsing Remitting Multiple Sclerosis (RRMS). Its action prevents the binding between VLA-4 with VCAM-1 implaying a reduction of the leukocytes migration through the endothelium, reducing the inflammatory infiltrate. However, the use of this monoclonal antibody appears to cause an increase in circulating stem cells in patients subjected to this therapy. The aim of the study is to investigate the alteration of hematological parameters, including cell population data, in patients with natalizumab therapy.
Materials and Methods: 443 peripheral blood samples were analyzed with the automated analyzer sysmex XN-9000 and the microscopy revision were done with the sysmex DI-60 digital morphology analyser. The outcomes of interest have been analyzed with a stratified statistical analysis for natalizumab use or not. The quantitative variables were evaluated as mean, standard deviation, median, interquartile range and the p-values were considered significnant only below $\alpha$=0,05.
Results: From the analysis of the results obtained for the leukocyte parameters it is emerging as the values are significantly higher in patients treated with natalizumab compared to the control groups. Erythroblasts ($0.02*10^9$/L), monocytes ($0.68*10^9$/L), lymphocytes ($4.17*10^9$/L), immature granulocytes ($0.04*10^9$/L), eosinophil ($0.26*10^9$/L), basophils ($0.06*10^9$/L) and the LY-WY parameter (993.30) are increased in treated patients compared to naives. The counting of monocytes and immature granulocytes is significantly increased in natalizumab patients compared to patients treated with other drugs (p-value 0.001 and 0.0001 rispectively). It has been observed that the treatment with natalizumab is the only that induce an increase of eosinophils, which decreases in patients with RRMS ($0.26*10^9$/L vs $0.14*10^9$/L) and basophils, that decrease into patients under others therapy ($0.06*10^9$/L vs $0.03*10^9$/L). The increase in leukocytes count in that sense finds confirmation in literature data and appears attributable to the inibition of VLA-4, which sequests the cells in the peripheral circle, preventing the correct homing.
Conclusions: The long-term consequences on hematopoiesis are not been defined yet so other studies are required in order to evaluate any other alterations.

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**DIAGNOSTIC PERFORMANCES OF BIOCHEMICAL TESTS FOR MULTIPLE SCLEROSIS**

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Aim In addition to the detection of oligoclonal bands, biochemical analyses (focused on intrathecal immune reaction) on cerebrospinal fluid (CSF) play a relevant role in diagnostic work-up of Multiple Sclerosis (MS). Aim of this work is to investigate their diagnostic performances in a large population of patients.Patients 386 patients were consecutively enrolled. They underwent lumbar puncture (LP) for CSF biochemistry from Jan. 2014 to Jan. 2019. Diagnoses were collected by a blind neurologist, and compared with initial suspicion. 127 (32,9%) were diagnosed of MS according to the 2017 McDonald criteria; 118 (30,6%) of other neurological inflammatory diseases (ID); 141 (36,5%) of non-inflammatory neurological diseases (NID). Methods All samples were immediately processed. Albumin, Immunoglobulin (IgG) and free light chains (FLC) were measured by nephelometry (BN II, SIEMENS). Statistical analysis (AUC, sensitivity and specificity) was performed on received-operating curve (ROC) using a VassarStat software. Absolute concentrations of IgG, KFLC and LFLC in CSF; the ratioes KFLC/LFLC, KFLC/IgG, LFLC/IgG in CSF; IgG index (IgG quotient/Albumin quotient), KFLC index (KFLC quotient/Albumin quotient), LFLC index (LFLC quotient/Albumin quotient), KFLC index/LFLC index were investigated.Results For each parameter AUC, sensitivity and specificity are sequentially reported: KFLC (0.924; 87.8%; 81.8%); LFLC (0.729; 60.8%, 70.9%); IgG (0.630, 68.9%, 51.0%); KFLC/LFLC (0.844, 82.4%, 78.4%); KFLC/IgG (0.943, 95.9%, 67.6%); LFLC/IgG CSF (0.669, 70.3%, 49.7%); IgG index (0.845, 79.7%, 73.6%); KFLC index (0.955, 98.7%, 70.1%); LFLC index (0.842, 90.0%, 56.8%); KFLC index/LFLC index ratio (0.840, 85.0%, 54.4%). KFLC/IgG and KFLC index were highly correlated for both all patients ($R^2$=0.711, p<0.0001) and MS patients ($R^2$=0.729, p<0.0001).Conclusion Among the tests investigated, KFLC index revealed highest sensitivity, followed by the CSF KFLC/IgG ratio. IgG index (Link-index), although still employed, showed a low sensitivity. This confirms the strong diagnostic power of KFLC index. Since both serum and CSF are required to performed this test, CSF KFLC/IgG ratio a good alternative, since it can be easily applied on stored CSF samples when serum is not available.

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**MISURA IMMUNOTURBIDIMETRICA DELLE kFLC LIQUORALI: L'ESTENSIONE DELLA CURVA DI CALIBRAZIONE PUÒ INCREMENTARE L'EFFICIENZA DIAGNOSTICA PER LA SCLEROSI MULTIPLA?**

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Introduzione: Il liquor (CSF) riflette meccanismi di attivazione del sistema immune nel distretto cerebrale e costituisce una preziosa fonte di biomarcatori di patologie infiammatorie del SNC. Numerosi studi hanno indagato le applicazioni della misura quantitativa delle catene leggere libere kappa (kFLC) liquorali e degli indici derivati (kFLC-Index) nell'iter diagnostico delle patologie infiammatorie demielinizzanti del SNC.
Razionale: Obiettivo dello studio è valutare le performances diagnostiche del kFLC-Index rispetto al metodo qualitativo gold standard (OCB).
Materiali e Metodi: Nella prima fase dello studio sono stati arruolati 66 pazienti afferiti all'UO di Neurologia dell'Ospedale San Raffaele nel 2018, suddivisi in tre gruppi: soggetti con SM e soggetti di controllo con patologie infiammatorie (IND) e non infiammatorie (NIND) del SNC. Le analisi sono state eseguite sul turbidimetro Optilite con il kit diagnostico kappa Freelite™ (The Binding Site) che utilizza un'unica curva di calibrazione per siero, urine e CSF con LoQ dichiarato di 0.31 mg/L. Mediante diluizioni manuali aggiuntive del calibratore del kit è stata costruita un'espansione della curva di calibrazione fino a un LoQ calcolato di 0.07 mg/L. Nella seconda fase dello studio è stato applicato il valore di cut-off del kFLC-Index per valutare l'eventuale presenza di sintesi intratecale (SI) in un sottogruppo di 21 pazienti SM con OCB negative.
Risultati e Discussione: Il 47% dei CSF analizzati con curva di calibrazione standard presenta concentrazione di kFLC < LoQ, pertanto è stata applicata l'estensione manuale della curva standard. Le misure di kFLC liquorali e dei relativi indici hanno evidenziato differenze statisticamente significative fra il gruppo SM rispetto a IND e NIND. Il valore di cut-off del kFLC-Index ottenuto mediante curva ROC è 5.8 (sensibilità 0.90, IC 95% 0.74-0.98 e specificità 0.77, IC 95% 0.60-0.90). Il kFLC-Index presenta maggiore sensibilità diagnostica rispetto alle OCB (90% vs 84%). In 11/21 pazienti SM con OCB negative (52%) è presente SI di kFLC.
Conclusioni: Dai risultati emerge l'opportunità di aumentare la sensibilità analitica del metodo applicato al CSF per impiegare il kFLC-Index come test complementare alle OCB nell'iter diagnostico della SM.

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## CALCITE KIDNEY STONE EXTRACTED DURING A URETERORENOSCOPY PROCEDURE, A CASE REPORT

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We report a case of a kidney stone composed by calcite (calcium carbonate), extracted during a urinary tract surgery procedure in a 47-years-old male patient who had no other comorbidities and had no ongoing therapy.
The patient was admitted in a large peripheral hospital where a flexible ureterorenoscopy was performed. The stone was extracted from the lower kidney calyx and no postoperative complications occurred.
The stone was then sent to our Laboratory Department and analyzed using a FTIR spectrometer Nicolet iS5 (Thermo Fisher Scientific); the resulting spectra are routinely compared to the Kidney Stone Basic library and Daudon library as spectral reference libraries. The patient's spectrum had a match of 90.57% with a spectrum in the Daudon library containing "95% calcite + gypsum + proteins".
Our laboratory receives stone samples both from our hospital and from hospitals of a wide area in the North-East of Italy. Kidney stones are usually composed by whewellite, wheddelite, monohydrate or dihydrate uric acid, dahlite, carbonite apatite or struvite and cystine.
Calcium carbonate is commonly found in rocks such as marble. For in-vivo organisms is the main component of, e.g., parts of marine organisms and husks of chicken eggs. Also, calcium carbonate can be found in human salivary and biliary stones. Despite calcium carbonate kidney stones can easily be found in herbivores such as horses and rabbits, they are really rare to find in humans, maybe because higher pH values than human reference values are needed to crystallize.
With a literature review we found only a limited number of cases reported in humans to have a pure calcite calculus. Moreover, calcium carbonate stones have been sometimes reported as artifacts, usually sent by patients with psychiatric diseases.

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## UROLITHIASIS IN NORTH-EAST ITALY, DATA ANALYSIS FROM FTIR SPECTROSCOPY RESULTS: AN UPDATE

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Introduction: Urolithiasis is estimated to have prevalence rates that vary from 1% to 20%, rates >10% are found in high income countries. Beside contributing disorders, stone incidence depends on several genetic, dietary, ethnic and environmental factors. The 2019 update of the European Association of Urology (EAU) Urolithiasis Guidelines emphasised environment by adding high ambient temperatures and chronic metal exposure as high-risk factors to stone formation.
Materials and methods: We collected data of kidney stone samples sent from a wide area of North-East of Italy to our Laboratory Department from January 2016 to May 2019; all of them were analysed using a FTIR spectrometer Nicolet iS5 (Thermo Fisher Scientific) and the resulting spectra were routinely compared to Kidney Stone Basic and Daudon's as spectral reference libraries.
We grouped samples upon the geographical position of the sending hospitals: group VETV (provinces of Venice and Treviso), group PDRO (provinces of Padua and Rovigo), group VRVI (northern Vicenza zone and western Verona zone), group BZ (province of Bolzano).
Results: A total of 4356 stones were evaluated. The overall most prevalent stones were made of Calcium oxalate (Whewelite, Wheddelite and their mixtures) 63.3%, Uric acid 14.9% and Dahlite 11.6%. Other stones were composed either singly or of a mixture of the previous materials and of Brushite, Struvite, Ammonium urate and Cystine. Calcium oxalate stones were statistically significant associated with geographical groups ($p = 0.006$), being 57.6% and 67.5 % the prevalence of VRVI and of BZ.
Discussion and conclusion: EAU guidelines recommend stone analysis should be performed in all first-time stone formers; as our laboratory receives all stones extracted in public hospitals of 7 different provinces in the North-East of Italy, a high volume of information might be used to produce inferences on prevalence of risk factors for urolithiasis in the overall population. Although we could not exclude a possible epidemiologic bias due to different hospitals' complexity, overall collected data showed a different prevalence distribution of Calcium Oxalate stones in Veneto region with respect to Bolzano province.

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## PLA2R ANTIBODY IN THE DIAGNOSIS AND MONITORING OF PRIMARY MEMBRANOUS NEPHROPATHY: STRENGTHS AND WEAKNESSES

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Membranous nephropathy (MN) is an organ-specific autoimmune disease in which autoantibodies are directed against podocyte antigens, resulting in widespread subepithelial immune deposits. Phospholipase A2 receptor (PLA2R) and thrombospondin type-1 domain-containing 7a (THSD7A) were identified as two major pathogenic podocyte antigens involved in idiopathic MN (IMN). Approximately 70% and 3% of patients with IMN have circulating anti-PLA2R and anti-THSD7A antibodies, respectively, which have been widely used as biomarkers in clinical practice for diagnosis, treatment and prognosis evaluation. We introduced the research of anti-PLA2R and anti-THSD7A antibodies in our laboratory on May 2018. At first, we detected serum anti-PLA2R and anti-THSD7A antibodies only by indirect immunofluorescence assay (IIF) with initially serum dilution 1:10. When positive, serum patient was diluted 1:100 and 1:100 in order to have a semiquantitative results. Recently, we have introduced also enzyme-linked immunosorbent assay (ELISA)for quantification of serum anti-PLA2R antibody and the results are considerate negative for <14 RU/mL, 14 ≤ equivocal ≥ 20 RU/ml and positive for >20 RU/mL. From May 2018 to May 2019 we analyzed 158 serum patient with diagnosis of MN or suspected MN by IIF. 43 patients were positive for anti-PLA2R antibody. From April 2019 we introduced also the ELISA kit for anti-PLA2R antibody and we are comparing the results with IIF. We analyzed 30 serum patients with IIF and ELISA: 24 were negative, 6 were positive for both tests, 6 were negative for ELISA but weakly positive with IIF. There are few studies about comparison for IIF and ELISA in the detection of anti-PLA2R antibodies and they show similar performance with advantage of quantitative result for ELISA, nevertheless with ELISA we can not detect anti-THSD7A antibodies. Moreover, there are some weaknesses about PLA2R ELISA cut-off especially for borderline results. We suggest the screening of MN by IIF in order to research both anti-PLA2R and anti-TSHD7A antibodies. If anti-TSHD7A antibody is positive we can continue follow-up by IIF, instead if only anti-PLA2R is positive we can perform follow up with ELISA paying attention to the cut-off and working closely with the nephrologists.

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## THE EFFECTS OF FIBROBLAST GROWTH FACTOR 23 AND PTH ON VITAMIN D METABOLISM IN PATIENTS WITH KIDNEY TRANSPLANT

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FGF23 is secreted in response to dietary phosphate load or increase in $1,25(OH)_2$VitD. Both in normal subjects and in patients with chronic kidney disease, FGF23 induces phosphaturia, reduces the net production of $1,25(OH)_2$VitD and targets the parathyroid glands to decrease PTH secretion. In this work, we analysed the relationships between the serum profiles of both FGF23 and PTH and the patterns of Vitamin D metabolites, in patients with kidney transplant. Ninety-four patients were studied. Fifteen healthy subjects(eGFR>90mL/min) were assayed to confirm normal values for FGF23. In plasma and serum, intact FGF23, intact PTH, 25(OH)VitD and $1,25(OH)_2$VitD were determined by immunometric assays (FGF23 and VitD on Liaison®, Diasorin; PTH on Immulite 2000Xpi®, Siemens). In order to investigate on the effects of both FGF23 and PTH on $1,25(OH)_2$VitD synthesis, out of 94 patients 47 were selected. The ratio between $1,25(OH)_2$VitD and 25(OH)VitD ("VitD ratio") was considered as index of active Vitamin D net production. In healthy subjects, FGF23 levels were 55.8±11.7 pg/mL (range 35.9-80.2). In all patients, serum FGF23 was 109.8±119.7 pg/mL (range 78.9-758.5) and correlated inversely with eGFR ($p<0.01$) and positively with serum $PO_4$ ($p<0.01$). FGF23 levels were within the normal range in 100% (5/5) of patients with eGFR≥90 mL/min, 74% (17/23) of patients with eGFR 60-89 mL/min, 43% (25/58) of patientts with eGFR 30-59 mL/min and 12.5% (1/8) of patients with eGFR<30mL/min. In the subgroup of 47 patients with eGFR≥30 mL/min and without VitD deficiency, serum FGF23 was 80±42.4 pg/mL and PTH was 106±65 pg/mL; $1.25(OH)_2$VitD levels were independent of those of 25(OH)VitD (p=ns). In a multivariate analysis, VitD ratio was positively associated with eGFR ($p<0.001$) and PTH ($p<0.001$), and negatively with FGF23 ($p=0.022$) and $PO_4$ ($p=0.028$). In patients with kidney transplant, serum $1,25(OH)_2$VitD levels were independent of those of 25(OH)VitD and were modulated negatively by both FGF23 and $PO_4$ and positively by PTH. About 54% of all patiets with kidney transplant showed an increase of serum FGF23 levels above normal limits. This may inhibit the activity of kidney 1$\alpha$-hydroxylase and affect the synthesis of calcitriol.

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## METABOLIC EFFECTS OF CHOLECALCIFEROL SUPPLEMENTATION IN KIDNEY STONE FORMERS WITH VITAMIN D DEFICIENCY

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Vitamin D (VitD) plays an important role in maintaining bone health and its deficiency was associated with many disorders. However, clinicians are still reluctant to treat VitD deficiency patients with kidney stones, fearing that this may increase intestinal calcium absorption, urinary calcium excretion and the risk of calcium stone formation. In this work we investigated whether cholecalciferol (CHO) supplementation may increase the risk of stone recurrence in patients with calcium nephrolithiasis and VitD deficiency. Thirty-three calcium stone formers with VitD deficiency were considered. Calcium excretion and urine supersaturation with calcium oxalate (ßCaOx) and brushite (ßbsh) were evaluated, both before and after CHO supplementation. 25(OH)VitD and 1,25(OH)2VitD were determined by immunometric assay on Liaison® XL (Diasorin SpA); PTH was determined on Immulite 2000Xpi® (Siemens HealthCare). In daily urine, total nitrogen (TNE) and sulphate were measured as indices of protein intake; sodium as index of its dietary intake; the difference between cations and anions as index of intestinal absorption of alkali (Alk) and net acid excretion (NAE) as index of acid-base balance. NE, sulphate, sodium, Alk and NAE did not change during the study (p=ns). After the CHO treatment, both serum calcium and phosphate did not vary significantly (p=ns); 25(OH)VitD increased from 11.8±5.5 to 40.2±12.2 ng/mL ($p<0.01$); 1,25$(OH)_2$ VitD increased from 41.6±17.6 to 54±16 pg/mL ($p<0.01$); PTH decreased from 75±27.2 to 56.7±21.1 pg/mL ($p<0.01$); urinary calcium increased from 2.7±1.5 to 3.6±1.6 mg/Kg b.w. ($p<0.01$). Eventually, after CHO, ßbsh increased from 0.9±0.7 to 1.3±1.3 ($p=0.02$) whereas ßCaOx did not vary significantly. Before CHO supplementation, only 6/33 patients (18.2%) showed hypercalciuria, whereas 13/33 patients (39.4%) had hypercalciuria after CHO supplementation ($pX^2=0.03$). In stone formers, CHO supplementation may increase calcium excretion, or reveal an underlying condition of absorptive hypercalciuria previously hidden by VitD deficiency. Eventually, that may increase stone forming risk. If VitD supplements are prescribed to kidney stone formers, a careful monitoring of urine metabolic profile is warranted, in order to customize the metaphylaxis accordingly.

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## BIOMARCATORI DI TURNOVER OSSEO IN PAZIENTI CON INSUFFICIENZA RENALE CRONICA

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I pazienti con insufficienza renale conica (CKD) hanno una densità minerale ossea inferiore rispetto alla popolazione generale con un aumento della probabilità di fratture e ricadute sulla qualità di vita e mortalità. Presentano, inoltre, un aumento delle calcificazioni vascolari e dei tessuti molli con aumento di ricoveri e decessi per eventi traumatici e cardiovascolari. E' quindi di notevole importanza clinica la disponibilità di biomarcatori di turnover osseo la cui concentrazione sierica non sia influenzata dalla funzionalità renale, facilmente applicabili alla pratica clinica, utili a stratificare il rischio clinico di questi pazienti. Il propeptide aminoterminale del procollagene di tipo I(PINP) è il marcatore più sensibile e specifico di formazione dell'osso; è utile osservarne le variazioni interindividuali nel tempo, in corso di terapia. La fosfatasi acida tartrato resistente (TRAcP5b) è un marker di riassorbimento osseo specifico per la matrice ossea e l'isoforma 5b è propria degli osteoclasti attivi. MGP è un potente inibitore della calcificazione tissutale. Presenta residui di gamma glutammato sottoposti a carbossilazione vitamina K dipendente e residui di serina sottoposti a fosforilazione. Le forme fosforilate e carbossilate sono quelle biologicamente attive. Abbiamo analizzato la concentrazione di questi markers in 75 pazienti (51 uomini e 24 donne), età media 64.9 anni, emodializzati. Il 28% dei pazienti ha riportato valori di MGP inferiori a 300 pmol/L (v.n. 300-824 pmol/L), il 35% degli uomini e il 64% delle donne in postmenopausa ha mostrato valori di TRAcP al di sopra del range (v.n. 1.4-6.1 U/L gli uomini e 1.2-4.8 U/L le donne); anche i valori di PINP nel 40% dei pazienti supera il range di normalità (v.n. 27.7-127.6 ng/ml). Complessivamente circa il 60% dei pazienti in emodialisi mostra i segni bioumorali di un intenso rimaneggiamento osseo, con associati elevati livelli di PTH e basse concentrazioni di inibitori delle calcificazioni vascolari. I nostri dati preliminari suggeriscono che MGP,TRAcP, PINP possano contribuire a caratterizzare queste alterazioni e a personalizzare il trattamento farmacologico con riduzione del rischio cardiovascolare e della mortalità dei pazienti in dialisi.

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**POST-STREPTOCOCCAL GLOMERULONEPHRITIS COMPLICATED WITH TRANSIENT RENAL FAILURE IN A 10-YEAR-OLD BOY - LABORATORY DIAGNOSTICS AT THE FOREFRONT**

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Introduction: Differential diagnosis of renal diseases in patients presenting with acute haematuria is challenging, especially in pediatric population. Acute post-streptococcal glomerulonephritis (PSGN) is the most common cause of nephritic syndrome in children. It is a glomerular immune-complex disease usually occuring two to three weeks after a group A beta hemolytic streptococcal (GAS) infection. Clinical manifestations include asymptomatic to gross hematuria, peripheral edema and hypertension. Laboratory investigations usually reveal hematuria, proteinuria and low serum C3 level, while the microbiological tests provide evidence of the previous GAS infection. We present a 10-year-old boy with macroscopic haematuria, who was diagnosed with PSGN complicated with acute renal failure.
Methods: A case report.
Results: A 10-year-old boy presented with an acute onset of macroscopic haematuria, dysuria and fever. Initial laboratory tests revealed high serum Creatinine (1.26 mg/dL), Blood Urea Nitrogen (36 mg/dL) and Potassium (5.26 mmol/L) and low eGFR (57 mL/min/1.73 m2). Those findings were consistent with the acute renal injury, so intravenous rehydration therapy was started. On urinalysis heamaturia (erythrocytes: 1555/μL), proteinuria (681 mg/L) and leukocyturia (286/μL) were detected. Urine erythrocyte morphology evaluation showed 45% of dismorphic red blood cells. Autoantibodies (Abs) against antinuclear antigens (ANA), anti-dsDNA Abs, anti-myeloperoxidase and anti-proteinase 3 Abs, were all negative. CRP and WBC were not significantly altered. High anti-streptolysin-O titer (989 UI/mL), low C3 serum level (40 mg/dL) and throat swab culture positive for GAS, led to the final diagnosis of PSGN. Promt antibiotic therapy was introduced, which was followed by marked clinical improvement over the course of one week. Regular clinical and laboratory follow-up including urinalysis was scheduled.
Conclusions: Most children with PSGN have an excellent prognosis when accurately diagnosed and treated. With this case we highlight how specialists in laboratory medicine can play an important role in guiding the diagnostic process through an appropriate use of the tests, speeding up the treatment management of the patients and improving the clinical outcome.

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**QUANTITATIVE EVALUATION OF URINARY BENCE JONES PROTEIN USING NEPHELOMETRIC AND IMMUNOTURBIDIMETRIC ASSAYS IN SPOT URINE SAMPLES**

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Aim: The detection of Bence-Jones protein in urine by qualitative methods is generally followed by the quantification of kappa or lambda free light chains (KFLC or LFLC) with quantitative (densitometric, nephelometric or immunoturbidimetric) assays and expressed as mg/24 hours. This implies that a 24 hours collection of urine should be performed, with all the associated pitfalls. Aim of this work was to evaluate the possibility that nephelometric and immunoturbidimetric assays performed in spot urine samples and corrected for urinary creatinine could replace the assays performed in 24 hours urine samples.
Patients and methods: Three groups of patients were investigated: BJ negative (BJ-), BJ/KFLC positive (BJK +), BJ/LFLC positive (BJL+). For each patient FLC concentration was measured in urine using both nephelometry (BNII, Siemens) and immunoturbidimetry (Optilite, Binding Site) in either a spot and a 24 hours samples. Urinary creatinine was measured using enzymatic assay (Siemens).
Results: KFLC (nephelometry) were 78.6±70.4, 1562±2298 and 57.2±45.5 mg/24 hours in BJ-, BJK + and BJL+ respectively; KFLC (immunoturbidimetry) were 83.0±76.1, 1860±3012, 53.9±37.8 mg/24 hours. LFLC (nephelometry) were 16.0±15.1, 28.4±56.1 and 396±438 mg/24 hours in BJ-, BJK+ and BJL+ respectively; LFLC (immunoturbidimetry) were 3.6±3.0, 8.0±18.1, 2046±4588 mg/24 hours. When corrected for urinary creatinine, KFLC (nephelometry) were 6.3±4.4, 154±243 and 9.5±9.1 mg/24 mg creatinine in BJ-, BJK+ and BJL+ respectively; KFLC (immunoturbidimetry) were 6.2±5.5, 178±331, 9.7±8.1 mg/mg creatinine. LFLC (nephelometry) were 1.3±0.9, 4.1±7.8 and 145±327 mg/mg creatinine in BJ-, BJK+ and BJL+ respectively; LFLC (immunoturbidimetry) were 0.29±0.27, 1.26±2.91, 1299±3547 mg/creatinine. A high correlation was found between FLC expressed as mg/24 hours and expressed as mg/mg creatinine (R2=0.793 and R2=0.802 for KFLC measured by nephelometry and immunoturbidimetry and R2=0.787 and R2=0.922 for LFLC measured by nephelometry and immunoturbidimetry; in all cases $p<0.0001$).
Conclusion: These results suggest that the evaluation of free light chains in spot urine corrected for urinary creatinine could be a rather good surrogate for the quantitative measure in 24 hours samples in BJ positive patients.

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**SERUM DJ-1 LEVELS IN ENDURANCE ATHLETES**

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Aim: DJ-1, a highly conserved protein, is involved in many biological processes such as Parkinson disease, cancer, mitochondrial regulation and regulation of oxidative stress, by directly quenching Reactive Oxigen Species (ROS). It has been demonstrated that running wheel exercise increased DJ-1 muscular plasma and brain expression in mice. Moreover serum DJ-1 level was found to be increased after 2 weeks of diet and exercise in Japanese women compared to controls, and this change was associated with reduction of biomarkers of metabolic syndrome. We hence designed this study for investigating the effect of a half-marathon on DJ-1 concentration in serum of healthy recreational athletes and sedentary controls.
Methods: 29 amateur runners and 28 sedentary controls were enrolled. Serum concentration of DJ-1 was measured wth ELISA kits developed by R&D (Minneapolis, USA). Differences were assessed with Mann-Whitney test or Wilcoxon signed-ranks test when appropriate, whilst correlation analyses were performed with Spearman's test. The results were corrected for the plasma volume (%PVC), calculated from pre- and post-exercise levels of hematocrit and hemoglobin.
Results: The baseline DJ-1 levels measured before the run (678.21 pg/mL; range, 189.94-9088.87 pg/mL) were nearly 5-fold increased compared to sedentary controls (104.86 pg/mL; range 33.53-4489.49 pg/mL; $p<0.0001$). DJ-1 levels were obviously higher in serum of patients immediately and after 3 hours of running than in the controls ($p<0.0001$). Immediately and 3 hours after the run, no significant variation of DJ-1 levels could be detected ($p=0.195$ and $p=0.875$ respectively).
Conclusions: The results of this study suggest that DJ-1 might chronically (but not acutely) change in response to exercise and lifestyle, perhaps as a consequence of oxidative stress. Reference: Yamane T, Murao S, Kozuka M, et al. Serum DJ-1 level is positively associated with improvements in some aspects of metabolic syndrome in Japanese women through lifestyle intervention. Nutr Res 2014;34:851-5.

P180
**DOSAGGIO AUTOMATIZZATO DEGLI OSSALATI PRESENTI IN UN CALCOLO URINARIO**

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Il nostro Laboratorio esegue attualmente sette dosaggi fotometrici (calcio, fosforo, magnesio, acido urico, ammonio, cistina e ossalato), effettua la visualizzazione dei carbonati e fa uso di un opportuno software in Excel estrapolante le più comuni componenti di un calcolo urinario. Si propone di dosare gli ossalati, presenti nel calcolo urinario, con una metodica a tre reagenti da noi adattata sull'analizzatore Viva E della ditta Siemens. Il principio biochimico si basa sull'azione dell'ossalato che decolora il complesso Ferro-solfosalicilico in ambiente tamponato (borato) ed evidenziato alla lunghezza d'onda di 505 nm.10 mg di calcolo frantumato sono trattati con 75 μL di acido solforico concentrato; dopo completa dissoluzione al vortex, si aggiungono 10 mL di acqua distillata. Dopo miscelazione e successiva centrifugazione, il sopra natante ottenuto si dosa sulla strumentazione biochimica sopra citata. A 220 μL di tampone borato (0,1 M pH 9,5) l'analizzatore aggiunge 30 μL di campione. Successivamente sono aggiunti 21 μL di cloruro ferrico esa-idratato (0,09 g/dL) e 21 μL di acido solfosalicilico (1 g/dL). Dopo un'attesa di circa 6,5 minuti si ricava la concentrazione dell'analita riferendoci ad una curva di calibrazione a 6 punti (modello spline cubico modificato) compresi tra 0 e 6,0 mmol/L di ossalato di sodio.La precisione 3x5 (3 replicati in 5 giorni) riferita ai controlli a titolo di 1,5 e 5,0 mmol/L di ossalato è di poco superiore al 2% . L'analisi di regressione di Passing-Bablok calcola l'equazione $y = -0,003 + 1,0183. x$ (la variabile indipendente rappresenta il metodo in uso Trinder della ditta LTA) senza evidenziare errore sistematico. L'elaborazione dati evidenzia un bias non significativo. Il metodo in prova è accettabile e presenta inoltre delle buone prestazioni, riferite alla concentrazione di 1,5 mmol/L, alla qualità analitica prefissata del 10% .La nuova metodica proposta, da noi costruita ed adattata su analizzatore biochimico, è affidabile, rapida, a costi ridottissimi e, rispetto alla metodica Trinder in uso, non necessita di diverse diluizioni del campione e principalmente non viene influenzata dalla eventuale contemporanea presenza dei fosfati nel calcolo. Vidali M, Tronchin M , Dittadi R. Protocollo per la comparazione di due metodi analitici di laboratorio. Biochimica clinica, 2016, vol. 40, n. 2: 129-42

P181
## CELL FREE DNA ISOLATION FROM PERIPHERAL BLOOD: IMPACT OF PRE-ANALYTICAL VARIABLES

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Background: Pre-analytical phase standardization is a key step in translation of ccfDNA analyses to clinic. We aimed to verify the effect on ccfDNA isolation of collection tube, processing time and method of extraction.
Methods: Blood from 10 donors was collected in $K_2$EDTA tubes (BD) and PAXgene Blood ccfDNA tubes (Preanalytix), kept refrigerated (4°C), centrifuged once after 2h or 72 h. ccfDNA extraction from plasma was performed by four commercial kits: Quick-cfDNA Serum & Plasma Kit (Zymo Research) (A), Maxwell RSC ccfDNA Plasma Kit (Promega) (B), QIAamp MinElute ccfDNA Midi Kit (QIAGEN) (C), Helix Circulating Nucleic Acid (Diatech Pharmacogenetics) (D). Starting plasma volume (4mL) and ccfDNA elution volume (80mL) were standardized for all kits. Extracted DNA quantity was determined by fluorimetric assay (Qubit® dsDNA HS Assay Kit, Thermofisher). Leukocyte gDNA contamination was evaluated as DNA integrity index by RealTime Alu PCR. ccfDNA recovery was measured by RealTime PCR, targeting exogenous DNA spiked in blood samples (TATAA Universal DNA Spike 166 bp kit, TATAA Biocenter AB).
Results: Extracted DNA concentration was 0.40 ± 0.45 ng/mL (mean ±SD) being significantly higher only in $K_2$EDTA tubes processed at 72 h (0.94 ± 0.58 ng/ mL) ($p<0.001$). DNA integrity index was 0.43 ± 0.26 (mean ± SD) being gDNA contamination significantly increased only in $K_2$EDTA tubes processed at 72 h (0.83 ± 0.18) ($p<0.001$).Mean ccfDNA recovery was 56.43 ± 31.55% being significantly associated with the extraction kit (A=38.14 ± 26.80%; B= 67.92 ± 31.27%; C=67.85 ± 31.98% and D=48.60 ± 25.96%) ($p<0.001$), independently from type of blood collection tube ($p=0.151$) and processing time ($p=0.468$).CONCLUSIONS. ccfDNA signals are stable ex vivo up to 72h before processing, independently from type of blood collection tube. ccfDNA contamination by leukocyte gDNA can be avoided by dedicated collection tubes. The protocol adopted for ccfDNA extraction influences its recovery.

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## HYDRASHIFT DARATUMUMAB ASSAY (DIRA) TO EVALUATE THE RESPONSE TO TREATMENT WITH DARATUMUMAB IN PATIENTS WITH MULTIPLE MYELOMA: CLINICAL CASES

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Background: Daratumumab, humanized IgG k monoclonal antibodies targeting CD38 (mAb) that has become increasingly in the treatment of Multiple Mieloma (MM), can interfere with serum electrophoresis (CZE) and Immunofixation (s-IFE). Clinical response criteria to treatment for MM include changes in serum and urine monoclonal protein (MP) levels evaluated by SPE and IFE, but the use of Daratumumab, which may result in a detectable MP, may cause misinterpreting in the response evaluation. A Daratumumab specific IFE reflex assay (DIRA) has been developed and can be utilized to remove interference and to promote a correct interpretation of clinical response to the treatment. We describe the interference by mAb in two patients affected by IgGλ MM and LCMM kappa respectively.
Methods: The first patient, 58 years old with IgGλ MM, received DARA-LENA treatment. After two cicles therapy MP strongly decrease. The second patient, 64 years old with LCMM kappa, after six cicles therapy with DARA-VD, showed appearance of MP in gamma fraction by CZE. The laboratory test used to value clinical response was: CZE, s-IFE, u-IFE, DIRA (SEBIA) and s-FLC (The Binding Site). Results: The CZE of first patient presents a light peak in gamma fraction confirmed by s-IFE to be IgGk e λ not quantifiable; DIRA confirmed the presence of IgGλ and eliminated interference by immunotheraphy; u-IFE negative, ratio k/λ 0,92: valuated as Very Good Partial Response. In the second patient s-IFE showed IgG kappa; DIRA confirmed interference by mAb; u-IFE negative; ratio k/λ 3,16: valueted as Complete Response.
Conclusion: The presented cases suggest that Daratumumab, dosed at therapeutic blood levels, can be detected as a monoclonal band in s-IFE and it may interfere with MP. The follow-up, that is part of the IMWG criteria to assess treatment response in patients with MM, can be misinterpreted. Moreover, the laboratory specialist needs to be informed when patients receive mAb in order to use strategies to eliminate s-IFE interference so as to favor a correct evaluation of response to the treatment. Bibliography Monitoring Multiple Myeloma patient treated with daratumumab:teasing out monoclonal antibody interference. Clin Chem Lab Med 2016.

P183
## DETERMINATION OF TETRAHYDROXYLATED METABOLITES OF CORTISOL AND CORTISONE BY LC-MS/MS: A COMPARISON BETWEEN TOTAL AND FREE COMPOUNDS

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Background and aim: The apparent mineralocorticoid excess (AME) is a rare genetic disorder caused by impaired activity of the enzyme 11β-hydroxysteroid dehydrogenase Type 2 (11βHSD2). The biochemical diagnosis is usually made by measuring total tetrahydroxylated metabolites of cortisol (THF and allo-THF) and cortisone (THE) expressed as THF+allo-THF/THE ratio after a step of hydrolysis and using home-made Gas Chromatography-Mass Spectrometry (GC-MS) techniques. We recently developed a LC-MS/MS method which was found to be a faster, reliable alternative to GC-MS for assessment of THF+allo-THF/THE. The aim of this study was to assess the possibility of quantifying free rather than total metabolites (i.e., free plus conjugated), so further simplifying the diagnostic work-flow of AME.
Methods: Thirty eight urine samples from patients with AME-like phenotype were analyzed in duplicate with and without applying a ydrolysis step. Non-hydrolyzed samples were simply extracted in acetonitrile by protein precipitation, whilst hydrolyzed specimens were incubated for 16 hours at 45°C with glucuronidase (Helix Pomatia, Merk KG, Germany), then dried under nitrogen flow and finally resuspended in water and acetonitrile. Treated samples were analyzed by our home-made LC-MS/MS method (Nexera X2 UHPLC-4500MD Sciex). Spearman test was used to assess the correlation between total and free THF and THE. Correlation analysis on allo-THF was not performed, since the levels of free allo-THFs fell under the detection limit of the method (LOQ=5 ng/ml).
Results: Median values of total and free THF were 1025 ng/mL (range 202-10634 ng/mL) and 8.7 ng/mL (3.4-209 ng/mL), respectively, whilst those of total and free THE were 1047 ng/mL (range 70-3211 ng/mL) and 5,29 ng/mL (0,47-103 ng/mL), respectively. Correlation was 0.854 ($p<0.0001$) for THFs and $r=0.766$ ($p<0.0001$) for THEs.
Conclusion: THF and THE values, measured as total or free metabolites obtained with or without deconjugation by enzymatic hydrolysis, appeared well correlated, thus making possible the quantification of free rather than total metabolites in clinical practice. Correlation analysis between total and free allo-THFs should also be assessed with higher sensibility MS for making definitive conclusions.
Reference: Cuzzola A, et al. A comprehensive study for the validation of a LC–MS/MS method for the determination of free and total forms of urinary cortisol and its metabolites: J Pharm Biomed Anal. 2014;94:203-9.

P184
## ECONOMIC EVALUATION RELATED TO THE USE OF PROFESSIONAL BLOOD GLUCOSE METERS IN AN ITALIAN REGION

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Objective: The aim of this Budget Impact Analysis is to evaluate the economic impact in the payer prospective of replacement of NP-BGM by P-BGM, taking into consideration the impact of LOS and the reduction on nurse's labor time.
Background: During hospitalization the 30% to 40% of inpatients are diagnosed with glycemic crisis. In these circumstances, the glycemic monitoring systems have a central and consolidated role since the concentration of plasma glucose higher than 140mg/dL or lower than 54mg/dL is strongly associated with mortality, morbidity and length of stay (LOS). There are two different scenarios of glycemic monitoring which depends on the type of glucose meters and its consequences accordingly to scientific literature: -Non-Professional Blood Glucose Meter (NP-BGM) without connectivity and frequently lower accuracy, which leads to 1.8% of post analytic errors on patient's electronic records.- Professional Blood Glucose Meter (P-BGM) with proven accuracy and connectivity, which is connected to data management system avoiding the manual reporting and post-analytic errors.
Method: It is adopted a model decision tree to project health and economic outcomes with 5 years of time horizon, in a medium-large populated Italian region.The model takes into account the following variables:- 4.496 Number of patients undergoing blood glucose testing by a P-BMG per day;- 6.564.340 tests carried out per year;- Replacement of ≈6.000 NP-BGM by 1.759 P-BMGThe other costs included in the model are: direct acquisition costs, direct medical costs (400€ ward room cost per day), labor cost and hospital productivity of delivering care to patients. Results: The economic impact of P-BGM use instead of NP-BGM represents a positive of ≈8.000.000 euros every year taken into, and a significant decreasing of 67% in nurse time required to manage glucose tests. Additionally, was registered a reduction of ≈900 bed days with full occupancy per year, gathered from a decrease of the patients LOS.
Conclusions: The use of P-BGM significantly improves the saving in a medium-large populate Italian region, the quality of work and the reduction of costs related to adverse event.

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## INTERFERENZA DELL'ANTICORPO MONOCLONALE DARATUMUMAB NELL'ELETTROFORESI DELLE PROTEINE DEL SIERO E NELL'IMMUNOFISSAZIONE DI PAZIENTI CON MIELOMA MULTIPLO: PRIME ESPERIENZE AL POLICLINICO S. MATTEO DI PAVIA

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Introduzione: Anticorpi monoclonali umanizzati (mAbs), utilizzati per la terapia del mieloma multiplo (MM), possono interferire con gli anticorpi nativi del paziente nei test di elettroforesi proteica (EFS) e di immunofissazione (IF) raccomandati dalle linee guida IMWG per valutare la risposta completa al trattamento.I protocolli per la terapia di recidive o di mieloma multiplo refrattario prevedono l'utilizzo di un anticorpo monoclonale denominato Daratumumab: un'immunoglobulina umana G1 (IgG1) $\kappa$ che si lega al recettore CD38. Risultati falsi positivi indotti da Daratumumab sono particolarmente problematici nei pazienti con MM poiché le linee guida attuali designano come criterio di remissione completa (CR) l'assenza di proteina M rilevabile da EFS o IF.
Obiettivo: Discriminare la proteina endogena dal farmaco.
Materiali e metodi: Campioni di siero di 10 pazienti con MM sono stati utilizzati per confrontare i risultati dell'EFS e di IF prima del trattamento con Daratumumab, durante la terapia e al termine della somministrazione del farmaco. Per discriminare la proteina endogena dal farmaco, è stato usato un saggio basato sulla formazione di un legame daratumumab/anti-daratumumab che determina, su gel d'agarosio, lo spostamento di tale immunocomplesso nella zona alfa-1 (HYDRASHIFT 2/4 DARATUMUMAB, Sebia).
Risultati: Nei pazienti in terapia, sul gel di agarosio usato per l'immunofissazione è osservabile una banda sui tracciati IgG e K al di fuori della zona delle gammaglobuline e a livello della zona alfa-1.Tale banda indica la presenza del complesso daratumumab/anticorpo anti-daratumumab.Questa metodica ha permesso di confermare in tre pazienti trattati la persistenza di proteina M endogena e la sua assenza negli altri sette.
Discussione e conclusioni: Con il crescente utilizzo di anticorpi monoclonali per il trattamento del MM, diventerà sempre più importante lo sviluppo di nuovi test validati per il monitoraggio clinico della risposta del paziente al regime terapeutico. Saggi in grado di superare l'interferenza prodotta dal farmaco, distinguendolo dalla proteina M endogena, consentono di monitorare correttamente la risposta alla terapia evitando di incorrere in errori di interpretazione dei risultati dell'EFS e dell'IF.

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## CONFRONTO ANALITICO E VALUTAZIONE DELL'IMPATTO CLINICO DI ESTRADIOLO, PROGESTERONE E VITAMINA D 25OH ESEGUITI SU DUE PIATTAFORME ANALITICHE DI IMMUNOCHIMICA

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Introduzione: Le determinazioni immunochimiche soffrono ancora della mancanza di standardizzazione, quindi può risultare abbastanza difficile confrontare i risultati di diversi sistemi analitici. Scopo di questo studio è una valutazione clinico-metodologica del dosaggio di Estradiolo (E2), Progesterone (PG) e Vitamina D 25OH (vitD) eseguito su due sistemi automatizzati, Liaison XL (Diasorin, Saluggia, Italia) e DXI800 (Beckman Coulter, Brea, CA, USA). Metodi: Sulle due piattaforme analitiche sono stati analizzati rispettivamente 57 campioni di PG, 67 di vitD e 66 di E2. La comparazione dei metodi analitici è stata condotta attraverso una analisi di Passing-Bablock. Il coefficiente di correlazione ($R^2$), e l'analisi di regressione lineare sono state calcolate con MedCalc Statistical Software (version 18.11.3). Risultati: Le analisi di comparazione evidenziano una buona correlazione tra i due analizzatori relativamente al dosaggio di vitD (y = 1x + 2,0, $R^2$ 0,83) e E2 (y = 1,02-0,41, $R^2$ 0,98), mentre la correlazione del PG (y = 2,3x-0,11, $R^2$ 0,74) è risultata accettabile evidenziando la presenza di un bias significativo. Considerando la vitD, la valutazione relativa all'interpretazione clinica ha mostrato, utilizzando 20 ng/ml come valore discriminate per definire il deficit, una buona concordanza tra i due metodi (91% dei pazienti, 61/67. Conclusioni: La valutazione del dosaggio vitD e E2 ha dimostrato una buona correlazione clinica e analitica tra i due metodi. Il metodo E2 di Beckman presenta una linearità più elevata rispetto a Diasorin (1.000 vs 5.000 pg/ml), rappresentando un vantaggio importante nel caso di donne seguite per la riproduzione assistita, in cui il livello di E2 può aumentare in modo importante. Relativamente alla misura del PG la valutazione dei due sistemi ha evidenziato una discreta correlazione analitica, tuttavia i livelli costantemente più alti misurati su analizzatore Beckman possono modificare l'inquadramento clinico delle pazienti rappresentando motivo di preoccupazione soprattutto in caso di riproduzione assistita, in cui un cut-off di 1,5 ng/ml viene utilizzato per la valutazione dell'outcome del trasferimento embrionale. Ciò suggerisce la necessità di una definizione di un limite specificamente basato sul metodo.

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## EVALUATION OF SOY-BASED LIPID EMULSION TO SIMULATE LIPEMIA IN INTERFERENCE STUDY

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Lipemia often causes interference in assay using optical systems for detection, increasing light absorbance. Decrease in intensity of the light is erroneously attributable to sample concentration. Indeed, lipemia interferes by altering light scattering which intensity is affected by the number of particles, size and wavelength of the light. Determinations of lipemic index or triglyceride (TRG) concentrations are commonly used to quantify lipemia. However, since the intensity of scattering is greater at low wavelength and proportional to the size of the particles, often there is no correlation of absorbed light with lipid concentration. Soy-based lipid emulsions (Lipofundin or Intralipid), are used to simulate lipemia in interference studies, but they do not correlate with VLDL and chylomicrons.In this study we propose a simple and inexpensive method to simulate intermediate and large VLDL and a method to evaluate their size avoiding use of Dynamic Light Scattering (DLS). Dose-response interference study was done by comparing a mix of sonicated soybean oil and phospholipids (SOP) with Lipofundin (LIP) vs 6 routine markers (uric acid, URA; urea, URA; glucose, GLU; creatinine, CRE; total bilirubin, BT and alkaline phosphatase, ALP) covering the largest wavelength range (340-550 nm). Control samples of normal and pathological sera were spiked with the emulsions (200-2000 mg/dL nominal triglycerides). Particle sizes of SOP and LIP were measured by DLS and related to z coefficient calculated as the slope of the linearized, $\ln ABS=\ln(\lambda^{-1})$, spectrum of absorbance from 300 to 700 nm. TRG concentration was determined for SOP and LIP. For SOP the coefficient z was 2.6 corresponding to particle 100-150 nm (250-350 nm for LIP) as determined by DLS. Coefficient z was used to evaluate the stability of SOP over the time and as reference during the preparation of fresh SOP. No interference from SOP along the range was observed for URE, ALP CRE an BT with SOP, while LIP interferes with URE and ALP from 800 mg/dL, with CRE and BT from 200 mg/dL. About GLU and URA both SOP and MIX interferes even at the lowest concentrations. SOP size is much more closed to intermediate and large VLDL than LIP and the presence of glycerol in LIP overestimates TRG concentration in LIP samples.

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## ANTITOSSOIDE TETANICO: VALUTAZIONE DI UN NUOVO METODO AUTOMATIZZATO

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Introduzione: Il tetano è causato dal batterio C. tetani responsabile della produzione di una neurotossina. La prevenzione della malattia si basa sulla vaccinazione che conferisce una protezione con efficacia superiore al 95%. La copertura nel tempo è di almeno 10 anni ed è prolungata dai richiami. La misurazione degli anticorpi (Ab) del paziente risulta quindi fondamentale per gestire eventuali vaccinazioni di richiamo e di profilassi. In questo lavoro abbiamo valutato un sistema di misura immunoturbidimetrico (Optilite, The Binding Site) confrontandolo con il metodo Elisa (Technogenetics) in uso nel nostro laboratorio.
Metodi: Lo studio di comparazione è stato condotto analizzando 71 campioni di siero. La concordanza tra i valori ottenuti in turbidimetria ed in Elisa è stata valutata mediante analisi statistica (Bland e Altman, griglia di Shermok).
Risultati: Bland e Altman: Optilite [Ab] media 2,25, max 9,24, min 0,15 IU/mL, differenza media (Elisa – Optilite) 0,39, Limits of Agreement (LOA) 2,90 e -2,11 IU/mL.
Griglia di Shermok: Sono stati utilizzati 4 snodi decisionali che definiscono, in base alla misurazione degli Ab, inquadramenti diagnostici differenti. La percentuale di osservazioni che implicherebbe una decisione clinica discordante rispetto ad Elisa varia a seconda del range di titolo anticorpale considerato: <0,1 IU/mL 2,8%, 0,11-0,5 IU/mL 4,2%, 0,51-1,0 IU/mL 7%, 1,1-5,0 IU/mL 11,3%.Conclusioni: Il grafico Bland e Altman evidenzia che le differenze dei risultati rientrano all'interno dei LOA fino alla concentrazione di 3,5 IU/mL, sono indipendenti dal titolo di Ab e non mostrano errori sistematici di sovrastima o sottostima rispetto allo metodo Elisa. Rispetto agli snodi decisionali della Griglia di Shermok la % più alta di osservazioni (11,3), che porterebbe ad un differente inquadramento clinico, si colloca nel range >1,0 IU/mL: tale concentrazione garantisce immunità a lungo termine rendendo meno urgente il richiamo. Per valori inferiori a 0,1 IU/mL, concentrazioni alle quali il vaccino/richiamo è necessario a causa di immunità incerta o assente, la % di osservazioni con impatto clinico discordante scende al 2,8%. I due metodi mostrano quindi performances comparabili all'interno dei ranges clinici più rilevanti per la protezione immunitaria.
"World Health Organization. Tetanus vaccines: WHO position paper. Wkly Epidemiol Rec. 2017 Feb 10;92(6):53-76."
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## A NOVEL MULTIPLEX FLUORESCENT CELL BARCODING TECHNIQUE USING LIPOPHILIC TRACER DYES

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Fluorescent cell barcoding (FCB) is a multiplexed high-throughput flow cytometry technique in which samples can be acquired collectively minimizing technical variability. Lipophilic carbocyanine dyes have low cytotoxicity and are used as tracers in living and fixed cells. Here, we developed and optimized a novel FCB protocol with lipophilic dyes and without the use of permeabilization buffers. Our protocol allows surface marker staining without affecting epitope and cell integrity while increasing flow cytometry data throughput and reliability.Peripheral blood mononuclear cells were isolated by Ficoll-Paque gradient centrifugation from whole blood of healthy donors. Cells were frozen in 90% RPMI and 10% dimethyl-sulfoxide (DMSO) and stored at -80°C until use. The Vybrant™ Cell-Labeling Solutions was used for FCB, and working solutions were freshly prepared from stocks by dilution with DMSO. FCB was carried out in a U-bottom 96 well plate in a final volume reaction of 40 µL/well by combining 30 µL of sample and 5 µL of each dye at appropriate dilutions. For a 4-sample barcoding, a 2x2 matrix was designed using two dilutions of DiO and DiI or DiD; while, for 9-sample FCB, a 3x3 matrix was made using three dilutions of DiO and DiD. Briefly, after thawing, $2x10^7$ cells were fixed with 3 mL of 4% paraformaldehyde for 15 min at room temperature (RT), centrifuged, and then suspended in phosphate buffer saline (PBS) in order to have $1x10^6$ cells/30 µL. Afterwards, 30 µL of samples were added to appropriate wells, incubated at RT for 20 min, washed twice with PBS, and then combined in one tube. Acquisition was performed using a BD FACSVerse cytometer equipped with blue and red lasers, and BD FACSDiva software was employed for sample acquisition and analysis. Fluorescence values were reported as median fluorescence intensity (MFI), CV (CV = SD/mean of population), and MFI fold change calculated as $[MFIpeak_2 - CVpeak_2]/[MFIpeak_1 + CVpeak_1]$ and used to assess FCB efficiency. MFI fold increase ranged from 9.1 to 9.8 achieving a complete peak separation between barcoded populations without any overlap.Our protocol provides implementation of FCB technique making this method suitable for multiplex immunophenotyping especially when handling low cell number or frail cell type samples.

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## AN UHPLC-DAD METHOD FOR SIMULTANEOUS DETERMINATION OF 10 ANTIBIOTICS FOR THERAPEUTIC DRUG MONITORING OF CRITICALLY ILL PEDIATRIC PATIENTS

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The practice of Therapeutic Drug Monitoring (TDM) involves the measurement of plasma concentrations at a specific time in a dosing interval, depending on drug's pharmacokinetic (PK) and pharmacodynamic (PD) properties and subsequently a dose optimization. Some patients require special dosing considerations as a result of intense and complex pathophysiological changes, especially critically ill pediatric and neonatal patients for which often the PK/PD knowledge is limited and incomplete. TDM is particularly relevant for antibiotics management because together with MIC (minimum inhibitory concentration) evaluation contributes to formulate an effective dosage regimen avoiding therapeutic failures (1). However, TDM should rely on analytical methods such as liquid chromatography characterized by fast detection, accuracy and data robustness. Therefore, we have developed and validated a new Ultra High Performance Liquid Chromatography coupled to Diode Array detection (UHPLC-DAD) method for simultaneous quantification of ten antibiotics in plasma samples of pediatric and neonatal patients. This analytical procedure uses the same chromatographic conditions (extraction procedure, analytical column, mobile phases, chromatographic run) to simultaneously quantify Ceftazidime, Ceftriaxone, Meropenem, Ciprofloxacin, Tigecycline, Cefoxitin, Ampicillin, Levofloxacin and Piperacillin, plus the beta-lactamase inhibitor Tazobactam. Validation parameters were tested: selectivity, accuracy, precision, limits of detection and quantification, recovery and stability. This new method is accurate, reproducible, has a short analysis time and has been applied to the analysis of plasma samples from critically ill pediatric and neonatal patients admitted to the intensive care units of our hospital and treated with one of ten antibiotics analysed. In fact, another important aspect of our method is the analysis of microsamples volumes (100 µL). Indeed, thanks to a heel stick capillary (HSC) device (2), we managed to get enough plasma to dose these analytes, even in critically ill pediatric patients from which only a small volume of sample is available. In conclusion, we believe that this developed method could be a useful tool for TDM strategy in clinical practice.

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**ONLINE INFORMATION ON LABORATORY MEDICINE TESTS: FRIEND OR FOE?**

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Backgrounds: The widespread use of internet has prompted patients to get medical information from specialized web-sites and social media. However, these sources often include non-documented and/or unreliable information. Aims were to evaluate the content quality of search engines results, considering three common laboratory tests, urinalysis, cholesterol and PSA.
Methods: Searches were performed by Google Trends in Italian, using Italy as region and the following settings: 5 years, web search and all categories. For each test, the first five related queries were considered and searched using Google engine. Considering the first three web-page results of each query, quality of the reported information was evaluated by means of the availability of: cited sources, scientific board revision/approval of contents.
Results: Only PSA showed an increased "interest over time" of about 40% in the last 5 years, while cholesterol showed a yearly seasonal trend, with a minimum at December. Cited sources were present in 40%, 47% and 7% of urinalysis, cholesterol and PSA first three reported web-pages, respectively. Scientific board revision/approval were present in 87%, 53% and 47% of urinalysis, cholesterol and PSA first three reported web-pages, respectively. Web-sites owners were: publishing groups (35%), public/private institutions (27%), encyclopedias (11%), bloggers (11%), sellers (7%) and private laboratories (7%).
Conclusions: Scientific board revision/approval was the most used method for guaranteeing information quality by web-resources. Among the evaluated tests, PSA presented the poorest information accuracy. Google Trends may help in understanding people information needs regarding laboratory testing.

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**MESSA A PUNTO DEL DOSAGGIO DELL'ACIDO LATTICO NEL LIQUIDO CEFALO RACHIDIANO SU STRUMENTAZIONE ADVIA CHEMISTRY XPT**

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Introduzione: E' noto che il dosaggio dell'acido lattico può essere utilizzato per differenziare le meningiti batteriche da quelle tubercolari fungine e virali.
Sullo strumento ADVIA Chemistry XPT (Siemens) la metodica per la determinazione dell'acido lattico è validata solo per plasma umano. Utilizzando il protocollo CLSI EP17-A2, sono state valutate le performance analitiche del dosaggio del lattato anche su matrice liquorale.
Obiettivo: Implementare il dosaggio del lattato nel liquido cefalo rachidiano (LCR) sulla strumentazione ADVIA Chemistry XPT.
Materiali e metodi: Le performance analitiche del dosaggio su LCR sono state valutate mediante calcolo di LOB, LOQ, CV% intra ed inter serie. Il LOB è stato determinato dosando la concentrazione dell'acido lattico in 3 campioni di fisiologica: 60 ripetizioni in 3 run. Per determinare il LOQ, da un calibratore a concentrazione nota sono state preparate diluizioni scalari con calcolo di media, deviazione standard e CV%. Il CV% inter ed intra serie è stato ottenuto utilizzando un controllo di terza parte (Liquichek Spinal Fluid Control Bioard).
Risultati: Il LOB è stato calcolato con la formula LoB = media + 1.645* deviazione standard ed è risultato pari a 0.07 mg/dL. Nei 3 campioni di fisiologica (60 ripetizioni) la media dei valori di lattato è risultata 0.025 mg/dL, la ds 0.028, CV 1.12%; la media delle assorbanze 0.000063, la ds 0.00013, CV% 2.1.Il profilo di imprecisione ha permesso di determinare il LOQ al CV 10% che è risultato pari a 7.4 mg/dL. Con un controllo di terza parte è stato determinato il CV% intra ed inter serie: livello 1 intra 4.79%, livello 2 intra 2.11%; livello 1 inter 2.65%, livello 2 inter 3.37%.
Discussione e conclusione: Il dosaggio del lattato su LCR può essere implementato su ADVIA Chemistry XPT senza costi aggiuntivi per il laboratorio, avendo dimostrato che il metodo è robusto, veloce ed affidabile anche su matrice liquorale.
Bibliografia: Clinical and Laboratory Standards Institute (formerly NCCLS). Evaluation of DetectionCapability for Clinical Laboratory Measurement Procedures; Approved Guideline—SecondEdition. CLSI document EP17-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2012

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## HOW TECHNOLOGY CAN SUPPORT THE HARMONIZATION OF PROCESSES IN LABORATORY MEDICINE: AN EXAMPLE OF A SPREADSHEET FOR ESTIMATING MEASUREMENT UNCERTAINTY

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Backgrounds: In the last decade, advancements in technology has provided a positive development for laboratories and further technological advancements may aid in overcoming the challenges of between-laboratory harmonization. A feasible example can be the implementation of systems for performing calculations on different complex scenarios. The aim was to propose a technological model that allows between-laboratory harmonization of measurement uncertainty (MU) estimation.
Methods: Technological tools were evaluated for the applicability to the model for estimating MU and for their availability in medical laboratories. Google sheets (Google, Mountain View, CA, USA), MS Excel (Microsoft, Redmond, WA, USA) and LibreOffice (The Document foundation, Web Community) were evaluated for: a) the possibility of implementation of complex calculations such as that required by MU, b) compatibility with different operating systems; c) possibility of team work and of d) online shareable work.
Results: Google sheets (GSheet) was found to fit the purpose. With respect to the other systems, this presents the following advantages: calculations feasibility, full compatibility with all operating systems (it requires only a web browser), team and online working capabilities. A GSheet of four worksheets was prepared for MU estimation. The first and the second worksheets require users to entry data. Internal quality control data of 6 six months data, of two different levels, can be entered for the estimation of imprecision component of MU. Moreover, twelve EQAs samples can be entered for the estimation of bias component. Another worksheet automatically generate calculation for the uncertainty of bias component by using given data. The last worksheet contains the resulting MU, calculated with 95% confidence, expressed both as absolute or relative (%) uncertainty. MU are reported with or without the inclusion of bias components.
Conclusions: Gsheet was ideal for approaching the development of a technological model for performing complex calculations in different laboratories, with the possibility of being able to work online on shared data. The use of this model for MU estimation by laboratories, complying with ISO 15189:2012 accreditation, supports harmonization processes.

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## DEVELOPMENT AND VALIDATION OF AN HPLC-MS/MS METHOD FOR THE DETERMINATION OF BUSULFAN IN PEDIATRIC PATIENTS

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In collaboration with Chromsystems has been developed an HPLC-MS/MS method for the determination of Busulfan concentration in patients' blood.The company produces the "Basic Kit TDM series A", which is a generic extraction method that works on both serum and plasma and it's shared between different drugs class. It has been tested and confirmed by the company research and development division about its compatibility with busulfan molecule. We used the busulfan standard as certified reference material (1 mg/mL) in methanol (Cerilliant) and the corresponding deuterate as certified reference material too (internal standard D8, 100 µg/mL in methanol, Cerilliant). Then, in our laboratory, we identified the transitions for the two molecules and defined the chromatographic gradient, adapting the information reported on the certificate of analysis. Once verified the others parameters for HPLC-MS/MS setup we proceeded with the method validation to introduce it in routine analysis for the measurement of busulfan in patients' blood. The calibrators and controls have been made "in-house" using a serum blank matrix, to guarantee the matrix effect. The values have been chosen using as reference what has been described by Soo Young Moon (Soo Young Moon et al. Annals of Laboratory Medicine 34(1):7-14 January 2014). For the validation step we followed the CLSI guidelines; the document C50A which provides a flow chart for methods development and validation, with the references to the others CLSI guidelines; as well as the document C62A which goes deeper into the fundamental aspects about method setup. The EP10A3 has been used for the evaluation of the method qualitative performance; the LoB, LoD and LoQ determination has been done following the specification of CLSI EP06A. The LoQ determination is specified in CLSI C62A too, where it defines the signal to noise ratio has to be at least 20:1, while the LoD at least 10:1 ratio. Finally, we have evaluated some samples from pediatric patients analyzed in an external laboratory as confirmation for the developed method.

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**ANTI-MÜLLERIAN HORMONE MEASUREMENT: COMPARATIVE EVALUATION OF THREE COMMERCIAL IMMUNOASSAYS**

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Anti-Müllerian hormone (AMH) is a homodimeric glycoprotein produced by granulosa cells of growing ovarian follicles and it is considered to be a valid marker of ovarian reserve. Recent studies have challenged the reliability of the Gen II assay (mainstay method for AMH measurement in the past years) and currently a few new more reliable AMH measurement methods have been developed. Aim of this study was to evaluate the analytical performances between three different methods for the determination of AMH: we compared the Ultra-Sensitive AMH/MIS ELISA kit (Ansh Labs) versus two automated chemiluminescent immunoassays, the Elecsys® AMH (ECLIA, Roche Diagnostics) and the Lumipulse® G AMH (CLEIA, Fujirebio Diagnostics).In laboratory of Tumor Markers of Policlinico Umberto I (Rome) we evaluated 92 serum samples from consecutively enrolled women: 45 affected by endometriosis (aged 24-47), 11 affected by autoimmune diseases (aged 30-46) and 36 healthy women (aged 20-49). All AMH assay results were reported in ng/mL . Statistical analyses were performed with MedCalc version 15.8. Values measured by the AMH/MIS ELISA kit were significantly higher than those from the Elecsys® AMH and the Lumipulse® G AMH ($P < 0.02$). Comparison between assays was analyzed using Passing–Bablok regression: AMH values measured by the three methods have good correlations ($R > 0.9$ for all pairwise correlations). The intra-assay and inter-assay CVs of Ultra-Sensitive AMH/MIS ELISA were significantly higher than those of both Lumipulse® G AMH and Elecsys® AMH ($P < 0.05$). Ultra-Sensitive AMH/MIS ELISA showed lower precision and linearity compared to the two automated assays, while the comparison between Elecsys® AMH and Lumipulse® G AMH in terms of imprecision (Intra-assay and Inter-assay), LOB and LOD showed acceptable differences. In our study Elecsys® AMH and Lumipulse® G AMH revealed better analytical performances than Ultra-Sensitive AMH/MIS ELISA. The two new automated assays should be preferred to the manual one with the aim of reducing costs, have greater standardization and give prompt AMH result to the clinicians. Elecsys® AMH and Lumipulse® G AMH perform in a similar way so the decision to use one assay can depend on individual requirements of a particular institution.

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**CITRATI URINARI: CONFRONTO FRA METODO ENZIMATICO/COLORIMETRICO E CROMATOGRAFICO IN HPLC/UV**

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Introduzione: nelle calcolosi urinarie, il dosaggio dei citrati urinari ha un ruolo importante in quanto permette di stabilire l'ipocitraturia, causa di calcoli urinari. I citrati hanno un ruolo protettivo: si combinano con il calcio nel lume dei tubuli renali e formano un complesso solubile, così si riduce la disponibilità del calcio a formare cristalli con l'ossalato e si limita la successiva formazione di aggregati di questi cristalli a formare calcoli. In più in carenza di citrato, l'organismo preleva basi dalla struttura ossea, nostro principale magazzino di alcali al fine di mantenere il pH ematico al suo valore fisiologico di 7,4 indebolendo così la trama ossea. Lo scopo del nostro studio è stato quello di confrontare le prestazioni di due metodi commerciali disponibili: metodo HPLC con rivelatore UV e metodo enzimatico colorimetrico.
Materiali e metodi: sono stati analizzati contemporaneamente con i due metodi 26 campioni di urine delle 24 ore conservati a -20° fino al momento dell'analisi di pazienti provenienti da diversi reparti dell'Azienda Ospedali Riuniti di Ancona. Il metodo cromatografico (Citrati urinari in UV, Eureka, sensibilità 20 mg/L) prevede un HPLC/UV a $\lambda$=210nm, colonna Hi-Plex H, 300x7,7mm; il metodo enzimatico colorimetrico (Acido citrico,LTA, sensibilità 20 mg/L), sfrutta l'ossidazione del NADH in NAD e la diminuzione dell'assorbanza a 340nm,delle reazioni di riduzione dell'acido citrico.
Risultati e conclusioni: 26 campioni di pazienti sono stati analizzati per la validazione del metodo; accuratezza e precisone del nuovo metodo, sia intra-day che inter-day, sono accettabili essendo sempre inferiori al 15%, il recupero del metodo in HPLC è del 100%, il Mann- Whitney Rank Sum test ha mostrato una distribuzione normale dei valori dei citrati. Il range di concentrazioni misurate andava da 60 a 770 mg/L. L'analisi di regressione ha mostrato che i due metodi sono ottimamente correlati con R=0.989. Il bias tra i due metodi è risultato non significativo. Il metodo in HPLC/UV può quindi rappresentare una alternativa al metodo enzimatico per la determinazione dei citrati urinari.
Bibliografia: Mollering,H.&Gruber,W.(1996) Determination of citrate with citrate lyase. Anal.Biochem.17,369-376.

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**ANALYTICAL PERFORMANCE OF THE NEW ACCESS PCT ASSAY**

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Introduction: Procalcitonin (PCT), a calcitonin pro-hormone, is considered up to now the better sepsis biomarker, contributing to early diagnosis, helping in risk stratification and infection prognosis. For this reason, PCT requests significantly increased in emergency conditions, with development of several assays on different analytical platforms. All these assays have non-competitive architecture and apply patented B·R·A·H·M·S antibodies.
Study purpose: to evaluate the new Access PCT assay, with chemiluminescent tracer, paramagnetic particles for immunocomplex separation, available on Access and DxI platforms on serum and plasma, in comparison with B·R·A·H·M·S VIDAS PCT routinely used in the laboratories of Hospitals in Asolo disctrict, ULSS 2 Marca Trevigiana.
Materials and methods: Our laboratories processes on average 10.100 PCT tests per year, 36% from Emergency Department and 16% from Intensive Care Unit. 105 anonymous samples were analyzed on VIDAS and DxI within four hours. PCT positive patient records (unique patient identification number) were checked for PCR, lactate and blood culture.
Statistical analysis: concordance table, correlation coefficient (r) Passing Bablok (PB), Bland Altman (BA).
Results and discussion: 21 of 105 VIDAS results were negative (<= 0.5 µg/L) and 84 positives (> 0.51 ug/L), of which 40 > 2.1 µg/L and 44 between 0.51 and 2.0 µg/L. Concordance analysis showed 100% agreement on negative values and 94% on positive values. r= 0.99, (IC 95% 0.9912-0.9951) was perfectly in line with that reported in the Access PCT datasheet; PB: intercept -0.035 (95% IC) and slope 0.96 (95% IC) with a non-significant deviation from linearity (P = 0.55); BA: absolute bias 0.4 µg/L, upper limit 4.1 and lower limit -3.3.
In conclusion, Access PCT proves performances adhering to the reference one, with an excellent adaptability to the process in emergency both track implementation capability and the possibility to use the same platform of other emergency tests. Moreover, we observed a reduced sample volume (30-35 µL, plus 60 µL dead volume, instead of 200 µL) and time to first result (19 minutes). All this contributes to optimize times, flows and technical human resources.

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**MESSA A PUNTO E UTILIZZO IN DUE LABORATORI LOMBARDI DI UNO STRUMENTO INFORMATICO PER LA VERIFICA DELLA QUALITA' ANALITICA DI STRUMENTI DI NUOVA INTRODUZIONE**

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Introduzione: Gli avvicendamenti strumentali rappresentano uno dei momenti più impegnativi nella vita di un Laboratorio Clinico, in quanto richiedono l'espletamento di numerose attività, fra cui la verifica della qualità analitica della strumentazione di nuova introduzione, sia da un punto di vista strettamente statistico, sia da quello dell'appropriatezza rispetto alla destinazione d'uso ("fit for purpose"). Per valutare il primo aspetto, lo strumento messo a disposizione dal GdS SIBioC "Statistica" (Biochim clin 2016;40:129-42) rappresenta un punto di riferimento imprescindibile; per valutare il secondo aspetto abbiamo creato un nuovo foglio elettronico, integrando i test esistenti con quelli necessari per verificare il rispetto o meno delle specifiche di qualità basate sulla variabilità biologica (VB).
Metodi: Il foglio elettronico è stato realizzato con Microsoft Excel, implementando formule statistiche e codice VBA per il calcolo dei parametri di imprecisione (DS e CV), inesattezza (Bias) ed errore totale (TEs) e per la verifica della loro accettabilità sia sulla base di criteri statistici che clinici.
Risultati: Il foglio elettronico è stato utilizzato presso i Laboratori Analisi dell'ASST Spedali Civili di Brescia e dell'ASST Valcamonica per verificare le prestazioni analitiche di 2 strumenti Roche Cobas 8000 (moduli c702 ed e801; 98 misurandi di chimica clinica e immunometria) e di 2 strumenti Abbott Alinity ci (71 misurandi), subentranti a precedente fornitura. Applicando lo schema 5X5 proposto nel documento SIBioC su due tipologie di campioni (pool di siero/plasma umani e materiali di controllo commerciali), è stato possibile effettuare confronti sia prettamente analitici (imprecisione rispetto alle specifiche dichiarate dalle ditte produttrici, inesattezza rispetto al valore assegnato per consenso ai materiali di controllo, errore totale rispetto al traguardo analitico scelto per il monitoraggio delle prestazioni), sia clinici (confronto dei parametri precedenti con i traguardi di accettabilità basati sulla VB).
Conclusioni: L'implementazione di strumenti informatici creati direttamente dai professionisti di Laboratorio ne agevola in modo efficace ed efficiente l'espletamento delle numerose attività di competenza.

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## EVALUATION OF THE ANALYTICAL PERFORMANCES OF MINDRAY CI-2000i: A COMPARATIVE EXPERIMENTAL STUDY

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Background: we performed a comparative experimental study to evaluate the analytical performances of the Mindray CI-2000i system compared to our automated immunoassay platforms, the Siemens ADVIA Centaur XP and DiaSorin LIAISON systems. We measured serum levels of ferritin, a marker of total body iron stores, alpha-fetoprotein, in adults associated with acute viral hepatitis, chronic active hepatitis and cirrhosis, carcinoembryonic antigen, a tumor associated antigen also measured in a variety of benign conditions and serum levels of thyroglobulin, detected in small quantities during synthesis and transportation. The aim of this study was to analyze the degree of data overlapping obtained by different analyzer platforms.
Methods: we detected serum ferritin levels in 124 samples, alpha-fetoprotein in 122 samples and carcinoembryonic antigen in 90 samples using our ADVIA Centaur immunoassay system and Mindray CI-2000i system. We also measured thyroglobulin levels in 138 serum specimens using both DiaSorin LIAISON and Mindray CI-2000i chemiluminescent immunoassay analyzers. We used a manufactured third-part control as internal quality control (IQC). Statistical analysis was performed using Passing-Bablok's regression and Bland-Altman different plots.
Results: ferritin, alpha-fetoprotein and carcinoembryonic antigen serum levels detected by the two different immunoassay platforms showed an optimal comparability between Mndray CI-2000i system and ADVIA Centaur system with respectively $R_{pearson}$ values of 0,9843, 0,9994 and 0,9045. Passing-Bablok's regression was respectively of 0,9686, 0,9989 and 0,8181. Analysis of thyroglobulin serum levels demonstrated that Mindray CI-2000i system and DiaSorin LIAISON system are also comparable at high levels with $R_{pearson}$=0,9449 and $r^2$=0,8928 (Passing-Bablok's regression).
Conclusions: the purpose of this experimental study was to compare the analytical performances of Mindray CI-2000i to other immunoassay analyzers, ADVIA Centaur XP and DiaSorin LIAISON systems, measuring serum levels of four analytes in a number of serum specimens collected during our routine laboratory analysis. Statistical analysis results revealed high levels of comparability of the Mindray CI-2000i automated system and chemiluminescent immunoassay methods.

P200
## VALUTAZIONE DEI SISTEMI ADVIA 2120 PLASMA APPLICATION E SYSMEX UF-1000I PER IL CONTEGGIO DELLE CELLULE RESIDUE NELLE UNITÀ DI PLASMA PER IMPIEGO TRASFUSIONALE

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Background: Le normative vigenti e le linee guida europee richiedono che il plasma fresco congelato contenga meno di $6.0x10^9$/L di emazie residue (RBC) e meno di $0.1x10^9$/L di leucociti (WBC) prima del congelamento. Il conteggio di un numero estremamente basso di RBC e WBC è tecnicamente complicato ma obbligatorio per il controllo di qualità su plasma per impiego trasfusionale.
Materiali e metodi: L'analizzatore per ematologia ADVIA 2120 (Siemens MI) dispone di un'applicazione "Plasma" (ADVIA-PA) approvata per i liquidi biologici con una linearità dichiarata per RBC fino a $10x10^9$/L. Sono state valutate le performance analitiche di ADVIA-PA per il conteggio dei RBC residui fino a valori di $0.69x10^9$/L in modo da poter soddisfare le linee guida attuali.Inoltre, sono stati eseguiti due studi comparativi: uno per RBC e uno per WBC residui confrontando ADVIA-PA e l'analizzatore di urine Sysmex UF 1000i-Dasit MI (UF 1000) con i metodi di riferimento. Sono stati utilizzati per il confronto 79 campioni reali di plasma ottenuti dal frazionamento di sangue di donatori sani, prima del congelamento. Sono state utilizzate la regressione di Passing Bablok e il metodo di Bland-Altman per l'analisi dello studio comparativo.
Risultati: Le performance analitiche di ADVIA-PA hanno dimostrato un'ottima linearità ($R^2$>0.99) per concentrazioni di RBC da 88.3 a $0.69x10^9$/L. Le prove di precisione hanno dimostrato coefficienti di variazione massimi di 15.78%.Una buona correlazione è stata dimostrata tra la conta dei RBC manuale con camera di Nageotte, considerata il gold standard e UF 1000 (r=0.89, Bias=0.003) mentre ADVIA-PA dimostra una sovrastima nella lettura dei RBC residui (r=0.62, Bias=-1.332).Buone correlazioni sono state dimostrate per la conta dei WBC residui tra il metodo di riferimento citofluorimetrico BD Leucocount™ (Beckton-Dickinson MI) e UF 1000 (r=0.85, Bias=0.011) come pure per ADVIA-PA (r=0.81, Bias=0.012).
Conclusioni: ADVIA-PA, nonostante fornisca ottime performance analitiche, dimostra una sovrastima nella determinazione dei RBC residui. UF 1000 dimostra un'ottima correlazione con i metodi di riferimento sia per RBC che per WBC e può potenzialmente essere utilizzato per il conteggio delle cellule residue nelle unità di plasma per impiego trasfusionale.

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**SYSMEX UF-5000 BODY FLUIDS MODE: PERFORMANCE OF NUCLEATED CELLS, AND DIFFERENTIAL COUNT IN BODY FLUIDS**

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Introduction: Cellular analysis of Body Fluids (BF) provides important diagnostic information in various medical conditions. The reference method for cell counts in BF is the manual method by optical microscopic (OM) in counting chamber; this procedure is time-consuming, labour intensive and requires experienced laboratory workers. For this reason, in the last decade, the use of automatic haematological analysers improved the accuracy and the workflow of BF examination. Sysmex UF-5000 automated urine analyser uses a fluorescent flow cytometry technology and an hydrodynamic focusing to identify and enumerate cells. It has a dedicated module for Body Fluids analysis (UF-BF) that provides the following parameters: total nucleated cells (TC-BF), leukocytes (WBC-BF) polymorphonuclear (PMN #; PMN %) and mononuclear (MN#; MN%) cells. The aim of this study was to evaluate the application of Sysmex UF-5000 Body Fluid mode in cytometric analysis of BF compared to OM.
Methods: 100 BF samples (40 Ascitic Fluid, 15 Pleural Fluid, 28 Pericardial Fluid, 17 Synovial Fluid) with a TC-BF ranging from 11 to 46833x$10^6$/L, collected in $K_3$EDTA tubes (Becton Dickinson), were simultaneously analyzed with UF-BF and OM. The agreement between UF-BF and OM was assessed with Passing Bablok (PB) regression and Bland-Altman Bias analysis. Statistical analysis was carried out with Analyse-it software 3.90.5 (Leeds, UK).
Results: For TC-BF the comparison between UF-BF and OM showed a PB regression of y=1.04x+26.98, Bias of 76.5x$10^6$/L and 7.3%. For WBC-BF, PB regression was y=1.05x + 226.8, Bias was 520.1x$10^6$/L and 42.7%. For MN-BF and PMN-BF, PB regression were y=1.30x +140.7 and y=0.99x + 26.47, absolute Bias were 510.0x106/L and 85.8x106/L, relative Bias were 52.9% and 46.3% respectively
Conclusion: The use of UF-5000 Body Fluid mode can provide an effective and automated alternative to OM in routine BF analysis, also enhancing laboratory workflow, thanks to its ability to perform direct analysis on untreated BF samples in a single run. The rapid visualization of instrumental data can gives important hints on the prevalent cell population whenever a high-count sample is present, thus being an effective way to make rapid therapeutic decisions and save time and manual labor.

P202
**PERFORMANCE EVALUATION OF NEW SENTINEL ATELLICA ALLIANCE APPLICATIONS COPPER AND ZINC, AND OF KAPPA LIGHT CHAINS, LAMBDA LIGHT CHAINS, ON THE SIEMENS ATELLICA CH ANALYZER**

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Background: The purpose of the study was to evaluate the performance on a Siemens Atellica CH Analyzer of Sentinel CH reagents Kappa Light Chains (K), Lambda Light Chains (L) as Open Channel, and Copper (Cu) and Zinc (Zn) as Atellica Alliance Applications. K and L are used in the diagnosis and monitoring of patients with a monoclonal gammopathy. Cu deficiency is linked to heart disease, bone and joint osteoarthritis, osteoporosis and decrease in antioxidant protection. Zn is necessary for cell replication. Its deficiency causes skin lesions, growth retardation and impaired immunological functions.K and L tests are based on an immuno turbidimetric reaction between anti-K (L) polyclonal antiserum and antigen. The turbidity formed is proportional to the amount of the analyte present. Both Zn and Cu form a stable colored complex with a specific complexant, the intensity of the color being proportional to the level of Zn or Cu present.
Methods: Evaluation of: LoB, LoD, LoQ, inter-assay imprecision (BR), on board reagent stability (OB), lot calibration interval (LCI), linearity (LIN), instrument correlation (COR), sample carry over (CO) and hook effect (HE) following CLSI guidelines. Analyse-it software was used for the statistical analysis.
Results: LoB*: K 0.2 mg/dL; L 1.6 mg/dL; Zn 2.6 mg/dL; Cu 1.4 mg/dL.
LoD*: K 0.4 mg/dL; L 2.0 mg/dL; Zn 3.9 mg/dL, Cu 3.3 mg/dL.
LoQ*: K 2.1 mg/dL; L 3.9 mg/dL; Zn 3.9 mg/dL; Cu 6.4 mg/dL.
BR [CV%]: K 1.6-4.2; L 1.4-6.7; Zn 0.4-1.1; Cu 0.6-2.4.
OB (30 days + 10%) [Bias %]: K -6.5-5.3; L -5.3-10.6; Zn -1.7-2.4; Cu -9.8-6.3.
LCI (180 days; Zn and Cu up to 80 days, in progress) [Bias %]: K -9.6-7.2; L -11.1-3.8; Zn -9.2-4.8; Cu -4.8-9.4.
LIN [up to]*: K 661 mg/dL; L 355 mg/dL; Zn 2055.3 mg/dL; Cu 504.4 mg/dL.
COR: K y = 1.007x+1.756 mg/dL r = 0.989; L y = 0.992x +3.451 mg/dL r = 0.987; Zn y = 1.014x -4.419 mg/dL r = 1.000; Cu y = 1.006x-3.372 mg/dL r = 0.999.
CO [Bias %, (Bias % considering the gap between the two concentrations)]: K 1.22 (0.03); L -3.13 (-0.05); Zn -2.20 (-0.09); Cu -0.99 (-0.17).
HE [No HE up to]: K 1352 mg/dL, L 2112 mg/dL.
*: performed on 2 batches.
Conclusions: All assays tested were able to meet performance requirements on the Atellica CH Analyzer.

P203
**VOLUMETRIC ABSORPTIVE MICROSAMPLING (VAMS) AS A VERSATILE SAMPLING APPROACH FOR THERAPEUTIC DRUG MONITORING IN CLINICAL SETTINGS**

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Volumetric absorptive microsampling (VAMS) is a novel methodology for a simple fixed-volume single-drop blood collection (10, 30 or 50 μL). When combined with liquid chromatography tandem mass spectrometry (LC-MS/MS), VAMS is a valid tool for therapeutic drug monitoring (TDM) studies, because small blood volumes can be collected at frequent time points in pharmacokinetic analysis by avoiding venipuncture. However, several technical issues need to be accurately investigated to minimize pre-analytical variability for process standardization before routinely use these devices in clinical practice. In this work, we evaluated the influence of different parameters on the extraction yield obtained on three model compounds with different polarity, such as amoxicillin, caffeine and paclitaxel. Pre-analytical variables that were investigated included: comparison between capillary and venous blood on extraction yield; influence of anticoagulants and haematocrit values on calibration curve reliability; stability of adsorbed samples and extracts at different temperatures and time points. For our experiments, 10 μL VAMS (MITRA®, Neoteryx LLC, CA, USA) were employed for blood collection. After drying, VAMS were rehydrated with ultra-pure grade water and placed in a water-bath at 37°C for 10 min. Samples were then extracted with methanol and 1% formic acid, sonicated and centrifuged. Supernatants were transferred to appropriate vials and injected in a LC-MS/MS system for single-reaction monitoring (SRM) analysis mode. Our results showed good stability of the molecules after absorption on VAMS, either at room temperature or at 4°C. The optimized extraction method was efficient for our selected compounds and might be also applied to other drugs. The LC-MS/MS method showed good linearity for all compounds. In conclusion, our results confirm that VAMS has several technical advantages compared to other dried microsampling techniques, in which accuracy is more influenced by sampling volume or hematocrit values.

P204
**VERIFICA DELLA COMPARABILITA' DEI RISULTATI DI CHIMICA CLINICA SUL SIERO FRA I SISTEMI ANALITICI ADVIA® CHEMISTRY 2400 ED ATELLICATM SOLUTION CH930 PRESSO IL LABORATORIO SMEL2 DELL'ASST PAPA GIOVANNI XXIII DI BERGAMO**

S. Gelsumini, S. Buoro, C. Saiaci, L. Cerutti, M. Parimbelli, S. Apassiti Esposito, C. Bizzoni, A. Cesani, G. Agnolet, G. Bolzoni, S.R. Romeo, A. Lucente, G. Colombo, M. Fortino, E. Lochis, M. Bombardieri, N. Della Malva, A. Caracciolo, G. Caldara, G. Merlo, R. Pallotta, A. Beluzzi, G. Bettoli, F. Capocefalo, S. Castelletti, A. Ferlita, P. Gherardi, S. Resmini, G. Guerra

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Nel 2019 lo SMeL2 dell'ASST Papa Giovanni XXIII di Bergamo, certificato ISO 9001:2015, ha subito un aggiornamento tecnologico che ha previsto la sostituzione dei sistemi analitici ADVIA® Chemistry 2400 con Atellica™ Solution CH930 (Siemens). Prima dell'introduzione in routine di Atellica™, il laboratorio ha verificato la comparabilità dei risultati di tutti i test di Chimica Clinica siero tramite valutazione iniziale (software MetComp) con il grafico Bland Altman sui campioni dei pazienti e, in presenza di un bias rilevante, una correlazione Passing-Bablok (PB), coprendo l'intervallo di misura clinicamente rilevante. Dei 30 test in uso: 11 mostravano un bias non significativo (ALT, Bilirubina Diretta e Totale, Colinesterasi, Cloro, Creatinina, GGT, HDL, LDL, Magnesio e Fosfato – 37%); 11 mostravano un bias significativo con PB negativa (Albumina, ALP, Amilasi Pancreatica, AST, Calcio, Colesterolo Totale, Potassio, Lattato, Sodio, PCR, Urea – 37%). Di conseguenza, questi 22 test (74%) sono risultati comparabili in modo soddisfacente per il criterio statistico ed introdotti in routine. I restanti 8 test (Amilasi, CK, Fe, Glucosio, Lipasi, Urato, Proteine Totali e Trigliceridi – 26%) invece, hanno mostrato un bias significativo con PB positiva, quindi si sono rese necessarie altre valutazioni. Per autorizzare l'ingresso in routine di questi test, dati i continui fermi strumentali degli ADVIA (dovuti alla loro usura), abbiamo verificato sia l'assenza di misclassificazione dei risultati tra i due strumenti, sia che il coefficiente di determinazione $r^2$ fosse ≥0,99. Nel primo esercizio di VEQ (per Regione Lombardia, AOU Careggi), i risultati di questi 8 test sono risultati all'interno del Limite di Accettabilità applicato nella valutazione dell'Errore Totale. Terminata positivamente la verifica iniziale, è obiettivo del laboratorio perseguire periodicamente gli obiettivi di miglioramento delle prestazioni di tutti gli analiti, monitorandole secondo le indicazioni societarie SIBioC, implementando la definizione di una procedura specifica, un'istruzione operativa interna, le modalità di gestione delle situazioni non comparabili e la registrazione di dati ed azioni intraprese in seguito all'analisi dei risultati ottenuti dalla verifica della comparabilità dei dati.

P205
**COMPARISON EVALUATION OF NEW AUTOMATED MAGLUMI FREE TESTOSTERONE ASSAY®**

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Background: Testosterone and its metabolites are the major active androgens. Most testosterone circulates bound to sex hormone-binding globulin (SHBG), whilst a minor amount circulates in free form, and this is believed the metabolically active fraction. Many techniques are now available for free testosterone measurement, including the reference method (equilibrium dialysis and ultrafiltration), analogue radioimmunoassays and innovative fully-automated chemiluminescence immunoassays. Accordingly to the recommendations of the ISO 15189, clinical laboratories should validate the quality specifications of new tests according to defined protocols. The aim of this study was to verify the analytical performance of the new MAGLUMI free Testosterone® (SNIBE, Shenzhen, China).
Methods: The study population consisted of 130 patients (45 women and 85 men; mean age, 51±22 and 30±12 years, respectively). Two paired serum aliquots were assayed with a reference radioimmunoassay (DSL ACTIVE Free Testosterone RIA assay, Pantec, Italy) and with the new commercial chemiluminescent MAGLUMI free Testosterone® on Maglumi 800 (SNIBE, Shenzhen, China).
Results: The within-coefficients of variations (CVs) of free Testosterone with low (10.2 pmol/L), 3.2%. Results of Serum samples (n=130) were compared with those of the reference commercial DSL ACTIVE Free Testosterone RIA assay. The median values (2.5-97.5 percentiles) of the samples were: 16,8 pmol/L (4,1-160,7 pmol/L) for Maglumi free testosterone® and for 11,7 pmol/L (1,5-56,3 pmol/L) DSL ACTIVE Free Testosterone RIA assay. The nonparametric regression according to Passing & Bablok and the Spearman's correlation coefficient confirmed optimal performance for Maglumi free testosterone® (Maglumi free testosterone® = 2,50 x DSL ACTIVE Free Testosterone RIA - 3,48; r= 0.95, p<0.001). The mean percentage bias of Maglumi free testosterone® versus DSL ACTIVE Free Testosterone RIA assay was +63,5% (95% CI: +57,2% to +69,8%, p<0.0001) in Bland and Altman plots analysis.
Conclusion: We conclude that the analytical performance and technical features of new Maglumi free testosterone® makes it a suitable assay for rapid quantification of free testosterone in clinical laboratories. Due to inter-assay discrepancy , laboratories shall define locally validated reference ranges.

P206
**ANALYTICAL EVALUATION OF THE NEW HPLC INSTRUMENT TOSOH HLC-723 G11 FOR DETERMINATION OF GLYCATED AND VARIANT HEMOGLOBIN**

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Background: High-performance liquid chromatography (HPLC) is a technique extensively used for diagnostics of diabetes and hemoglobinopathies. The advantages of HPLC mainly entail excellent resolution, reproducibility and rapid quantification of several hemoglobin subtype. This study was aimed to verify the analytical performance of the new Tosoh HLC-723 G11 for assessment of glycated and abnormal hemoglobins before introduction in routine clinical practice.
Methods: EDTA whole blood samples for HbA1c and HbA2 assessment were simultaneously tested (paired aliquots) with the currently used HPLC assay analyzer (HLC-723 G8, TOSOH Italy) and with the new HLC-723 G11 (TOSH, Italy).
Results: The within- and between-run coefficients of variation (CVs) of samples with low (37 mmol/mol), and high (84 mmol/mol) HBA1c was were 0,7-1,7%, and 0,5-2,6%, respectively. The within- and between-run CV of samples with low (2,6%), and high (4,6%) HbA2 were 1,9%-1,2% and 2,6-2,8% respectively. In 40 EDTA whole blood samples the median values (2.5-97.5 percentiles) , were 48 mmol/mol (31-85 mmol/mol) for HbA1C and 2,6% (1,6-6,9%) for HbA2 on HLC-723 G8, and 47 mmol/mol (28-82 mmol/mol) for HbA1C and 2,9% (1,9-8,9%) for HbA2 on HLC-723 G11. The Passing & Bablok regression and Spearman's correlation coefficient were excellent, being [HbA1C G11] = 1,0 x [HbA1C G8] - 3,0 (r=1.00, p<0.001) and [HbA2 G11] = 1,36 x [HbA2 G8] – 0,52 (r= 0.94, p<0.001). The mean bias of G11 for HbA1c and HbA2 versus G11 was -6,1% (95% CI: -4,1% to -8,2%) and +15,2% (95% CI: +8,3% to +22%, p<0.001).
Conclusion: The analytical performance and the technical features of new HPLC-723 G11 make it a suitable instrument for reliable quantification of HBA1C and HbA2 in routine clinical practice.

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**CONFRONTO FRA DUE SISTEMI PER L'ANALISI AUTOMATIZZATA DELLE URINE E L'ESAME MICROSCOPICO DEL SEDIMENTO**

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L'esame chimico-fisico e del sedimento urinario viene abitualmente eseguito con sistemi automatizzati che usano tecnologie basate sulla citometria a flusso o su analisi di immagini. In questo studio sono state confrontate le prestazioni degli strumenti Iris IQ200 (Beckman C., Brea, USA) e Sysmex UF-5000 (Sysmex Co, Kobe,Giappone) con l'analisi microscopica manuale. Sono stati selezionali 362 campioni della routine che dopo l'analisi automatizzata sui due sistemi sono stati sottoposti a centrifugazione (400g per 5 minuti) ed esaminati al microscopio sia a 200X che a 400X.Sono stati valutati i confronti della misura di globuli rossi (RBC), globuli bianchi (WBC), cellule squamose e non squamose, cilindri e miceti. RBC e WBC sono stati suddivisi in 6 classi (≤20, 21-40, 41-80, 81-200, 201-500, >500 elementi/µL). Le concordanze sono state valutate mediante l'analisi della k di Cohen. Le percentuali di concordanza parziale si riferiscono alla concordanza anche con le classi adiacenti a quella in considerazione.Risultati.RBC. UF5000 vs Microscopio: k= 0.557 (concordanza moderata: 61.3%). La concordanza parziale è di 81.2%. Confronto IRIS vs Microscopio: k= 0.483 (concordanza moderata : 55.3%). La concordanza parziale è di 78.2%WBC. UF5000 vs Microscopio: k= 0,634 (concordanza buona: 52.8%). La concordanza parziale è di 80.4%. Confronto IRIS vs Microscopio: k Cohen = 0.551 (concordanza moderata: 46.1%). La concordanza parziale è di 74.9%Cilindri. Concordanze deboli per UF5000 (k= 0.37) e per Iris (k= 0.256). Il confronto migliora (k=0.544) per UF5000 alzando il cut-off della positività da 1 a 4 cilindri, ma restano il 46% di falsi positivi e non vengono individuati il 30% dei cilindri rilevati al microscopio. Aumentando il cut-off con IRIS il confronto invece peggiora. Miceti e Cellule epiteliali non squamose: positività molto basse, la statistica non può essere affidabile, ma le classificazioni degli strumenti non risultano comunque soddisfacenti, particolarmente per IRIS. Cellule squamose: concordanza modesta (cut-off 20) con IRIS (k: 0.45) e con UF (k: 0.37).Complessivamente le prestazioni dei due strumenti sono sufficienti per la misura di RBC e WBC, specie per UF, mentre restano ancora scadenti per l'individuazione in prima battuta di altri elementi, in particolare per IRIS.

P208
**EVALUATION OF A CAPILLARY ZONE ELECTROPHORESIS SYSTEM FOR SCREENING AND DIAGNOSIS OF MONOCLONAL GAMMOPATHIES, CARBOHYDRATE-1 DEFICIENT TRANSFERRIN, GLYCATED AND HEMOGLOBIN VARIANTS**

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Background: Capillary zone electrophoresis (CZE) assay has progressively taken the place of agarose gel electrophoresis in laboratory practice giving the possibility to perform high number of samples in brief time with a good analytical quality. At the moment it represents the gold standard for serum protein electrophoresis (SPE).
Objective: CZE also finds fields of application in determination of 33 carbohydrate-deficient transferrin (CDT), HbA1c glycated hemoglobin and hemoglobin variants. Here we compared data obtained by a CZE test method with those obtained by conventional methods used for detection of these analytes.
Methods: Serum or whole blood samples (n. 200 for SPE; n. 100 for immunosubtraction; n. 120 for CDT, n. 213 for HbA1c glycated hemoglobin and n. 81 for hemoglobin variants) from patients consecutively admitted at "Sandro Pertini" Hospital of Rome were collected and concordance between two CZE-based systems (V8 Nexus vs Capillarys 2) specifically designed for SPE and immunosubtraction were assessed. Differences between CZE and high performance liquid chromatography (HPLC) were also assessed in CDT (V8 Nexus vs Agilent Series 1200 HPLC System), HbA1c glycated and hemoglobin variants dosages (V8 Nexus vs Tosoh G8 HPLC).
Results: A good correlation was obtained in measurement of percentage of γ-globulin with respect to total serum proteins and both systems showed a measure of inaccuracy extensively within desirable specification for imprecision. Concerning immunosubtraction, test method allowed the identification of an additional monoclonal component that was undetectable using reference method or IFE. In order to identify any difference between HPLC and CZE the graph of linear regression and the Bland-Altman plot were used. Moment correlation coefficients and linear regressions were calculated using Pearson correlation for CDT, HbA1c glycated hemoglobin and hemoglobin variants.
Conclusions: The fully automated CZE tested method resulted fully concordant with reference methods currently in use in our laboratory. With reference to immunosubtraction, it is confirmed as an additional method, not alternative to IFE useful for a subset of samples that are difficult to interpret with conventional technology. CZE also provides precise, quick, and very easy quantification of CDT and glycated hemoglobin and hemoglobin variants. We believe it is very reliable and suitable for routine investigation.

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## FREE LIGHT CHAINS URINARY LEVELS IN THE FOLLOW-UP OF PATIENTS WITH MONOCLONAL GAMMOPATHY: COMPARISON BETWEEN DENSITOMETRIC AND TURBIDIMETRIC METHOD

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Introduction: Bence Jones protein (BJ) is crucial in the management of patients with monoclonal gammopathy (GM). Guidelines suggest to quantify BJ by densitometry (DM). The purpose of the work is to assess: 1) the agreement between BJ by DM and urinary free light chains (uFLC) levels by immunoturbidimetry (ITA); 2) if uFLC measure by ITA is a good marker in the follow-up of patients with GM; 3) uFLC levels in patients with GM - with or without BJ - compared to healthy controls.
Materials and methods: 139 samples were analysed: 40 negatives, 46 with GM but BJ negative, 53 with GM and BJ positive (28 BJ-Kappa and 25 BJ-Lambda). The parameters analysed were: BJ (Hydrasys, Sebia, France) serum FLC (sFLC) and urine (Optilite, Binding Site, UK) and total urinary protein (uPT) (AU680, Beckman Coulter, USA).
Results: The results (average ± standard deviation) in mg/L were the following. 40 healthy controls: sFLC-K 19±7; sFLC-L 16±4; uFLC-K 23±28; uFLC-L 2±3; uPT 85±5446. Samples with GM but BJ negative: sFLC-K 56±155; sFLC-L 53±150; uFLC-K 56±59; uFLC-L 4±5; uPT 225±405;28. Samples with GM BJ-K: sFLC-K 744±845: uFLC-K 7723±13954; BJ-K DM 543±932 (8 not measurable); uPT 817±1078;25.
Samples with GM BJ-L: sFLC-L 2011±6545: uFLC-L 1654±6841; BJ-L DM 551±1851 (7 not measurable); uPT 1561±3535.
Comparison between BJ DM and uFLC, linear regression (r): BJ-K: y=15x-284; r= 0.99 (p< 0.001); BJ-L: Y=3.6x-352; r=0.98 (p<0.001).
Conclusions: DM is a technique highly operator-dependent, time-consuming and with a low sensitivity: 28% of BJ were not detected because of low or high proteinuria, or comigration with Ig or beta zone proteins. The turbidimeter Optilite allows to detect uFLC in a wide range of measure (from 1 mg/L) and it is automatically able to manage the antigen excess. We found a very good correlation between uFLC measured by Optilite and BJ quantified by DM, thus, uFLC by ITA could be useful in follow-up of GM patients. In patients with GM but BJ negative it should be noted a little increase of uFLC especially regarding uFLC-k in comparison to healthy patients. Lastly, to be noted is the extremely high value of uFLC-k in patients with BJ-k, up to more than 10 times the value of uPT and sFLC-K, data on which it is necessary to reason about.

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## MISURA DELLE FREE LIGHT CHAINS URINARIE E QUANTIFICAZIONE DELLE PROTEINA DI BENCE JONES (PBJ) IN ELETTROFORESI CAPILLARE (CZE) IN SOGGETTI AFFETTI DA DISCRASIE PLASMACELLULARI: CONFRONTO TRA METODI ANALITICI

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Background: Le discrasie plasmacellulari sono un gruppo di patologie caratterizzate dalla proliferazione clonale di plasmacellule e/o linfociti B che producono proteina monoclonale: Immunoglobulina intatta, catene leggere libere k e/o λ, o catena pesante. L'IMWG suggerisce per la diagnosi, in particolare Amiloidosi AL, monitoraggio e risposta alla terapia, la quantificazione della Proteina di Bence Jones(PBJ). Il metodo raccomandato è la quantificazione mediante scansione densitometrica del picco monoclonale evidenziato sul tracciato elettroforetico delle urine 24h rapportato alla proteinuria totale e alla diuresi. Si vuole confrontare la quantificazione (QBJ) con metodo raccomandato e la misura immunochimica delle Free Light Chains urinarie (u-FLC).
Metodi: Sono stati confrontati 65 campioni di urine 24h di soggetti con MM, LCMM, AL e LCDD, diagnosticati con: elettroforesi proteica (CZE), immunofissazione siero (s-IFE), immunofissazione urine (u-IFE) (SEBIA) e s-FLC (The Binding Site). La QBJ è stata eseguita con metodo CZE, per la misura di u-FLC sono stati utilizzati i kit diagnostici Freelite Mx kappa e lambda free (The Binding Site). Infine, è stato considerato eGFR per la valutazione della funzionalità renale. I dati sono stati elaborati con metodica statistica non parametrica di Spearman.
Risultati: I campioni con PBJ k hanno mostrato con metodo CZE una QBJ da 0,1 a 2484 mg/24h, la misura u-FLCk da 23,44 a 22014 mg/24h. I campioni di pazienti con PBJ λ da 0,1 a 5538 mg/24h con CZE, mentre la misura u-FLC λ da 13,2 a 33373 mg/24h. Il confronto statistico tra i due metodi ha mostrato per PBJ k una buona correlazione statisticamente significativa (p<0,001; r= 0,719); per PBJ λ la correlazione migliora significativamente (p<0,001; r=0,922). La correlazione parziale corretta per eGFR migliora significativamente per PBJ k e λ: r=0.956 e r= 0,957 rispettivamente.
Conclusioni: La misura delle u-FLC correla con il metodo raccomandato, ma ulteriori studi sono necessari per valutare se la misura immunochimica delle u-FLC possa essere una valida alternativa. La quantificazione immunochimica della PBJ potrebbe migliorare la fase preanalitica di preparazione del campione, che ora risulta essere indaginosa e di non facile utilizzo nei laboratori di elevata routine.

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**TROPONINA CARDIACA AD ALTA SENSIBILITA': UN NUOVO POCT A CONFRONTO CON I CONSOLIDATI ANALIZZATORI DEI LABORATORI CLINICI**

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Introduzione: PATHFAST™ (P) è un POCT, che utilizza le tecnologie CLEIA e Magtration®, sul quale è stato di recente applicato e reso commercialmente disponibile, un nuovo metodo ad alta sensibilità per la determinazione quantitativa della troponina cardiaca I (hs-cTnI).
Scopo: Valutare le caratteristiche analitiche e cliniche di P presso i Laboratori analisi di Padova (Lab 1) e di Mestre (Lab 2) in confronto a quelle dei metodi che misurano la hs-cTnI sulle strumentazioni piu' diffuse nei laboratori clinici.
Materiali e Metodi: Valutazione analitica: sono stati utilizzati materiali di controllo del commercio e pool di plasma da litio eparina. Valutazione clinica: le determinazioni di hs-cTnI sono state eseguite in campioni (plasma da litio-eparina; basale e a 3 ore) di pazienti ammessi in Pronto Soccorso per dolore toracico e confrontate con quelle degli analizzatori Architect PLUS $i2000_{SR}$ (Abbott) (A), Dimension Vista (Siemens) (D), Advia Centaur XPT (Siemens) (AC), DXI 800 (Beckman Coulter) (B). Utilizzando il 99° percentile specifico per sesso dichiarato da ciascun produttore, è stata valutata la % di concordanza (CON=POS/POS+NEG/NEG) e discordanza (DIS=NEG/POS+POS/NEG) nella classificazione del risultato (POS>99° perc.; NEG≤99° perc.).
Risultati: Tutte le concentrazioni sono espresse in ng/L. Valutazione analitica: range-concentrazioni (range-CV%): (Lab 1)=14.6-12227.3 (5.0-9.8); (Lab 2)=36.6-1633.9 (4.8-7.8). Valutazione clinica: periodo di arruolamento=12-14/11/17; pazienti arruolati=133 (69 maschi, 64 femmine; 18-96 anni; 89 (66.9%) dimessi dal PS, 44 (33.1%) ricoverati. Campioni analizzati=191. CON=%, DIS=% (NEG/POS, POS/NEG): A vs P=88.5, 11.5 (0.0, 11.5); AC vs P=96.3, 3.7 (0.5, 3.2); D vs P=94.8, 5.2 (2.6, 2.6); B vs P=76.8, 23.2 (0.0, 23.2). La prevalenza di risultati discordanti è stata osservata sul prelievo basale (range%=59.1-83.3).
Conclusioni: Le caratteristiche analitiche osservate risultano conformi a quanto dichiarato dal produttore e adeguate per i metodi definiti ad alta sensibilità. Le prestazioni cliniche hanno evidenziato un grado di concordanza (% range=76.8-96.3) complessivamente soddisfacente con tutte le piattaforme analitiche utilizzate, ampiamente validate in letteratura per la misura di cTnI con metodi ad alta sensibilità.

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**VALUTAZIONE ANALITICA DELLA DETERMINAZIONE DEL PSA TOTALE (TPSA) SU DUE ANALIZZATORI AUTOMATICI, COBAS E801 E CENTAUR XP**

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Background: Anche se la comparabilità fra metodi commerciali per la determinazione di tPSA è migliorata, l'armonizzazione tra i metodi è ancora scarsa. Scopo del lavoro è stato valutare la precisione di due metodi per il PSA totale, in chemiluminescenza (CLIA) su analizzatore Centaur XP, e in elettrochemiluminescenza (ECLIA) su analizzatore COBAS e801 (ROCHE). E' stata anche valutata la comparazione tra i due metodi soprattutto nel follow-up di pazienti sottoposti a trattamento chirurgico o a terapia.
Materiali e Metodi: Per la valutazione della precisione analitica, sono stati utilizzati controlli (QC) a tre livelli di concentrazione di PSA, forniti da Biorad e testati seguendo il protocollo EP5A. Per lo studio di comparazione tra i metodi, sono stati selezionati 30 campioni di siero di pazienti con valori di PSA totale compresi tra 0,01 e 0.999 ng/ml. I dati sono stati valutati con l'analisi di Bland and Altman.
Risultati: Studio di precisione. Centaur XP: QC livello 1, CV% tra-serie=1.89 (media=0.75ng/ml), CV % intra-serie=1.08 (media=0.73 ng/ml); QC livello 2, CV% tra-serie=2.08 (media=3,02ng/ml), CV% intra-serie=1.49 (media=2.9ng/ml); QC livello 3, CV% tra-serie=2.96 (media=18.7ng/ml), CV% intra-serie=1.32 (media=18.2ng/ml); COBAS e801: QC livello 1, CV % tra-serie=1.48 (media=0.065ng/ml), CV% intra-serie=0.98 (media=0.063ng/ml); QC livello 2, CV % tra-serie=1.96 (media=3,25ng/ml), CV% intra-serie=1.01 (media=3.22ng/ml); QC livello 3, CV% tra-serie=2.42 (media=14.33ng/ml), CV% intra-serie=1.32 (media=14.2ng/ml). Studio di comparazione. Bias assoluto CENTAUR XP vs COBAS e801: 33,92 ng/ml, CI 95% (14,48 to 53,36); 95% limit of agreement = -68,10 (lower), 135,95 (upper) (P<0,05).
Conclusioni: I dati ottenuti evidenziano per i metodi CLIA ed ECLIA risultati riproducibili tra- e intra-serie per i 3 livelli di controlli testati con una imprecisione totale rispettivamente pari a CV% totale tra 1.08 e 1.32, e tra 0.98 e 1.32. I metodi si confermano idonei ad un uso routinario per la misurazione del PSA totale anche in pazienti in follow-up post-chirurgico o terapeutico. Tuttavia, un bias statisticamente significativo tra i metodi indica la necessità di utilizzare, durante il monitoraggio, lo stesso metodo analitico al fine di ridurre il rischio di errori clinici.

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**VALUTAZIONE ANALITICA DELLA DETERMINAZIONE DELLA TIREOGLOBULINA SU DUE ANALIZZATORI AUTOMATICI, COBAS E801 E LIAISON XL**

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Background: L'introduzione di nuovi strumenti nei laboratori richiede studi di validazione per assicurarsi che i nuovi metodi incontrino standard di performance accettabili. Scopo del lavoro è stato valutare la precisione di due metodi per la Tireoglobulina (TG), in chemiluminescenza (CLIA) su analizzatore LIAISON XL (DIASORIN), e in elettrochemiluminescenza (ECLIA) su analizzatore COBAS e801 (ROCHE). Inoltre è stata valutata la comparazione tra i due metodi per verificare la performance analitica soprattutto per valori molto bassi di TG nel follow-up di pazienti post-chirurgici o in terapia.
Materiali e Metodi: Per valutare la precisione analitica dei metodi CLIA ed ECLIA, sono stati utilizzati controlli (QC) a due livelli di concentrazione di TG, forniti da Diasorin e Biorad rispettivamente; entrambi sono stati testati seguendo il protocollo EP5A. Per lo studio di comparazione e la valutazione della concordanza tra i metodi, sono stati selezionati 30 campioni di siero con valori di TG compresi tra 0,04 e 0.956 ng/ml. I dati sono stati valutati con l'analisi di Bland and Altman.
Risultati: Studio di precisione. LIAISON XL: QC livello 1, CV% tra-serie=1.89 (media=5.38ng/ml), CV% intra-serie=0.97 (media=5.21ng/ml); QC livello 2, CV% tra-serie=2.97 (media=170,46ng/ml), CV% intra-serie=1.25 (media=167.52ng/ml); COBAS e801: QC livello 1, CV% tra-serie=1.37 (media=12.21ng/ml), CV% intra-serie=0.86 (media=12.36ng/ml); QC livello 2, CV% tra-serie=2.49 (media=111,16ng/ml), CV% intra-serie=1.32 (media=112.6ng/ml). Studio di comparazione. Bias assoluto LIAISON XL vs COBAS e801: 19,32ng/ml, CI 95% (-16,53 to 55,18); 95% limit of agreement = -168,89 (lower), 207,54 (upper) ($P<0,05$).
Conclusioni: I dati evidenziano per i metodi CLIA ed ECLIA risultati riproducibili tra- e intra-serie per i 2 livelli di controlli testati, con una imprecisione totale rispettivamente pari a CV% totale tra 0.97 e 1.25, e tra 0.86 e 1.32. I metodi presentano adeguate performance analitiche relativamente alla precisione. Inoltre un bias statisticamente non significativo suggerisce che la valutazione di pazienti in follow-up post-chirurgico o terapeutico, nei quali variazioni di valori molto bassi migliorano il management del paziente, possa non risentire del passaggio da una all'altra metodica.

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**V8 NEXUS : VALUTAZIONE PRELIMINARE NELLA DIAGNOSTICA PROTEICA URINARIA**

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Introduzione: La diagnostica proteica urinaria deve comprendere una valutazione quali-quantitativa delle proteine escrete. L'elettroforesi urinaria è utilizzata nella routine di laboratorio per la classificazione ed il monitoraggio della proteinuria e per la rilevazione e quantizzazione delle componenti monoclonali.
Scopo del lavoro: valutare le performance analitiche e strumentali del nuovo strumento in tecnologia capillare V8 Nexus della ditta Helena Biosciences.
Metodi: Elettroforesi e immunosottrazioni urinarie sono state eseguite utilizzando urine delle 24 h precedentemente dosate nefelometricamente. I campioni urinari processati sono stati preventivamente desalificati utilizzando colonnine cromatografiche NAP-5 Columns in combinazione con V8 Urine Preparation Buffer SP6 Zoom kit, in alternativa ai dispositivi da centrifuga.
Sensibilità: si è provveduto a verificare e calcolare la sensibilità del metodo, utilizzando un' urina avente i seguentiparametri : 44 mg/l Kappa Urinaria, Lambda Urinaria < 50 mg/l, Alfa1 micro : 42.1 mg/l e microalbuminuria 4.9 mg/l. Il campione presentava una componente monoclonale precedentemente tipizzata in agarosio come C.M. Kappa libera. Effettuando diluizioni a scalare il picco in zona Gammariferibile C.M. Kappa Libera era rilevabile ad una concentrazione pari a 11 mg/l.
Risultati: Sono state eseguite 30 elettroforesi con proteinuria variabile da <150 mg/24h sino a 35 g/l. L'ottima qualità dei tracciati ha reso possibile la classificazione delle proteinurie in glomerulare, tubulare e mista. In presenza di picchi omogenei, è stata eseguita l' immunosottrazione al fine di tipizzarli.Il metodo si è dimostrato efficace nel quantizzare le componenti monoclonali urinarie presenti ed elimina le interferenze insite nella scansione densitometrica e nel processo di colorazione e decolorazione che si possono riscontrare con il gel d'agarosio.
Conclusioni: I dati preliminari ottenuti, hanno evidenziato un'ottima qualità analitica. Lo strumento dimostra semplicità d'uso. Inoltre, il sistema di desalinizzazione del campione urinario si è rilevato di ottima qualità e di facile utilizzo (circa 10 minuti di preparazione).

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**HYALURONIC ACID: A NEW BIOMARKER IN SYSTEMIC AL AMYLOIDOSIS**

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The systemic amyloidoses are a group of complex diseases caused by tissue deposition of misfolded proteins that results in progressive organ damage. Immunoglobulin light chain amyloidosis (AL) is caused by misfolded light chains produced by a small, dangerous B-cell clone. Hyaluronic acid (HA) is a marker of fibrosis, used, together with other markers, to evaluate the severity of fibrosis in the liver. We hypothesised that fibrosis can have a role in the reversibility of organ involvement in AL amylodosis. Thus, we evaluated the correlations of HA at baseline with markers of organ involvement and its role as a possible predictor of organ response in 90 consecutive patients with systemic AL amyloidosis, using the ELFTM Test ADVIA Centaur® Kit (Siemens Healthcare Diagnostics, Germany). Organ response (OR) was evaluated with current validated criteria based on biomarkers. Mann Whitney test was used to evaluate differences in HA between subgroups. Logistic regression analysis was used to assess the ability of HA to predict OR. Best cut-off of HA predicting OR were identified with ROC analysis. Median age was 67 years (range: 59-73 years) and 58 (64%) patients were males. Heart was involved in 76 (83%) and kidney in 58 (63%) cases. Among 49 patients evaluable for cardiac and renal response, 21 (43%) achieved OR 6 months after treatment. In the overall population the median serum HA level was 80.1 ng/mL (range 46.6-142.2 ng/mL) higher than that reported in healthy volunteers (34.6 ng/mL ± 8.8; Polyzos SA et al 2019). Median HA concentration was 83.7 ng/mL (IQR 56-160.4 ng/mL) in patients with cardiac involvement and 60.3 ng/mL (IQR 30-70.6 ng/mL) in patients without cardiac involvement (P<0.003). HA level at baseline predicted OR after treatment (P<0.002). The best HA cut-off at baseline best predicting subsequent OR was 84 ng/mL (sensitivity of 76% and a specificity of 64%). Patients with HA concentration <84 ng/mL at baseline had a higher probability to achieve OR (33% vs. 10%; P=0.005). In conclusion, HA is a promising marker to predict reversibility of organ involvement in AL amyloidosis. Further larger studies are necessary to confirm and validate these favorable results.

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**PROTOCOLLO PER LA DEFINIZIONE DEGLI ESAMI INTERFERITI DA EMOLISI E DEI LIMITI DI ACCETTABILITA': APPLICAZIONE SU ATELLICA-CH**

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La gestione dei campioni emolizzati su analizzatori automatici è oggi facilitata e standardizzata dalle possibilità della misura diretta dell'interferente nel campione. Questi sistemi permettono di definire dettagliatamente, anche nell'ambito delle procedure operative, oltre che la natura delle non conformità dei campioni e le procedure utilizzate per identificarle, la tipologia di analisi influenzate dalla presenza di una specifica interferenza e i limiti di entità dell'interferenza oltre i quali l'analisi non è più attendibile. Scopo di questo lavoro è la descrizione di un protocollo per la definizione degli esami interferiti da emolisi e dei relativi limiti di accettabilità, su una strumentazione analitica di chimica clinica recentemente introdotta sul mercato.
Materiali e Metodi:
Sono stati valutati tre moduli Atellica-CH, integrati in due Atellica Solution collegati ad un sistema Aptio (Siemens Terrytown, USA). Atellica CH utilizza la pre-diluizione del campione 1:5. Questo consente volumi di campione molto ridotti e una attenuazione delle interferenze per diluizione degli interferenti. Dal sangue di un volontario sano si sono ottenuti plasma libero da emoglobina e 7 campioni con emolisi crescente, mediante il mescolamento del plasma e relativo dell'emolisato dello stesso campione. L'emolisi è stata provocata meccanicamente per passaggio ripetuto attraverso un ago sottile. La concentrazione di emoglobina libera nell'emolisato e stata misurata con contaglobuli XN-1000 (Sysmex, Cobe, Japan). Su ogni campione sono state misurate le concentrazioni degli analiti: NA, K, Cl, Mg, Ca, P, AST, ALT, GGT, COL-T, HDL-C, TG, BT, BD, ALP, AMI, LIP, CHE, PCR, CREA, Ac. Urico, BUN, Prot. tot., Alb.,Fe, TRF, LDH, NH4, LA, CK. La differenza critica della misura nel campione rispetto alla misura nel plasma, è stata calcolata secondo Fraser (Fraser CG. et al. Crit Rev Lab Sci 1989;27:409-37), sulla base della variabilità biologica individuale (dati di letteratura) e la variabilità analitica calcolata in laboratorio dai controlli di qualità interni.
Risultati: Il campione emolisato [E] aveva una concentrazione di 20,7 g/L di emoglobina. Su Atellica-CH l'indice di emolisi, su una scala da 0 a 6, per questo campione era H6 (2270,4). Le diluizioni scalari (1:1) sono risultate rispettivamente: [1] 10,35 g/L (H6-1127,2); [2] 5,175 g/L (H4-542,5); [3] 2,588 g/L (H3-289,8); [4] 1,294 g/L (H2-147,9); [5] 0,647 g/L (H1-74,7); [6] 0,323 g/L (H1, 14,8); [7] 0,162 g/L (H0-0,0); [plasma] 0,0 g/L (H0-3,5). Hanno mostrato interferenza maggiore della differenza critica gli esami: LDH H1-[5]; Potassio H2; AST H3; CK H3; GGT e FE H6-[1]. Non è stato valutato il limite di accettabilità per il glucosio, per il decadimento delle concetrazioni a causa della glicolisi in vitro. Non si sono

rilevate differenze significative nell'indice di emolisi tra i tre strumenti valutati.
Conclusioni: Il protocollo utilizzato consente di identificare la tipologia delle analisi influenzate dalla presenza di emolisi e i limiti di entità dell'interferenza oltre i quali l'analisi non è più attendibile.
La differenza critica è forse un parametro troppo stringente per la valutazione dell'accettabilità del potassio. La diluizione dei campioni nei sistemi Atellica-CH riduce il numero di esami interferiti significativamente dalla presenza di emolisi.

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**IL "LIBRO BIANCO" DEI GIOVANI PROFESSIONISTI DI MEDICINA DI LABORATORIO: RISULTATI DELL'INDAGINE DEL GRUPPO DI STUDIO (GdS) SIBioC YOUNG SCIENTISTS**

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Il panorama della Medicina di Laboratorio (MdL) è stato profondamente modificato dall'avvento di nuove tecnologie e sistemi, come l'automazione, i Big Data e le scienze Omiche. Il profilo dei Professionisti deve adattarsi a questi cambiamenti, sviluppando le competenze necessarie in ambito tecnologico, clinico e manageriale. Il GdS SIBioC Young Scientists ha promosso un questionario rivolto ai giovani professionisti under 40, con lo scopo di valutare i percorsi formativi e la condizione lavorativa. Il questionario (54 domande divise in 7 sezioni: anagrafica, iscrizione SIBioC, competenze, condizione lavorativa, specialisti, specializzandi, percezione del futuro) è stato preparato con Survey Monkey e diffuso ai soci SIBioC, per mezzo delle segreterie delle Scuole di Specializzazione, e attraverso i canali social del GdS. In circa 3 mesi sono state raccolte 283 risposte di giovani (età media 33 anni), distribuiti in tutt'Italia. La figura più rappresentata è quella dei Biologi (46%), affiancata da tutte le altre categorie (Medici 24%, Biotecnologi 14%, TLSB 10%, Chimici 2%, Farmacisti 2%, altro 2%). Tra i 194 soci, il 58% si sente poco coinvolto nelle attività societarie, il 35% è iscritto ad almeno un GdS. La maggioranza (70%) consulta abitualmente la rivista Biochimica Clinica, conosce LabTestsOnline e partecipa alla formazione FAD o residenziale SIBioC. Tra gli specialisti (45%), quasi tutti in Patologia e Biochimica Clinica, il 37% ha un contratto a tempo indeterminato, il 23% a tempo determinato, il 17% pratica la libera professione e il 17% ha una borsa di studio/assegno di ricerca; 2 su 3 afferiscono a strutture pubbliche. Il giudizio degli specializzandi (27%) sulla qualità formativa delle scuole è eterogeneo. Il 52% è inserito attivamente nell'attività clinica. Il 29% svolge attività di ricerca. Il 31% non percepisce alcuna retribuzione. Il questionario ha permesso di fotografare la situazione formativa e lavorativa di un campione di giovani laboratoristi, soci e non soci SIBioC, e di evidenziarne le diverse esigenze. Sono emerse la propensione all'innovazione, elemento chiave per interpretare i nuovi modelli professionali, e anche le molteplici competenze, sulle quali è importante investire per l'innovazione della MdL.

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## LIVELLI DI OMOCISTEINA E DEGLI ALTRI TIOLI A BASSO PESO MOLECOLARE IN SOGGETTI AFFETTI DA BPCO

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La broncopneumopatia cronico ostruttiva (BPCO) è una patologia dell'apparato respiratorio caratterizzata da ostruzione non completamente reversibile delle vie aeree. Il fattore di rischio principale per lo sviluppo di questa malattia è il fumo di sigaretta, ma anche l'esposizione cronica ad altri irritanti respiratori, come fumi e polveri, può contribuire allo sviluppo della BPCO. Essa rappresenta attualmente la quarta causa di morte nel mondo e questo è dovuto anche alla presenza di comorbidità, come le patologie cardiovascolari. Visto il ruolo rilevante dell'omocisteina (Hcy) in queste patologie[1], lo scopo di questo progetto è stato quello di valutare i livelli di questa molecola e degli altri tioli a basso peso molecolare nei soggetti dello studio.
Lo studio è stato effettuato su 54 soggetti affetti da BPCO, suddivisi per stadio di gravità della patologia, e 54 soggetti di controllo, appaiati per età e genere. Entrambi i gruppi di studio sono stati sottoposti all'analisi dei biomarcatori di stress ossidativo, come i tioli a basso peso molecolare (cisteina, Hcy, glutatione, cisteinglicina e glutamilcisteina). I risultati ottenuti evidenziano come i soggetti affetti da BPCO mostrino livelli elevati di Hcy (mediana: 16 μmol/L; IQR: 12.7–17.8 μmol/L vs 12.9 μmol/L; IQR: 10.6–15.6 μmol/L; $p<0.01$) e cisteinglicina (mediana: 28.9 μmol/L; IQR: 24.5–35.2 μmol/L vs 26.4 μmol/L; IQR: 21.5–31.5 μmol/L; $p<0.05$) rispetto ai soggetti di controllo. Non sono state riscontrate differenze significative nei livelli di glutatione, cisteina e glutamilcisteina. Con la suddivisione della patologia in stadi di gravità, i livelli di Hcy e cisteinglicina si sono mostrati significativamente più elevati con la progressione della BPCO (trend lineare: $p=0.012$ e $p=0.003$ rispettivamente, tramite ANOVA).
I risultati ottenuti in questo studio mostrano una correlazione significativa tra la severità della patologia e i livelli di Hcy e cisteinglicina. In considerazione della nota relazione tra omocisteina (ed in minor misura cisteinilglicina) e patologie cardiovascolari, il riscontro di elevati livelli di questi composti possono essere utilizzati per spiegare, almeno in parte, l'elevata frequenza di patologie cardiovascolari nella BPCO.
[1] Mangoni AA, Jackson SH. Am J Med 2002, 112: 556-565

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## SYNERGISTIC EFFECT OF COMBINED ADMINISTRATION OF AN ONCOLYTIC ADENOVIRUS AND BIFIDOBACTERIUM SPP. SUPPLEMENTS IN A MOUSE MODEL OF MELANOMA

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In the last decade, cancer immunotherapy has delivered impressive results in clinical settings. However, efficacy has not been consistent probably because of several e several environmental and genetic factors influencing the outcome. Many studies showed that intestinal microbiota can affect checkpoint inhibitors-based immunotherapy outcome both in animal models and patients. In particular, Bifidobacterium seems to have a role as a positive regulator of antitumor immunity in vivo by promoting pro-inflammatory signals in innate immune cells.We hypothesized that modulation of host microbiota could also synergize with active immune therapy, such as oncolytic viruses. Oncolytic viruses can infect and lyse tumor cells, causing the release of tumor-associated antigen, therefore, stimulating an antitumoral immune response. We decided to investigate whether administration of Bifidobacterium could positively affect response to oncolytic vaccines. So we administered a mix of Bifidobacterium to C57BL/6J mice inoculated with syngeneic B16-OVA melanoma cells and then treated them with either oncolytic vectors or PBS and compared melanoma growth rate with groups that did not receive bacterial supplementation. Mice treated with Bifidobacterium supplements and oncolytic viruses showed a reduction of tumor size, compared to control groups. By using 16S rRNA sequencing of stool sample, we have indirectly observed Bifidobacterium sp. and Faecalibaculum sp. abundance-featured gut microbiota, in mice that effectively respond to the viral therapy. Because of possible correlations between perturbation of gut microbiome and systemic immune responses to the different treatment, we determined the CD4+ and CD8+ T cell dependent IFN-γ production, obtaining specific antigen expression pattern correlated to therapies.

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## EFFECTS OF PHYSICAL ACTIVITY AND NITRATE INTAKE ON METABOLIC SYNDROME

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Background: Metabolic syndrome is a cluster of serious health conditions that occur together increasing the risk of type II diabetes, stroke and cardiovascular diseases. The causes of this syndrome include obesity, physical inactivity, genetic factors and ageing. Metabolic syndrome is associated with chronic inflammatory status and vascular endothelial dysfunction due to an imbalance in adipocytokine production by the adipose tissue. Moreover, it is characterized by an increase in the degree of oxidative stress. In this study, the effects of physical activity and nitrate intake in patients with metabolic syndrome were evaluated.
Methods: Patients, male with metabolic syndrome, where divided in two groups: control and nitrate supplemented (double blind crossover study). Nitrate was 8mmol in 500mL of an almond beverage, administered before the physical activity test. Blood samples from subjects were collected before and after having performed 30 minutes of physical activity in controlled conditions. The expression of antioxidant and mitochondrial biogenesis related proteins in peripheral blood mononuclear immune cells (PBMCs) was measured.
Results: After the physical activity in control patients, the expression of antioxidant proteins and mitochondrial biogenesis related proteins increased. On the other hand, in patients treated with nitrate the increasing trend of these proteins was prevented.
Conclusions: In conclusion, physical activity induces oxidative stress condition, and consequently the increased expression of the antioxidant defences was observed. There were some evidences that nitrate intake can modulate the physical activity induced redox imbalance and the expression of the mitochondrial biogenesis related proteins.

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## REDOX PROFILES OF MITOCHONDRIAL BREAST CANCER SUBTYPES

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Background: The capacity of cancer cells to growth and disseminate are profoundly influenced by the adaptation of their metabolism to extra- and intracellular oncogenic signals. This property underlies the heterogeneity of tumour subtypes and the development of chemoresistance. Here, mitochondrial breast cancer subtypes, previously identified in our laboratory, were characterized for their redox profiles. We focused on glutathione and thioredoxin systems, directly involved in the modulation of thiol-redox signaling.Methods: MCbiclust (1) was used for breast cancer sample stratification [TCGA (2) and METABRIC (3)] according to mitochondrial gene expression profiles. On breast cancer cell lines, we evaluated mitochondrial function, metabolic profile by functional imaging, biochemical approaches and mass spectrometry. The thiol-redox profile was analyzed by quantification of total thiol groups, glutathione concentration and redox state, together with measurements of enzymatic activity and protein levels of the glutathione and thioredoxin systems.Results: We have found that the mitochondrial subtypes were characterized by differential glutamine utilization, associated with adaptive changes in mitochondrial function. Moreover, we found specific redox properties of the mitochondrial subtypes. Intriguingly, the glutamine addicted mitochondrial subtype was more susceptible to oxidative stress following glutamine restriction, as shown by the decrease of thiol levels and concomitant increased glutathione oxidation. In addition, enzymatic activities and protein expression analysis carried out on glutathione and thioredoxin systems revealed different pattern of enzymes involved in cellular redox control in the mitochondrial breast cancer subtypes.Conclusions: Metabolic gene expression profiling has stratified breast cancer patients into mitochondrial subtypes. These subtypes had different redox phenotypes, which could be exploited to develop new personalized cancer treatments. References: 1 Bentham RB, et al; Nucleic Acids Res. 20172 Cancer Genome Atlas Network. Nature. 20123 Curtis C, et al; Nature. 2012 Acknowledgements: Supported by the BBSRC, British Heart Foundation, UCL COMPLeX, Wellcome Trust, Francis Crick Institute and AIRC Italy.

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## OPPORTUNITÀ OFFERTE DALLA ACQUISIZIONE DI UN UNICO FORNITORE DI SISTEMI PER L'ESECUZIONE DI ESAMI PER LO STUDIO DELL'EMOSTASI IN REGIONE TOSCANA

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Premessa: Il settore di Coagulazione rappresenta uno dei settori più critici della Medicina di Laboratorio per numerosi problemi legati a vari aspetti delle tre fasi di indagine, preanalitica, analitica e post-analitica. Nonostante la pubblicazione di numerose Linee Guida esiste ancora una estrema eterogeneità di comportamento per la gestione delle tre fasi, con particolari criticità soprattutto nella fase pre- e post-analitica. La recente gara per l'assegnazione ad un unico fornitore per l'intera Regione Toscana di sistemi (strumenti e reagenti) per le indagini delle patologie emorragiche e trombotiche, offre l'opportunità di armonizzare e riorganizzare su base regionale le numerose e diverse procedure attualmente operative all'interno dei 41 Laboratori che eseguono esami di coagulazione nella Regione Toscana.
Metodologia: E'stato recentemente costituito un Gruppo di Studio Emostasi della Regione Toscana con lo scopo di prendere in esame le varie problematiche del Settore, suddividendo i compiti all'interno delle tre Aree Vaste (Nord-Ovest, Centro e Sud-Est). Le problematiche che inizialmente sono state affrontate sono le seguenti: Gestione della fase preanalitica in coagulazione (Area Vasta Nord-Ovest); Valori di riferimento e modalità di espressione di PT e APTT (Area Vasta Centro); Preparazione e gestione del POOL (Area Vasta Sud-Est).
Obiettivi: Riorganizzazione delle procedure della fase pre-analitica su tutto il territorio della Regione Toscana attraverso l'adozione di identiche procedure di raccolta, trasporto, manipolazione e conservazione dei campioni di sangue per gli esami di studio dell'emostasi. Gestione della fase analitica con procedure di CQI idonee ed omogenee, anche attraverso l'uso di materiali preparati in centri di riferimento regionali. Modalità di espressione dei risultati e valori di riferimento dei vari test di primo e di secondo livello identiche in tutti i laboratori della Regione.
Conclusioni: L'acquisizione di un unico fornitore di sistemi di studio dell'emostasi offre la grande opportunità di risolvere, almeno in parte, le numerose criticità del settore di coagulazione; le proposte presentate dai tre gruppi di lavoro dovrebbero portare ad operare e a refertare in maniera omogenea sull'intero territorio regionale.

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## FLOW CYTOMETRIC ANALYSIS OF BLOOD LYMPHOCYTE SUBSETS IN PRETERM INFANTS AND MOTHERS AFTER PREGNANCIES COMPLICATED BY PREMATURE PROLONGED RUPTURE OF MEMBRANES

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Background and aimIn preterm newborns innate and adaptive immunity may be compromised by factors associated with preterm birth, such as pPROM, which is responsible for approximately one third of all preterm deliveries and may present with chorioamnionitis in half cases. The present study was designed to evaluate lymphocyte subsets of all preterm deliveries and to detect possible changes in the neonatal and/or maternal immune system in different clinical conditions.MethodsThe study enrolled 16 women with diagnosed pPROM and 19 preterm babies born between 23$^{rd}$ and 36+6$^{th}$ weeks of gestational age at the Department of Women's and Children's Health of Padua University Hospital vs a control group (11 mothers and 13 newborns). We obtained maternal and neonatal blood samples on a routine assessment in the first 3 days following the delivery, testing lymphocyte subpopulations by flow cytometry (TBNK panel plus some populations involved in T cell maturation and activation), WBC count, IL-6 and C-Reactive Protein levels. EDTA whole blood and serum samples were analyzed within 24h after sampling. Sample preparation was performed according to the CLSI H42-A2 guideline.ResultsStatistically significant differences in lymphocyte subset distribution between the two newborn groups were detected for: NK cells, naïve helper T cells and memory helper T cells.Preliminary data analysis showed that naïve CD4+ T lymphocytes were lower in preterm babies born from mother with pPROM ($p<0.005$), while memory CD4+ T lymphocytes were higher in preterm babies born from mother with pPROM ($p<0.005$). The other lymphocyte populations did not show statistically significant differences between the two groups.There weren't any statistically significant difference in the total WBC count, T and B lymphocytes count between mothers and babies as well as between mothers with diagnosed pPROM and mothers without pPROM. C-Reactive Protein levels were significantly different both between mothers with and without pProm, being lower in the first group compared to the second, and also between newborns and mothers.Naïve CD8+ T lymphocytes were lower in preterm babies born before 28th weeks of gestational respect to preterm babies born after 28th weeks of gestation. Also preterm babies who developed bronchopulmonary dysplasia showed a significant decrease of naive CD8+ T lymphocytes.From the comparison between preterm newborns with and without chorioamnionitis and preterm newborns with and without early onset sepsis, we observed an increase both in T helper cells absolute count and in PCR levels.The comparison between vaginal delivery and cesarean section showed statistically significant differences in T helper cells absolute count, CD4/CD8 ratio and leukocytes.Finally, newborns were stratified according to the latency time (period between diagnosis and delivery), considering 4 weeks as cut-off. Between these two groups, we observed a significant difference in T and NK cells distribution.Conclusions In the comparison between mothers and newborns, independently from the maternal pathology, we detected an expected maturational trend with regard to non-MHC restricted cytotoxic T cells, naïve and memory CD4+ T lymphocytes, naïve and terminal effector memory CD8+ T cells, activated (CD3+ CD38+ and CD3+ HLA-DR+) T lymphocytes, regulatory T cells and C-Reactive Protein. In the analysis of newborns with various clinical conditions, the most interesting result is the decrease of naïve and the contemporary increase of memory CD4+ T lymphocytes in pPROM newborns, suggesting a more protracted antigenic stimulation compared to controls. In pPROM newborns, an immune modulation seams to need for a longer latency time to be established. Further investigations are needed to define the role of reduced naïve CD8+ T cells in BPD and increased helper T cells in early onset sepsis.

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## A Retrospective Study on the Relationship between Autonomic Neuropathy and Incretin-based therapies in Patients with Diabetes Mellitus type 2

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Background: Type 2 diabetes is characterized by a lower insulinotropic activities by incretin hormones, glucose-dependent insulinotropic polipeptide-1 (GIP) e Glucagon-like peptide-1 (GLP-1), and consequently a reduced incretin effect and abnormalities of gastric emptying, critical determinant of postprandial glycaemia. In the clinical practice, the presence of autonomic dysfunction is determined by the Ewing tests, evaluating the parasympathetic and the sympatethic nerve damage. Diagnosis of same damages in the gastrointestinal region includes invasive methods, unfeasible in a routinely practice of a Metabolic Disease Center.Aim: To analyse the possible significativeness of autonomic neuropathy diagnosis, performed by the Ewing tests, in order to evaluate the response to the incretin-based therapies, acting on the enteroinsular axis.Methods: We performed a retrospective study, analysing a cohort of 134 patients, treated with increti-based therapies. All the patients had been evaluated for autonomic neuropathy by the Ewing tests. We compared the response to the incretin-based therapies of the patients resulted negative to the Ewing test with the response to the same treatment of the patients positive to the Ewing tests, analysing the glycated haemoglobin level shifts, over one year of therapy. The clinical report of each patient was analysed to evaluate the possible confounder factors on the glycated haemoglobin values.. Statistical analysis of the data was performed by Analyse.it. software by using two-tailed t-test.Results: We observed decreasing levels of the glycated haemoglobin values in all the patients negative to the Ewing tests, treated by incretin-based therapies, over one year of therapy. That decrease resulted statistically significant. No decrease of glycated haemoglobin levels was registered in the patients positive to the Ewing test, treated with the same therapies. The correlations between the glycated haemoglobin values registered and the confounders factors did not appear statistically significant. Conclusions: Our retrospective analysis showed that the patients positive to the Ewing tests, were not responsive to the incretin-based therapies. At the first level of analysis, the statistical significativeness of this result appears to positively answer to our aim. A retrospective analysis does not allow a conclusive result, but it is the useful tool to evaluate the next prospective study to confirm our important findings.

# 51° Congresso Nazionale della Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica (SIBioC - Medicina di Laboratorio)

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