Special Supplement 1 SS1

BC biochimica clinica

RIASSUNTI 50° CONGRESSO NAZIONALE SIBioC

SIBioC - Medicina di Laboratorio
membro di
International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)
European Federation of Clinical Chemistry and Laboratory Medicine (EFLM)

BIOMEDIA
La condivisione del sapere

ISSN 0393 - 0564

volume 42

sommario

SS[

biochimica clinica

Rivista fondata da Norberto Montalbetti
e già diretta da Carlo Franzini

Rivista della Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica - Medicina di Laboratorio

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International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)
European Federation of Clinical Chemistry and Laboratory Medicine (EFLM)

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Biochimica Clinica is indexed in Scopus (www.info.scopus.com), EMBASE (www.info.embase.com) Engineering Village (www.ei.org), Reaxys (www.info.reaxys.com) and ESCI (www.wokinfo.com/products_tools/multidisciplinary/esci)

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Publisher
Biomedia srl
Via L. Temolo 4
20126 Milano
www.biomedia.net

Amministrazione e Pubblicità
Business Office and Advertising
Biomedia srl
Via L. Temolo 4 - 20126 Milano
Tel. 0245498282 - Fax. 0245498199

utilizza un Sistema di Gestione Qualità Certificato per l'attività di

Fornitura di servizi per la progettazione, realizzazione e distribuzione di prodotti editoriali

Autorizzazione del Tribunale di Milano n. 40 del 2.02.1987

Grafica e impaginazione
Biomedia srl
Via L. Temolo 4 - 20126 Milano

Stampa
GRAFICA BRIANTEA srl
20040 Usmate (MI)

Abbonamento annuo
Euro 50 per Italia/Privati
Euro 75 per Italia/Enti
Euro 75 per Estero/Privati
Euro 90 per Estero/Enti

Prezzo di un fascicolo Euro 5,16

Spedizione in abbonamento postale D.L. 353/2003 (conv. in L. 27/02/2004 n. 46) art. 1, comma 1, LO/MI

Associato all'USPI Unione Stampa Periodica Italiana
Tiratura di questo numero 2000 copie
2000 copies of this issue have been printed
ISSN 0393-0564

Volume 42
Special Supllement SS1

50° Congresso Nazionale della Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica (SIBioC - Medicina di Laboratorio)

Napoli, 16-18 ottobre 2018

Riassunti Sessioni Scientifiche

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Legenda:

SP	Sessione Plenaria
SS	Sessione Parallela
CO	Comunicazione Orale

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

SS01-01
LABORATORY MEDICINE: "COST" OR "ADD VALUE"?

G. Lippi

Verona

The panorama of laboratory medicine, as part of the growing complexity of health care in the third millennium, is variegated. Laboratory medicine has been an irreplaceable element in the clinical decision-making throughout its almost millennial history. Despite its essential role, the general perception of laboratory medicine, especially by politicians, administrators and some clinicians, is not always favourable because this "science" is often considered on the basis of its costs and not according to the added value generated. The increased complexity and volume of testing, coupled with an increasing aspiration for efficiency and quality by patients, may even compromise the survival of clinical laboratories. Nonetheless, it is now clearly demonstrated that the economic impact of laboratory diagnostics on general healthcare expenditure is very modest in the most advanced countries, usually comprised between 1.4% and 2.8%. Nevertheless, the comparison between global cost (personnel, consumables, services, ordinary operating expenses) and revenues, clearly shows that laboratory diagnostics can generate twofold economic resources than those consumed, thus representing one of the areas with higher profitability not only in healthcare, but also in comparison with other human activities, wherein the net profit of the most profitable global industries does not usually exceed 30%. Realistically, laboratory diagnostics can therefore guarantee a net profit almost seven times greater than any other human sector. Alongside a genuine economic perspective, the added value that laboratory diagnostics offers in diagnostic and treatment pathways, especially in the era of personalized medicine, emerges strongly, since precision medicine strongly relies on results of genetic, epigenetic and biochemical tests. The role of laboratory medicine in the field of diagnostic and therapeutic care pathways is unavoidable, especially in some areas of medicine in which the diagnosis or therapeutic management are subsidiary to result of laboratory tests (e.g., anticancer therapies, diagnostics of acute coronary syndrome, and so forth). A major awareness of the added value of laboratory diagnostics is now unavoidable to reaffirm the role of the clinical laboratory in the modern healthcare system.

SS01-02
APPROPRIATENESS IN LABORATORY MEDICINE

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Laboratory Medicine plays a central role today in the entire decision-making process in Medicine, from diagnosis and prognosis definition to therapy monitoring. Laboratory Medicine is not limited to analytical aspects but has the primary objective of improving patient outcomes.
In this context, appropriateness is a very relevant issue. In fact, the inappropriate use of laboratory tests induces the deterioration of quality of care since the lack or excess of information related to the clinical problem can often be misleading or even harmful. Moreover, an inappropriate test involves a significant waste of resources. For these reasons appropriateness has become a strategic topic today, when the containment of costs for health services has became mandatory.
Appropriateness consists in requesting the right test, for the right patient, at the right time, thus avoiding the overuse of laboratory tests with the aim of providing the most effective intervention for a specific individual. It also should be noticed that the appropriateness of a specific test can be evaluated only in the context of the specific clinical question, and for the individual patient. Thus, in most circumstances, appropriateness depends by pre-test probability of having the disease.
Some examples of appropriateness that are often overlooked in the field of Laboratory Medicine are:

- Free PSA should be proposed only to patients with total PSA ranging from 3 to 10 ng/ml. Laboratories should implement adequate instruments, such as reflex test, to limit inappropriate requests.
- The use of AST, LDH, CK or myoglobin in patients suspected of acute myocardial infarction is not appropriate. For the diagnosis of acute myocardial infarction the only effective marker is troponin measured with high sensitivity assays.
- Glycosuria for diabetes monitoring is not appropriate. For this purpose HbA1c should be used and it should be repeated only twice per year in patients with type 2 diabetes with stable glycemia well within target.
- The basic evaluation of thyroid disease requires the execution of TSH only. The dosage of fT4 or antithyroperoxidase (anti-TPO) antibodies should be performed only when an alteration of TSH levels is detected.
- Genetic tests for thrombophilia screening, namely Factor V Leiden and Factor II G20210A, should be performed only when a personal and family history of thromboembolic diseases is ascertained and they must not be repeated during the life.

Great efforts are needed to achieve appropriateness in laboratory tests requesting. Intervention strategies include educational programs; development of Evidence Based Medicine; sustain to large randomized clinical trials; promotion of formulation and dissemination of guidelines and consensus documents.
In conclusion, appropriateness is a cornerstone in high quality medical practice. Inappropriate use of laboratory testing induces high medical costs due to unnecessary diagnostic test, patients discomfort, and definitely poor outcomes for patients.

SS01-CO01

INNOVATION IN TYPE 2 DIABETES PATIENTS CLINICAL PATHWAY: THE ROLE OF GLYCATED ALBUMIN

L. Ferrario[1], E. Foglia[1], F. Schettini[1], A. Avogaro[2], C. Bellia[3], F. Bertuzzi[4], G. Bonetti[5], A. Ceriello[6,7], M. Ciaccio[3,8], M. Corsi Romanelli[9,10], E. Dozio[10,11], L. Falqui[12], A. Girelli[13], A. Nicolucci[14], G. Perseghin[15,16], M. Plebani[17], U. Valentini[13], M. Zaninotto[17], D. Croce[1,18]

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Aims: The study proposed a multi-dimensional assessment, exploring clinical, economic, ethical, social and organisational implications, related to an innovative glycemic marker introduction (glycated albumin – GA), in the Italian clinical practice, as an add-on technology to the traditional glycemic monitoring systems (Hb1Ac and FPG), for insuline-naïve patients affected by tipe 2 diabetes, assuming oral therapy.
Methods: The HTA approach considers the Italian NHS perspective. All the 9 dimensions derived from EUnetHTA Core Model, were deployed considering: i) a systemic literature review, ii) administration of qualitative questionnaire filled by 15 healthcare type 2 diabetes experts (evaluation scale, ranging from -3 to +3) and iii) quantitative approaches, useful for the economic evaluation of the clinical pathway and for budget impact analysis.
Results: Literature review showed that AG implementation could reduce the risk to develop diabetes complications (such as diabetic retinopathy, nephropathy and cardiovascular problems) and improve the number of patients achieving a therapeutic success (97% vs 72%).
Professionals' perception confirmed the superiority of AG, in terms of safety (0.11 vs 0.65) and effectiveness (0.53 vs 1.80) profiles. At the 12-month time point to the base-case scenario for market penetration, AG introduction would lead to fewer amount of patients experiencing a therapy switch (-89%), with a significant economic saving equal to 1.06% (€3,551,275), for the NHS, thus presenting a better cost-effectiveness trade-off with respect to the comparator (CEV: 225.53 vs 237.74). Whilst the use of traditional systems would be more advantageous from an equity point of view (0.13 vs 0.72), due to GA limited accessibility, GA would improve both patients (1.33 vs 2.17) and caregivers (0.83 vs 1.50) quality of life. Despite, in the short-term, GA required training courses and equipment update, in the long-term it would be the preferable technology, from an organizational point of view (0.01 vs 0.30), freeing up organizational resources.
Conclusions: Results demonstrates the strategic relevance related to AG introduction into the Italian clinical practice, its economic sustainability and feasibility, as well as the potentialities in clinical pathway improvement.

SS01-CO02

INVESTIGATION'S MANAGEMENT PROTOCOL IN THE COAGULATIVE FIELD ON THE PREOPERATIVE PATHWAY

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The request of the basic coagulation tests (PT and aPTT) falls within the normal preoperative pathway. The aim of the work is to highlight the effectiveness of the management of patients with altered screening tests through a protocol shared among the Intensive Care Unit, which coordinates the preoperative pathways, Hematology Unit, which performs the consultations, and the Laboratory, which performs the I and II level coagulation tests. The protocol causes a role reversal of the laboratory, from a passive element to the proposer of the diagnostic path. After the first finding of alteration of one or both PT and aPTT tests, patient's blood is collected in 3 sodium citrate tubes on which the laboratory performs both first level and mixing test. The results, according to the evaluation of the laboratory specialist and a specific algorithm, trigger the most suitable second level tests. At the end of the diagnostic procedure the Haematologist, on the basis of the results, decides whether the haematological examination is

needed. In case of negative bleeding history and LAC positive or factor XII defect, the patient undergoes surgery. If an indicator of hemorrhage, such as non-LAC inhibitor, a factorial defect or vWF desease is found the haematologist collects a thorough anamnesis of the patient for a correct evaluation of the bleeding risk. In 2017 the protocol was applied to 37 cases of altered PT/aPTT. In 8% of cases the alteration was not confirmed, in 11% of cases the investigations didn't find a cause of the PT/aPTT prolongation, but in 81% of cases an explanation of the prolongation was found. In some patients there was not haemorrhagic risk-associated defects/alterations but whose definition was useful for the evaluation of the patient. With the new protocol, two or three hospital admissions are required instead of the previous five, with patient's stress and resources saving. Furthermore the implementation of the mixing test-based diagnostic algorithm allows the execution of appropriate tests suggested by hemostasis laboratory with the decrease of useless tests.

SS02-01

THERAPEUTIC MONITORING OF TNFA INHIBITOR DRUGS AND AUTOANTIBODIES: SIBioC RECOMMENDATIONS

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Abstract: Tumor necrosis factor alpha (TNFα) is a proinflammatory cytokine involved in the pathogenesis of chronic inflammatory disease, such as rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, Chrons disease and ulcerative colitis. TNFα inhibitors (anti-TNFα) are monoclonal antibodies drugs directed against TNFα. They are adalimumab, infliximab, etarnecept, golimumab and certolizumab (1). Their effect consists in reducing the inflammatory response of autoimmune diseases. We evaluated evidence about the therapeutic efficacy of these drugs on patients affected by chronic inflammatory disease, highlighting their clinical benefit, but also a high risk of injection site reactions and infections (2). Furthermore, these drugs, due to their immunogenicity, can cause the formation of anti-drug antibodies (ADA). ADA could interfere with drugs compromising their effects, and resulting in a loss of clinical response (2). The determination of serum TNFα inhibitors and ADA levels could improve the patient's management, allows improving the treatment and reducing the risk of adverse effects. Even if the loss of therapeutic response, due to ADA production and the variation of TNFα inhibitors concentration are well documented, the clinical benefit of their serum determination remains unclear due to lack of standardization of laboratory methods (3, 4). The drug and ADA monitoring could be useful to reduce the loss of response and to avoid immunogenicity in patients' either responders or not responders to TNFα therapy, but the monitoring implementations needed of standardization of determination methods and agreement in the interpretation of results (5).
Members of SIBioC Study Group "Autoimmunità e Immunologia Clinica" drafted a documented showing the potentiality and level of criticality of TNα inhibitors and ADA monitoring. There are any indications about the use of immunogenicity test to guide the therapy, but there is the need of more information before implementing this test in clinical practice.

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SS02-CO03

VITAMIN D PLASMA LEVEL IS ABLE TO AFFECT NIVOLUMAB DRUG EXPOSURE IN A COHORT OF PATIENTS WITH NON SMALL CELL LUNG CANCER

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Immune-checkpoint inhibition using programmed cell

death-1 and its ligand drug inhibitors have improved survival among patients with advanced non small cell lung cancer: nivolumab (NV) is one of the most used and the presence of its antiantibody is considered a negative prognostic factor. Vitamin D (VD) deficiency (<20ng/mL) is frequent in lung cancer patients and studies showed this hormone modulates the expression of genes involved in drug metabolism/elimination and in the immune system regulation. No data are present in literature concerning NV and its relationship with VD. For these reasons, aim of this study was to quantify 25-hydroxyVD, NV and its anti-antibody in patients' plasma before starting (baseline) and at 15, 45 and 60 days of therapy. Molecules were quantified through enzyme-linked immunosorbent assay.
We enrolled 45 patients: median NV concentrations were 12.48ug/mL (interquartile range, IQR:9.54-17.13), 22.31ug/ mL (IQR:18.30-34.88) and 27.05ug/mL (IQR:17.43-39.38) respectively at 15, 45 and 60 days. No anti-NV antibodies were found. VD median concentrations were 12.78ng/mL (IQR:10.09-16.55), 13.62ng/mL (IQR:10.86-16.11), 11.78ng/mL (IQR:10.13-18.87) and 12.92ng/mL (IQR:10.79-17.03) respectively at baseline, 15, 45 and 60 days. Correlations were observed between NV concentrations at 15 days and VD levels at baseline (p=0.024, Pearson's coefficient (PC) 0.451) and at 15 days (p=0.017, PC=0.542); NV exposure at 60 days was correlated with VD at baseline (p=0.001, PC=0.730), at 15 (p<0.001, PC=0.858), 45 (p=0.001, PC=0.779) and 60 days (p<0.001, PC=0.900). Furthermore, in a sub-population we stratified patients according to VD deficiency: baseline VD levels<20ng/mL were associated with lower NV concentrations at 15 (p=0.103, a trend without statistical significance), 45 (p=0.018) and 60 days (p=0.021); 15 days VD<20ng/mL with 15 (p=0.019), 45 (p=0.019) and 60 days (p=0.028) NV lower concentrations; finally, 60 days VD<20ng/mL with 60 days lower NV levels (p=0.030). This is the first study showing VD is able to predict NV concentrations and describing nivolumab levels in real-life context of non small cell lung cancer. Further studies in bigger and different cohorts are needed to clarify these aspects and to relate them to clinical features.

SS03-01

FROM COMPLETE BLOOD COUNT TO MOLECULAR BIOLOGY: YESTERDAY, TODAY AND TOMORROW

S. Buoro

Bergamo

The qualitative and quantitative cells evaluation on the peripheral blood smear by optical microscopy was the first step taken by the scientific community towards the current concept of liquid biopsy.
The cellular component in the peripheral blood is the result of fine regulation of hematopoiesis on the bone marrow that depends of the ability of the stem cell and other cell precursors to replicate, to differentiate and to migrate into the peripheral blood. The evaluation of the cells in the blood allows verifying the alteration of homeostasis of the hematopoiesis due to infections, nutritional alterations, neoplasia or paraneoplastic reactions etc.
The number and type of peripheral blood and bone marrow cells, associated with the patient's clinical condition, have been the basis of the development of modern hematology. Cell morphological evaluation was a fundamental criterion to FAB classification of hematological diseases (1). Another important step of laboratory hematology has been the development of flow cytometry with the availability of the first complete blood count profile (CBC). This method of analysis allows the cells count and characterization, obtaining robust results in a short time, at low cost and all day (2). The development of flow cytometry in the new hematology analyzers has been very rapid in the last decade. Today, the new CBC-profile, integrated with the latest generation erythrocyte, platelet and leukocytes parameters provides increasingly detailed quantitative and qualitative information about the cells (3), allowing the recognition also of poorly represented neoplastic cells or alterations induced by solid neoplasms. The development of multiparametric flow cytometry, with the classification of cells based on the protein biomarkers expressed on their surface, as a differentiation cluster (CD), allowed the "immunological" classification of cells (4). The last two decades have been also characterized by the rapid development of molecular biology including the evaluation of gene expression with "Next Generation Sequencing" (NGS), and the improvement of methods for detection of free circulating DNA (5). This technology progress have allowed a better definition of etiopathogenesis, diagnosis, prognosis and therapy of hematological and non-hematological neoplasms (5). The high quantity and the increasingly detailed information from cells analysis, up to the data of gene expression, have revolutionized the classification of hematological diseases (Hematology Tumors Classification of 2016 proposed by the World Health Organization). Thanks to a more refined characterization of neoplasms, we are seeing the development of personalized therapies, with biological drugs aimed at correcting the gene defect that causes the disease. The reference model is chronic myeloid leukemia (CML), a clonal neoplasm caused by t(9;22) translocation with the generation of the BCR/ABL fusion gene that generally leads to the increase of neutrophils and immature granulocytes in peripheral blood, with the presence of blasts. In this case, the knowledge about of the genetic lesion led to the development of a targeted biological drug that allowed to chronic the disease.

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SS03-04

MOLECULAR BIOMARKERS IN ACUTE AND CHRONIC LEUKEMIAS

B. Izzo

Napoli

The real prevalence of the apparent treatment-resistant hypertension (TRH) is difficult to be measured since very often TRH depends on poor therapeutic adherence (TA). Therefore, by extending the application of Therapeutic Drug Monitoring (TDM) to antihypertensive drugs could be useful to assess the TA, especially for those patients that are candidates to surgery. We hence developed and validated a UHPLC-MS/MS method for the simultaneous TDM of ten antihypertensive drugs in urine samples, with a reduced invasiveness and a great compliance: this can be suitable for a rapid adherence screening before surgery. Amlodipine, atenolol, clonidine, chlortalidone, doxazosin, hydrochlorothiazide, nifedipine, olmesartan, ramipril and telmisartan were tested. This method has been validated according to FDA and EMA guidelines. A volume of 100 µl of urine sample, calibration standard and quality control was added with 40µl of internal standard (IS, 6,7-dimethyl-2,3-di(2-pyridyl)quinoxaline) and 860 µl of a mixture of water:acetonitrile 90:10 (v:v, +0.05% formic acid). After vortexing and centrifuging the samples, the resulting supernatant was analyzed through a UHPLC-MS/MS system (Shimazdu, Kyoto, Japan). This method was tested on real samples from patients with apparent TRH, upon informed consent. Results have been than verified with the TDM of plasma sample from the same patients, through an already validated method. Method performances fitted FDA and EMA guidelines for all analytes. Thirty-six patients have been enrolled: 39% of them resulted fully adherent, 39% were only partially adherent and 22% resulted completely non-adherent. TDM of urine samples resulted congruous with TDM of plasma samples about the complete non-adherence but could misunderstand full and partial adherence (lack of one or only a part of prescribed drugs). Concluding, the very low invasiveness and the fast “dilute-and-shot” extraction procedure make this method suitable for screening the adherence of a large population and when it is impossible to perform blood sampling. Nevertheless, the TDM of plasma samples will probably represent a “gold standard”, especially when an accurate and precise quantification of drug concentrations is needed, even considering the poor urinary excretion of some molecules.

SS04-01

EMERGENCY TESTS MANAGEMENT: SEPARATED OR CONSOLIDATED LABORATORY SECTOR?

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Clinicians are interested in a laboratory service that encompasses analytical quality, full availability and timeliness; among these characteristics, timeliness of result reporting is considered the most relevant. The main causes of laboratory turnaround time (TAT) delay can be ascribed to order entry, responsible for about 30% of all reports that are delayed. Sample collection causes 27% of delays, analytical phases 28%, report transmission 2%; the remaining 13% was not determined. There are three options to manage and perform “emergency" tests: a) in dedicated (“emergency”) laboratories in the central laboratory, together with other tests, b) in the central laboratory, together with other tests, and c) at point-of-care (POC). The first model has been certainly the most popular until the nineties, but during the past decades, the healthcare systems have rapidly changed and today hospital care is primarily advocated for critical patients and acute treatments, for which laboratory test results are crucial and need to be always reported in predictably short. However, not only hospital care need of short TAT. For both the “acute care” and outpatient offices, laboratory services must ensure a short TAT (usually less than 1 hour) to guarantee timely patient care. In addition, outpatients being cared for by general practitioners require rapid response for test requests since most of these patients are affected by chronic conditions which require continuous monitoring in order to guide effective and prompt adjustments to therapy (i.e., Oral Anticoagulant Therapy, Diabetes, Heart failure). Therefore, in the current healthcare system, most laboratory tests should be considered “STAT”. POCT for immediate results, and consolidate corelab, for a high throughput have been progressively considered as possible alternative solutions. Consequently, laboratories in the hospital setting as well as in territorial service can face this challenge by changing their organization from a compartmentalized laboratory department toward a decision making-based laboratory department. This requires the implementation of a core laboratory, that exploits total laboratory automation (TLA) using technological innovation in analytical platforms, track systems and information technology, including middleware, and a number of satellite specialized laboratory sections cooperating with care teams for specific medical conditions. In this laboratory department model, the short TAT for all first-line tests performed by TLA in the core laboratory represents the key paradigm, where no more stat testing is required because all samples are handled in real-time and (auto) validated results dispatched in a time that fulfils clinical needs. To optimally reach this goal, laboratories should be actively involved in managing all the steps covering the total examination process, speeding up also extra-laboratory phases, and such sample delivery. Furthermore, to warrant effectiveness and not only efficiency, all the processes, e.g. specimen integrity check, should be

managed by middleware through a predefined set of rules defined in light of the clinical governance. We are now moving towards a new model of healthcare systems, where every patient has a critical pathology and treatment is "urgent" for everyone. In this scenario, laboratories need to consider every test as a Stat test, so that the separation between routine and stat organizations will be abolished. Automation, particularly TLA, represents a formidable tool to meet the increasingly more demanding critical needs and, even more importantly, improve patient outcomes. POCT could be variously integrated into Stat test activities, usually (but not solely) in the evaluation of vital functions.

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SS04-02

WHICH BIOLOGICAL MATRIX TO USE FOR BLOOD TESTING: SERUM OR PLASMA?

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Clinical laboratories can routinely use different biological matrices even if the majority of tests are performed on serum or plasma. Serum represents the liquid part of the blood originating from the clotting process that is activated by the transformation of fibrinogen in fibrin, a meshwork that can trap inside some cellular components (e.g. platelets). Plasma is obtained by the centrifugation of blood treated with anticoagulants and contains all the intact coagulation factors. Different anticoagulants agents are commercially available, such as EDTA salts, sodium citrate and lithium heparin, but their specific utilization largely depend on the test type, as they can interfere with specific analytical reactions. For example, EDTA salts, being a chelating agent, is known to interfere with several biochemical reactions, while lithium heparin, with a non-competitive mechanism, is one of the most inert anticoagulant. Because serum does not contain anticoagulants, it is free of platelets and clotting-derived proteins, but contains clotting metabolites. Both serum and plasma are usually collected in test tubes containing a gel barrier, which allowed to separate the cellular elements by centrifugation. Historically, serum has been the preferred assay material for determining measurands concentrations in blood specimens and it is still widely used. Today, many clinical laboratories are facing a change from serum to plasma, as the plasma constituents are thought to better represent the patient's pathological situation than serum (1). However, both matrices present advantages and disadvantages. Firstly, plasma samples can be centrifuged immediately after collection, while serum needs 30 minutes for completing coagulation. This time saving property of plasma is particularly relevant for those situations, e.g. emergency departments, in which physicians need urgent testing. Plasma allowed also to obtain a higher volume yield than serum (15%-20% or more), and prevents the coagulation-induced interferences, such as the decreased glucose, the increased lactate dehydrogenase, etc… On the other hand, sample centrifugation and the usage of gel barrier tubes are not sufficient for removing all blood cells and the presence of plasma residual cells, even if pelleted over the gel, may alter some constituent concentrations. Serum is almost completely lacking in cellular constituents. Further, the widespread usage of serum over plasma, has contributed to the development of many analytical methods validated for serum but not for plasma.
In conclusion, despite the wider usage of serum over plasma, the advantages of plasma are encouraging clinical laboratories moving from serum to plasma. However, manufactures should validate analytical methods also for plasma as this step is expensive and time consuming for clinical laboratories.

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SS04-CO04

A COLLABORATIVE STUDY PLANNED BY THE WORKING GROUP ON HEMOSTASIS AND THROMBOSIS OF THE ITALIAN SOCIETY OF CLINICAL BIOCHEMISTRY AND CLINICAL MOLECULAR BIOLOGY (SIBIOC) ON HEMOLYSIS INTERFERENCE ON FIVE ROUTINE HEMOSTASIS TESTS

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Introduction: Hemolysis is the leading cause of sample rejection in laboratory hemostasis testing. Many studies demonstrate the effect of hemolysis on coagulation tests using artificially hemolyzed samples. New coagulometers are able to detect optical interferences replacing visual inspection of the sample. The aim of this study was a prospective assessment of spontaneous hemolysis during sampling on hemostasis tests, by comparing results of hemolyzed (H) versus new, nonhemolyzed (NH) specimens, collected within 4 hours afterward, from the same patient. Moreover, visual assessment of hemolysis by two operators was also compared with instrumental hemolysis index.
Materials And Methods: The study was planned by the Working Group on Hemostasis and Thrombosis of the Italian Society of Clinical Biochemistry and Clinical Molecular Biology (SIBioC), and thus involved 15 hospital laboratories allocated in 6 different Italian Regions. A total of 269 pairs of plasma samples, mostly from inpatients, were analyzed using ACL TOP 750-CTS (IL, Bedford, USA), for the following tests: prothrombin time (PT), activated partial thromboplastin time (aPTT), DDimer (DD), fibrinogen (Fib) and antithrombin (AT). The critical difference (CD) and bias between H and NH was calculated for each test. Differences between paired samples were evaluated by non-parametric paired sample test and Bland-Altman analysis. Results: Mean bias was -0.1s for PT (P=0.057), -1.1s for aPTT (P<0.001), 1025ng/mL for DD (P<0.001), -0.04g/L for Fib (P=0.258) and 1.4% for AT (P=0.013). Bias exceeding the CD varied according to the method, with larger differences for aPTT (36.1%) and DD (17.1%). No correlation was found between free hemoglobin values and difference in hemostasis tests between H and NH samples. The agreement of visual hemolysis assessment among different operators, and between visual and instrumental assessment, evaluated by Cohen's kappa, was 0.85 and 0.62, respectively. Conclusion: According to our data, PT seems scarcely influenced by spurious hemolysis. Nevertheless, a larger unfavorable impact of spurious hemolysis was observed for other routine coagulation tests, manly for aPTT, DD and lesser extent for AT and Fib, suggesting that these test results should be suppressed in hemolyzed plasma samples. A good correlation of hemolysis assessment was found between the two operators, but only moderate compared to instrumental hemolysis evaluation. The use of instrumental hemolysis index seems today more raccomandable.

SS04-CO05

CHOISE OF THE RIGHT SAMPLE TYPE FOR PLASMA GLUCOSE DETERMINATION IN ORAL GLUCOSE TOLERANCE TEST: AN IMPORTANT PRE-ANALYTICAL TOOL FOR A CORRECT CLASSIFICATION OF GESTATIONAL DIABETES MELLITUS

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Background-Aim: The aim of the present study was to evaluate the effect of the use of acidified mixture of NaF, EDTA and citrate, as suggested in recently published Italian recommendations of WG Diabete of SIBioC-SIPMeL and SID, on OGTT for screening and subsequent diagnosis of gestational diabetes mellitus (GDM).
Methods: A total of 82 pregnant women were submitted to OGTT 75 g for screening of GDM in Vicenza [n=51, mean age (range): 33 (18-44) years] and in Brescia [n=31, mean age (range): 32 (22-43) years] were included in the study. All subjects signed written informed consent to participate to it. Glucose determination was performed in currently in use NaF containing tubes and in FC-MIX tubes from reiner BioOne, with a lyophilized ternary mixture, in Vicenza and in GlucoEXACT tubes from Sarstedt, with a liquid one, in Brescia, using an hexochinase method on Dimension Vista systems from Siemens Healtheers. Screening for GDM was considered positive if at least one of the three blood samples exceed clinical cut-off derived from HAPO study.
Results: Using NaF 6/51 (11.8%) and FC-mix tubes 12/51 (23.5%) women, respectively, were diagnosed having GDM in Vicenza. Using NaF 2/31 (6.5%) and GlucoEXACT tubes 5/31 (16.1%) women, respectively, were diagnosed having GDM in Brescia. Median (IQR) of glucose in NaF tubes was: 76 (70.3-83.8) at T0, 130.0 (109.3-150.5) at T60' and 106.0 (91.3-125.5) mg/dL at T120', respectively in FC-MIX tubes was: 81.0 (77.0-89.0) at T0, 131.0 (115.3-154.0) at T60' and 109.0 (96.5-129.8) mg/dL at T 120' in Vicenza. Median (IQR) of glucose in NaF tubes was: 74.3 (71.2-77.5) at T0, 122.2.0 (102.4-136.8) at T60' and 106.7 (98.4-128.5) mg/dL at T120', respectively; in GlucoEXACT tubes was: 83.0 (78.7-85.4) at T0, 126.9 (111.1-143.8) at T60' and 116.5 (105.8-137.1) mg/dL at T120' in Brescia. There was a statistically significant difference (P<0.05) between NaF and ternary mixture at all time points.
Conclusions: The use of the new tubes containing a mixture of NaF, EDTA and citrate is a useful and necessary pre-analytical tool for a right OGTT for the

screening GDM. NaF containing tubes should no longer been used for screening of GDM because their use results in underdiagnosis of GDM.

SS05-01

PRESENTATION OF THE CONSENT DOCUMENT ON THE BLOOD AS BIOLOGICAL MATRIX TO EVALUATE THE DRIVING UNDER THE INFLUENCE OF DRUGS AND PSYCHOTROPIC SUBSTANCES

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The prohibition of driving under the influence of drugs and psychotropic substances is governed by art. 187 (Legislative Decree No. 285/1992 and subsequent amendments and additions) which establishes both illicit conduct and the sanctions. With the Law of 23 March 2016, n. 41 new types of crimes were introduced in the penal code with reference to articles 589-bis and 590-bis, respectively entitled "vehicular homicide" and "serious or very serious road personal injury".
Among the multiple problems of interpretation and practical application of the legislation one of the most difficult concerns the lack of uniformity on the methods of investigations to establish the state of psycho-physical alteration of a driver due to the use of drugs and psychotropic substances and the interpretation of the toxicological-analytical result. For these reasons it was necessary to establish a technical table at the Istituto Superiore di Sanità with the experts of the same Institute, the Ministry of Health, the Health Commission and representatives of the major Italian Scientific Societies, competent in the subject, whose task is being to elaborate a technical document on analytical protocols and on the cut-off values that can be used for the analysis of drugs and psychotropic substances in the blood, in order to evaluate the psychophysical alteration of the guide due to the use of these substances. Hence the need of a document which treats four basic points:
1) The choice of the biological matrix: the blood is the only biological matrix to evaluate the driving under the influence of drugs and psychotropic substances.
2) The timing: the time between the road check, the request to carry out the blood sampling and the carrying out of a medical facility of the sample must be as short as possible and traced.
3) The methodology used for the analysis of drugs and psychotropic substances in the elective matrix; once the collection has been carried out, the laboratory, where it is possible, proceeds with screening tests, followed, in the case of positive results, by a confirmation analysis with a toxicological-forensic value in chromatography coupled to mass spectrometry.
4) The choice of the cut-off values for the different drugs and psychotropic substances in the elective matrix correlated with driving disability. The methods for identifying cut-off values in the context of driving under the influence of drugs and psychotropic substances are different and based on different criteria. The Technical Table has therefore established some minimum cut-off values that can be correlated with an inability to drive.

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SS05-04

THE CONSUMPTION OF AMAZING SUBSTANCES FOR THERAPEUTIC OR RECREATIONAL PURPOSE: THE CRITICALITY RELATED TO DEPENDENCE AND TOLERANCE

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The concern to determine a condition of complex dependence or addiction induced by drugs that can be used for analgesic purposes in patients suffering from chronic non-cancer pain, represents an impediment that is not infrequent in everyday clinical practice. In fact, opioid drugs or cannabis, in addition to having an antalgic effect, are able to modulate the brain system of gratification and reward of any individual. These effects can be "evaluated" and experienced by the subject in different ways: in some cases they are not of significant importance and do not determine any alteration of the psychic and behavioral balance of the subject in others, instead, they can represent the basis for the beginning of a misuse of the drug, of the worsening of the phenomenon of tolerance and of the progressive establishment of a state of addiction proper. Therefore, although both opioid drugs and cannabis are able to determine a iatrogenic dependence related to their pharmacodynamic characteristics, this does not in itself represent a pathological condition, but a normal physiological process linked to the progressive establishment of the phenomenon of tolerance that inevitably, these drugs determine, in the same way as other molecules that insist on any type of brain receptor. Therefore, being affected by a condition of "dependence" during the treatment of pain, does not mean becoming "sick" nor, even less, being in the precondition of becoming "addicted" subjects. The same

condition of pseudoaddiction, generally determined by low non-therapeutic dosages of the prescribed antalgic treatment, does not represent a risk of addiction and, it becomes, only if incorrectly maintained, as responsible for the misuse of the prescribed drug that the patient starts independently, looking for a "real" antalgic dose, in total absence of a control and a series of specialized tests. All this would also be supported by recent studies of a neurobiological nature in which a reduction of dopamine production and, therefore, of the "reward" effect of opiates in animal models affected by neuropathic pain and treated with an antalgic therapy would have been demonstrated based on opioids. They must therefore be specific organic, psychic and social factors, related to the additive effects of opioids or cannabis, to make patients undergoing analgesic treatment more vulnerable to the additive effects proper of these drugs.

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SS06-03
HELICOBACTER PYLORI: FROM BENCH TO BEDSIDE

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Helicobacter pylori infection, which affects more than 50% of the human population, is usually acquired during childhood, lasting a lifetime if not treated. The prevalence of infection varies greatly worldwide being higher in the developing, where it is estimated at 70%, than in developed countries, where it affects about 30 to 40% of population. H. pylori is not spontaneously cleared from the infected stomach, and can survive for decades in this ecologic niche, causing gastric damage and disease. All H. pylori infected individuals have mild to severe gastric mucosal inflammation but, in a subset of subjects, it can also cause peptic ulcer, gastric adenocarcinoma or MALT lymphoma. Ninety-five per cent of duodenal and 70% of gastric ulcers are associated with H. pylori while from 75% to 90% of non-cardia gastric cancers and 75% to 100% of gastric diffuse large B cell lymphomas are attributable to the infection. However, these severe diseases are present in only 10 to 20% of infected patients, those affected by malignant disease being a very small minority (1-2%).
Many factors, often interplaying, have been associated so far with these severe H. pylori-associated clinical outcomes: the duration of the infection, host related factors (both genetic and non genetic) and bacterial related factors (virulence factors). Moreover, the human stomach is known to have its own microbiota regardless of H. pylori colonization, being the most relevant phyla Proteobacteria, Firmicutes, Actinobacteria, Bacteroides and Fusobacteria as in many other site of human host. H. pylori presence and its infection density are important in modifying microbiota relative composition. Progression to severe H. pylori associated disease is accompanied by a series of histopathological changes (Correa's pathway) that modify gastric niche reducing progressively H. pylori colonization density, modifying microbiota relative composition and exiting in some cases to dysbiosis particularly in gastric cancer patients. The possible role of microbiota changes in gastric carcinogenesis is therefore an important medical question. Studies on humans, however, have not allowed scientists to verify whether the observed specific changes in microbial communities along Correa's pathway are a cause or simply an effect of disease evolution. Studies on well established H. pylori mouse models with H. pylori infected INS-GAS mice showed that gastric atrophy, metaplasia and dysplasia were more severe and gastrointestinal intraepithelial neoplasia (GIN) more frequent in animals hosting commensal flora than in germfree animals underpinning a role of microbiota at least in magnifying H. pylori effect. Multiple interactions taking place between H. pylori and other bacterial species might modulate the overall inflammatory response to H. pylori infection and induce inflammatory processes related to cancer development at late stage of carcinogenesis when H. pylori density is dramatically reduced. These interactions might be a potential target for manipulation aiming to prevent disease and improve outcome.

SS06-04
MICROBIOTA AND OBESITY

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Napoli

Obesity represents a leading health problem worldwide that fundamentally arises from a positive energy balance under a complex interaction of genetic and environmental factors regulating weight gain. Although obesity is highly heritable, genetic variants so far identified only explain a small proportion of the obesity risk. Moreover, the genetic and epigenetic contribution to obesity are profoundly influenced by behavioral and environmental aspects, such as a reduction in physical activity and/or the high availability of low-cost and high-energy foods. In the last years, the gut microbiota has been proposed as a relevant factor connecting genes

(and their functions) and environment. In humans, the gastrointestinal tract contains approximately 1014 bacteria, belonging to more than 1000 different species that represent a potential genetic space of more than three million bacterial genes. The microbiome (the totality of all the genomic elements of a specific microbiota) retains a high degree of plasticity and its composition changes adaptively with age, diet and the use of medications as well as in relation to the healthy status of the host in a bi-directional way. Accumulating evidences suggest that composition and functions of gut microbiota differ between healthy lean subjects and obese patients. The mechanisms that have been proposed to link the gut microbiota to obesity include energy extraction capacity from food, influence on the integrity of the gut barrier, modulation of the immune system and production of specific metabolites. These latter not only have a local effect on the gut-associated immune system and intestinal barrier, but also function as signals to other tissues and organs including brain, liver and adipose tissue, where they can regulate hunger/satiation stimuli and energy metabolism. To date, the vast majority of the studies conducted in obese patients only report data concerning microbiota composition evaluated on fecal samples and few data are available regarding the functional activity of microbiota especially at level of small intestine, in particular in duodenum, that has a critical physiological and pathophysiological role in metabolic homeostasis. To shed light on the functional correlation between microbiome and obesity, as well as on the regulatory mechanisms involved in this complex interplay, we performed a metatranscriptomic analysis of duodenum samples obtained both from obese patients and normal weight subjects. By next generation sequencing we analyzed the duodenal bacterial composition (analysis of 16S rRNA), as well as the whole bacterial and host transcriptome. Bioinformatic tools evidenced different bacterial composition as well as gene expression levels between obese and control groups. In particular, genes involved in energy production and conversion resulted to be up-expressed in obese patients suggesting that microbial energetic metabolites could be mediators between gut microbiota and obesity. The possibility to modulate the gut microbiota composition by dietary interventions, or by the use of probiotics or lastly by a fecal microbiota transplantation, may offer new possibility to treat obesity.

SS06-CO06

GUT MICROBIOTA FUNCTIONAL PROFILES IN SARDINIAN CENTENARIANS

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It's widely recognized that Sardinia is a longevity hotspot, also the basis of longevity in this region is currently unknown. Gut microbiota may hold a clue. The change in taxonomic composition for gut microbiota with aging leads to altered metabolic activities of microbes in gut. We investigated the correlation between the gut microbiota and health status in various age groups including centenarians. We recruited 65 subjects as a part of the AKEA Study, divided into three age groups: 19 healthy young, 24 healthy elderly 22 centenarians. The metagenomic sequencing was used to explore the gut microbiota composition and functional profile variation between different age groups in a cohort of Sardinians. Metagenomic sequencing data revealed that gut microbiota of the healthy elderly and young in Sardinia population share similar taxonomy composition and metabolic function profile, but a distinct composition was found for most of the centenarians.
In centenarians the proportion of dominant gut microbes and gut microbial genes was decreased, while an accumulation of sub-dominant species and genes was observed. We detected specifically high prevalence of Methanobrevibacter, Bifidobacterium, Lactobacillus and Escherichia in Sardinia centenarians. Amino acid biosynthesis potential was found to be declined, and menaquinol biosynthesis was enhanced. Interestingly healthy aging didn't cause sharp changes of the composition and function profile of gut microbiota, rather, gut microbiota in Sardinia centenarians show a unique taxonomy and metabolic feature. Gut microbes in centenarians appear to cooperate to present a special energy harvest pattern that restricts the carbohydrate intake by the host and release beneficial metabolites. This may be a benefit that impacts the likelihood of longevity. Sardinia centenarians' unique gut microbiota suggests that gut microbiota could be a promising target for pro-aging intervention.

SS07-01

ADVANTAGES AND LIMITATIONS OF DIGITALIZED MORPHOLOGY

F. Dima

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The blood count test and the differential leukocyte count is one of the most requested tests and requires considerable time commitment from the staff. After introduction of automated hematology analyzers, and development of automatic validation rules, the number of microscopic revisions has decreased considerably. In the vast majority of clinical laboratories, the largest cost is represented by the staff who, in many cases, has not increased but has often declined. The latest generation technologies of automated hematology analyzers encompasses instruments adaptable to individual realities of the various laboratories according to both the type of user and workloads. In the presence of atypical or immature cells, the analyzers generate alarms or flags which, together with other reasons, may require microscopic revision. And it is precisely the microscopic examination that includes both the preparation of the slide and the ensuing analysis which is the most expensive part of the process, also due to the need for highly qualified personnel for morphological evaluation. The current automated morphological analysis systems are capable to provide significant improvements of workflow. There are systems integrated into the hematologic chain capable of preparing and staining the slides for subsequent digitized analysis. The digitized morphology analyzers consist of an optical unit consisting of a microscope, a camera and an information system containing cell acquisition and classification software. The images are analyzed by an artificial neural network based on a database of cells and pre-classified according to the leukocyte classes. The cells are then shown on a computer screen for confirmation or possible reclassification by the operator, which also allows revision of the morphology of erythrocytes and platelets. In this study, we aimed to highlight the advantages potential and the limitations of digitalized morphology, including abnormal and immature cells. Many published studies have highlighted the good accuracy and precision of these systems for identifying normal cells and blasts, with some notable exceptions regarding inaccurate classifications of small blasts and for lymphoma cells. In general, the most recent evidence from the literature has shown a larger number of false positive results and a lower burden of false negative results in leukocyte classifications, along with a significant reduction of inter- and intra-observer variability. We hence conclude that digitized images offer many advantages compared to traditional microscopy, can be memorized for training activities, external quality evaluations and can be transmitted from remote locations for expert advice with optimization of turnaround time.

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SS07-02

NEXT GENERATION FLOW CYTOMETRY IN THE ANALYSIS OF MINIMAL RESIDUAL DISEASE

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In recent years remarkable advancements have been made in the flow cytometric (FCM) analysis of rare cellular events, with the development of technical guidelines applicable to a series of clinically relevant applications (i.e. hematological malignancies, PNH, circulating tumor cells and microparticles, rare blood cell subsets). The FCM analysis of minimal residual disease (MRD) in hematological malignancies is now a standardized approach and is included in the clinical routine evaluation of therapeutic courses as well as a surrogate endpoint of outcome in clinical trials. To date FCM MRD assessments are in use with at least four hematological malignancies: B-Cell Chronic Lymphocytic Leukemia (B-CLL), Acute Lymphocytic Leukemia (ALL), Multiple Myeloma (MM), and more recently also Acute Myeloid Leukemias (AML).
The mandatory technical prerequisites to study MRD include: careful cleaning of instrument fluidics, optimized staining to ensure the maximal specificity, the concentration of samples by bulk lysis, the usage of sample pooling and the acquisition of very large cell datafiles to ensure the proper levels of sensitivity. With the advent of MRD studies, the concepts of LLOD and LLOQ, commonplace in clinical chemistry, have been adapted and translated to the peculiar FCM event analysis, and statistical tables have been specifically developed to guide operators. The calculation of MRD events (i.e. malignant cell events over the total nucleated cell denominator) is not a mere arithmetical operation, rather is a strict statistical procedure in which the level of sensitivity (LLOD) varies according to the total number of acquired cells, ideally in the 10^5 - 10^6 range. To comply with such stringent requirements, at least 30-50 MRD events must be captured, usually over several millions of 'clean' nucleated cell events. The concept of a detection sensitivity level that is not predefined as in other analytical fields, but is variable according to the outcome of each cell capture procedure sounds rather

new, and still requires consolidation among FCM users. Next generation FCM is therefore a new technical approach rather than a new instrumentation. Any ordinary benchtop 8-color FCM can be used, provided all the cited requirements are carefully met and the acquisition of bulky cell datafiles is ensured. It is commonly stated that event numbers count here more than color channels. Each hematological malignancy needs its special technicalities for MRD evaluation, includes different consensus MRD levels and requires disease-specific timepoints for monitoring, so 'one size does not fit all' in this case. Because of its crucial clinical importance, FCM MRD evaluation in hematological malignancies is today included the UKNEQAS external quality assurance programs, with the ALL scheme fully ISO17043 accredited and the others at present still in the accreditation pipeline.

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SS07-CO07

PRELIMINARY DATA OF SURVEY ON THE QUALITY OF THE COMPLETE BLOOD COUNT REPORTING WORKING GROUP ON DIAGNOSTIC HEMATOLOGY (WGDH) AND WORKING GROUP ON EXTRA-ANALYTICAL VARIABILITY (WGEAV) OF THE ITALIAN SOCIETY OF CLINICAL CHEMISTRY AND CLINICAL MOL

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The Working Groups on Diagnostic Hematology (WGDH) and on Extra-Analytical Variability promoted a survey about quality of the complete blood count reporting. The survey included 36 questions and it was sent to all SIBioC members by mail and published on the website for 2 months. Twelve days after the publication,125 laboratories (Labs) representing 90% of the Italian regions responded. About 60% of Labs are basic with specialized sections and with inpatient and outpatient patients. Microscopic revision by digitalized morphology was carried out only 4% of Labs. In the report among the quantitative parameters, there is a high degree of concordance for leukocytes, erythrocytes, hemoglobin, hematocrit, MCH, MCHC, RDW, Platelet and leukocytes differential count, while MPV and PDW are reported by 70% of Labs. International units of measure are used on 60% of Labs. The presence of blasts and immature granulocytes are not reported by 20% of Labs and the presence of plasma cells, prolymphocytes and erythroblasts are not reported by 30%. About 10% of Labs do not perform reticulocyte counting. The erythrocyte and leukocyte news parameters are not reported by 70% of Labs and reticulocyte parameters, mainly the immature fraction, are reported by 38%. Specific reference intervals for gender and age were adopted by 68% of Labs, but only 50% have instrument-specific intervals. The qualitative comments were included in the report by 83% of Labs, but only in 45% the comments are harmonized. The SIBioC-document was adopted by 66% of Labs, with adaptive modifications in 50%. Only 60% of Labs defined the criteria for choice of qualitative comments; they are often shared only verbally and only in 6% shared with the clinician.For the management of critical values 83% of Labs have a shared document. Critical values are communicated to the requesting physician by 90% of Labs and 63% have received the SIBioC indications. Preliminary data show that the activities promoted by the WGDH to harmonize the report have been effective on the "classic" parameters with an improvement compared to the 2014 survey, but much still needs to be done, especially for the new parameters. The management of comments still shows considerable heterogeneity, while the management of critical values is generally good.

SP08-01

RECENT ADVANCES IN LABORATORY MEDICINE FOR PRECISION MEDICINE IN NEONATOLOGY AND PEDIATRICS

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Clinical mass spectrometry is a rapidly expanding area of laboratory medicine. It is a particularly valuable technology for the diagnosis of biochemical genetic inherited disorders. In this presentation, I will describe the use of targeted and untargeted metabolomic analysis which identifies metabolic disorders in the newborn and in all age-groups.

Targeted metabolite analysis for metabolic disorders was first initiated in the 1950's with an agar plate-based bacterial growth assay for measuring high levels of phenylalanine in newborn blood spots for the diagnosis of phenylketonuria. This process was initiated by Robert Guthrie and Dr Guthrie's name is still frequently associated with the newborn screening process (the Guthrie test). The success of early diagnosis of phenylketonuria is well established.

Throughout the latter part of the 20th century more than 700 single gene defects were identified which could be diagnosed by metabolite analysis. This process was particularly enhanced by the development of clinical mass spectrometry and the ability to measure the abnormal biomarkers associated with these diseases. One of the most valuable diagnostic tools available was the use of gas chromatography-single quadrupole mass spectrometry to measure the organic acid content of patient urine samples. This represented an untargeted approach to diagnosis where the biomarkers of potential interest were mostly unknown prior to the testing process. Urine organic acid analysis can help in the diagnosis of around 100 single gene defects and is a very early example of the omics revolution in laboratory medicine. Interestingly, this process also measures many biomarkers of the gastrointestinal microbiome. There are ongoing studies of microbiome influence on conditions such as Inflammatory Bowel Disease for which pathogenesis has not yet been identified nor have good early diagnostic biomarkers.

Tandem mass spectrometry has also enhanced our ability to increase the number of metabolic disorders that can be diagnosed by a blood spot in the newborn period. David Millington and coworkers at Duke University identified the role of acylcarnitines as diagnostic biomarkers for multiple nuclear encoded mitochondrial disorders of organic acid and fatty acid metabolic pathways, in particular medium-chain acyl-CoA dehydrogenase which in many populations was found to be as common as phenylketonuria. Using multiple reaction monitoring for up to 30 acylcarnitine species and many amino acids it is possible now in a single blood spot to identify the biomarkers for about 30-40 of the known single gene defects resulting in a biochemical genetic disease. Additional biomarkers are being added for other conditions that could not be screened for previously. Clinical data on outcomes from early diagnosis of many of the screened disorders is very positive Enhanced targeted and untargeted metabolomics processes are used to assist in the diagnosis of many of the non-screened conditions. There is particular growth in the area of disorders of post-translational protein glycosylation for which in recent years over 130 new metabolic disorders have been identified by the measurement of glycosylation profiles on circulating proteins such as transferrin and also for both n-linked and O-linked glyco-profiles using accurate mass time of flight mass spectrometry.

Most biomarkers for disease diagnosis have been identified in body fluids, yet metabolism itself is taking place primarily within cells. I will present some biomarker data for cellular metabolism as proof of principle. It is likely that intracellular metabolic testing will give better insight into the metabolic processes and disease. To this end I will present a novel assay that simultaneously measures all acyl-CoA species in a tissue sample and demonstrate a proof of principle that the assay has potential value.

SP08-02

GENETIC PROFILE AND AGGRESSIVE BEHAVIOR

M. Ruoppolo, F. Salvatore

Napoli

The advent of tandem mass spectrometry allowed to enlarge consistently the spectrum of metabolic diseases that might be easily and quickly detected. The main advantage of this technology is that it can detect several disorders by a single injection: LC-MS/MS, applied to newborn screening programs, can detect more than 70 different metabolic diseases on a single DBS collected within 48-72 hours of birth. Currently, many of the countries around the world have MS/MS-based NBS Programs to detect a variable range of genetic disorders. In Italy, only in recent years some regions issued legislative acts to promote expanded newborn screening with MS/MS. In May 2007 we initiated an expanded newborn screening for inborn errors of metabolism in Campania region by analyzing amino acids and acylcarnitines in DBS samples using tandem mass spectrometry. Here we report the most recent results of our experience from this program.

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SP08-03

NEONATAL SEPSIS IN NEONATOLOGY: CONVENTIONAL AND EMERGING BIOMARKERS

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The assessment of the early diagnosis, the clinical course and the long-term outcomes of neonatal sepsis remain notable laboratory medicine challenges. Commonly used non-culture based diagnostic tests include the total and differential white blood cell (WBC) count, the absolute and immature neutrophil counts, and the ratio of immature to total neutrophils. Tests for evaluating the inflammatory response include C-reactive protein (CRP), procalcitonin (PCT), fibrinogen, haptoglobin, inflammatory cytokines, proteomic markers in amniotic fluid, and cell surface biomarkers, such as neutrophil CD64 and soluble CD14 subtype (presepsin). Recently, the potential role of metabolites has emerged as key issue for the early diagnosis and prediction of neonatal sepsis. Metabolomics – the omic approach investigating in biofluids and tissues the molecular phenotype and its changes over time in health and disease - seems to provide an early etiological diagnosis together with the severity and the progression of the infection. Metabolomics represents the new frontier for evaluating the individual response to the therapy, including any organ damage due to drug toxicity, and the role of microbiome in the early stage (attack of pathobionts to the organism) and in the final phase of sepsis (collapse of the microbiome). Until today, nearly 2,000 patients (including adults, children and babies) with sepsis have been evaluated by the metabolomic approach. Their biofluids (serum, plasma, blood, urine, BALF) were investigated by using either nuclear magnetic resonance (NMR) spectroscopy or mass spectrometry coupled with gas or liquid chromatography (GC-MS and LC-MS, respectively). Data from the literature showed that a number of metabolic pathways may be altered in neonatal sepsis, including those involved in the acute phase response, hypoxia, oxidative stress, energy supply. In particular, it was found a reduced level of ATP, balanced by an increase in fatty acids oxidation, an increase glucose turnover and a switch of glucose consumption from the mitochondrial oxidative phosphorylation to other metabolic pathways such as lactate and pentose phosphate pathways. By comparing the urine metabolome of septic newborns 48 - 72 hours before the onset of clinical signs and symptoms with that of healthy newborns, we found a significant increase of glucose, lactate and acetate and a significant decrease of tamarind 4-Hydroxybenzoic acid (THBA), ribitol, ribonic acid and citrate. In a study of individualized medicine, we monitored the urinary metabolome of a very low birth weight (VLBW) newborn with a fungal infection; interestingly, at the end of the antifungal therapy the metabolome was far from that of non-infected babies. Metabolomic studies in newborns born from mothers affected by chorioamnionitis and in babies with necrotizing enterocolitis showed that gluconic acid is strongly involved, being significantly increased. Indeed, gut microbiota dysbiosis due the overgrowth of certain destructive bacterial species like Str. faecalis, Pseudomonas spp. and E. coli, led to the Entner-Doudoroff bacterial pathway, an overlooked glycolytic route. With the analysis of urine metabolites on admission in neonatal intensive care unit, metabolomics seems to promise impressive improvements in managing sepsis by: characterizing, measuring and distinguishing human metabolites from microbial metabolites (Rosetta Stone of microbiomics); monitoring the effectiveness and the toxicity of the antibiotic treatment; predicting the clinical outcome and the mortality of septic babies.

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SP08-04

EARLY DIAGNOSIS OF AUTISM SPECTRUM DISORDER: BEYOND THE GENETIC

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Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by impairment in social-communication skills, restricted and repetitive interests. Epidemiological studies show a

recent increase in worldwide prevalence rates of ASD, currently estimated over 1% with a male to female ratio 4:1. Despite the progress in understanding the neurobiology of ASD, the causes remain still unknown. A complex relationship between genetic, epigenetic and environmental factors contributes to ASD etiopathogenesis and is responsible of the clinical phenotypic heterogeneity (1). Actually, the diagnosis is still clinical, based on standardized neuropsychological assessment (Autism Diagnostic Observation Schedule –ADOS; Autism Diagnostic Interview Revised-ADI). No valid and specific biomarkers for ASD have been identified yet.
Metabolomics explores the molecular complexity of ASD and the relationships among phenotypes related to external agents. As an emerging tool of network medicine, metabolomics provides a direct functional read-out of the phenotype by the detection, identification, and quantification of metabolites in biological fluids in order to recognize metabolic alteration between comparative samples. Recent evidences show a different urinary metabolomic profile between ASD children and their unaffected siblings(2-5). More in detail, a high level of mammalian-microbial cometabolites, an alteration in nicotinic-acid metabolism, a mitochondrial dysfunction, and increased oxidative stress and amino acid metabolism have been observed in ASD individuals. These findings suggest a potential role of gastrointestinal (GI) dysbiosis, perturbation of antioxidant status, excitatory/inhibitory imbalance in the etiopathogenesis of ASD comorbidities such as GI disorders, epilepsy and sleep problems. Identifying a specific ASD “metabolomic signature” related with the clinical phenotype (severity of core symptoms, developmental trajectory) represents the main future goal in assisting clinicians with earlier diagnoses.

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CC01

A NEW CASE OF METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) DEFICIENCY: THE CONTRIBUTION OF EXPANDED NEWBORN SCREENING AND GENETIC TEST

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Methylenetetrahydrofolate reductase (MTHFR) deficiency is the most common inherited disorder of folate metabolism, transmitted in an autosomal recessive fashion, caused by mutations in the MTHFR gene. The disease is characterized by severe neurological signs and an early treatment, with high betaine doses and methylfolinic acid, could prevent clinical manifestations, but poor experience exists in the literature.We report a new case of MTHFR deficiency, who underwent expanded newborn screening (NBS), performed by LC-MS/MS technology, that showed hypomethioninemia 3.9 µmol/ l (rv: 6-20 µmol/l). The second tier-test showed hyperhomocysteinemia (Hcy 106,7 µM, rv: 5-15 µM). There was no increases of urinary methilmalonic acid excretion. These results raised the suspicion of a folate metabolism disorder and the asymptomatic newborn, 12 days old, started empirical treatment with high dosed betaine, methylfolinic acid and vitamin B12.The genetic test of MTHFR deficiency revealed the presence of two missense mutation: c.176G>C (p.Trp59Ser)
and c.1769T>G (p.Leu590Arg), in MTHFR gene, inherited from the father and mother respectively. The c.176G>C was previously reported as disease causing mutation while the c.1769T>G is a novel variant which can be classified as likely pathogenic according to the ACMG criteria. Furthermore, the sequencing of the MTHFR gene showed the presence of two polymorphisms, c.665C>T (p.Ala222Val) and c.1286A>C (p.Glu429Ala), known as genetic risk factors for thrombophilia, being in homozygous or compound heterozygous state. Parents’ genotyping showed that both carry a genetic risk factors for thrombophilia, being the father homozygous for c.665C>T polymorphism and the mother compound heterozygous for c.665C>T and c.1286A>C. During follow-up levels of methionine, detected by HPLC, have normalized and homocysteine levels have reduced. In conclusion, this case report highlights the importance of NBS, which identified an asymptomatic baby affected by a disorder of folate metabolism. However, the molecular analysis is necessary to reach the precise diagnosis and to provide the appropriate counseling to the parents, also for the possibility of a prenatal diagnosis.

CC02

"WHEN LABORATORY MEDICINE MAKES THE DIAGNOSIS": CASO CLINICO DI ANEMIA EMOLITICA AUTOIMMUNE A CALDO.

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INTRODUZIONE
L'anemia emolitica autoimmune (AIHA) è la manifestazione di patologie poco comuni ed è causata da auto-anticorpi (A-Ab) che selettivamente attaccano e distruggono gli eritrociti del paziente provocando la sintomatologia dell'anemia.I due tipi principali tipi di AIHA sono correlati alla temperatura di reazione degli A-Ab: da agglutinine fredde e a caldo (WAIHA).Riportiamo un caso di WAIHA in cui la diagnosi è stata fatta dagli specialisti in medicina di laboratorio (LAB) attraverso test appropriati.
DESCRIZIONE CASO CLINICO
Uomo caucasico di 36 anni esegue esami di LAB prescritti dal medico di medicina generale (MMG) per astenia e segnalazione di "urine scure" da 2 giorni. Gli ematochimici evidenziano anemia severa, bilirubina (totale e indiretta) e LDH aumentate e marcata reticolocitosi. Si approfondisce con aptoglobinemia, fortmente diminuita, ed esame microscopico che presenza di eritroblasti e di numerosi sferociti suggestivi per AIHA. Viene pertanto eseguito Test di Commbs Diretto (DAT) che si conferma positivo (IgG a 37°C). Avvisato immediatamente il MMG per l'invio al Pronto Soccorso (PS) del paziente. Giunto in PS, dopo circa due ore, il quadro dell'anemia peggiora.Inizia il trattamento con Metilprednisolone (1 mg/Kg per 2 volte al die), idratazione con soluzione fisiologica (500 mL a 125 mL/ora), protettore gastrico (1 compressa/die), terapia con Folina (1 compressa/die) e Dobetin 1000 (1 flacone). Successivamente ricoverato presso la Medicina Generale (MGen) in attesa di trasferimento presso centro ematologico di riferimento.
RISULTATI
Ematochimici routine:
Hb:6.3g/dL;RET:27% (430000/mm3); NRBC:20% dei WBC
T-Bil:4.74mg/dL;I-Bil:4.13 mg/dL;D-Bil:0.61 mg/dL
LDH:682UI/L
Aptoglobina:3mg/dL
DAT a 22°C: positività aspecifica per tutti gli antisieri
DAT a 37°C: positività 4+ per IgG
Ematochimici in PS:
Hb:5.8 g/dL
DAT: positivo confermato
Gruppo:0/D (CcDee)
Ematochimici in MGen:Hb:4.8g/dL nei giorni di ricovero.
DISCUSSIONE
La Medicina di LAB attraverso un corretto ed appropriato utilizzo dei test è un servizio imprescindibile che può fare diagnosi ed indirizzare precocemente il pazient per l'opportuno trattamento terapeutico.
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CC03

UTILITY OF PERIPHERAL SMEAR IN RAPID DIAGNOSIS OF PERTUSSIS

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A 1 month- old girl with no prior illness, presented to the Emergency Department for cough and shortness of breath.
The blood analysis revealed an increased peripheral white blood cell count with lymphocytosis: WBC: 28,19 * 109 /L (75% lymphocytes, 21% neutrophils, 2,7% monocytes, 0,4% eosinophils and 0,2% basophils); hemoglobin level was 10,8 g/dL and platelet count was 518 * 109/L. Biochemistry was normal including normal C – reactive protein. Because of the leukocytosis and lymphocytosis, on the basis of the rules set to morphological evaluation with Cellavision system, a peripheral blood smear was automatically performed. Morphological evaluation of the peripheral blood smear revealed numerous mature lymphocytes with scant cytoplasm, condensed chromatin, and clefted nuclei, characteristic of Bordetella Pertussis lymphocytosis. On the basis of these findings and then of this clinical suspicion, a real time PCR was performed on a respiratory sample that was positive for B pertussis and Rhinovirus. The patient was treated with azithromycin and cortisone showing a progressive clinical improvement. Pertussis is an acute illness of respiratory and infants in pre-vaccination age are the most vulnerable group with the highest rates of morbidity and mortality. The clinical presentation can be atypical and the disease is often misdiagnosed. Analyzing culture is the gold standard for diagnosis but this takes a few days. Polymerase chain reaction is a rapid and more sensitive test but it is not available in all hospitals. Studies of pertussis in children show absolute lymphocytosis in >50% of patients, and characteristic small, mature lymphocytes with hyperchromatic, cleaved nuclei may account for as much as 56% of total lymphocytes. Therefore the presence of these lymphocytes can provide a strong diagnostic suspicion. This case emphasizes, the utility of peripheral blood smear evaluation as a diagnostic tool until other results become available or when more sophisticated diagnostic methods are not available.

CC04

BLASTI LINFOIDI SU LIQUIDO DI VERSAMENTO PLEURICO: CASE-REPORT

F. Sirianni

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Introduzione
Nell'ottobre del 2017 una giovane donna di 18 anni, fumatrice, con anamnesi remota e familiare negativa e, negli ultimi due mesi, rari episodi di dispnea, si è presentata in Pronto Soccorso per edema facciale insorto due giorni prima. Sono stati eseguiti i prelievi per emocromo ed esami ematochimici, l'RX del torace e la toracentesi per la presenza di un versamento pleurico
Materiali e metodi
Il liquido pleurico è stato raccolto in EDTA. Il conteggio delle cellule è stato eseguito con analizzatore automatico Sysmex XN 9000 in modalità Body Fluid; l'emocromo è stato eseguito con strumento automatico Sysmex XN 9000; gli esami ematochimici sono stati eseguiti con lo strumento automatico Beckman Coulter DxH. Sul campione di liquido pleurico è stata eseguito lo studio dell'immunofenotipo linfocitario con citofluorimetro Beckman Coulter Navios
Risultati
L'esame emocromocitometrico e lo striscio periferico risultavano nella norma; gli esami ematochimici dimostravano un lieve aumento dell'LDH (386 U/L, valori normali 136 - 260 U/L) e del CA125 (112 .7 U/mL, R.I. < 35). Sul liquido pleurico i valori di LDH erano 716 U/L, con Ratio LDH liquido/LDH sierico = 1.8 (essudato). La citologia microscopica dimostrava alcuni elementi atipici suggestivi di natura linfoide; sulla base di questo dato è stato subito eseguito lo studio dell'immunofenotipo linfocitario, che dimostrava una popolazione suggestiva di linfoma/leucemia acuta T
Discussione e conclusioni
La modalità Body Fluid permette di esaminare i liquidi cavitari in meno di un minuto e con elevata accuratezza; in questo caso le cellule blastiche sono state riscontrate, dopo attenta osservazione microscopica, prima della loro comparsa nel sangue periferico, cosa che può avere importanti ricadute sull'outcome del paziente. E' infatti stato possibile allertare l'Ematologo, e di programmare tempestivamente la biopsia linfonodale e l'esame del midollo osseo, confermando la diagnosi e istituendo la terapia

CC05

CEREBROSPINAL FLUID ANALYSIS: A RARE CASE OF NEUROLOGICAL LOCALIZATION OF MULTIPLE MYELOMA

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Multiple myeloma (MM) is a neoplastic proliferation of plasma cells (PC). Involvement of central nervous system (CNS) is a rare event (1% of cases), associated with poor prognosis: median overall survival, from the moment of diagnosis of
CNS localization of MM, is only 1.5 months(1,2).
On September 2017, cerebrospinal fluid (CSF) of a 64 years old male patient was sent from hematology unit to laboratory for cell count and morphology, chemical-physical examination and microbial serology. CSF was clear, colourless and with increased proteins (1.01 g/L [0.10-0.45]); in microscopy count (Bürker's chamber, Turk diluent) were observed abnormal cells (40/µL). Our laboratory protocol establishes that CSF must be analyzed on automatic cell counter (Sysmex XN 9000, body fluid module): it showed a few white blood cells (3/µL), but, surprisingly, high number of total cells (64/µL). The morphological evaluation, with cytocentrifugation and May-Grunwald Giemsa stain, revealed characteristic appearance of mature PC. This result was then confirmed by immunophenotyping: PC were CD138+/CD56+/CD19-/CD20-/CD45-/CD117-. Microbial serology was negative.These findings were indicative of MM neurological localization. Patient on January 2017 had been diagnosed as IgA lambda MM (monoclonal component: 46 g/L), with serious bone involvement that caused progressive loss of walk. At the time of analysis, patient had a worsening of neurological symptoms, developing dysphagia. Positron Emission Tomography revealed hyperdenses areas, but it wasn't able to distinguish between lymphoproliferative disease and thrombotic phlogosis.Patient received seven intratecal chemotherapy, not completely successful: CSF PC disappeared, but Magnetic Resonance Imaging didn't show improvement and patient became unable to control movements. His neurological conditions worsened till irreversible coma. He died in November.
We present a rare case of MM neurological localization. Laboratory CSF analysis quickly diagnosed the CNS involvement, in particular with morphological evaluation of cells. Despite rapid diagnosis and therapy, patient died within two months.

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CC06

EFFICACY OF INTRAVENTRICULAR AMIKACIN TREATMENT IN PAN-RESISTANT PSEUDOMONAS AERUGINOSA POST-SURGICAL MENINGITIS

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Data about management of neurosurgical device-associated infections are scanty, and no standardized procedures are available. Intraventricular colistin was associated with positive clinical outcome and doses are empirically chosen.

Pharmacokinetic models for CNS drug disposition are not available; intraventricular (IVT) dosage was not studied in prospective, randomized trials, and empiric dosage of IVT amikacin 5-50 mg daily is recommended for meningitis.

A 66 year-old woman underwent surgical excision of cavernous angioma. Surgery was complicated by cerebral ischemia with a prolonged stay in ICU. At 3rd day hydrocephalus occurred and we placed an external ventricular device (EVD). On day 12, patient had meningitis WBC 1600 cell/μL, glucose 10 mg/dL, total proteins 566 g/L in CSF. As Gram staining showed Gram(-) rods, and due to P. aeruginosa, we started an empiric therapy with intravenous (IV) cefepime, IV and IVT colistin. CSF culture revealed a MDR P. aeruginosa only sensitive to colistin with a MIC value of 0,75 mg/L. We optimized therapy by IV colistin, meropenem and IVT colistin but, after an initial improvement of CSF parameters, there was an increase of CSF WBC count and a decrease of CSF glucose. CSF culture showed a growth of P. aeruginosa with colistin MIC from 0,75 to 4-8 mg/L. Another change occurred: IVT amikacin 30 mg daily and IV cefepime plus IV amikacin 1 g/daily. We chose IVT amikacin instead of colistin because, although resistant, amikacin MIC level (32 mg/L) was the nearest to breakpoint (16 mg/L). From day 28, P. aeruginosa became pan resistant.

Amikacin intrathecal concentration, 1 h after infusion end, exceeded 200 mg/L, and it was adequate to amikacin MIC breakpoint. We observed variable colistin MIC values with 2 distinct analytical methods. We verified activity of different therapeutic regimens by bactericidal activity of colistin and amikacin IVT treatments in CSF by microtiter dilution method. With IVT colistin, bactericidial activity was not optimal for trough (trough <1:2, peak =1:64) while it was better with amikacin (trough = 1:8; peak = 1:128).

The success of our strategy was objectively measured by CSF culture and by progressive improvement of CSF parameters, considering the complex clinical scenario of this fragile ICU patient.

CC07

INFEZIONE NEONATALE DA HUMAN PARECHOVIRUS

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Introduzione

I Parechovirus sono virus appartenenti alla famiglia dei Picornaviridae, suddivisi in due specie, Parechovirus A e Parechovirus B, di cui il primo è noto come "Human Parechovirus o HPeV" in quanto patogeno per l'uomo. Sono stati identificati 19 sierotipi di HPeV. L'HPeV-3 si associa a gravi condizioni patologiche quali sepsi, encefalite, meningite ed epatite nei bambini di età <3 mesi. Recenti studi su liquor proveniente da bambini con sospetta patologia al sistema nervoso centrale (SNC) hanno dimostrato che l'HPeV ha una prevalenza del 3-17%. I bambini che sopravvivono a patologie del SNC da HPeV mostrano, con l'andare del tempo, danni alla materia bianca del cervello e anomalie dello sviluppo. Caso clinico. Un neonato di 31 giorni viene ricoverato in urgenza in Pediatria. Viene richiesto l'esame emocromocitometrico che ha riportato i seguenti valori: leucociti 9.82x103/mmc (IR 4-10x103/mmc), piastrine 147 x103/mmc (IR 150-400x103/mmc) ed emoglobina 11.5 g/dl (IR 11.5-16). Gli esami ematochimici di PCR, pro-calcitonina, AST e ALT sono stati rispettivamente di 1.2 mg/dl (IR 0.0-0.6), 10.3 ng/ml (IR 0.00-0.05), 69 U/l (IR 0-40) e 22 U/l (IR 0-40). Il giorno successivo vengono richiesti emocoltura da catetere venoso periferico ed esame colturale delle feci per la ricerca di Salmonella e Shigella risultati entrambi negativi. L'urinocoltura ha rilevato la presenza di Pseudomonas aeruginosa. Viene richiesto anche l'esame chimico-fisico del liquor. Il liquor si presentava torbido, ematico e l'esame chimico mostrava: proteine 232 mg/dl (IR neonati 15-170), glucosio 43 mg/dl (IR neonati 34-119), lattato 13,5 mg/dl (IR 9-26) e 247 cellule/mcl (IR neonati <30) con prevalenza di cellule polimorfonucleate (58%). In urgenza su liquor viene quindi eseguita una multiplex PCR mediante tecnologia FILMARRAY® (Biomerieux) che rileva la presenza di Human parechovirus. Conclusione. La rapida identificazione di HPeV nel liquor ha svolto un ruolo chiave in quanto ha permesso di ridurre i tempi di refertazione, di migliorare la qualità della diagnosi e la tempestività terapeutica. Ha permesso inoltre di programmare un adeguato follow up del bambino. In caso di sospetta meningite nei bambini di età <3 anni sarebbe quindi auspicabile introdurre la ricerca di HPeV.

CC08

ANTICORPI ANTI-DFS70 FORSE ASSOCIATI AD ANTI-DNA: UN "COMPLESSO" CASO DI LABORATORIO

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Introduzione
la ricerca di anticorpi anti-nucleo (ANA) in immunofluorescenza indiretta (IFI) è di grande utilità nella diagnosi delle malattie autoimmuni sistemiche ANA-associate (MAIS-AA). Nell'ambito delle specificità ANA, è recentemente emersa l'importanza di distinguere gli anticorpi anti-DFS70 che, soprattutto se presenti isolatamente, tendono ad escludere piuttosto che a rafforzare l'ipotesi di MAIS-AA (1).Caso clinico: per la ricerca di ANA per una donna di 62 anni viene eseguito lo screening con IFI su cellule Hep-2 (Euroimmun AG, Lübeck, Germany) che evidenzia un pattern suggestivo per anti-DFS70, la cui presenza viene confermata mediante blot (profilo ANA a 16 antigeni, Euroimmun AG). Negativo il resto delle indagini, anticorpi anti-dsDNA in particolare; il quadro su Crithidia Luciliae (CL) (Euroimmun AG) evidenzia tuttavia quella che viene in prima battuta definita come "verosimile positività". Gli esami sono stati ripetuti in un secondo laboratorio, che utilizza linee cellulari Hep-2000 come substrato per lo screening ANA (ImmunoConcept, Sacramento, CA); inoltre l'indagine blot è stata effettuata mediante profilo ANA a 19 antigeni quantitativo (Alifax, Polverara, Italia). I risultati ottenuti nei due centri si sono dimostrati completamente sovrapponibili; la ricerca di anticorpi anti-dsDNA sono risultati negativi anche con metodo CLIA (A. Menarini Diagnostics, Bagno a Ripoli, Firenze). Dalle informazioni cliniche disponibili è emerso un quadro di astenia degli arti inferiori sensibile ai corticosteroidi, accompagnata da episodi di rash cutaneo per lo più concentrati nel periodo primaverile-estivo. Alla luce di questi elementi, il quadro CL è stato riclassificato come "dubbio positivo".Discussione: la complessità e la peculiarità del caso presentato sono costituite dal quadro ottenuto con CL nel contesto del risultato negativo della ricerca degli anticorpi anti-dsDNA con altre metodiche e la contestuale rilevazione della presenza di anticorpi anti-DFS70. Una possibile spiegazione potrebbe essere che la fluorescenza riscontrata su CL sia derivata da nucleo ed organello basale piuttosto che dal nucleo e cinetoplasto, oppure da una interferenza esterna. Il quadro clinico, poco suggestivo di MAIS-AA, rende più complessa l'interpretazione dei risultati. Degna di nota la sovrapponibilità dei risultati ottenuti nei due laboratori, a confermare la presenza di un quadro di laboratorio ben definito, pur se di complessa interpretazione.

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CC09

A CASE REPORT OF ENCEPHALOMYELITIS ANTI-MOG ANTIBODIES RELATED

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Background. Antibodies to myelin oligodendrocyte glycoprotein (anti-MOG-Ab) are serum immunoglobulins related to a demyelinating central nervous system (CNS) diseases which include a wide spectrum of neurological presentations. The research of anti-MOG-Ab is linked to anti-aquaporin-4 antibodies (anti-AQP4-Ab) for the diagnosis of optical neuromyelitis (NMOSD). Recently a new immunofluorescence technique (IFA) was introduced: in a single test there are two specific chips for anti-AQP4-Ab and anti-MOG-Ab. It can be performed both in liquor and in serum using cells transfected with MOG and AQP-4 and fluorescence conjugate. Case Report. We describe a case of aggressive adult anti-MOG-IgG encephalomyelitis. A thirty-two years old maroccan woman presented to our Neurology Unit with progressive worsening bi-frontal headache and bilaterally reduced visual acuty followed by disequilibrium dysartria and moderate ataxia. Cerebrospinal fluid (CSF) analysis documented pleyocitosis with prevalence of mononuclear cells and rare neutrhopilis, slight CSF-blood-barrier damage and absence of monoclonal bands. Laboratory test showed negativity of anti-nuclear, anti-extractable nuclear antigen and antiphospholipid antibodies as well as specific neuronal antibodies. IFA for anti-AQP4-Ab and anti-MOG-Ab was perfomed both in liquor and in serum: while it was totally negative in liquor, IFA in the serum reveled the only presence of anti-MOG-Ab. After our results, the patient was treated with methyprednisolone with a clinical improvement and reduction of brain lesion and maintenance of therapy with rituximab.Discussion. This case shows clinical characteristics of acute disseminated Encephalomyelitis (EM) and at the same time it is concordant with NMOSD diagnosis criteria. Most experts now consider anti-MOG-Ab-associated EM a disease entity in its own right, immunopathogenetically distinct from NMOSD and the only presence of anti-MOG-Ab can help physicians to correct discrimination of this disorder. We want to underline the utility of this new IFA the contemporary research anti-MOG-Ab and anti-AQP4-Ab is more useful and appropriate tool of correct diagnosis of neural diseases defining a new nosological entity.

CC10

LABORATORY TESTS, GHD AND ADHERENCE TO RHGH THERAPY

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Growth hormone (GH), is a natural hormone secreted by the pituitary gland, with important roles in human physiology. Growth hormone deficiency (GHD) is a disorder characterized by the inadequate secretion of GH, which results in short stature and maturation delays reflected by the delay of lengthening of the bones of the extremities that is inappropriate to the chronological age of the child.Testing is very important in determining whether the child with growth retardation does indeed have growth hormone deficiency. Various agents may be used including insulin, arginine and clonidine. These tests are meant to stimulate the pituitary gland to secrete GH, allowing the measuring of its levels in blood at timed intervals. IGF-1 is a protein produced in response to GH stimulation, which can be measured to screen for GHD, but is also used to titrate rhGH (recombinant human growth hormone) therapy.Treatment for GHD requires, in fact, daily injections of rhGH for several years, until the final stature is reached. Poor adherence to prescriptions is the main cause of the ineffectiveness of the therapy.A 7-year-old male arrived at our attention for stature growth delay. Laboratory tests excluded short stature due to hypothyroidism and celiac disease. The IGF-1 serum levels were normal, but two provocative tests used to assess growth hormone secretory status (clonidine and arginine) were positive, confirming the diagnosis of GHD. The child started the rhGH therapy and, after one year, had a satisfactory stature recovery until the age of 12. After this period there was no more increase in the growth speed, with the suspicion of a poor adherence to the treatment. Therapy monitoring was then performed by measuring the plasma levels of IGF-1, whose values confirmed the lacking compliance of the child.The patient suspended the therapy at the age of 16 since the statural growth speed was <3 cm/year and the bone age was correspondent to 18 years, with a final height below the genetic target.Treatment adherence is a very important clinical issue. As in the reported case noncompliance can have important consequences, leading to additional diagnostic tests and worse outcomes in terms of therapeutic efficacy, representing a damage for patients, health system and society.

CG01

ASSESSMENT OF IN VIVO 11B-HSD2 ACTIVITY: A STUDY OF METHODS COMPARISON

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Background. Apparent mineralocorticoid excess is an autosomal recessive disorder caused by the 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) enzyme deficiency. Traditionally, 11β-HSD2 activity is assessed by measuring the cortisol metabolite ratio (tetrahydrocortisol/tetrahydrocortisone, THF+5αTHF/THE) in 24h urine by gas chromatography (GC-MS). Recently, the ratio of urinary free cortisol/cortisone (UFF/UFE), measurable by liquid chromatography tandem mass spectrometry (LC-MS/MS), has been proposed as a technically easier alternative, though the few available results are controversial.Aim. To compare the measurements of THF+5αTHF/THE and UFF/UFE ratio in both spot and 24h urine samples as markers of vivo 11β-HSD2 renal activity in a family carriers of the A221G mutation (one homozygous affected proband, two heterozygous, consanguineous parents). Methods. THF, 5αTHF and THE were quantified with a home-made GC-MS method. Measurement of UFF and UFE was performed with LC-MS/MS (ISBN-BSN). Two cut-offs from the literature were tested for each parameter: 1.5 and 2 for THF+5αTHF/THE ratio, 0.5 and 1.0 for UFF/UFE.Results. THF+5αTHF/THE ratios were 9.38, 2.03 and 2.69 for proband, mother and father in the 24h urine samples and 6.17, 2.90 and 3.17 in urine spots, respectively. UFF/UFE ratios were 1.94, 0.77, 0.78 in 24h urine samples and 1.60, 1.79 and 0.74 in urine spots. THF+5α-THF/THE ratio displayed satisfactory diagnostic sensitivity for detecting both homozygous and heterozygous defects when either cut-off was applied. The UFF/UFE ratio could detect both homozygous and heterozygous at the 0.5 cut-off, whilst it lost sensitivity for heterozygous deficiency at the 1.0 cut-off. Results obtained in 24h urine samples were comparable to those obtained in spot urine samples using both methods.Conclusions. The assessment of UFF/UFE and THF+5αTHF/THE ratio may be suitable in spot urine samples. Among the two thresholds suggested for UFF/UFE ratio, the 0.5 cut-off enables increased diagnostic sensitivity for heterozygous 11β-HSD2 deficiency. Further studies evaluating the specificity of the test at this cut-off are warranted.Reference: Pizzolo F, et al. J Clin Endocrinol Metab. 2015;100:E1234-41; Danese E, et al. Biochem Med. 2017;27:031001.

CG02

LA VALUTAZIONE "ALLARGATA" DEI CONTROLLI DI QUALITÀ DEI LIQUIDI BIOLOGICI MEDIANTE SOFTWARE SNCS IQAS (SYSMEX)

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SCOPO

L'obiettivo di questo lavoro è quello di illustrare l'utilità del nuovo software Sysmex Network Comunication System (SNCS) per il miglioramento delle procedure di gestione del controllo di qualità dei liquidi biologici. L'esame citometrico comprende il numero degli elementi nucleati totali(TC-BF), il numero di Leucociti(WBC-BF) e il conteggio di altre linee cellulari(HF-BF).

MATE RIALI E METODI

Mediante il software SNCS è possibile monitorare e confrontare a livello mondiale le performance degli analizzatori. Le prestazioni analitiche sono giornalmente controllate da un Controllo di Qualità Interno (CQI) XNCHECK body fluid level 1 e 2. La determinazione di tutti i parametri è stata eseguita utilizzando l'analizzatore SYSMEX XN Module (Kobe, Japan), secondo le specifiche del costruttore. Per ogni singolo parametro di misurazione, WBC, RBC,TC,MN e PMN sono stati calcolati i coefficienti di variazione(CV) dei due livelli di controllo(1).

RISULTATI

I CV dei CQI di un mese di osservazione (31 determinazioni)(CV-LAB), sono stati confrontati con i CV del gruppo omogeneo (CV-O) elaboratori dal software SNCS.QC XNCHECK body fluid level 1: WBC CV-LAB di 7,82% vs CV-O del 8,3%; RBC CV-LAB di 5,68% vs CV-O del 5,35%; TC CV-LAB di 7,82% vs CV-O del 8,3%; MN CV-LAB di 20,6% vs CV-O del 15%; PMN CV-LAB di 6,82% vs CV-O del 9,5%. QC XNCHECK body fluid level 2: WBC CV-LAB di 1,7% vs WBC CV-O del 5,6%; RBC CV-LAB di 2,21% vs CV-O del 3,8%; TC CV-LAB di 1,7% vs CV-O del 5,62%; MN CV-LAB di 2,64% vs CV-O del 9,4%; PMN CV-LAB di 2,35%vs CV-O del 6,5%. Dall'analisi dei dati si può osservare come i CV del laboratorio risultano essere perfettamente allineati con i CV dei controlli del gruppo omogeneo e ben al di sotto di quelli dichiarati dalla ditta produttrice.

CONCLUSIONI

Il nuovo software SNCS permette di gestire a livello multicentrico i dati di laboratorio. L'utilità risiede nella possibilità di poter confrontare le prestazioni interne con quelle di un gruppo di laboratori di confronto, consentendo la quantificazione del bias analitico. Inoltre l'elaborazione real-time del CQI permette di eseguire interventi preventivi prima del verificarsi dell'errore in quanto i report del programma SNCS, accreditati ISO/IEC 17043, segnalano tempestivamente eventuali derive e trend (2).

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CG03

PREDICTIVE MEDICINE FOR PSORIASIS GENETIC RISK USING MULTI-GENE PANEL VARIANT ANALYSIS

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Psoriasis is a chronic multifactorial immune-mediated genetic skin disorder. Red, flaky, crusty patches of skin covered with silvery scales characterize psoriasis. These patches normally appear on elbows, knees, scalp and lower back, but can appear anywhere on the body surface with different degrees of severity (1). Most patients present small patches that involve a milder type of psoriasis known as "plaque psoriasis" (1). While the role of environmental triggers (stress, mechanical trauma and streptococcal infections) is well described in psoriasis onset, recent epidemiological studies have demonstrated that this condition has an important genetic background (2). Our aim was to use next-generation sequencing (NGS) technologies to better understand the complexity of this multifactorial disease and consequently to facilitate its early diagnosis and improve the management of patients and their families. To this aim, we designed a multi-gene panel of 96 genes constituted by: 46 genes related to the immune response and inflammatory processes, 11 genes with antimicrobial and antiviral activity, 11 genes involved in the regulation of the horny layer of the skin, 9 genes involved in cellular adhesion, growth and cellular cycle, 11 genes implicated in metabolism regulation and cellular signalling and 8 genes known to be associated to psoriasis. A total of 49 patients were enrolled in the study: 24 plaque psoriasis patients underwent NGS multi-gene panel testing, 18 subjects were family members of patients, clinically healthy and 7 individuals were healthy and without familiarity for psoriasis. We found 12 clinical variants in the NAT9, CDSN, APOE, CCHCR1, PGLYRP3, MICA, ACEand SLC12A8genes in the patients. In addition, we identified three variants already associated with the onset of psoriasisin TRAF3IP2, IL23R,andIL13genes. These preliminary data show that extended molecular analysis can reveal germline variants associated to psoriasis.

However, technical limitations related to the clinical value of the identified variants

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CG04

THE LABORATORY ASSESSMENT TO DIAGNOSE MALE ACCESSORY GLAND INFECTIONS IN MALE UNDERGOING TO IVF PROGRAM

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Infections of the male genital tract, which represent 15% of infertility cases, are mainly due to germs sexually transmitted or to certain/occasional pathogens. The etiopathogenetic mechanisms are multiple and act at different levels but all result in a potential damage of the sperm cells. The Male Accessory Genital Infection (MAGI) is diagnosed when abnormal sperm parameters are found associated with positive history for urinary infection, epididymitis and/or sexually transmitted disease, abnormal prostatic fluid and signs of ejaculate alteration. The purpose of our study is well characterize MAGI in male fertility diagnosis, by 1) definition of a diagnostic workflow for the characterization of MAGI; 2) implementation of the classification of pathogens that cause MAGI; 3) evaluation of MAGI effects on sperm cells, including the sperm DNA fragmentation. In this study, 40 samples from men (27-44 years) who had requested semen analysis were assessed. Before collecting the semen sample, the patients proceeded with urine collection in order to better differentiate the infection of the seminal tract from urinary tract infection. Once performed a dilution procedure, the seminal samples were spread on appropriate enriched, differential or selective culture media. Standard semen analysis was carried out according to WHO protocol after 2–4 days of sexual abstinence. An aliquot of semen sample was assessed using the Sperm Chromatin Dispersion Test (SCDt) for Sperm DNA Fragmentation(%SDF). The spermioculture allowed highlight the presence of pathogens in 47% of cases (24% not significant pathogens and 29% significant pathogens). 71% of these showed an alteration of sperm fluid viscosity conventionally recognized as warning light for MAGI diagnosis. In addition, the SDF% in semen samples, positive for pathogens, resulted between 50°-95° (25.5%-56.4%) percentile. The data, although preliminary, as well as confirming the implication of MAGI in male infertility, provide opportunities to investigate the significance of other pathogens in addition to those currently recognized in MAGI. Furthermore, the correlation between SDF and MAGI encourages in-depth studies on the mechanism of action of pathogens on spermatozoa.

COD. CG06

PREDICTIVE SFLT-1/PLGF CUT-OFF VALUE IN PATIENTS WITH PRE-ECLAMPSIA AT THE END OF PREGNANCY WITH OR WITHOUT PRE-ECLAMPSIA AT THE TIME OF FIRST ADMISSION

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Introduction
Pre-eclampsia (PE), a condition characterized by hypertension and proteinuria, is reported in 3% of pregnancies and it is associated with substantial perinatal morbidity and mortality in mothers and infants. The management of pre-eclampsia is also associated with significant healthcare costs.Pre-eclampsia is an inflammatory vascular systemic syndrome related to placental oxidative stress; some anti-angiogenic factors, such as protein sFlt1 (fms-like tyrosine kinase-1), are typically improved while angiogenic placental factors, such as PlGF (Placental Growth Factor) are reduced.Quantification of the ratio between two angiogenic placental factors, sFlt-1/PlGF , involved in the formation of new blood vessels, has provided valuable diagnostic information and forms the basis of the first automated biomarker test for pre-eclampsia.Methods. We have enrolled, in this study, 37 pregnant women referred to High Risk Pregnancy Center in the 2nd U.O. of Gynaecology and Obstetrics, University of Bari, Italy, identified through the following risk factors: Age, Previous pre-eclampsia, Family history of pre-eclampsia, Multiple pregnancy, Insulin dependent diabetes (IDDM), Chronic hypertension, Renal disease, Autoimmune disease, Antiphospholipid syndrome, Medically assisted procreation, Body mass index (BMI), Hypertension and Proteinuria.We have measured in 37 pregnant women, with median vital age 34 and with median weeks gestation 35, serum levels of sFlt-1 and PlGF and the value of the sFlt-1/PlGF ratio by Elecsys chemiluminescence assay (Cobas E 411 Roche Diagnostics).Result. There was a

significant association of sFlt-1/PlGF ratio > 66.78, showed an area under the Roc curve (AUC) value of 0.745 (95% confidence interval (CI), 0.554 to 0.886), P 0.0145, sensitivity of 75.00% and specificity of 72.22%, for pre-eclampsia at the end of pregnancy.Conclusions . Angiogenic markers, in particular sFlt-1/PlGF, have been shown to be useful in the differential diagnosis of hypertensive disorder of pregnancy and in predicting the development of PE. These preliminary findings in these select group of patients with or without PE at the time of first admission highlight the need for additional studies of an appropriate cut-off to elucidate the diagnostic/prognostic value of these assays.

CG07

THE ROLE OF VON WILLEBRAND FACTOR IN SEVERE MITRAL VALVE REGURGITATION. A NEW BIOMARKER AFTER TRANSCATHETER MITRAL VALVE REPAIR?

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Background: An association between the acquired von Willebrand syndrome (aVWS) and aortic valve stenosis has been established in the past and the valve replacement shown to lead to factor recovery. Severe forms of aVWS are associated with loss of high-molecular-weight multimers (HMWM) due to the shear stress produced by the blood turbulence in the stenotic valve. No association has ever been demonstrated between aVWS and mitral regurgitation. The aim of this study is to investigate a possible connection between severe mitral insufficiency and von Willebrand factor and whether shear stress due to the valve regurgitation decreases after transcatheter mitral valve repair.Methods: We enrolled 22 patients with severe mitral regurgitation and high surgical risk admitted for elective transcatheter mitral valve repair with Mitraclip system. All patients were successfully treated by TAVI, using only transfemoral/transeptal approach. In every patients we evaluated von Willebrand factor (VWF) antigen (VWF:ag), VWF activity with ristocetin cofactor (VWF:Rco), coagulation factor VIII (FVIII), ADAMTS13 and VWF multimer analysis. Blood samples were collected at time 0 (T0) before the treatment and 24 hours (T1) and 48 hours (T2) after valve repair.Results: Vwf:ag value was significantly increased compared to baseline at T1 (p<0.0064) and T2 (p<0.0032). Also VWF:rco value was significantly increased compared to baseline at T1 (p<0.0024) and T2 was significantly (p<0.0004). No difference was demonstrated in ADAMTS13. Western Blot analysis showed a reduction of HMWM at baseline and confirmed the increase of HMWM expression after mitral valve repair.Conclusions: This study for the first time shows that, similar to aortic valve, severe mitral insufficiency produces a stress shear that reduces the von Willebrand factor. Acquired von Willebrand syndrome due to mitral valve regurgitation can successfully be corrected by mitral valve repair with Mitraclip system without implanting a valve prosthesis. The molecular analysis of von Willebrand multimers showed that after Mitraclip HMWM increase, reducing the risk of bleeding.

CG08

BIOMARKERS BASED STAGING OF TRANSTHYRETIN WILD TYPE (ATTRWT) AMYLOIDOSIS.

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Wild type transthyretin amyloidosis, formerly known as senile systemic amyloidosis, is an increasingly recognized and fatal cardiomyopathy affecting ~20% of males >80 years old. The role of cardiac biomarkers was explored by different groups but has not been validated so far.We report the outcome of 185 subjects diagnosed at the Pavia Amyloid Center.The diagnoses were performed between 2007-2012 in 20 (15%), 2013-2015 in 65 (35%) and 2016-2018 in 100 (54%). Median age was 75 years [IQR: 71-80] and 169 patients (91%) were males. Median NT-proBNP was 2701 ng/L (IQR: 1468-4551 ng/L), cTnI 0.097 ng/mL (IQR: 0.068-0.153 ng/mL) and median estimated glomerular filtration rate (eGFR) was 62 mL/min (IQR: 50-78 mL/min). Median survival of living patients was 50 months. At univariate analysis, NT-proBNP >3000 ng/L (HR: 2.9, P<0.001), cTnI >0.1 ng/mL (HR: 2.44, P=0.002), eGFR <45 mL/min (HR 1.97, P=0.002) were significant prognostic determinants. We tested the Mayo Clinic staging system based on NT-proBNP (>3000 ng/L) and cTnI (>0.1 ng/mL). The cTnI cutoff was obtained with a ROC analysis based on death at 2 years. Median survival was 57 months in stage I patients (n=50, 27%), 52 months in stage II (n=66, 36%) (P<0.001), and 36 months I stage III (n=69, 37%), P=0.08. We assessed the UK staging system based on eGFR (<45 mL/min) instead of cTnI: median survival was 56 months in stage I patients (n=93, 50%), 40 months in stage II patients (n=68, 37%) (P=0.001), and 29 months in stage III patients (n=24, 13%), P=0.230. We then evaluated the THAOS staging system based on quartiles of NT-proBNP and cTnI (1-3 vs 4 quartiles for both) and we found that the median survival was 56 months in stage I patients (n=113, 61%), 37 months in stage II patients (n=43, 23%) (P=0.001), and 23 months in stage III patients (n=25, 16%), P=0.430. The

Mayo Clinic staging system has the best discriminating abilit

CG09

INTEGRATING DIAGNOSTIC APPROACHES TO EVALUATE THE DISCREPANCY OF BRAF AND NRAS MUTATIONAL PROFILES IN METASTATIC MELANOMA

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Oncogenic mutations in BRAF and NRAS genes are the most frequent alterations in melanoma. Considering that different treatment algorithms are based on the melanoma mutational profile, it is mandatory that all melanoma patients with metastatic disease are tested for the presence of BRAF and NRAS mutations. Therefore, finding the best diagnostic methods to identify BRAF and NRAS mutations with a high sensitivity and specificity is necessary for accurate patient selection for target therapy. In addition, there is uncertainty whether the mutational profile usually investigated in the primary melanoma accurately re#ects the mutational status of the metastatic lesions from the same patient. In this study, we aimed first to assess the frequency and distribution of BRAF and NRAS mutations in the primary melanoma and related metastasis of the same patient; secondly, we attempted to evaluate the combination of molecular methods and immunohistochemistry for melanoma mutational testing in clinical practice. Seventy-two FFPE paired samples of primary melanomas and related metastasis were obtained from 31 patients and were analyzed by both molecular methods (by Real-Time PCR and Sanger
Sequencing) and IHC (by the VE1 and SP174 antibodies) to test the BRAFV600 and NRASQ61 mutations. According to molecular testing, BRAFV600 mutations were detected in 46% of melanomas and NRASQ61 in 15%. Mutational somatic profile (BRAF, NRAS) was concordant between the primary melanoma and related metastasis in 77% of patients. Based on IHC, 44% of melanomas showed positive immunostaining with anti-BRAFV600E antibody, while 15% was positive for anti-NRASQ61R. There was substantial agreement between the molecular testing and IHC both for BRAFV600E (Cohen's kappa = 0.74; 89% of agreement) and NRASQ61R (Cohen's kappa = 0.76; 94% of agreement). In conclusion, our results support the intra-patient discrepancy of molecular profiles between primary and metastatic melanoma lesions, with major implication in clinical practice due to the difficulties of treating patients with appropriate target therapies. In addition, we demonstrated that combining molecular analysis with IHC for BRAF and NRAS mutational testing was a reliable diagnostic tool to face challenging samples of melanomas

50° Congresso Nazionale della Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica (SIBioC - Medicina di Laboratorio)

Napoli, 16-18 ottobre 2018

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

P001

ANALISI LEAN DEL SISTEMA DELLE EMOGASANALISI IN POCT. L'ESPERIENZA DELL'OSPEDALE DI MONZA.

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Introduzione: L'U.O. Laboratorio Analisi Chimico Cliniche, dell'Ospedale San Gerardo di Monza, ha in carico da 15 anni la gestione della diagnostica decentralizzata, che include al suo interno anche le tecnologie POCT deputate alla misura dell'emogasanalisi (19 emogasanalizzatori), collocate in 13 differenti UU.OO. L'aggiornamento tecnologico implementato dall'attuale fornitura (Siemens) è servito da trigger per estendere e migliorare un'analisi LEAN preliminare svolta in precedenza.
Scopo del lavoro: Analizzare, con approccio LEAN, la nuova organizzazione dei processi legati all'esecuzione dell'emogasanalisi in POCT, allo scopo di ottenere evidenze necessarie a:
- valutare gli effetti delle strategie messe in atto finora per assicurare l'efficienza e l'appropriatezza delle prestazioni di laboratorio volte al paziente a livello decentrato, con gli stessi standard di qualità del laboratorio centrale.
- progettare nuovi eventi di miglioramento (RIE).
Metodi: L'analisi LEAN è stata condotta seguendo le quattro fasi del ciclo di Deming. Dopo una prima fase di pianificazione delle attività da intraprendere, si è passati ad una fase di esecuzione, presso i reparti con: osservazione diretta dei processi (Go to Gemba), interviste strutturate agli operatori coinvolti e raccolta dati. Le evidenze collezionate hanno permesso la creazione di una mappa di processo (Value Stream Map) per ogni reparto. Nella fase di verifica, i processi sono stati misurati rispetto a 6 KPIs qualitativi, tramite l'utilizzo di una griglia di valutazione a cinque punti (RapidTool). I risultati emersi da questa fase sono stati poi rielaborati in Radar Charts, fondamentali per la progettazione di nuovi RIE. I RIE proposti sono stati comunicati ai reparti con report A3.
Sono stati coinvolti 50 stakeholders (medici, coordinatori infermieristici, infermieri, tecnici di laboratorio, consulenti del fornitore).
Risultati: L'analisi LEAN ha permesso di rilevare il successo delle strategie finora messe in atto, in particolare: raggiungimento di uno score medio complessivo di 3,5 per i sei KPIs di qualità, riduzione delle operazioni manuali dal 70% al 40% in termini di FTE/ giorno. Sono stati riscontrati degli spazi di miglioramento che si prevede di colmare attraverso l'implementazione dei RIE progettati.
Conclusioni: Questa nuova analisi LEAN ha creato l'opportunità di promuovere all'interno dell'organizzazione la diffusione di una cultura collaborativa, atta al miglioramento dei processi, fondamentale per il raggiungimento di uno score di 4 da parte di ogni reparto, entro il prossimo anno. Come prossimo passo, ci proponiamo di valutare non solo gli aspetti qualitativo/ organizzativi del processo, ma anche quelli economico/ gestionali, associando all'approccio LEAN l'approccio HTA.

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P002

LA CORRETTA IDENTIFICAZIONE DEL PAZIENTE: INDICATORE DI QUALITÀ (I.Q.) NEL MONITORAGGIO DELLA PERFORMANCE DEGLI OPERATORI NELLA GESTIONE DEI GLUCOMETRI NELL'OSPEDALE BAMBINO GESÙ

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Scopo dello studio: Il processo di formazione degli operatori rappresenta un punto chiave nella gestione di una corretta governance di un sistema PoCT, in considerazione del fatto che la strumentazione è utilizzata da personale senza specifiche esperienze di laboratorio.
Lo standard ISO 22870 afferma che "solo gli operatori che hanno completato la formazione possono gestire la strumentazione PoCT" e che è necessario "stabilire l'intervallo per il re-training".
Poiché risulta difficoltoso monitorare le performance di un elevato numero di operatori, abbiamo individuato come indicatore di qualità (IQ) "la corretta identificazione del campione/paziente".
Materiali e metodi: Nel periodo Ottobre-Dicembre 2016 sono stati installati e collegati in rete un totale di 62 glucometri Stat-Strip della Menarini Diagnostics in 46 Unità Operative, tutti gestiti da remoto con il software "Netcare". Abbiamo attivato una serie di corsi di formazione sul corretto utilizzo della strumentazione coinvolgendo oltre 600 unità di personale infermieristico.
Risultati: Nel mese di Ottobre dell'anno 2016 la percentuale dei test eseguiti sui glucometri con il barcode identificativo del paziente inserito correttamente è stata del 21,2 %.
A distanza di 18 mesi (Marzo 2018) questa percentuale è risultata del 98,6%.
I referenti PoCT del Laboratorio Analisi, oltre ad effettuare gli audit periodici, hanno monitorato l'indicatore di Qualità (I.Q.) per ogni singola U.O., in modo da effettuare corsi di formazione "retraining" per il personale infermieristico in 19 Unità Operative su 42 totali (45 %).
Conclusioni: La creazione del Gruppo Multidisciplinare dei PoCT composto oltre che dai referenti del Laboratorio Analisi anche da figure professionali appartenenti a Direzione Sanitaria, Bioingegneria, Informatici ha permesso una costante azione di formazione e "vision" sulle procedure PoCT secondo le normative di riferimento, nonché quelle indicate dall'accreditamento Joint Commision International (JCI).
A tale proposito, la "corretta identificazione del paziente" come Indicatore di Qualità nel monitoraggio della performance degli operatori che utilizzano la strumentazione PoCT è risultato sicuramente affidabile per la garanzia della qualità e sicurezza del dato analitico del paziente.

P003

DIAGNOSI DI LABORATORIO PER INFEZIONE NEL DISTRETTO PARODONTALE, DALLA PCR AL "MOLECULAR BEACON ARRAY"

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I dati forniti dall' Organizzazione Mondiale della Sanità riportano che a fronte del 30% della popolazione italiana che presenta salute parodontale, il restante 70 % è affetta da una forma di patologia che può essere lieve (80%) e grave (5-20%). Si tratta dunque di una patologia che ha un forte impatto sociale, e quindi una notevole incidenza nei costi della spesa sanitaria. L 'eziologia della malattia parodontale e di tipo infettivo-infiammatorio ma rispetto ad altre patologie infettive la parodontite presenta notevoli peculiarità che vanno ricercate nelle caratteristiche dei patogeni parodontali. In particolare nel parodonto sono descritte almeno 800 specie differenti, la maggior parte anaerobie e non ancora identificate, si impone pertanto un corretto approccio in termini della diagnosi clinica e di laboratorio. Attualmente, un aiuto importante alla diagnostica di laboratorio in parodontologia, viene dalla biologia molecolare. Le tecniche più efficaci e moderne attualmente in uso sono la Polymerase Chain Reaction (PCR), e la sua versione quantitativa, la PCR real-time. Queste metodiche hanno come principio lo specifico riconoscimento di una sequenza di DNA/RNA appartenente al microorganismo patogeno. I vantaggi principali vengono rilevati nella sensibilità (un incremento di 10-100 volte) e nel tempo del risultato (1- 4 ore). Nonostante questi vantaggi notevoli, i metodi citati possono presentare una bassa specificità, dovuta a sequenze di DNA molto simili appartenenti a specie microbiche affini, per cui il rischio di falsi-positivi risulta elevato. Recentemente un ulteriore passo nella diagnostica microbiologica molecolare è stato raggiunto con i "Molecular Beacon" (MB), Tyagi et al., 1996, sonde molecolari fluorescenti a folding circolare ad elevata specificità. Nel presente lavoro è stato progettato un pannello analitico con MB in grado di discriminare i principali patogeni parodontali già descritti in bibliografia, Socransky et al., 1998. In questo studio sono stati analizzati campioni di placca sottogengivale di 200 pazienti Italiani con parodontite clinicamente evidente. L' indagine ha evidenziato i seguenti risultati: l'87,5 % dei pazienti presentava un'infezione da Tannerella forsythia, il 18,7% da Prevotella intermedia, e il 6,2% Porphyromonas gingivalis. Le infezioni miste, sostenute da almeno 2 specie batteriche, erano il 7%. L'utilizzo dei MB in un pannello analitico contenente tutte le specie rappresentative della malattia parodontale "molecular beacon array", potrebbe disporre per il clinico elementi più oggettivi e, soprattutto scientificamente più significativi, su cui basare la diagnosi di infezione per malattia parodontale.

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P004

GENETIC TEST FOR LACTASE NON PERSISTENCE BY MEANS OF BUCCAL BRUSH AS ACCURATE AND NON-INVASIVE SUBSTITUTE FOR VENIPUNCTURE

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Background: adult-type hypolactasia is a frequent condition of lactose malabsorption; in Italy the frequency of 62,3% was detected (1). The lactose breath test is a simple tool for diagnosis but the need for prolonged monitoring of hydrogen excretion has led to a genetic test proposal. Genotyping for LCT-13910 C>T polymorphism has been proposed as a useful diagnostic marker of adult-type hypolactasia. The aim of this study was to evaluate DNA extraction and subsequent genotyping for lactase non persistence using buccal brush as accurate and non-invasive substitute for venipuncture.
METHODS: twenty-three consecutive functional patients underwent lactose breath test and lactase genetic polymorphism analysis. Genomic DNA was isolated from EDTA-uncoagulated blood and from epithelial cell obtained through buccal brush collection. From all samples, DNA was extracted using Eu-Gen extraction kit (Eurospital, Trieste, Italy), followed by R.T. PCR amplification, specific for T/T, C/C and C/T genotype (LactoGen kit-Eurospital, Trieste, Italy).
Results: DNA isolated from cells collected by means of buccal brush yielded a good quality and sufficient quantity of DNA to perform the analyses of lactase genotype. Genotyping for LCT-13910 C>T polymorphyism obtained from buccal cell-derived DNA were identical to those from blood-derived DNA. All patients showing positive lactose breath test (19/23) had homozygosis for LCT-12910 C>T polymorphism, being C/C. Among the four patients with a negative breath test result, three showed heterozygosis (being C/T) and one showed homozygosis (being T/T).
Conclusion: the introduction of the buccal brush for genetic test for lactase non persistence can omit the current invasive venipuncture; there is a need for a validated protocol to be implemented in the routine diagnostics.

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P005

L'APPROPRIATEZZA NELL'AMBITO DELLE SINDROMI MIELOPROLIFERATIVE CRONICHE PHILADELPHIA NEGATIVE

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Introduzione: Le sindromi mieloproliferative croniche Philadelphia-negative (MPNs) sono malattie neoplastiche clonali della cellula staminale emopoietica caratterizzate dall'assenza della traslocazione cromosomica t(9;22).Il WHO ha incluso tra le MPNs la policitemia vera, la trombocitemia essenziale e la mielofibrosi idiopatica. Le mutazioni alla base di queste patologie riguardano i geni JAK2(V617F), Calreticulina(CALR), MPL (W515K, W515L). La caratteristica di queste mutazioni è di essere mutualmente esclusive.
Scopo dello studio: Dato il numero elevato di richieste di esami, nel nostro laboratorio è stato condotto uno studio sull'appropriatezza, al fine di promuovere il miglioramento della qualità dei servizi e dell'assistenza erogata nel rispetto della spesa sanitaria. Al giorno d'oggi le Aziende Ospedaliere infatti si trovano nella situazione in cui da un lato vi è un aumento del numero di esami richiesti e dall'altro la difficile sostenibilità di tale spesa.
Materiali e metodi: Nel 2017 presso il Laboratorio sono giunte 847 richieste di analisi per JAK2. Di queste,172 presentavano richieste di JAK2, CALR, MPL. I casi sono stati così analizzati: è stato effettuato uno screening per l'identificazione della mutazione del JAK2 mediante l'utilizzo della tecnica della real-time PCR; i positivi allo screening venivano successivamente quantizzati, permettendo per appropriatezza di non eseguire gli altri due esami richiesti. I campioni con JAK2 negativo sono stati analizzati tramite corsa su gel di agarosio dei frammenti di DNA amplificati tramite PCR classica per ricercare le mutazioni della CALR. Nei casi che non presentavano la mutazione del gene veniva eseguita l'analisi dell'MPL tramite ASO-PCR.
Risultati e conclusioni: Dalle analisi è emerso che 20,1% e il 27,3% delle richieste, rispettivamente di CALR e di MPL, giunte nel nostro laboratorio sono inappropriate dal punto di vista diagnostico ed economico. Questi risultati sottolineano la necessità di una maggiore comunicazione tra medico e laboratorista, soprattutto in casi clinici di particolare complessità.

P006

OMICS IN THE ANALYSIS OF SOLID AND LIQUID BIOPSIES FOR PERSONALIZED MEDICINE: THE OMITERC PROJECT

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OMITERC (Applicazione degli OMIcs dalla biopsia solida alla liquida per una TERapia personalizzata del Cancro) is a data-sharing project sponsored by Regione Toscana, that aims to develop an electronic registry or “real world” dataset that aggregates and links cancer genomic and pharmacogenetics - pharmacogenomics data with clinical outcomes from wild-type BRAF metastatic melanoma and KRAS-mutated metastatic colorectal cancer treated at Azienda Ospedaliera Universitaria Senese (AOUS) and Azienda Ospedaliera Universitaria di Careggi (AOUC). The goal of the project is to provide a tumour genomic signature for the two types of cancer and, additionally, to realize a prototype of “regional real word” data set that will power novel clinical and translational research. The project also aims to aggregate, harmonize and share clinical and molecular data obtained during routine medical practice. Ten melanoma and twenty colon cancer patients have been enrolled and serial blood sampling and clinical follow-up have been performed. Here we present data deriving from the analysis of the Liquid Biopsy before and during therapeutic treatment. In particular, this study reports the results of the Circulating Tumor Cell (CTC) counting and characterization and the analysis of cell free DNA (cfDNA) by targeted next generation sequencing.

P007

VITAMIN D SIGNALLING ALTERATIONS INCREASE THE RISK FOR GESTATIONAL HYPERTENSION

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Gestational hypertension (GH) is one of the most common complications (1:10 pregnant women), and one of the predominant causes of perinatal complications, maternal, fetal and neonatal mortality worldwide. Epidemiological studies show that the prevalence of GH is around 2% -7%. Recent studies have revealed an association between vitamin D deficiency and the increased risk of pregnancy complications. The FokI and BsmI single nucleotide polymorphisms (SNPs) of vitamin D receptor (VDR) gene have been associated with decreased receptor activity or receptor amount in tissues, in the presence of wild-type allele f or mutated allele B, respectively, and significantly contribute to reduce the effectiveness of vitamin D action. In this study we aimed to assess the distribution of VDR SNP FokI and BsmI, as well as the circulating levels of vitamin D, in a population of 143 pregnant women (85 GH, age 31.5 ± 6.3 years; 58 controls, age 34 ± 5.6 years), recruited at the moment of hospitalization for childbirth, and analyze the relationships of these biomarkers with the development of GH. Genomic DNA was isolated from peripheral whole blood samples, quantified by spectrophotometric methods and checked for integrity by agarose gel electrophoresis. VDR FokI (rs 10735810) and BsmI (rs1544410) SNPs were screened by Real-time PCR allele discrimination. Plasma levels of 25-hydroxy-vitamin D3 were measured by HPLC. Vitamin D levels were deficient in both groups, and lower in GH patients than in controls (23.27 ± 13.38 vs 24.63 ± 14.38 ng/ml, p>0.05). The VDR FokI FF mutated genotype was the most frequent in both populations (GH 49.4 vs CTR 46.6%), and no significant differences were found between the two groups. The VDR BB mutated genotype was significantly more frequent in GH women than in healthy ones (p=0.039), and was found to be a risk factor for GH (OR=3.052, CI= 1.068-8.717). Notably, GH BB women had vitamin D deficiency (27.9 ± 3.4 ng/ml) while healthy BB pregnants had normal vitamin D levels (36.1 ± 5.5 ng/ml). These data suggest that the VDR BB genotype may not be a risk factor for GH when vitamin D levels are normal. Screening for VDR polymorphisms and assessment/supplementation of vitamin D might be useful for the implementation of preventive strategies for the onset of GH.

P008

DNA VARIANTS INTERPRETATION IN THE NEXT GENERATION SEQUENCING ERA: THE CASE OF EVAI TOOL

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Last years have been featured by rapid technological advances that have escalated our ability to study human genomes at single base resolution. In particular, the development of next generation sequencing technologies (NGS) has impacted every field of molecular research, including the study of human diseases. NGS-based protocols are now widely used also for molecular diagnostics enabling the fast analysis of multiple genes in several patients with high sensitivity. However, more our ability to produce high quantity of genomic data increases, more it is clear that specific infrastructures and bioinformatics pipelines are required to catch from the ensemble of the information obtained the only relevant for the clinical phenotype of the tested individual. Thus, a number of specialized bioinformatic tools are being developed to fill-in this gap. In this context, we tested the Expert Variant Interpreter (eVAI) developed by enGenome. eVAI automates variant interpretation by applying ACMG/AMP guidelines and prioritizes variants of unknown significance assigning a pathogenicity score based on the collected evidences. Evidences are built following guidelines criteria, use different omics resources and are evaluated in the light of every possible disease associated to a certain gene (e.g. variant allele frequency in population databases are evaluated considering disease incidences).Eighty-eight samples were analyzed with eVAI. DNA libraries were obtained by custom gene target enrichment (Haloplex, Agilent Technologies) and sequenced on the MiSeq instrument (Illumina). Obtained sequences were analyzed with both Agilent's SureCall and eVAI pipelines. eVAI was able to identify 15 disease-related variants of which 14 were classified as pathogenic and one is reported as of unclear significance in ClinVar database. Interestingly, 2 known pathogenic variants were not aligned by SureCall software risking to produce false negative results. Furthermore, novel deleterious variants, were not called by SureCall while eVAI tool highlighted them based on their effect on translated proteins. Thus, eVAI shows a higher diagnostic sensitivity coupled with a user-friendly interface that makes its use easy also for not experienced users and for routine diagnostic applications.

P009

EVALUATION OF TARGETED NEXT GENERATION SEQUENCING IN FORMALIN-FIXED, PARAFFIN-EMBEDDED MELANOMA-DERIVED DNA

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The capability to perform molecular profiles of cancers greatly contributed to the development and improvement of new anti-cancer drugs as well as to refine the prediction of response in subgroups of patients harboring specific alterations. Since the identification of somatic mutations plays a central role in the era of personalized medicine, the development of new molecular methods is a challenge of the research field and an important improvement for the diagnostic laboratories.The implementation of Next Generation Sequencing (NGS) into clinical setting is a promising application to improve target detection and molecular profiling suitable for the selection of a specific treatment or to predict the response to therapy in oncologic patients.Recently, NGS methods based on amplicon sequencing represent an appropriate analytical alternative able to merge the high specificity of the sequencing analysis with the sensitivity proper of the allele-specific molecular assays.To verify the potential impact of a targeted NGS approach (Ion AmpliSeq Cancer Hotspot Panel v2), selected melanoma samples (N=21) were retrospectively chosen and analyzed on the IonS5 platform (Thermo Fisher Scientific) in order to compare the system performance with the conventional methods adopted in our routine clinical setting (Sanger sequencing, Sequenom MassARRAY system, Real Time Allele-Specific PCR). In the first instance, the NGS capability in the identification of rarer and low abundant mutations in the main genes involved in melanoma (BRAF and NRAS genes) was evaluated and integrated with the results from the test panel covering hotspot regions of 50 oncogenes and tumor suppressor genes. The evaluation of the proposed NGS method was performed by the analysis of DNA derived from artificial samples (obtained by mixing cell lines carrying the specific somatic mutations under study) and FFPE melanoma samples to verify that the resolution of non-common mutations and low-frequency variants was suitable to meet the technical and clinical requirements. The assessment of the analytical quality parameters (i.e. uniformity, quality score and coverage) and the comparison of the results with orthogonal routinely-used methods suggest that the NGS diagnostics can be implemented in the clinical setting.

P010

GENETICS IN GESTATIONAL DIABETES MELLITUS: MUTATIONS IN GLUCOKINASE GENE CAN DIFFERENTLY INFLUENCE THE BIRTHWEIGHT OF AFFECTED FETUSES

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Maturity Onset Diabetes of the Young (MODY) designates a group of heterogeneous disorders characterized by diabetes onset in young, no signs of autoimmunity, autosomal dominant inheritance and dysfunction of the β cells due to mutations of at least 14 different genes involved in the homeostasis of glucose. The effect of MODY mutations on fetal and neonatal outcomes was previously investigated, suggesting that many critical effects could be highlighted at a careful evaluation of all MODY forms and many benefits can be obtained by an early diagnosis of MODY. Monogenic diabetes due to heterozygous mutations of glucokinase (GCK) (MODY2) has a prevalence up to 6% in women with gestational diabetes. To date we know that an heterozygous GCK mutation reduces the birthweight of the carrier fetus by more than 500 gr and this reduction is more consistent if the maternal hyperglycemia has been intensively treated during pregnancy. In fact, the maternal hyperglycemia represents, for the defective beta cells of the affected fetus, the main trigger for the insulin secretion and the insulin is the main determinant of neonatal birthweight. MODY2 affected women during pregnancy develop both fasting and postprandial high glycaemia, but many authors do not recommend treatment in case of affected fetus, because maternal hyperglycemia is needed to stimulate a normal fetal insulin secretion and consequently a normal fetal growth. We retrospectively studied in our database the birthweight of 48 MODY2 children and we found 3 large for gestational age. It is interesting to observe that all the three LGA children had the same GCK mutation, a microdeletion (c.1373_1385del13) in the exon 10. We speculate that probably this mutation could have determined a severe hyperglycemia in pregnancy to induce hyperinsulinism even in the fetus with GCK deficit, or, on the contrary, it could be possible that GCK dysfunction is so mild that fetal insulin secretion is near normal and increases, as usually in response to maternal hyperglycemia. In conclusion we hypothesize that not all MODY2 affected fetuses need to be exposed to the same levels of hyperglycemia to have an appropriate growth and this could be related to different kinds of GCK mutations that result in different phenotypes.

P011

IMPACT OF ADENOSINE A2A RECEPTOR POLYMORPHISM RS5751876 ON PLATELET REACTIVITY IN TICAGRELOR TREATED PATIENTS

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Dual antiplatelet therapy constitutes a key point in the management of patients with acute coronary syndromes. In particular, ticagrelor, an ADP-antagonist, can provide a more potent and predictable platelet inhibition as compared to clopidogrel, and adenosine-mediated pathways have been involved in its beneficial effects on mortality and myocardial perfusion. However, a quote of patients still displays a suboptimal platelet inhibition on ticagrelor, and, while the role of genetics in conditioning clopidogrel resistance is well established, few data have been reported for ticagrelor. We investigated the impact of rs5751876 C#>#T polymorphism of adenosine A2a receptor (ADORA2a) on platelet reactivity in patients during chronic treatment with ticagrelor. We included patients treated with ASA and ticagrelor for a recent ACS or elective coronary revascularization. Platelet reactivity was assessed at 30-90#days post-discharge by multiple-electrode aggregometry. HRPR for ticagrelor was defined as ADP-test results >417 AU*min. Genetic analysis was performed to assess the presence of rs5751876 C#>#T polymorphism of ADORA2a receptor. We included 244 patients in our study, 174 (71.3%) patients carried the polymorphism (T allele), 51 (20.9%) of them in homozygosis (T/T). C-allele carriers (homozygotes C/C and heterozygotes C/T) showed no difference in baseline characteristics but for lower HDL-cholesterol (p#=#0.01). An absolute lower rate of HRPR on ticagrelor was observed in homozygotes T/T (p#=#0.03). At multivariate analysis, C allele carriage was independently associated with the rate of HRPR on ticagrelor (adjusted OR[95%CI]#=#4.63[1.02-21.01], p#=#0.048). Our study results showed a significant independent association between rs5751876 allele C carriage and a higher rate of high residual platelet reactivity in patients on ticagrelor after a recent ACS or PCI.

P012
LA RICERCA DELLA MUTAZIONE JAK2 V617F: UN VALIDO SUPPORTO NELLA DIAGNOSI DEI DISORDINI MIELOPROLIFERATIVI

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I disordini mieloproliferativi (MPDs) sono patologie neoplastiche caratterizzate dalla proliferazione clonale di una o più tipologie cellulari della linea mieloide.
Tradizionalmente i MPDs includono patologie quali Leucemia Mieloide Cronica (LMC) e patologie BCR-ABL negative quali Policitemia Vera (PV), Trombocitemia Essenziale (ET) e Mielofibrosi Idiopatica (IMF). Recenti studi hanno mostrato una stretta associazione tra la mutazione V617F (G>T nell'esone 12) nel gene JAK2 (Janus Kinase 2) e i disordini mieloproliferativi cromosoma-Philadelphia negativi (Ph-MPDs).
Il gene JAK2, localizzato sul braccio corto del cromosoma 9, codifica per una proteina tirosin-chinasi citoplasmatica coinvolta nella trasduzione del segnale indotto da fattori di crescita emopoietici. La presenza della mutazione V617F comporta un aumento dell'attività della proteina tirosin-chinasica con conseguente aumento del segnale e proliferazione delle cellule emopoietiche. La rilevazione della mutazione JAK2 V617F è stata inserita tra i criteri maggiori WHO 2008 per la diagnosi dei Ph-MPDs. Tale mutazione è stato riscontrato in letteratura essere associata nel 65-95% dei casi a PV, nel 23-57% dei casi a ET e nel 35-50% dei casi a IMF.
Nel Laboratorio Analisi Ospedali Riuniti di Ancona la ricerca della mutazione JAK2 V617F è stata introdotta a partire da giugno 2016; i campioni di sangue intero, che pervengono da tutta la Regione, sono accompagnati da un modulo di richiesta compilata dal medico richiedente in cui sono riportati i dati clinico-anamnestici del paziente.
Ad oggi sono stati esaminati 1405 campioni con un trend in crescita nel numero di richieste/anno a dimostrazione del sempre maggiore interesse verso questo test.
Dall'analisi delle schede clinico-anamnestiche pervenute, nei campioni risultati positivi per la mutazione JAK2, la prevalenza delle patologie associate era del 47% per ET, 26% per PV e 25% per IMF.
In questi due anni, la percentuale di casi positivi per la mutazione riscontrata è stata compresa fra il 17% e il 20%; questo testimonia una appropriatezza prescrittiva pressoché costante nel tempo.
Alla luce dei dati ottenuti si evince che la ricerca della mutazione JAK2 possa essere impiegata dal clinico come test dirimente in caso di sospetto di patologie Ph-MPDs.

P013
LA RIVALUTAZIONE DEL CARIOTIPO COMPLESSO NELLA LEUCEMIA LINFATICA CRONICA

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La leucemia linfatica cronica (LLC) è la più comune leucemia dell'adulto. Lo studio delle lesioni citogenetiche nella LLC a partire dagli anni 2000 è stato condotto generalmente tramite FISH che però consente di rilevare solo le alterazioni cromosomiche più frequenti: trisomia 12, delezione del braccio lungo del cromosoma 11(q22-23), delezione del braccio lungo del cromosoma 13(q14), delezione del braccio corto del cromosoma 17(Tp53). I risultati sia della citogenetica che delle FISH, invece, consentono la stratificazione dei pazienti in gruppi prognostici e predittivi di malattia. La recente introduzione di nuovi mitogeni, in grado di migliorare l'indice mitotico,ha rivalutato il ruolo del cariotipo complesso (CC) che rappresenta una condizione a significato prognostico sfavorevole. Recenti studi di diversi AA. hanno evidenziato che, anche se la FISH è negativa, con la citogenetica convenzionale si possono riscontrare anomalie genetiche nel 25-37% dei casi altrimenti non identificabili e che nel 20% dei casi queste sono inquadrabili in un CC. Si è documentata, così, una possibile sottostima del rischio citogenetico. Nell'anno 2017, nella nostra Struttura, abbiamo analizzato n. 86 LLC, riscontrando n. 28 FISH negative e tra quest'ultime n. 4 CC. Tra le alterazioni più frequenti di tutti i pazienti esaminati abbiamo documentato n. 15 casi di trisomia 12, n.15 casi con del13q-, n. 10 casi con contemporanea presenza di 11q- e 13q-. L'introduzione della target therapy ha riportato all'attenzione clinica il ruolo prognostico del CC, evidenziandone un significato sfavorevole indipendente nel pazienti con LLC recidivata o refrattaria ed identificando un sottogruppo con outcome molto sfavorevole. L'identificazione di nuove alterazioni molecolari grazie alla valutazione delle anomalie evidenziabili mediante CC e FISH potrebbe consentire la creazione di un modello integrato, stratificando i pazienti con LLC in sottogruppi precisi, ottenendo così indicatori prognostici più affidabili.

P014

MODULATORI INTRAGENICI COINVOLTI NELLA REGOLAZIONE DELL'ESPRESSIONE DEL GENE CFTR DELLA FIBROSI CISTICA

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Alterazioni nel gene CFTR causano la Fibrosi Cistica (FC), una malattia le cui complicazioni portano a morte per insufficienza respiratoria. Il CFTR produce un trascritto primario di 6132 basi, con una complessa regolazione trascrizionale dipendente dall'intero locus del gene. La correlazione tra genotipo e fenotipo risulta ancora di difficile comprensione. Abbiamo valutato i possibili meccanismi coinvolti nella modulazione intragenica dell'espressione tramite uno studio del CFTR basato su tre approcci sperimentali: analisi di aplotipi intragenici in popolazioni FC e FC-like; analisi di tratti varianti (TG)mTn ed effetti sullo splicing dell'mRNA; analisi del 3'UTR per potenziali siti di riconoscimento di miRNA. Abbiamo effettuato l'analisi strutturale sul DNA tramite sequenziamento e quella funzionale sull'RNA tramite reverse transcriptase PCR, analisi densitometrica e real-time PCR. Tra gli aplotipi caratterizzati, se ne evidenzia uno con una frequenza significativamente maggiore nelle popolazioni di interesse (FC e FC-like) rispetto alla popolazione generale e con un andamento che varia in base alla gravità delle manifestazioni fenotipiche. E' stato evidenziato come nessuno splicing anomalo sia prodotto dalle singole variazioni di sequenza dell'aplotipo; i prossimi studi andranno a valutare se la contemporanea presenza in cis di tutte le variazioni che compongono l'aplotipo influenzi i livelli di mRNA del CFTR. Alcuni tratti varianti (TG)mTn risultano essere più frequenti nelle popolazioni FC-like che in quelle FC o nei controlli, dato che correla con il limitato effetto funzionale di questi tratti e con il fenotipo clinico di questi pazienti. L'analisi funzionale dell'RNA ha confermato una maggiore percentuale di splicing prodotta da questi specifici tratti; sono attualmente in corso ulteriori approfondimenti quantitativi tramite real-time PCR. Nel 3'UTR del CFTR non abbiamo evidenziato variazioni di sequenza che possano influire sul legame dei miRNA attualmente conosciuti in letteratura; stiamo valutando che effetto possano avere le altre variazioni da noi trovate in questa zona.

P015

VALUTAZIONE DELLA CLONALITÀ NEI DISTURBI LINFOPROLIFERATIVI DELLE CELLULE T: SEQUENZIAMENTO NGS ED ELETTROFORESI CAPILLARE TRADIZIONALE

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Introduzione: In presenza di una proliferazione T linfocitaria in cui l'immunoistochimica e la citofluorimetria non sono sufficienti a distinguere tra neoplasia e proliferazione linfocitaria reattiva, i test molecolari di clonalità linfocitaria risultano fondamentali per il completamento della diagnosi. Negli ultimi decenni la PCR e la visualizzazione degli amplificati con elettroforesi capillare è stata la tecnica di elezione per la ricerca della clonalità. Oggi, il sequenziamento mediante Next-Generation Sequencing offre la possibilitá di sequenziare ampie regioni genomiche con maggiore accuratezza e in tempi ridotti rispetto al passato.

Scopo dello studio: Lo studio mette in evidenza il percorso metodologico seguito nel laboratorio per pazienti con sospetta malattia T-cell. I test TCRβ e γ vengono eseguiti in primis con diversa metodologia, a seguire, se necessaria, la valutazione del TCRδ.

MATERIALI E METODI

Sono stati analizzati una serie di campioni di sangue midollare prelevati al momento della diagnosi e nei successivi follow-up. L'analisi TCRβ e δ è stata eseguita tramite PCR ed elettroforesi capillare, lo studio del TCRγ è stato eseguito con il kit LymphoTrack TCRG Assay Panel MiSeq. Il kit fornisce una singola multiplex master mix per coprire le regioni conservate V J del gene TCR, i primers progettati con adattatori Illumina e con 24 diversi indici permettono l'esecuzione di una sola reazione di PCR, il pool degli ampliconi e il caricamento sulla cella a flusso MiSeq.

Risultati: Per i 4 campioni testati alla diagnosi, entrambi i metodi hanno rilevato la presenza di clonalitá T. La concordanza di assenza/presenza di clonalitá si conferma con i due metodi anche nei follow-up. Il vantaggio offerto dalla tecnologia NGS ci ha permesso di evidenziare per un paziente la modifica della sequenza nella popolazione clonale durante il trattamento, assente negli altri pazienti analizzati.

Conclusioni: L'analisi del TCRG in NGS è adatto per lo studio routinario della clonalità T, in aggiunta ai tradizionali metodi, può fornire una visione più approfondita della popolazione linfocitaria esistente e consentire una valutazione quantitativa e qualitativa dell'eventuale sequenza clonale offrendo un grande vantaggio anche nel monitoraggio della malattia minima residua.

P016

CONTEMPORARY PRESENCE OF ALPHA AND BETA-THALASSEMIC TRAIT: RESULTS OF A FAMILY SURVEY

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At the request of gynecology, a woman (S.M.R.) of 34 years, pregnant at the 10th week, received a prenatal diagnosis from chorionic villus because both her and her husband (S.F.) are healthy carriers of beta-thalassemic trait. Mr. F.S. presents a biochemical framework compatible with beta-thalassemic trait (erythrocytes:6.48x10^6/ ul, hemoglobin:14g/dl, MCV:65.6fl, MCH:21.6pg, HbA2:5.9%, HbF:0.8%). Also the biochemical phenotype of Mrs. S.M.R. is compatible with beta-thalassemia trait (erythrocytes: 5.99x10^6/ul, hemoglobin: 12.8g/dl, MCV:66.9fl, MCH: 21.4pg, HbA2:5.2%, HbF:2.3%). Molecular analysis of the globin beta genes carried out on Mrs S.M.R. highlighted the codon39[C>T] mutation in heterozygosis, while the mutation found in the husband (F.S.) is IVS1.1G>A in heterozygosis. Moreover, from the pre-test genetic counseling it emerged that the father of Mr. F.S. appears to be a healthy carrier of alpha-thalassaemic trait. For this reason we have extended the molecular investigation of alpha globin genes to Mr. F.S. The molecular study of alpha globin genes has established that the latter presents the α2IVS1[-5nt] mutation in heterozygosity that determines the loss of a single α gene and a α+ phenotype (αIVS1$^{[-5nt]}\alpha/\alpha\alpha$) also called α2Tal. For the above reasons we have extended the molecular investigation of the alpha globin genes also to Mrs. S.M.R, which has been found to present the mutation -α3.7 in heterozygosis. From what has been said, it can be concluded that the spouses S.M.R. and F.S. have a 25% risk of generating children affected by beta thalassemia and 25% of conceiving children with a α1TAL. This condition is often mistaken for a martial deficit and treated inappropriately with iron. The case described by us shows that, in the face of subjects presenting a defect in the beta globin gene and a defect in the alpha globin gene (α2TAL), the biochemical screening alone is insufficient. From this arises that an exact identification of the healthy carrier of the simultaneous presence of alpha and beta-thalassemic trait is indispensable, with subsequent in-depth investigation in relation to the consanguinees and the spouse. In our case, a careful family history and the subsequent molecular study of the alpha and beta globin genes allowed first of all to make a correct diagnosis about the genetic structure of the alpha and beta globin genes and secondly allowed to identify couples at risk. to conceive children with beta thalassemia and / or α1TAL.

P017

CYP2C19 GENE COPY NUMBER VARIATIONS AND CLOPIDOGREL PHARMACOGENETICS: ASSOCIATION OF GENE DOSAGE AND CARDIOVASCULAR EVENTS IN SARDINIAN AND ITALIAN CONTINENTAL PATIENTS WITH CORONARY ARTERY SYNDROMES

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Variability in pharmacokinetics and drug response accounts for single-nucleotide variants/polymorphisms (SNVs/SNPs) as well as copy-number variants (CNVs). While the role of SNVs/SNPs on drugs metabolism has been extensively studied, little is known about the CNVs. Cytochrome P450 2C19 (CYP2C19) gene variants and their overall effects on the clinical outcomes of patients with Acute Coronary Syndromes (ACS) treated with Clopidogrel in a dual antiplatelets therapy, remain still controversial although bed-side genetic-driven care has been shown to be feasible. We sought to evaluate the impact of CYP2C19 CNVs on the clinical outcomes in Sardinian patients who underwent percutaneous coronary interventions (PCI) and received clopidogrel therapy having as control population Italian continental (Sicilian ancestry included). The prevalence of CYP2C19 CNVs were assessed by means of three dedicated TaqMan assays (Hs05148033_cn, Hs02932336_cn and Hs05107177_cn) in 100 Sardinian patients who underwent PCI. The control population was made of 200 Italian continental patients (of whom 60 individuals of Sicilian ancestry). Clinical relevant outcomes (adverse cardiovascular events, stent thrombosis and bleeding) and CYP2C19 CNVs were then associated in these two groups. The primary observation was the identification of CYP2C19 gene CNVs in the Sardinian population at higher rate: 7.2% of deletion and 3.2% of duplication alleles respectively in Sardinian vs 1.2% and 0.7% in the control group. The second finding showed that the CYP2C19 deletion allele is at increased risk of a composite of cardiovascular death, myocardial infarction, symptom-driven revascularisation compared with non-carriers (10.58% vs 6.07%, OR: 1.99, 95% CI, pz<0.001). Stent thrombosis (ST) is also more frequent in the deletion allele carriers (2.22% vs 0.44%, OR: 4.77, 95% CI, p<0.001). The risk of bleeding is higher in the duplication allele carriers. In conclusion, genetic testing including the search for CNVs, may be helpful to personalize patients' care being the dual antiplatelets therapy pivotal for patients undergoing PCI.

P018

DIAGNOSTIC YIELD OF SEQUENCING LIPOPROTEIN LIPASE GENES PATHWAY IN PATIENTS WITH SEVERE HYPERCHOLESTEROLEMIA

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Background: Elevated concentrations of LDL cholesterol and triglyceride-rich lipoproteins are correlated with risk for coronary artery disease (CAD) and can be caused by a wide range of genetic, lifestyle, and environmental factors. Gene sequencing allows for the identification of causative mutations in the Familial Hypercholesterolemia (FH) and lipoprotein lipase genes, all belonging to the LPL pathway which is thought to be associate with risk for early-onset CAD.
Methods: We sequenced the coding exons of the LDLR, PCSK9, APOB and LPL genes in 88 probands presenting with hypercholesterolemia (LDL cholesterol ≥190 mg/ dl), with previous history of CAD (N=67; 76.13%) or without (N=21; 23.24%). By means of a custom-based IonAmpliSeq panel, we meant to annotate rare (allele frequency <1%) damaging mutations in the above genes included loss of function variants (i.e., nonsense, canonical splice site, and frameshift) and missense variants annotated as pathogenic in a clinical genetics database or predicted to be damaging by each of five computer prediction algorithms.
Findings: Across all participants, 41 damaging mutations in the LDLR, PCSK9, APOB and LPL genes were identified in 40 probands (one was a carrier of a double heterozygosity). Compared to non-carriers, heterozygous carriers displayed higher LDL cholesterol (22% higher, 95%CI 11 – 23; p = 3 x 10-12) and plasma triglycerides (19% higher, 95%CI 12 – 25; p = 3 x 10-12) as well as increased risk for CAD (Odds Ratio 2.84; 95%CI 1.35 – 2.51; p = 0.0001). Beyond rare mutations, FH mutation carriers had higher cumulative exposure to LDL cholesterol than noncarriers and an additional analysis of 6 common LPL variants noted a 51% increase in odds of CAD (95%CI 39 – 64; p = 1.1 x 10-22) per standard deviation increase in triglycerides.
Interpretation: In our series, about 46.6% carry a damaging mutation in the LPL pathway genes that are associated with higher plasma triglycerides as well as increased risk for CAD. Impaired clearance of triglyceride-rich lipoproteins by the LPL gene appears to be a causal mediator of human atherosclerosis, the burden for coronary artery diseases.

P019

A NEW MULTI-GENE PANEL TAILORED FOR HEREDITARY BREAST AND OVARIAN CANCER PATIENTS

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Introduction. Genetic predisposition to Hereditary Breast and Ovarian Cancer Syndrome (HBOC) is well documented. BRCA1/2 genes are known as the major susceptibility genes in HBOC but additional genes have been associated to an increased risk of disease development. A majority of HBOC patients that undergo the gene testing for BRCA1/2 remain unexplained and this is a challenge for genetic counselling. New advances in Next Generation Sequencing (NGS) provide efficient method for the evaluation of multiple genes simultaneously. In this study, we selected n=38 HBOC to be genotyped by mean of a new multi-gene NGS kit in order to investigate the contribution of several HBOC-associated genes.
Method. A total of 38 patients with a diagnosis of HBOC or at high-risk for HBOC development were investigated using a new 6-gene panel (BRCA Complete™, EntroGen) on Illumina MiSeq platform. Germline mutations of BRCA1, BRCA2, CHEK2, PALB2, RAD51C and TP53 genes and CNV of BRCA1/2 were tested.
Results. In this cohort we found: n=11 BRCA1-mutated subjects (30% of the 38 patients) with 9 pathogenic variants and 2 variant of unknown clinical significance (VUS); n=2 pathogenic variant in BRCA2 (5%); n=1 VUS in CHEK2 (2%) and n=1 VUS in RAD51C. Finally, we identified n=23 (60%) HBOC patients without clinically relevant alteration for the target genes investigated.
Discussion: The hereditary risk associated with HBOC is emerging as a consequence of the contribution of several genes, principally involved in genome stability pathways. Consequently, an extended investigation involving other key role genes is now mandatory to gain a more comprehensive overview on the genetic status of these patients. In this study we assayed, for the first time, a new multi-gene panel NGS kit in order to characterize the mutational landmarks of HBOC patients. Among the mutated patients, BRCA1/2 genes were found to be the principal genetic drivers of the disease, with a prevalence of BRCA1 alteration (SNPs and indels). To note, other two important target were identified in affected patients. They carried respectively two VUS in CHEK2 and RAD51C genes. 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 a more appropriate information about the genetic status of each patient.

P020

CHARACTERIZATION OF THE MICROBIAL TRANSCRIPTOME OF DUODENAL MUCOSA FROM ADULT SEVERE OBESE AND CONTROL SUBJECTS BY NEXT GENERATION SEQUENCING

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Background: The gut microbiome contains trillions of bacteria that are crucial for the well-being of the host. Several evidences in animals and humans showed that alterations in gut microbiome can lead to many diseases, such as obesity. Major microbiota studies were performed on fecal samples that identified differences in the relative abundances of bacterial communities in the gut. Meanwhile, very little is known about the microbiota of the duodenum, a tract of intestine that plays a major role in harvesting energy from nutrients. Unknown is also the functional correlation between microbiome and obesity, as well as the regulatory mechanisms involved in this complex interplay. Aim: Our aim was to characterize the microbial transcriptome of duodenal mucosa from adult severe obese and control subjects in order to identify specific microbial functional metabolic pathways and networks associated with obesity. Materials and methods: We analyzed 2 groups of subjects undergoing gastro-duodenal endoscopy: A) 24 obese patients (BMI=35-40 kg/m^2), 12 females ; B) 23 normal weight age- and sex-matched control subjects (BMI 20.0-24.9 kg/m^2), 10 females. Duodenal biopsies were collected from each patient. Total RNA was isolated and sequenced by Next Generation Sequencing (Illumina, HiSeq1500-2500). Bioinformatic tools were applied to highlight different gene expression levels between obese and control groups. Results: Sixteen Cluster of Orthologus Groups of genes (COGs) were found differently expressed between the two studied groups at a statistical significant level (adjusted $p<0.05$). In particular, genes involved in carbohydrates metabolism, such as Pyruvate-formate lyase, Enolase, Glyceraldehyde-3-phosphate dehydrogenase and Phosphoenolpyruvate-protein kinase, several genes encoding for ribosomial proteins involved in translational processes as well as genes that regulate aminoacid metabolism were up-regulated among obese patients. Conclusions: Microbial transciptomic analysis revealed that genes involved in energy production and conversion are up-expressed in obese patients. This finding could suggest that microbial metabolites could be mediators between gut microbiota and obesity. Our studies could help to clarify the network between microbiome and host.

P021

COMPARATIVE GENOMIC HYBRIDIZATION (CGH) ARRAY E FIBROSI CISTICA

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La Fibrosi Cistica (FC), malattia genetica grave più comune nella popolazione Caucasica, è causata da mutazioni nel gene che codifica per la proteina Cystic Fibrosis Transmembrane conductance Regulator (CFTR) espressa negli epiteli secretori di vari organi dove, un mal funzionamento, causa secrezioni mucose dense e disidratate che generano infezioni e infiammazioni. Nonostante le oltre 2000 lesioni molecolari del CFTR note e lo sviluppo di protocolli di ricerca mutazionale con Detection Rate (DR) sempre più elevate, resta una certa percentuale di alleli FC non identificati perché caratterizzati da riarrangiamenti genomici (grandi delezioni, inserzioni, duplicazioni) che sfuggono alle normali tecniche di analisi basate sulla PCR. Da questo deriva la necessità di migliorare la ricerca mutazionale utilizzando tecniche high throughput, come la Comparative Genomic Hybridization (CGH). Per questo studio, svolto in collaborazione, 89 pazienti FC con forma classica, con una o nessuna mutazione individuata dopo sequenziamento, sono stati analizzati tramite saggio custom array-CGH. I campioni risultati positivi a tale analisi, sono stati ulteriormente caratterizzati tramite MLPA e analisi dell'RNA estratto da cellule dell'epitelio nasale prelevate mediante brushing. L'analisi condotta ha permesso di individuare 3 diverse delezioni, già note in letteratura, in 3 pazienti e 2 diverse duplicazioni, una già nota e una mai caratterizzata in precedenza, in 4 pazienti non imparentati. La duplicazione di 5336 bp non nota e riguardante l'esone 22 (esone 19 in vecchia nomenclatura) è stata caratterizzata, confermando il dato del saggio CGH, sia a livello di RNA che a livello genomico, dove è stato possibile individuare il breakpoint interno della duplicazione. Infatti, poiché l'array custom CGH utilizzato contiene un'alta densità di sonde all'interno del gene CFTR, i punti di interruzione dei riarrangiamenti possono essere localizzati facilmente e con precisione, facilitando notevolmente il compito di caratterizzazione dei breakpoint a livello di sequenza. Si può concludere che grazie a tale metodica è possibile ottenere una fine mappatura del gene CFTR e una caratterizzazione accurata dei riarrangiamenti individuati.

P022

PRELIMINARY STUDY OF THE MICROBIOME IN THE GUT, SKIN AND ORAL MUCOSA OF PATIENTS AFFECTED BY PEMPHIGUS VULGARIS AND BULLOUS PEMPHIGOID. AN OVERVIEW

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Introduction: The study of the human microbiome is one of the most dynamic current topics in biomedical research. Association between diseases and imbalance of the microbial populations are today well investigated. Pemphigus vulgaris (PV) and Bullous Pemphigoid (BP) are rare autoantibody-mediated blistering skin diseases. Flaccid blisters and tense bullae characterized PV and BP patients, respectively. Also oral mucous membranes are commonly affected, especially in PV. The aim of this study is the investigation of the microbiome pattern in gut, lesional skin and lesional oral mucosa in PV/BP patients in order to evaluate the impact of the bacterial populations in this two skin disorders.
Method: High-throughput sequencing of the V3-V4 hypervariable regions of 16S rRNA (Arrow Diagnostics) was used to compare the bacterial community composition of stool, skin and oral mucosa swab collected in a cohort of patients with PV (n=12) and BP (n=8). The dedicated bioinformatics pipeline (SmartSeq) was used to collect bacterial composition from Phylum to Species level. A specific in-house pipeline was applied to collect, aggregate and compare the dataset from the two disease group.
Results: The analysis of intestinal microbiome showed a concordance of bacterial phyla in both PV and BP patients, with a prevalence of Firmicutes and Bacteroidetes. We also observed the presence of Actinobacteria at low frequency (10%) in the only BP stool samples. The evaluation of skin lesions revealed the presence of Proteobacteria (15%) only in BP patients. On the other hand, this phylum characterizes the oral mucosa swab samples of the PV patients (over 20%) and is absent in BP sampling site (<5%). Additionally, we observed a higher diversity in bacterial composition in oral mucosa samples compared to skin swabs.
Discussion: A deeper knowledge of microbiome composition and microbe-host interactions in skin disorders will contribute to clarify the mechanism of these rare diseases. In this pilot study, we evaluated for the first time, the microbiome compositions of stool, lesional skin and oral mucosa of PV/BP, two rare

blistering dermatological conditions. In order to get a better comprehension of the involvement of the bacterial community in the pathophysiology in PV/BP patients, the results obtained from this standardized NGS protocol would be reinforced by correlation with other clinical parameters, which are actually under investigation.

P023

MOLECULAR ANALYSIS OF CIRCULATING DNA (CTDNA) FROM TUMOR PATIENTS USING A TARGETED SEQUENCING APPROACH BY NEXTSEQ 500/550 PLATFORM: A PRELIMINARY DATA

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Introduction: Cancer molecular profile evolves over time in response to a wide variety of endogenous and exogenous selective pressures. Tumor dynamic plasticity could not be captured in its complexity by the single molecular source offered by a one-time tissue biopsy. In recent year, liquid biopsy based on ctDNA analysis has shed a new light on the molecular diagnosis and monitoring of cancer. In this study we performed liquid biopsy strategy on ctDNA to define a mutational landscape of 16 patients with different type of solid tumor. Furthermore, we reported the preliminary data obtained from the use of a new kit highlighting its lights and shadows, in order to consolidate its use in clinical molecular diagnostics. Methods: We evaluated the The AVENIO ctDNA Analysis Targeted kit (Roche Diagnostic GmbH, Germany) on 16 tumor patients. This panel contains 17 genes, includng those in U.S. NCCN guidelines. The kit includes cfDNA extraction from up to 5ml plasma input, optimized reagents and workflow for NGS library preparation and target enrichment. Sequencing libraries were subjected to NGS using the NextSeq 500/550 platform (Illumina Inc.). The kit is also accompanied with an intuitive software and data analysis package to easily obtain results. In addition, the raw data were also analyzed with an "ad hoc" bioinformatic tool. Results: Of 16 tested samples, 3 were excluded from the analysis while 2 were negative. The genes most frequently mutated were APC, EGFR, BRAF, KRAS and TP53. The AVENIO software provides a final clinical report in PDF format with the variants found by applying a default filter set, for each sample. The report includes all the variants present in COSMIC and TCGA databases, as well as those of tabella "loci of interest" associated with the kit used. Our bioinformatics pipeline has confirmed the data of the AVENIO software and moreover it was performed the data aggregation. The extended analysis ha showed a pathogenic variant in BRCA1 (p.Gln1806*) and new variants, excluded in the final report of AVENIO. Conclusions: The AVENIO ctDNA Analysis Kits provide researchers with robust, reliable, highly sensitive and ease to use liquid biopsy assays. Nevertheless there are shadows on the bioinformatics tool as it does not

support comprehensive overview of discovered variants (frequency distribution, aggregated data). Further studies are needed to validate our bioinformatic workflow in order to consolidate this NGS approach in our clinical molecular setting.

P024

RELIABILITY AND PERFORMANCE OF A BISULFITE-NEXT GENERATION SEQUENCING ASSAY TO DISTINGUISH LESIONS AT HIGH RISK OF DEVELOPING ORAL SQUAMOUS CELL CARCINOMA FROM ORAL BRUSHING

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Introduction:
Oral squamous cell carcinoma (OSCC) is the sixth most common cancer with 275,000 new cases/year worldwide. It is usually diagnosed in an advanced stage (2/3 are III-IV), which is associated with 5-year survival rate of 50% and higher radio- and chemotherapy morbidity. On the contrary 80% of stage I-II patients survive at 5 years after diagnosis. Oral cancers mostly develop from Oral Potentially Malignant Lesions (OPML) such as Oral Leukoplakia (OL) and Oral Lichen Planus (OLP) which have an increased risk for malignant transformation ranged from 3.5 to 37%. In this study we evaluated the clinical reliability and performance of a non-invasive molecular method that we developed for the early detection of OSCC (Morandi et al. Clin Epigenetics 2017).
Methods:
Oral brushing samples were collected from 38 histopathological defined OSCC, 1 OSCC with sarcomatoid features, 30 OL, 18 OLP, and a series of oral mucosa around the site of intervention from 39 patients treated for OSCC during follow up. We enrolled also 54 healthy donors, 10 periodontitis and 6 fibromas as negative controls. The DNA methylation level of ZAP70, GP1BB, KIF1A, ITGA4, LINC00599, MIR193, MIR296, TERT, LRRTM1, NTM, EPHX3, FLI1 and PARP15 was evaluated by quantitative Bisulfite-Next Generation Sequencing (NGS). Bioinformatic analysis was performed in a GalaxyProject environment. Each sample was considered positive or negative in relationship to a pre-definite cut off value based on our developed algorithm.
Results:
Positive scores were detected for 38/38 OSCC, and 2/10 periodontitis. The only OSCC with sarcomatoid feature was negative, as well as 54/54 healthy controls and 6/6 fibromas samples (sensitivity: 0.974; specificity: 0.971; Positive Predictive Value: 0.95). Among OPML, 21/30 OLs, 3/18 OLPs, and 14/39 OSCC surgically treated during follow up showed positive scores. In OL patients, the presence of high grade dysplasia was significantly

associated to a positive score (9/9), with respect to 12/21 OLs without dysplasia (Chi 5.510 p<.05).
Conclusions:
Our novel non-invasive bisulfite-NGS assay could represent a promising method to early detect OSCC or high risk OPML in order to increase lifespan of patients with these tumors and to reduce the related economic burden.

P025

HIGH-RESOLUTION MELTING ANALYSIS TO SCREEN THE ST18 GENE FUNCTIONAL RISK VARIANT FOR PEMPHIGUS VULGARIS

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Introduction: Pemphigus vulgaris is the most frequent and representative form of autoimmune bullous disease with intraepithelial lesions involving the skin and the Malpighian mucous membranes. Many evidences support the role of genetics factors in PV development. The ST18 –497-65050T>C polymorphism (rs17315309) exhibit a very strong association in the pathogenesis of PV and could represent a new potential molecular target for the treatment of disease. The aim of this study is to set up a High Resolution Melting Analysis (HRMA) using the Magnetic Induction Cycler (MIC) (Bio molecular Systems), an innovative magnetic induction rotor-based platform recently become commercially available, to screen the ST18 rs17315309 variant in patients with PV addressed to our laboratory. Methods: The method was set-up "in blind" on 25 samples, including 5 patients with diagnosis of PV and 20 DNA healthy volunteer. Each samples analyzed by HRM was then genotyped by Sanger sequencing as confirmatory test. Results: HRMA assay was able to identify easily and unambiguously the c.–497-65050T>C genotypes evaluating melting curve shape and melting temperature (T_m) with a full consistency between Sanger results. Heterozygous samples exhibited a unambiguous melting profile while homozygote genotypes for the variant were easily identified by a Tm sfhit of 0.7 ±0.03°C compared to the wild-type ones. Conclusions: We established a highly sensitive and reliable HRMA approach for the identification of ST18 rs17315309 variant and we showed as it performs with high sensitivity and accuracy in genotyping the variant of interest, being 100% concordant with DNA sequencing. Using of our HRMA approach, which is cheaper, easy and rapid to use, could facilitate large cohort studies on ST18 rs17315309 variant in order to determine the impact of this variant in the disease development and to promote the innovative molecular treatment, above all in the emerging era of targeted therapy.

P026

FROM SURGERY ROOM TO PARP INHIBITOR TREATMENT IN HIGH-GRADE SEROUS OVARIAN CANCER (HGSOC) PATIENTS: TRENDS IN BRCA TESTING AND AN EXAMPLE OF FULL INTEGRATION WITH THE LAB

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Introduction: The high-grade serous ovarian cancer (HGSOC) is the most frequent type of ovarian cancer and somatic pathogenic variants (PVs) in BRCA1/2(BRCA) genes are some of the possible causes. The patients with germline or somatic PVs may benefit of the therapy with poly ADP-ribose polymerase inhibitors (PARPi). The analysis of tumor DNA can be used as a prescreen, the reason why the aim of this our study is to validate and standardize a faster analysis workflow that ensure a complete integration between the surgery room and the lab. Materials and Methods: 150HGSOC patients were chosen for the BRCA testing through the pre-op histological diagnosis or during the surgery. The patients had been submitted to ovarian biopsy and the sample request for BRCA testing was loaded on the management system of the lab directly from the surgery room. The sample was kept in the biobank and it was sent to the lab only when the written informed consent was available.The first step in molecular analysis of patient tissue was the preparation of the purified tissue DNA. About 30 mg of the tissue was homogenated, lysated, digested and the DNA was extracted with an automated device (MagCore HF16 Plus, Diatech Lab Line).The concentration and quality of the DNA were determined by using Qubit® fluorometer (Thermo Fisher Scientific).The Sequencing reactions were carried out on the MiSeq instrument (Illumina, CA) in order to determine the mutation status of BRCA genes.The data were processed byAmplicon Suite software (SmartSeq s.r.l). Results: In this study we assessed the sample suitability, in according with the biobank criteria and we verified the sample traceability of entire process. Secondly, the analytical procedure was successful on all samples: they were analyzed without possible losses and the acceptability of each result was confirmed. Thanks to this improvement we reduced reporting time from 1-2 months to 15-20 working days. Discussion. With this study we produced a standardized operating procedure that demonstrates the complete integration between the surgery room and the lab. Until now, the Aztrazeneca platform assured us a fast track BRCA testing as part of a clinical trial. In the future, however, we trust that this workflow could be approved by the National Health System. Furthermore, the correct identification of somatic BRCA PVs allows considering PARPi as a preferred paired therapy to the traditional treatment of platinum-sensitive ovarian cancer.

P027

MALDI-TOF/MS PEPTIDOMIC PROFILING FOR THE DIAGNOSIS OF INFLAMMATORY BOWEL DISEASES

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Backgrounds: Crohn's disease (CD) and ulcerative colitis (UC) represent the two main forms of inflammatory bowel diseases (IBD). Fecal calprotectin is the most useful biomarker for IBD diagnosis currently available, despite it suffers limitations in both sensitivity (80%) and specificity (65%)1. This study was focused on the identification of new diagnostic MALDI-TOF/MS peptidomic profiles for improving IBD diagnosis, thus reducing the number of unnecessary colonoscopy.
Methods: Stool samples obtained from subjects without (C) or with IBD were collected from the Department of Occupational Medicine and from the Department of Gastroenteroloy of the University-Hospital of Padova, respectively. Samples were resuspended in water 1000:1 (w/v), vortexed and ultracentrifuged for removing residual debris. Supernatants were mixed with acetonitrile 1:1 (v/v), allowing the precipitation of abundant proteins. After ultracentrifugation, supernatants were evaporated, resuspended in 0.1% TFA and desalted. MALDI-TOF/MS analyses were performed in a m/z ranging from 1000 to 4000 Da.
Results: Thirty-three and 133 stool samples from C and IBD, respectively, were collected. After MALDI-TOF/MS analyses, by evaluating all mass spectra, a total of 438 features were identified. In C, 67 of the 79 identified features were shared with IBD. In IBD, 359 features, in single or in combination, were present in 111/133 patients. The overall analyses of all mass spectra allowed diagnose IBD with 83% sensitivity and 100% specificity. Moreover, 34 and 25 peptides were closely correlated with CD and UC respectively, allowing distinction between the two diseases with 80% sensitivity and specificity. A patent was deposited (Patent Number: 102018000005689, 24/05/2018, Ministero dello Sviluppo Economico - Ufficio Italiano Brevetti e Marchi).
Conclusions: The MALDI-TOF/MS peptidomic profiling not only represents an inexpensive, rapid, high throughput and sensitive method for analysing stool samples but it also allows to achieve better characteristic than fecal calprotectin for the diagnosis of IBD and for distinguishing CD from UC.

[1]Padoan A, et al. Improving IBD diagnosis and monitoring by understanding preanalytical, analytical and biological fecal calprotectin variability. Clin Chem Lab Med. 2018.

P028

A COMPREHENSIVE BRCA1/2 NGS PIPELINE FOR AN IMMEDIATE COPY NUMBER VARIATION (CNV) DETECTION IN BREAST AND OVARIAN CANCER MOLECULAR DIAGNOSIS.

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Background: In the last years, the improvements of methodologies has led many diagnostics laboratories to test NGS-based platforms as the main technology for clinical testing. Today, the main challenge is the development of pipelines, able to provide CNV information and sequence data using single platforms, fulfilling the requirement of quality control for diagnosis. At this purpose, we report a NGS workflow for a fast and safe screening of BRCA1/2 variants. Patients and Methods: 287 consecutive unrelated Italian women, affected by breast or ovarian cancer, were investigated. Devyser BRCA NGS kit (DEVYSER, Hägersten, Sweden) was used for library preparation. The Illumina MiSeq System (Illumina, San Diego, CA, USA) and Amplicon Suite Software (SmartSeq, Novara, Italy) were employed for NGS analysis. In order to validate the CNV prediction data provided by NGS analysis, all samples were in parallel investigated using MAQ (Multiplex Amplicon Quantification) assay. Novel LGRs detected, were characterized using alternative techniques as Array-CGH and Long-range PCR. Results: Deleterious sequencing variants were identified in 80 patients (53 in BRCA1 gene and 27 in BRCA2). 11 samples, carrying pathological LGRs, showed NGS CNV prediction analysis results in agreement with MAQ technique. Therefore, the sensitivity of our NGS approach was 100%. Finally, concordant negative results were obtained for the remaining samples (100% specificity). The prevalence of LGRs in BRCA1/2 genes was the 12% of all disease-causing variants detected in our patients. In particular, BRCA1 rearrangements were the 14.5% of all BRCA1 causing variants identified. Differently, BRCA2 large deletions was only the 6.9% of all mutations occurring in this gene. Conclusion: Our integrative NGS-based approach fully satisfied the sensitivity and specificity parameters required on the BRCA1/2 LGRs detection. The workflow represents a robust and easy-to-use method for full BRCA1/2 screening, which can be easily implemented in routine diagnostic testing.

P029

THE IMPROVED DIASORIN Q-LAMP ASSAY FOR THE ACCURATE AND ULTRA-FAST DETECTION OF COMMON AND RARE ISOFORMS OF THE BCR-ABL1 TRANSLOCATION

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The molecular detection of BCR-ABL1 transcripts by RT-PCR is mandatory for the diagnosis of Ph+ Leukemias at onset. Recently a faster and reliable assay based on the Q-LAMP technology developed by DiaSorin has entered in laboratory routine. This assay detects in one hour BCR-ABL1 p190 (e1a2) and p210 (e13a2, e14a2) isoforms. In this study, we evaluated the new improved Q-LAMP formulation designed to detect also less frequent isoforms of the BCR-ABL1 transcripts p190 and p210 (e1a3, e13a3, e14a3). In addition, clinical studies demonstrated that the assay is capable of detecting also the rare isoform p230 (e19a2, e19a3). Methods: The new Q-LAMP technology consists in a multiplex assay for the differential detection of p190 and p210 transcripts and the amplification of the GUSB endogenous RNA. The assay has been tested on 185 clinical samples including 95 p210 positive (57 e13a2 and 28 e14a2, 8 e13a3 and 2 e14a3), 38 p190 positive (33 e1a2 and 5 e1a3) and 50 BCR-ABL1 negative samples. Additional 2 p230 rare isoforms were also included in this study. All samples were previously tested by RT-PCR, considered as the reference method. Results: The new BCR-ABL Q-LAMP assay showed 100% concordance with the RT-PCR, with an expected delayed amplification time for rare isoforms respect to the common ones. The average amplification time of p210 common isoforms were 22,24 and 25,03 min compared to the p210 and p190 rare isoforms that showed 26,54 and 36,84 min, respectively. The 2 p230 (e19a2) rare isoforms were also tested and resulted valid although, due to the very long transcript, they showed a very high average amplification time (50 and 48 min). Moreover, we observed an interesting discrimination between e13a2 and the e14a2 isoform in terms of amplification times (20,21 versus 26,36 min) likely associated to the different length of the two transcripts, with low coefficients of variability (0,15 and 0,11 respectively). Conclusions:

The enhanced BCR-ABL Q-LAMP assay well proved to detect both common and uncommon isoforms of the BCR-ABL1 translocation. This improved performances, combined with the speed and the close tube format, allow laboratories to optimize their workflow and represent a reliable solution for molecular diagnosis of Philadelphia Positive Leukemias.

P030

THE ROLE OF CIRCULATING MIRNAS IN MUSCULAR TRAINING AND PHYSIOLOGY

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A large portion of the genome contains RNA sequences that do not code for any proteins, the microRNAs (miRNAs). These RNA products can regulate gene expression at the post-transcriptional level. Importantly, it has been also confirmed that to exert their function miRNAs enter the circulation system (circulating miRNAs: c-miRNAs), transported in blood stream associated to proteins, lipids or vesicles such as exosomes, and apoptotic bodies. This leads to consider these molecules as possible markers in standard laboratory medicine.
Several miRNAs have been described as involved in skeletal and cardiac muscle differentiation and regeneration (myomiRs), starting also the study about their regulation in exercise physiology. Our preliminary results in the analysis of subjects that underwent in physical exercise, demonstrated that there are c-myomiRs, like miR-1, miR-133a, and miR-206, that show a positive modulation of their expression after exercise, and that can be easily detected isolating RNA from subject's plasma. Among the regulated c-miRNAs, miR-133a showed an upregulation in all patients analysed, suggesting that this miR could be importantly regulated during physical exercise. The analysis of the possible target genes of miR-133a showed as candidate the gene of tropomyosin 4 (TMP4). In the 3' UTR of this transcript there is present a putative target site for miR-133a, conserved in different species. The Real-Time amplification of TMP4 in the individual used for the test, showed a down-regulation of its expression, indicating a correlation with the expression of miR-133a. Moreover, the in vitro testing using a luciferase-assay approach, performed using the TMP4-3' UTR, containing the putative responsive element, demonstrated the depletion of luciferase activity by miR133a.
Since the secretion of miRNAs in plasma could be due to the muscular cell damage related to the physical stress, we extracted exosomes from the plasma of trained individuals and analysed for the presence of miR-133a, obtaining a positive result. This leads to infer, that the release of miR-133a could not be due to only the cell damage, but to an active secretion mechanism, with a regulatory importance to be further investigated.

P031

A LARGE GENES PANEL TO IMPROVE THE DIAGNOSIS AND PREDISPOSITION ANALYSIS OF HEREDITARY CARDIOMYOPATHIES IN A SELECTED POPULATION FROM CARDIOLOGICAL HEALTH CENTERS

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Hereditary cardiomyopathies are primary myocardial disorders in which prevalently myocardial structural alterations compromise heart functions and result in a wide spectrum of phenotypes including cardiac death (1,2). These diseases are mainly due to mutations in sarcomeric genes, transmitted often in an autosomal dominant manner with incomplete penetrance (3). The most common inherited cardiac disorder is hypertrophic cardiomyopathy with a prevalence of 1/500 adults (4). Other forms are dilated cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, left ventricular noncompaction, restrictive cardiomyopathies and other minor forms (5,6). Given the genetic and phenotypic heterogeneity of these diseases, a wide genetic test should be used to identify the most possibly mutation carriers, to make a specific diagnosis and so initiate treatment (7). In this context, we have developed a large NGS cardiomyopathy-related gene panel and have analyzed 104 unrelated patients. This custom panel, designated MyoNext, consists of 111 genes associated with all forms of inherited cardiomyopathies, bound to the above-mentioned diseases including specific syndromes characterized by heart defects but not all the genes related specifically to arrhythmias for which we designed another panel of 76 genes. More than 30% of patients were found to be carriers of pathogenic mutations. Most mutations were found in the MYBPC3and MYH7genes (19% and 13%, respectively). Mutations were also found in the CAV3, MYL2, SCN5A, TNNT2, LMNA,RAF1, GPD1L, TGFB3, DMDand GAAgenes. The diagnostic sensitivity of our MyoNextpanel was 43%. In addition, our data indicate that molecular screening has to be extended beyond the “traditional” myocardial genes.

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P032

A STRATEGY FOR THE SELECTION OF QPCR REFERENCE GENES BASED ON PUBLICLY AVAILABLE TRANSCRIPTOMIC DATA SETS

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In the NGS era, quantitative PCR (qPCR) is still widely employed for both basic research and molecular diagnostics due to its ease of use, versatility and limited costs. Measurement of transcriptional levels through qPCR critically depends on reference genes used for normalization purposes.

Here, we devised a strategy to select valid reference genes for a specific clinical or experimental setting based on publicly available transcriptomic data sets. As a proof of principle, we applied it to identify and validate reference genes for transcriptional studies of bone marrow-derived $CD138^+$ plasma cells from patients with AL amyloidosis or control subjects.

We performed a systematic review of published literature to compile a list of 128 candidate reference genes for qPCR experiments with human samples. Next, we interrogated the Gene Expression Omnibus data repository to assess expression levels of these 128 genes in published transcriptomic studies on bone marrow (BM)-derived $CD138^+$ plasma cells from patients with different plasma cell dyscrasias and controls. For the top 15 genes with the lowest difference across examined cases, we iteratively used PrimerBlast, Ensembl, dbSNP and OligoAnalyzer to identify primer pairs on either exon-exon boundaries or on different exons, devoid of SNPs and lacking thermodynamically significant secondary structures and homo- and heterodimer formation. We used cDNA prepared from the amyloidogenic plasma cell line ALMC-2 and agarose gel electrophoresis and melting curve analysis to verify PCR specificity. Further technical validation identified 7 genes with a dynamic range spanning 5 orders of magnitude of cDNA dilution, with PCR efficiency between 91 and 109%, intra- and inter-assay variation coefficient between 0.3 and 1.3% and 0.1 and 0.6%, respectively. Next, we analyzed expression profiles of these 7 candidate genes in $CD138^+$ plasma cells isolated through MACS-sorting from diagnostic leftovers of BM samples of patients with AL amyloidosis and healthy BM donors. Use of GeNorm identified ACTR3, ALG9 and SDHA as the most suitable reference genes in this clinical setting.

The strategy presented here may be applicable to other clinical and experimental settings for which publicly available transcriptomic data sets are available.

P033

BEYOND BRCA: MULTI-GENE PANEL TESTING TO DEFINE THE EXTENT OF GERMLINE MUTATIONS IN A NUMBER OF RELATED GENES

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Breast cancer (BC) is the most frequent cancer in women worldwide and its incidence have increased in recent decades. Although the predisposing form of BC (hereditary breast and ovarian cancer, HBOC) accounts only for a restricted percentage of the total cases, it is an important issue due to the enhanced cancer risk in mutations carriers. Mutations in the BRCA1 and BRCA2 genes are commonly related to HBOC (1); however, they occur only in about 25% of HBOC cases. Indeed, a large number of other genes are involved in BC predisposition, with low, moderate or even high levels of penetrance (2). This heterogenic background makes difficult to reach an univocal diagnostic protocol by conventional Sanger sequencing. Next generation sequencing technologies enable a more comprehensive molecular characterization of hereditary BCs (3). In this context, we designed a custom multi-gene panel constituted by 44 BC-related genes. Next, we selected 56 patients with a significant familiarity for BC and other tumors, but who tested negative in previous BRCA1 and BRCA2 screening. A DNA library was prepared for each patient by selectively capturing all the exons plus 50 bp in the intronic boundering regions, the promoter and the 3' UTR. We found 6 mutations in 10 unrelated patients (18%): 3 pathogenic variants in the MUTYH gene [c.494A>G, p.Tyr165Cys (in 2 patients); c.1145G>A, p.Gly382Asp (in 2 patients); and c.891+3A>C,p.Gly264TrpfsX7]; 1 nonsense variant (c.793G>T, p.Glu265Ter) in the RNASEL gene, in 3 patients; and 2 nonsense variants respectively in the ATM (c.1463G>A, p.Trp488Ter) and MSH6 (c.892C>G, p.Arg298Ter) genes each one in a patient. Our data show the importance of extending multi-gene testing of hereditary BC molecular bases beyond BRCA testing. Once the significance of variants has been established, it will be possible to support clinical decision-making, thereby contributing to the choice of the most proper therapeutic approach and thus to reduce the lifetime BC risk and increase both the patient's quality of life and life expectancy.

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P034
EVALUATION OF CIRC_100219 AND MIR-135B IN SERUM AND EXOSOMES OF PREGNANT WOMEN

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Aim: Circular RNAs (circRNAs) are recently discovered and highly stable non-coding RNAs acting as gene regulators. These circRNAs can function as miRNA sponges, thereby upregulating or downregulating miRNA target gene expression.
MiR-135b is expressed in placenta tissue and can be found in the maternal circulation, thus playing a functional role in pregnancy. This miR is a target of hsa_circ_100219. The aims of our preliminary study were to evaluate the circ_100219 and miR-135b expression in pregnant and non-pregnant women and investigating the relationship between circ_100219 and miR-135b in both serum and exosomes.
Methods. Total RNA was isolated from serum and exosomes of 30 healthy pregnant women (32.9±5.1 years) at 23-27 gestational weeks and 30 healthy non-pregnant women (31.3±5.4 years), by using mirVana PARIS Isolation Kit (Thermo Scientific, Wilmington, Delaware, USA). Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) was used to quantify hsa_circ_100219 and miR-135b expression. GAPDH and U6 snRNA were chosen as reference for normalizing expression levels. The differences between pregnant and non-pregnant women were evaluated with Mann-Whitney test and correlation was assessed with Spearman's test. The level of statistical significance was set at $p<0.05$.
Results: The circ_100219 expression levels were significantly lower both in serum and exosomes of second trimester pregnant women compared to control group ($p<0.0001$). Mir-135b expression levels were found to be significantly higher in pregnant than in control group ($p<0.0001$). A significant negative correlation was observed between circ_100219 and miR-135b expression levels in both serum and exosomes ($r=-0.34$, $p=0.009$ and $r=-0.31$, $p=0.01$, respectively). The circ_100219:miR-135b ratio was significantly increased in the pregnant compared to control group, in both serum and exosomes (49.0 vs. 1.1, $p<0.0001$ and 2042.4 vs 28.5, $p<0.0001$, respectively).
Conclusions: Our results confirm a role for circ_100219 and miR-135b in physiological pregnancy. Further studies are needed to investigate the circ_100219:miR-135b ratio in pregnancy complications.

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P035
TELOMERE SHORTENING AND PCDH10 PROMOTER METHYLATION IN COLORECTAL CANCER MUCOSAE

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Aim: Telomerase activity and telomere length (TL) have important implications in several human diseases. Telomere shortening is associated with colorectal carcinogenesis. Moreover, recent studies showed that protocadherin 10 (PCDH10) plays a critical role in cancer cell growth, by negatively regulating telomerase activity. PCDH10 is frequently downregulated by promoter DNA methylation. The aim of this study was to investigate whether PCDH10 promoter methylation was associated with TL in colorectal cancer (CRC).
Methods: DNA was extracted from 35 CRC and 35 adjacent normal tissues with Gentra Purgene Kit (Qiagen, Hilden, Germany). A quantitative methylation-specific PCR (MSP) technique was used to analyze a selected CpG site in PCDH10 promoter. TL was evaluated with qPCR and expressed as telomere to single copy gene (T/S) ratio. Differences were assessed with Mann-Whitney test and correlation was 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: We found that TL was significantly lower in CRC than in adjacent non-cancerous tissues ($p=0.0002$). The area under the ROC curve (AUC) for TL was 0.76 (95% Confidence Interval: 0.64-0.88, $p=0.0001$). Aberrant PCDH10 promoter methylation was detected in 100% of CRC tissues, but in none of the paired non-cancerous tissues. The median methylation rate in CRC tissues was 55.7% (6.1-97.8%). TL was negatively correlated with PCDH10 methylation ($r=-0.42$, $p=0.0002$).
Conclusions: These results suggest a pivotal role of telomere shortening and PCDH10 methylation in CRC tissues. TL may be seen as a potential biomarker in CRC diagnostics

P036

ATIPICO AUMENTO DEI LIVELLI DI TRANSFERRINA DESIALATA (CDT) IN UN SOGGETTO APPARENTEMENTE NON ABUSATORE DI ALCOL

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Nel tentativo di ottenere la licenza di guida per i taxi, un ragazzo di 24 anni risulta positivo al controllo obbligatorio per legge sulla transferrina desialata (CDT). Per tale motivo, il soggetto si reca volontariamente al Centro Alcologico di riferimento della Regione Lazio, dichiarando di non essere un abusatore cronico di alcol e di voler essere sottoposto ad ulteriori controlli, al fine di individuare il motivo di tale alterazione. Si effettua un primo dosaggio di CDT in elettroforesi capillare che mostra un valore di 15.3%, 10.9% di disialo e 4.4% di asialo (positivo>1.6). Al fine di escludere che il marcato incremento dipendesse dall'alcol, il ragazzo viene richiamato a sorpresa e sottoposto a dosaggio della CDT (10.7%) e di un secondo marcatore di abuso alcolico, l'etilglucuronide (EtG) urinario, che risulta essere non dosabile poiché largamente inferiore al cut-off di 100 ng/ml (positivo >500 ng/ml). Tutti gli altri marcatori indiretti di abuso alcolico, MCV, GGT e transaminasi, risultano nella norma. A seguito di tali risultati, viene presa la decisione di estendere tutti i test biochimici alla famiglia e di eseguire alcuni test genetici sui geni che secondo la letteratura possono causare un incremento della CDT senza determinare patologie correlate severe: il gene della fosfomannosio isomerasi PMI (Helander et al. 2014) e il gene della transferrina Tf (Grahn et al. 2016). La madre, il padre e la sorella risultano avere tutti i valori analitici nella norma, mentre il fratello più piccolo mostra anche esso un incremento marcato della CDT pari a 5.2% ed EtG anche in questo caso largamente negativo (non dosabile). L'analisi per sequenziamento del gene PMI non mostra variazioni di sequenza che possano causare tale aumento e il gene della transferrina è attualmente in corso di studio.

P037

FIVE NEW CASES OF ACQUIRED HAEMOPHILIA A IN SIENA IN 2017: FORTUITY OR PREVIOUS UNDERESTIMATION?

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The development of inhibitors against clotting factors is related with alterations in coagulation tests associated or not with bleeding episodes of varying seriousness in patients without a personal or family history of coagulation disease. These antibodies are often directed against factor VIII (Acquired Haemophilia A, AHA) and occur in neoplastic and autoimmune diseases, in post-partum, following drug administration or surgery or are due to an unknown cause (50% of cases are idiopathic). Last year 5 cases have occurred in our hospital, Azienda Ospedaliera Universitaria Senese, which serves a catchment area of about 840000 people, while AHA is a rare disease, with an incidence of about 1 to 1.5 case per million/year in the general population.

We do wonder if last year's high incidence was just a fortuity or rather the result of previous underestimation because of undiagnosed or unreported cases.

In all cases (1 post-partum, 1 neoplastic, 2 idiopathic, 1 autoimmune) the clinical features and the prolonged aPTT suggested the suspicion of AHA, requiring a mixing study. We performed the aPTT of mixtures of the patient's plasma and normal pool plasma before and after incubation at 37°C for 2 hours (ACLTOP 500, Werfen). Only in one case did the direct mixing test highlight the presence of an inhibitor (Rosner Index=20%). In the other cases we needed to perform the 37°C 2-hours incubation in order to disclose the failure of correction. We ruled out nonspecific inhibitors, such as lupus anticoagulant.

The diagnosis was confirmed by the identification of reduced factor levels (FVIII in all cases) and by the subsequent titration of the inhibitor (Nijmegen modification of the Bethesda method).

Since FVIII inhibitors are often time and temperature dependent the diagnosis of AHA requires the appropriate laboratory procedures to perform a correct algorithm in order to rapidly identify and treat these patients, often at risk of fatal bleedings. Given the peculiarity of the laboratory diagnosis of this kind of patients it is also crucial a close collaboration between clinicians and clinical pathologists.

P038
FREE LIGHT CHAINS E PROTEINURIA DI BENCE JONES IN SOGGETTO AFFETTO DA MIELOMA MULTIPLO A CATENE LEGGERE (LCMM)

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Background: Il mieloma multiplo a catene leggere (LCMM) è una discrasia plasmacellulare caratterizzata da un'espansione neoplastica di linfociti di tipo B che producono catene leggere libere monoclonali di tipo kappa o lambda. L'immunofissazione urinaria (u-IFE) combinata con la quantificazione della Proteina di Bence Jones (PBJ) su urine 24h è stata da sempre considerata il test diagnostico più sensibile per la diagnosi e il monitoraggio della malattia. Oggi il percorso diagnostico di queste patologie è stato rivoluzionato dal dosaggio delle Free Light Chains (sFLC). Metodi: Soggetto di sesso femminile di 54 aa affetta da LCMM isotipo lambda è stata monitorata nel periodo febbraio 2014 – agosto 2015 con: elettroforesi sierica (CZE), Immunofissazione sierica, Immunofissazione urinaria, dosaggio della PBJ (Sebia) e dosaggio delle sFLC (The Binding Site). Risultati: Il follow-up del paziente ha evidenziato una tendenza alla diminuzione sia della concentrazione delle sFLC che della PBJ nei primi mesi terapia, con una significativa correlazione nella prima fase della malattia. Una remissione completa della malattia non si è mai verificata: dal dicembre 2014 al febbraio 2015 gli esami ematochimici evidenziavano un peggioramento della funzionalità renale (eGFR 8mL/min e PBJ 1504 mg/24h), ed aumento delle FLCλ 8971mg/L. Conclusioni: Il caso oggetto di studio ha evidenziato che il dosaggio delle sFLC e della PBJ dipendono entrambi dalla funzionalità renale. Diversi studi sono stati effettuati per valutare se il dosaggio delle sFLC potesse sostituire la ricerca e la quantificazione della PBJ, ma tutte le evidenze scientifiche concordano nel sostenere che i due esami debbano essere considerati complementari e non alternativi, contribuendo ciascuno nel proprio ambito a migliorare l'efficacia diagnostica, prognostica e di monitoraggio dei LCMM. Le sFLC, oggi, potrebbero rappresentare il metodo di scelta per la valutazione della risposta alla terapia, mentre la proteinuria totale e la misura della creatinina sarebbero utili per monitorare la funzionalità renale in questi pazienti. Bibliografia: Dejoie T,Corre Jill,Caillon H et al: Serum free light chains, not urine specimens, should be used to evaluate response in light-chain multiple myeloma. Blood 2016 128: 2941-2948.

P039
INTERFERENZA NEL DOSAGGIO TSH: CASE REPORT

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Si riporta il caso di una paziente di anni 70, giunta all'osservazione dell'Ospedale del Cuore di Massa per sindrome coronarica acuta e sottoposta a rivascolarizzazione percutanea. La paziente, portatrice di protesi valvolare meccanica, presentava insufficienza renale cronica, ipertensione arteriosa, anemia sideropenica, pregresso linfoma NH, lesione surrenalica. E' stata inoltre evidenziata la presenza di una componente monoclonale di lieve entità, caratterizzata con immunofissazione come IgM lambda. Il test di screening HIV effettuato su Abbott Architect i1000 (HIV ag/ab Combo ref 4J27) è risultato positivo (13 S/CO, v.n. <1). Il test di conferma per HIV, effettuato mediante test InnoLia HIV non ha dato esito certo, presentando sulla striscia reattiva un rumore di fondo molto elevato e confondente. Il test HIV (RNA) quantitativo ha dato esito negativo, con un quantità di copie/ml non rilevabile. Il risultato del dosaggio del TSH effettuato su Abbott Architect i1000 (TSH ref 7k62) è stato > 100 uUI/mL (v.n. 0.35-4.96 uIU/mL), con FT4 1.71 ng/dL (v.n. 0.70-1.48) e FT3 1.16 (v.n. 1.71-3.71) pg/mL: per il TSH il sistema ha effettuato la diluizione 1:5 in automazione, con risultato 3.299 uIU/mL. Per spiegare questa discrepanza è stata sospettata la presenza di un agente interferente, sospetto sostenuto anche dal risultato falso positivo ottenuto per il test HIV. Il dosaggio del TSH è stato quindi ripetuto su una piattaforma diversa (Tosoh AIA, ST AIA-pack TSH ref 025294). I risultati ottenuti sono stati: TSH 0.470 uIU/mL (v.n. 0.25-4.5), FT4 2.55 ng/dL (v.n. 0.7-1.7), FT3 3.75 pg/mL (v.n. 2-4). E' stato effettuato su entrambe le piattaforme un test di linearità, mediante diluizioni seriali del campione. La linearità non è perfettamente mantenuta per le analisi effettuate su Abbott Architect, mentre la curva è più lineare per i test effettuati su Tosoh. Conclusione: la discrepanza fra risultati ottenuti su piattaforma Architect i1000 Abbott su campione intero o dopo diluizione automatizzata del campione ha permesso di rilevare la presenza di un interferente non meglio identificato. A questo punto sarà necessario indagare sulla natura dell'interferente e sulle possibile cause tecniche legate alle caratteristiche del dosaggio.

P040

OCCURRENCE OF MULTIPLE MONOCLONAL COMPONENTS IN A B-CELL NON-HODGKIN LYMPHOMA PATIENT

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Non-Hodgkin lymphomas are clonal lymphoproliferative diseases that originate from B lymphocytes (80-85% of cases), from T lymphocytes (15-20%) or, rarely, from natural killer lymphocytes.
A 70-year-old female patient was admitted at the Department of Haematology in May 2017 reporting having B symptoms like serotine fever and weight loss (>10% in few months). The complete blood count (CBC) showed anaemia (Hb 8 mg/dL), leucocytosis and thrombocytosis. PET/CT revealed multiple mediastinal and lung lymphadenomegalies; then she performed cytological lymphonodal examination by fine needle aspirate, and bone biopsy that resulted in a small cell type CD19+, CD5+ and λ+ B cell lymphoma. Ann Arbor stage was IV B, in according with bone marrow biopsy.
Capillary zone electrophoresis (CZE) of serum proteins and immunosubtraction (ISE) performed in our laboratory using Capillarys 2 (Sebia), revealed four monoclonal components (MC): two IgA λ (8 g/L, 11 g/L); one IgM λ (16 g/L) and one IgG λ (8 g/L), all confirmed by serum immunofixation electrophoresis (IFE) performed with Hydrasys 2 Scan (Sebia). Moreover, we revealed altered levels of: PT 98 g/L (64-83), FLC k 23 mg/L (6.7-22), FLC λ 197 mg/L (8.3-27), with FLC k/λ 0.11 (0.31-1.56), IgA 7.24 g/L (0.7-4.0), IgM 18.9 g/L (0.4-2.3), while IgG 12.0 g/L (7.37-16.0) remained in the reference range.
The patient underwent a first R-CHOP (Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, Prednisone) chemotherapy cycle, a transfusion of red cell concentrates and a weekly administration of erythropoietin. In November 2017, the CBC and PT normalized, the four MC became <5 g/L and the clinical conditions of the patient improved; the further improvement of the patient's general conditions was observed after six R-CHOP chemotherapy cycles (April 2018); imaging assessment at final restaging showed aspecific lung uptake without deep and superficial lymphoadenomegalies; the patient refused final revaluation with bone marrow biopsy.
This clinical case describes an unusual electrophoretic pattern observed in a B-cell non-Hodgkin lymphoma patient, characterized by the presence of simultaneous four serum MC composed of three different Ig heavy chains (α, μ, γ) associated with λ light chain restriction.

P041

SINDROME DA ATTIVAZIONE MACROFAGICA (MAS): DESCRIZIONE DI UN CASO CLINICO

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Introduzione: La MAS è una rara patologia del sistema immunitario, derivante da un disordine delle capacità immunoregolatrici, caratterizzato da febbre, epatosplenomegalia, pancitopenia, ipertrigliceridemia ed iperferritinemia. Distinguiamo: una forma primaria, geneticamente determinata o linfoistiocitosi emofagocitica, che condivide con la forma secondaria aspetti clinici ed anomalie immunologiche, ed una forma secondaria, che può estrinsecarsi in corso di malattie infiammatorie sistemiche, di eventi neoplastici o di infezioni virali, batteriche e fungine. I meccanismi eziopatogenetici sono sconosciuti, ma pare che alla base di questa sindrome ci sia un'alterata regolazione acquisita dei linfociti T e NK, che causano iperattivazione di monociti e altre cellule ad attività fagocitica, inducendo un aumentato rilascio di citochine proinfiammatorie. Tali alterazioni immunologiche e gli incrementati livelli sierici di ferritina causano un'intensa reazione infiammatoria sistemica.
Esame obiettivo: Bambino di 1 mese giunge in Pediatria il 13.06.2018 con ipertermia persistente, difficoltà respiratoria, alterazioni dello stato di coscienza ed addome disteso. Vengono avviati monitoraggio clinico ed indagini laboratoristiche.
Risultati: Dagli esami ematochimici del 13.06: PCT = 0,14 ng/mL (v.n. < 0,05). Gli esami colturali su sangue e urine e la sierologia virologica risultano negativi. In data 15.06: emazie = 3,04 milioni/μL (v.n. 3,9) e Hb = 9,90 g/dL (v.n. 11,5), AST = 325 U/L (v.n. ≤ 34) e ALT = 92 U/L (v.n. ≤ 55), LDH = 835 U/L (v.n. 125-220), PCT = 1, 93 ng/mL. In data 18.06: linfociti = 5,89 mila/μL (v.n. 1,50-4,00) e monociti = 1,35 mila/μL (0,0-0,90), ferritina = 11531,4 ng/mL (v.n. 30-400). In data 21.06: linfociti = 9,42 mila/μL e monociti = 2,51 mila/μL e piastrine = 629 mila/μL (v.n. 150-400), trigliceridi = 631 mg/dL (v.n. ≤ 150). Sospettata la MAS, si avvia terapia corticosteroidea ad alto dosaggio. Dal 25.06 il bambino mostra un miglioramento delle condizioni cliniche parallelamente ad un miglioramento dei parametri laboratoristici.
Conclusioni: La MAS può comportare sepsi severa, shock settico con coinvolgimento multiorgano e morte. Il riconoscimento precoce di questa grave complicanza rappresenta una vera sfida diagnostica per il pediatra.

P042
UN CASO CLINICAMENTE RILEVANTE DI GAMMOPATIA MONOCLONALE IN ETÀ PEDIATRICA DOVUTA AD INTERFERENZA DA FARMACI BIOLOGICI

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Introduzione: L'indicazione diagnostica principale per la richiesta dell'elettroforesi sierica (EP) è la ricerca di possibili componenti monoclonali (CM) in presenza di una condizione clinica che ne giustifichi la sua presenza Pertanto la sua richiesta in età pediátrica trova poche ragioni di appopriatezza e il riscontro di CM rappresenta un evento eccezionale nella pratica di laboratório clinico ad eccezione di alcune malattie linfoproliferative maligne come la leucemia linfoblastica acuta o in un linfoma non–Hodgkin.Nei quadri reumatologici in eta giovanile tuttavia i segni clinici possono essere molteplici all'interno della stessa patologia di conseguenza l'inquadramento nosologico e la diagnosi differenziale sono complessi e richiedono l'utilizzo di esami di laboratorio il cui risultato deve essere interpretato alla luce del sospetto clinico e del quadro sindromico generale.Caso clinico: Paziente di 6 anni con diagnosi pre-natale di tretralgia di FALLOT a cui viene diagnostica all'età di un anno "artrite idiopatica giovanile ad esordio sistemico". La paziente inizialmente viene sottoposta a terapia steroidea ed con methotrexate. In seguito data la persistenza della sintomatologia con successivo interessamento articolare a carico delle caviglie, polsi, gomiti e piccole articolazioni delle mani, è stata intrapresa una terapia con farmaco biologico etanercept (Enbrel) da 10 mg. Successivamente sospesa terapia con Etanercept e avviata terapia con Humira (adalimumab). La bimba viene sottoposta a giugno 2018 ad esami di routine laboratoristici di controllo tra cui l'elettroforesi proteica che evidenzia la presenza in zona gamma di due picchi la cui monoclonalità è confermata dall'immunofissazione sierica, due componenti monoclonali rispettivamente IgG catene leggere Kappa e IgG catene leggere Lambda.Conclusioni:il caso qui presentato, descrive un contesto clinico infrequente, ma non spoaradico in cui l'esecuzione delle EF in età pediatrica non è solo appropriata ma anche raccomandabile soprattutto in questa patologia dove la terapia farmacologica prevede l'utilizzo di farmaco biologici.

P043
UN CASO CLINICO DI STRONGYLOIDES STERCORALIS

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Introduzione: La strongiloidiasi è una parassitosi comunemente presente nelle aree tropicali e subtropicali in zone rurali, sono stati riportati casi anche nel Nord-Europa. I sintomi specifici sono rash cutaneo, dolori addominali, diarrea, asma, tosse e marcata eosinofilia.
Viene descritto un caso di eosinofilia causata da infestazione di un Nematode Intestinale in un paziente arrivato in Pronto Soccorso nel mese di ottobre 2017.
Materiali e metodi: Uomo di 53 anni R.T. proveniente dal Bangladesh giunge in Pronto Soccorso il 16/10/17 con dolori addominali, nausea e vomito. Il paziente vive in una comunità di immigrati da tre mesi in Italia. Vengono eseguite analisi di routine (emocromo, coagulazione, chimica), ECO addome e TAC addome.
Risultati: L'emocromo presentava Leucociti=8.800/ uL, Emazie=4.500.000/uL, Emoglobina=13g/ dL, Neutrofili=53%, Linfociti=8%, Basofili=0.7%, Eosinofili=29%, Proteina C Reattiva=1,2.
ECO addome: milza ingrossata; TAC: zone di addensamento nella milza e nei polmoni. Il paziente viene ricoverato in Broncopneumologia, presenta sindrome asmatica e lesione orticarioide sulla gamba destra. Per la persistenza di dolori epigastrici viene sottoposto a una gastroscopia che rileva un'ulcera gastrica. Viene trattato con antibiotici e cortisonici.
La sintomatologia persiste e si invia in laboratorio un campione di feci per l'esame coproparassitologico. L'esame viene eseguito a fresco dove si evidenziano alla lettura microscopica numerose larve di Strongyloides Stercoralis. Il reparto trasferisce il paziente presso struttura specializzata con trattamento specifico (Albendazolo, Ivermectina non reperibile in Italia).
Conclusioni: La strongiloidiasi è una parassitosi di difficile diagnosi perché spesso l'infezione è asintomatica ed alla quale si deve pensare nei casi di leucocitosi con eosinofilia marcata in pazienti provenienti da aree endemiche. I metodi utilizzati possono essere colturali (Baermann, Harada e Mori) e sierologici. L'esame microscopico resta l'esame più idoneo nel riconoscere le larve del parassita la cui presenza permette la diagnosi certa

P044

UN'EFFICACE ASSOCIAZIONE TRA TECNOLOGIA E TRADIZIONE CON SIGNIFICATIVI BENEFICI CLINICI

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C.F. (6 anni) arriva al Pronto Soccorso del nostro ospedale per emissione di urine color cola ad una settimana da un'otite trattata con Amoxicillina/ Clavulanato. All'esame obiettivo risulta in buone condizioni generali, presenta edema palpebrale bilaterale, contrazione della diuresi e riscontro di valori di pressione sistolica e diastolica superiori al 99°centile per età ed altezza. L'esame del sedimento urinario eseguito con strumentazione Beckman Coulter IQ200 Sprint evidenzia reperti compatibili con nefrite con screzio nefrosico (tappeto di emazie, presenza di cilindri ialini, ialino-granulosi, granulosi e cilindri con inclusioni eritrocitarie, confermati anche all'osservazione microscopica del campione, presenza di emazie dismorfiche); proteinuria > 150 mg/24 ore, negativo l'esame microbiologico delle urine. Gli esami ematici rivelano un modesto incremento di azotemia (70 mg/dL) e dei livelli di potassio (5.3 mEq/ L), ipoalbuminemia (2.9g/dL), un estremo consumo delle frazioni del complemento, specie C3, e un alto valore del TAS (1734 UI/mL; vn ≤ 150 UI/mL). L'esame chimico fisico dell'urina ai successivi controlli dopo l'esordio mostra ancora microematuria in assenza di proteinuria e un sedimento non reattivo. La Glomerulo-Nefrite Acuta post-streptococcica (GNA), una delle più comuni nefriti in età pediatrica, per la cui diagnosi è necessaria la dimostrazione di un'infezione streptococcica precedente, è il classico esempio di una sindrome nefritica caratterizzata da improvvisa insorgenza, di ematuria macroscopica, edema, ipertensione e, nei casi più gravi, insufficienza renale. Esordisce 7-15 giorni dopo una faringo-tonsillite o un'infezione cutanea da ceppi "nefritogeni" di Streptococco ß-emolitico di gruppo A. La diagnosi di GNA richiede l'esame del sedimento urinario che mostra la presenza di ematuria tipo glomerulare e di cilindri polimorfi e una riduzione della frazione circolante C3 del complemento. E' stato importante riuscire a individuare tempestivamente un sedimento reattivo mediante l'analisi automatizzata del campione con la strumentazione in dotazione presso il nostro laboratorio. L'esperienza degli operatori impegnati nella lettura del sedimento automatizzato è un requisito fondamentale per poter garantire una performance ottimale al paziente.

P045

UN CASO DI MALATTIA DA CATENE PESANTI: IL RUOLO DEL LABORATORIO

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Background: Le malattie da catene pesanti (HCD) sono sindromi sistemiche, rare, clinicamente e morfologicamente distinte, tipicamente associate ad una neoplasia a cellule B. La caratteristica principale è la produzione di una catena pesante immunoglobulinica (di tipo γ, α, o μ) non legata alla catena leggera, a formare una immunoglobulina (Ig) intera. Le HCD rappresentano una variante di linfoma e non una vera malattia plasmacellulare. La forma α è la più comune, seguita dalla forma γ (~120 casi) e infine dalla μ. Caso clinico: Una donna di 53 anni, trattata 20 anni prima con trapianto di cellule staminali, chemioterapia (CT) e radioterapia per un LNH, è stata ricoverata per parestesie e astenia agli arti superiori; la pz lamentava da un mese febbricola, sudorazioni notturne, calo ponderale e linfoadenomegalia inguinale bilaterale (in fase di accertamento). Gli esami di laboratorio all'ammissione evidenziavano: GB 3010/mmc, PLT 134.000/mmc, Hb 98 g/L, creatinina 3,87 mg/dL, Ca2+ 7 mg/dL, NT-proBNP >30.000 pg/mL. L'elettroforesi (ETF) delle sieroproteine (eseguita con metodo CZE, Sebia) ha evidenziato un picco in zona β2 globulinica, che all'immunotipizzazione eseguita come reflex test, è risultato essere una componente monoclonale (CM) costituita da sole catene pesanti IgG (22 g/L), non leganti catene leggere. L'Immonofissazione sierica ha confermato la presenza di una banda costituita da sole catene pesanti. A completamento diagnostico sono stati eseguiti: ricerca della proteina di Bence-Jones (positiva per catene leggere λ free, segnalata anche la presenza della CM costituita dalle sole catene pesanti, non migrante in posizione contestuale alla prima), FLC (K 10,7 mg/L, λ 57,10 mg/L, K/λ ratio 0,19), IgG 8,78 g/L, IgA 0,14 g/L, IgM <0,4 g/L, proteinuria (2,66 g/24ore). Dopo l'inizio della CT, l'ETF ha mostrato una riduzione della CM IgG (7 gr/L) e la comparsa di due modeste CM, la 1° di tipo IgG λ (2gr/L) e la 2° IgG K (non quantificabile) dovuta ai farmaci biologici. A causa dell'aggressività della HCD è stato intrapreso un nuovo schema terapeutico con farmaci di seconda linea (Daratumumab) con conseguente "buona risposta parziale". Il confronto continuo Clinica-Laboratorio ha permesso una diagnosi rapida e una migliore gestione terapeutica della paziente.

P046
IFDs AND CONCOMITANT Ps. AERUGINOSA INFECTION IN MYELODYSPLASTIC SYNDROME: A CASE REPORT

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Introduction: Invasive fungal disease (IFDs) are a not neglegible complication in patients hospitalized and with multiple concomitant diseases as haematological disorders. In recent years a number of epidemiological studies have documented rising incidences of these infections, wich parallel increasing complexity and changing demographic characteristics of patients population. Among IFDs, the incidence of Candida infections is still rising. We present a case of IFDs with concomitant Pseudomonas aeruginosa infection in a patient with myelodysplastic syndrome (MDS) and atrial fibrillation.
Methods: A 90 years-old man with history of chronic anemia was admitted to Calcinate Hospital in January 2018, for tachicardya in atrial fibrillation, cachexia, total parenteral nutrition and recent surgery. Hematologic examination and biochemistry demonstrated coagulopathy, hyperbilirubinemia, elevated C-reactive protein and thrombocytopenia. Blood tests revealed a platelet count 33 x 10^9/L and a C reactive protein of 34 mg/L, hematocrit 30%. Hematology was consulted who agreed the most likely cause of the thrombocytopenia and anemia was as a myelodysplatic syndrome (MDS) in elderly patient. An urgent chest X-ray revealed a right middle zone and left lower lobe consolidation, opacity with pleural effusion.
Results: Blood colture were positive for Candida Alb. and Pseudomonas Aeruginosa. Were respected major criteria for diagnosis of invasive candidiasis (IC) as blood colture positive for Candida, Age ≥ 18 years, recent hospitalized in Internal Medicine, haematological disorder. Was started treatment with fluconazolo and gentamicina.
Conclusions: There are many complex variables in the treatment of elderly patients with MDS. Particularly important in the elderly MDS patients is the evaluation of the age-related decline in normal bone marrow function, including diminished capacity for response to stressors such as infection. Several studies were conducted to evalue the interaction of Candida Alb. and Pseudomonas Aeruginosa especially in elderly MDS patients. In our opinion, a multidisciplinary collaboration between haematologist, radiologist and cytologist is essential in order to obtain the diagnosis as soon as possible.

P047
PSEUDOIPERPOTASSIEMIA IN PAZIENTE CON FIBRILLAZIONE ATRIALE, FRATTURA PERTROCANTERICA, TROMBOCITEMIA ESSENZIALE: A CASE REPORT

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Introduzione: La pseudoiperpotassiemia in condizioni cliniche di aumentata conta piastrinica è causata da un aumento in vitro della concentrazione del potassio sierico durante la coagulazione del sangue intero, dovuta alla lisi delle piastrine e di altre componenti cellulari, in presenza di una normale funzione renale e normali livelli plasmatici di potassio. Per la conferma della pseudoiperpotassiemia sono necessarie le determinazioni di potassio sia nel siero che nel plasma per escludere la vera iperpotassiemia.
Caso clinico: Riportiamo il caso di una donna di 84 anni affetta da fibrillazione striale (FA), ipertensione, ipotiroidismo, artrite polidistrettuale, esiti di trombosi venosa profonda (TVP) al polpaccio sinistro, riscontro di lieve piastrinosi all'esame emocromocitometrico in tromboprofilassi con cardioaspirina 100 mg/die. A febbraio 2018 intervento di osteosintesi con vite placca in frattura pertrocanterica sinistra. Giungeva presso la nostra U.O. di Subacuti dell'Ospedale di Calcinate in data 14/02/2018 con esami ematochimici iniziali che mostravano WBC 13.330 x 10^9/L; RBC 3.47 x 10^12/L; Hb 10 g/dL, Hct 32.4%; PLT 649 x 10^9/L;glicemia 79 mg/dL; creatinina 0.94 mg/dL;Na 133 mEq/L; K 5.5 mEq/L; AST 28 U/L;ALT 35 U/L. Un emogasanalisi venosa mostrava Ph 7.42 PCO2 51 mmHg PO2 25 mmHg Na 133 mmoli/L K 4.8 mmoli/L; Hct 35% HCO3- 33.1 mmoli/L, confermando un iperpotassiemia. Il suo livello di potassio sierico era 5.6 mmol/L in presenza di una normale funzione renale (clearance della creatinina 78 ml/min) . Fu posto il sospetto diagnostico di pseudoiperpotassiemia in trombocitemia.
Risultati: Gli esami successivi mostravano WBC 7.860 x 10^9/L; RBC 3.84 x 10^12/L; Hb 11.1 g/dL, Hct 35.7%; PLT 355 x 10^9/L Glicemia 80 mg/dL creatinina 1 mg/dL Na 136 mEq/L K 5.9 mEq/L. Nei tre giorni successivi misurando il livello di potassio sia in una provetta con litio-eparina (campione plasmatico) e nel siero. Fu trovata una netta differenza tra i livelli sierici (in media 6.27 mmol/L) ed i livelli plasmatici di potassio. Per cui fu sospesa terapia con cardioaspirina 100 mg/die ed introdotta terapia con enoxaparina sodica (4000 U.I./die) in associazione a clopidogrel 75 mg/die; successivi esami ematochimici hanno confermato un abbassamento dei livelli di potassio sierico.

P048

SEVERE INTRACEREBRAL HEMORRHAGE, MUSCLES VASTUS PATERAL HEMATOMA AND THROMBOCYTOPENYA IN WARFARIN THERAPY: A CASE REPORT

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Introduzione: L'iperdosaggio o l'interazione di farmaci antibiotici e/o antimicotici durante terapia con warfarin può essere causa di emorragie di vario grado, quali emorragie cerebrali ed ematomi muscolari. Circa la metà di tutti i decessi tuttavia avviene entro 48 ore dalla presentazione. Presentiamo il caso di una donna di 80 anni con cefalea, nausea, vomito con vasto ematoma ventre muscolare del gluteo medio e vasto laterale sinistro.
Caso clinico: Giunge alla nostra osservazione, presso l'U.O. di Subacuti e Cronici di Calcinate (BG), paziente di 80 anni con anamnesi positiva per ipertensione arteriosa, decadimento cognitivo severo da circa 1 anno, fibrillazione atriale in terapia anticoagulante con warfarin (TAO), cardiopatia valvolare in esiti di valvuloplastica meccanica. Intervento chirurgico di sostituzione valvola mitralica circa 10 anni (2008). A novembre 2017 episodio febbrile, disuria e riscontro all'esame urine di leucocitosi ed abbondante flora micotica. La paziente assumeva senza consulto del medico, terapia antibiotica con chinolonici (dosaggio di 500 mg/die) e fluconazolo (dosaggio di 100 mg/die) per circa 7 giorni, con discreto miglioramento della sintomatologia urinaria. Dopo circa 5 giorni dalla fine della terapia la paziente incominciò a manifestare afasia, alterazione dello stato mentale ed ipomobilità all'arto inferiore sinistro. Nelle successive ore per concomitante cefalea, nausea e vomito giungeva in P.S. in urgenza ed eseguiva TC encefalo che evidenziava emorragia in corrispondenza del tronco encefalico, cervelletto, nuclei della base e TC coxo-femorale sinistra che mostrava vasto ematoma rifornito del ventre muscolare del gluteo e del vasto laterale sinistro.
Risultati: Gli esami di laboratorio iniziali davano i seguenti valori: emoglobina 14.2 g/dL, conta dei globuli bianchi 21.640/mm3, piastrine 20.000/mm3, creatinina sierica 1.03 mg/dL, potassiemia 3.2 mmol/L. Un ECG segnalava una fibrillazione atriale ad alta frequenza. La paziente ha assunto in urgenza vitamina K e concentrati protrombinici nel tentativo di ridurre il rischio di sanguinamento attivo e/o comparsa di ulteriori siti emorragici. Inoltre è stata instaurata terapia con digitale, verapamil, complessi multivitaminici contenenti potassio e trasfusione di concentrati piastrinici, per la severa trombocitopenia.
Conclusioni: L'identificazione precoce ed il trattamento dei disordini coagulativi nei pazienti in TAO con multipli siti emorragici spesso è di difficile diagnosi. La vitamina k, plasma fresco congelato, fattore VIIa ricombinante possono essere somministrati nel tentativo di limitare il sanguinamento, specie nei pazienti in terapia con warfarin.

P049

CASE REPORT: MACROAST IN A HEALTHY WOMAN

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Introduction: A serum aspartate aminotransferase (AST) increase may indicate hepatitis, alcoholic liver disease, cirrhosis, cholestasis, acute myocardial infarction or skeletal muscle trauma. Chronic and isolated elevation of AST in patients having no related clinical signs or symptoms is suggestive for the presence of macroaspartate aminotransferase (macroAST). MacroAST is a macroenzyme that circulates in the bloodstream as a high molecular weight complex, either by self-polymerization or by association with serum proteins such as immunoglobulins. The early and correct diagnosis is important to avoid unnecessary, time-consuming, expensive or invasive investigations.The following is a case of macroAST. Materials and methods: A 45 year-old female was admitted to the gastroenterology department of the Careggi Hospital in Florence, because of an isolated increase in AST activity levels. Physical examination revealed a healthy woman with no obvious abnormalities. The first investigation of laboratory, performed on 2017 September 19, showed normal hematological and biochemical parameters. Presence of macroAST was presumed by the gastroenterologist and the clinical laboratory was asked to perform additional tests to confirm macroAST diagnosis. For the detection of macroAST, the polyethylene glycol (PEG) precipitation method was used. AST activities, in the supernatant after PEG precipitation and in serum diluted in Phosphate Buffered Saline (PBS), were measured by Siemens VISTA Clinical Chemistry System. A percentage recovery (%R) of AST activity ≤40% indicates the presence of macroAST. Results: The AST activity in the patient's serum was 149 U/L. After the PEG precipitation, the AST activity was 8 U/L, in PBS it was 143 U/L, so the AST %R was 5.6%. Conclusion: The results confirm the clinical suspicion and are consistent and indicative of the macroAST diagnosis. The role of the laboratory in detection of macro-AST can be very important and laboratory test results should always be assessed together with the patient's clinical history and clinical examination. Clear communication between clinicians and the laboratory can lead to early detection of the macroAST that could avoid unnecessary diagnostic confusion, unnecessary, expensive and even invasive procedures and treatments.

P050
FALSELY ELEVATED THYROGLOBULIN AND CALCITONIN DUE TO HETEROPHILE ANTIBODIES IN NON-RELAPSING THYROID CARCINOMA: WHEN LAB TESTS LIE

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Approximately 40% of serum samples contain non-analyte substances that bind to the assay antibodies. Generally, the heterophilic antibodies (HAb) binds to both the capture and detection antibody, simulating the presence of analyte in its absence and resulting in a false positive result or a falsely increased measurement.
A 46-year-old woman undergone total thyroidectomy was referred to our center for tumor markers measurement. In external laboratories, rhTSH-stimulated Tg assay was negative (stimulated Tg 0.9 ng/ml), whereas increased values of calcitonin (CT) and CEA were detected. At our center, basal Tg, rhTSH-stimulated Tg and TgAb measurement performed on a fully-automated Modular platform (Modular analytic E170, Roche Diagnostic) were negative. We found repeatedly high values of CEA, Ca19-9, Ca125. Unexpectedly, basal and pentagastrin-stimulated CT values performed on Immulite 2000 (Siemens) were undetectable.
Thereafter, thyroglobulin measurement performed on ACCESS (Beckman Coulter) showed a value of 40.5 ng/ml, confirmed by two subsequent tests on the same platform. A neck ultrasound was negative and rhTSH-stimulated Tg assay showed no increase in time. Tg retested on Modular platform (Modular analytic E170, Roche Diagnostic) was 0.75 ng/ml and on Immulite 2000 system 1.01 ng/ml.
The disagreement between thyroglobulin and CT level obtained by different methods, the negative response to stimulation test and the discrepancy between the clinical and the biochemical observation, led us to suspect a laboratory error. We measured serum CT on the Liaison (DIASORIN) platform before and after treating serum samples in a heterophilic blocking tube (HBT) (Scantibodies Laboratory). After incubation in a HBT, a CT value of 4.5 pg/ml was found versus 506 pg/ml, confirming interference by heterophile antibodies. We found that our patient had elevated rheumatoid factor (RF) levels (>500 IU/ml), but there was no clinical picture compatible with rheumatoid arthritis.
HAb interference with tumor markers is particularly important as it may lead to inadequate patient clinical management. Close communication between the clinicians and laboratory staff is vital to recognize and confirm as soon as possible suspected cases of analytical interference.

P051
I LINFOCITI “PARLANO”: UN CASO DI PERTOSSE

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La pertosse è una malattia respiratoria acuta, causata dal batterio gram-negativo Bordetella pertussis. Si registrano 30 milioni di casi/anno; i bambini con meno di 5 anni muoiono nello 0.53% dei casi(1). I sintomi sono simili a quelli di un raffreddore; seguono poi violenti attacchi di tosse. La diagnosi avviene con real time PCR. Il trattamento antibiotico elettivo è costituito da azitromicina/ claritromicina(2). La prevenzione avviene soprattutto con il vaccino, somministrato a partire dall’ottava settimana di vita.

Nel febbraio 2018 arriva, presso il laboratorio di ematologia, l’emocromo di un bambino di sei settimane, proveniente dal pronto soccorso pediatrico. Si registra un aumento dei globuli bianchi (41.31 10^9/L [5.0-16.6]), di cui il 60% è costituito da linfociti, e delle piastrine (704 10^9/L [130-400]). Al microscopio ottico, dopo colorazione con May-Grunwald Giemsa, i linfociti appaiono di piccole-medie dimensioni e con rapporto nucleo/citoplasma >1. La maggior parte ha un nucleo clivato, con una profonda incisura; altri hanno un nucleo convoluto. La morfologia, unita ai dati dell’emocromo, fa pensare a una proliferazione linfocitaria causata da un’infezione.
Consultando la cartella clinica si scopre che al bimbo, portato in ospedale per insufficienza respiratoria e cianosi periorale, è stato effettuato anche un aspirato nasofaringeo per cercare, mediante real time PCR, DNA/ RNA batterici e virali. L’esame rivela la presenza di B.pertussis. Il paziente riceve supporto respiratorio, è trattato con azitromicina ed è somministrata un’infusione glucosalina. Dopo alcuni giorni si assiste a una buona ripresa clinica.

Il caso sottolinea l’importanza di un’attenta e precisa osservazione microscopica. In presenza di linfocitosi periferica, unita al riscontro di linfociti clivati, e di dati clinici opportuni, è possibile pensare a un caso di pertosse. Il laboratorio quindi, descrivendo la morfologia linfocitaria, può aiutare il clinico in una diagnosi rapida, portandolo a definire una corretta e tempestiva terapia antibiotica, ancora prima che siano disponibili i risultati molecolari.

1) del Valle-Mendoza J et al. BMC Res Notes 2018; 11, 318
2) Altunaiji MS et al. Cochrane Database of Systematic Reviews 2007, DOI: 10.1002/14651858.

P052

A CASE OF IgD LAMBDA MULTIPLE MYELOMA AND ACUTE RENAL FAILURE WITHOUT M-SPIKE IN SERUM ELECTROPHORESIS

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Background: IgD multiple myeloma (MM) is a rare subtype of this disorder, accounts for less than 2% of all myeloma. It usually affects a younger population and is characterized by their poor prognosis than other MM isotypes. The distinctive features are the predominant occurrence in males, predominance of λ light chains, frequent renal impairment and uncertain appearance of M-component in serum electrophoresis. A quick correct laboratory diagnosis means establishing appropriate treatment early and a better prognosis for patients. Methods: A 57 years old man with history blood hypertension was admitted to Renal Unit in August 2016 because hyperpyrexia, left-lower limb pain, weight loss and acute renal failure. In October the patient was admitted again to Renal Unit for flare-up of symptoms. The laboratory diagnostic workup was oriented both to the evaluation of renal failure 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: Laboratory investigation showed eGFR 20 mL/min, haemoglobin 9,4 gr/dl; the CZE showed absent M-spike; s-IFE, with standard antisera, showed one monoclonal band λ without corresponding heavy-chain band, a second s-IFE using antisera to IgD and IgE showed a monoclonal IgD no corresponding λ band. The immunoselection technique was used to establish the presence of IgD λ and λ free band. The FLCλ 2348 mg/L, FLCk 92,3 mg/L, ratio k/λ 0,04.; u-IFE yielded a Bence Jones proteinuria λ. Bone marrow biopsy showed plasmacellular infiltration > 20%; kidney biopsy confirmed diagnosis “light chain cast nephropathy”. Conclusions: The present case demonstrates that IgD MM may be associated with an extensive production of FLC which may be responsible for renal failure and may thus misdiagnosed as a LCMM. Knowledge of its typical laboratory feature is crucial to establish the correct diagnosis. It is important to perform s-IFE using antisera to IgD in case monoclonal light chain bands without corresponding heavy-chain bands at the first diagnosis.

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P053

AN UNUSUAL IgE/LAMBDA-SECRETING MULTIPLE MYELOMA

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Background: IgE-secreting multiple myeloma is a rare disease characterized by a high frequency of Bence-Jones proteinuria and plasma cell leukaemia when compared to other isotypes of monoclonal proteins. Less than 50 cases have been reported in literature, but the real number could be higher since monoclonal anti-D and anti-E antibodies are not usually employed in the immunofixation, when an exclusive band related to the free light chains lambda or kappa is present.
Patient and Methods: a 79-year-old man was hospitalized for urosepsis. Radiological images showed a spread osteolysis and an important femoral metaphysic osteolysis that caused the detachment of the small trochanter. Other biochemical findings evidenced a mild microcytic anemia (Hb 9.10 g/dL, MCV 67.2), a normal renal function and no hypercalcemia. Serum was tested for capillarys electrophoresis (CZE SEBIA) and immunofixation (IF) (Hydragel IF 2/4 and Hydragel 2IF/BJ HR, SEBIA).
Results: serum electrophoresis showed a monoclonal component (10 %) in the gamma zone that needed to be confirmed by IF in serum and urine. Serum IF was initially performed with most frequently employed antisera (IgG, IgA, IgM, kappa and lambda). In the agarose gel, a clear band was detected with the lambda light chain not corresponding to any band detected with heavy chain antisera. So it was necessary to procede with a subsequent immunofixation including other antisera (IgD, IgE, lambda and free lambda) in order to have a more complete typing of the monoclonal component. The final characterization was consistent with a monoclonal gammopathy IgE/lambda. Urine IF revealed a Bence Jones positive for lambda free.
Immunohistochemistry of bone-marrow biopsy confirmed the occurrence of Multiple Myeloma IgE/lambda (lambda monoclonal infiltrate 60%).
Conclusion: the diagnosis of rare multiple myelomas, like IgE/lambda gammopathy, needs a particular and deep approach. The presence of a monoclonal band in the light chain lane, without correspondence in the most common heavy chains (IgG, IgA and IgM) lanes, needs to be investigated for the less common IgD and IgE chains. This more accurate diagnostic investigation allows to obtain a correct diagnosis excluding a misdiagnosis of a microsecretory myeloma.

P054
L'IMPORTANZA DELLO SCREENING DEI DIFETTI EMOGLOBINICI

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Perviene al nostro laboratorio la richiesta per elettroforesi dell'emoglobina di un bambino di 7 mesi. Gli esami ematici evidenziano un quadro di anemia microcitica ipocromica con presenza di eritroblasti ortocromatici (Hb 8.2 g/dl, MCV 67.0 fL, MCH 20.7 pg, reticolociti 0.309 10^6/ul). L'elettroforesi dell'emoglobina con tecnologia capillare (Capyllaris 2 Flex Piercing Sebia) mostra un'alterazione delle frazioni emoglobiniche (A 6.5%, A_2 1.7%, F 91.8% assenza di varianti emoglobiniche). Pertanto il paziente ha eseguito consulenza con lo specialista e approfondimento con tecniche di biologia molecolare. Il sequenziamento diretto su DNA estratto da leucociti del sangue periferico ha evidenziato le seguenti mutazioni sul gene della β globina localizzato sul cromosoma 11: HBB:93-21G>A (IVS-I-110) allo stato eterozigote e HBB:c.118C>T p.(Glu40*) (codon 39) allo stato eterozigote. Il paziente viene quindi inquadrato come un soggetto eterozigote composto per β-talassemia. La β-talassemia major è la forma più severa di β-talassemia causata da totale mancanza di produzione di β-globina dovuta alla presenza di due alleli βo o ad un'eterozigosi composta (βo /β+) con conseguente eccesso di catene α-globiniche libere che formano precipitati nei precursori eritrocitari, causando una loro anomala maturazione e una prematura distruzione nel midollo osseo (eritropoiesi inefficace). Clinicamente la malattia si manifesta nei primi mesi di vita con anemia severa, scarso accrescimento, epatosplenomegalia, irritabilità ed episodi infettivi ricorrenti. La diagnosi precoce è pertanto fondamentale per grantire la sopravvivenza del paziente ed avviarlo ad un regolare regime emotrasfusionale; pazienti selezionati possono essere candidati ad una cura definitiva attualmente rappresentata dal trapianto di cellule staminali ematopoietiche e dalla terapia genica. L'incidenza annuale dei casi sintomatici si è ridotta moltissimo grazie allo screening delle donne portatrici in gravidanza. Si sottolinea l'importanza del ruolo principe dell'elettroforesi dell'emoglobina e degli esami di 1° livello nella prevenzione e nello screening dei difetti dell'emoglobina. Le Talassemie, in particolare le beta talassemie, costituiscono l'impegno diagnostico prevalente per il laboratorio di 1° livello.

P055
LA VALUTAZIONE DELL'Hba2 NEL PROGRAMMA DI PREVENZIONE DEI DIFETTI TALASSEMICI: LA SCOPERTA DELL'Hba2-SILE

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L'emoglobina A2 (HbA2) è una componente dell' Hb, costituita da catene α2-δ2, che in condizioni fisiologiche nell' adulto costituisce il 2-3% dell' Hb totale. L'HbA2 ha scarso rilievo fisiologico, ma è il marcatore fondamentale per la diagnosi del trait β-talassemico, condizione che comporta un aumento di HbA2 compreso tra 4-6%.
I fenotipi derivanti da diverse condizioni possono modificare la concentrazione dell'HbA2 e rendere difficile la diagnosi. La qualità analitica della misura dell' HbA2 deve quindi rispondere a esigenze cliniche di una corretta prevenzione.
Riportiamo un caso di scostamento minimo dalla norma dell'HbA2 non raro nella pratica quotidiana: a ottobre 2017 è giunta alla nostra osservazione una donna albanese gravida, il cui assetto emoglobinico eseguito con il metodo in uso in routine (HPLC, Bio-Rad, Beta-Thal DualKit) ha mostrato una minima diminuzione rispetto alla norma. Sono stati quindi eseguiti: emocromo, sideremia, ferritina. L' assetto emoglobinico è stato ripetuto con 2 metodi alternativi: HPLC (Trinity Biotech Premier Hb9210 Resolution) ed elettroforesi capillare (Capillarys 2 Flex Piercing Sebia). Infine è stato richiesto l'esame molecolare per la caratterizzazione dell' eventuale variante δ e la ricerca di trait talassemici.
I cromatogrammi ottenuti indicavano valori di HbA2 variabilmente diminuiti e quindi la presenza di un difetto dei geni δ globinici. L'analisi molecolare ha confermato l'ipotesi indicando solo la presenza, allo stato eterozigote, di una nuova variante delle catene δ globiniche (HBD:c.11T>C). Tale nuova variante è stata denominata Hb A2-Sile (delta3(NA3) Leu>Pro).
L'esame dell' assetto Hb è fondamentale per valutare i difetti quali-quantitativi dell' Hb. I metodi a disposizione possono essere basati su principi metodologici differenti, ma tali da fornire informazioni che si integrino.
Le raccomandazioni per gli esami di 1°livello dell'Hb della Società Italiana Talassemie ed Emoglobinopatie (SITE) suggeriscono di confermare i risultati con almeno due diversi metodi. Il caso riportato, pur non clinicamente rilevante, contribuisce alla conoscenza della variabilità dei geni globinici; ma l' eventuale diminuzione dell'HbA2 deve essere sempre valutata, per evitare diagnosi errate nella prevenzione delle talassemie.

P056

MISURA DELLE FREE LIGHT CHAINS PLASMATICHE NELLA VALUTAZIONE DELLA RISPOSTA AL TRATTAMENTO CON ANTICORPO MONOCLONALE ELOTUZUMAB IN SOGGETTO CON MIELOMA MULTIPLO A CATENE LEGGERE

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Background: Il mieloma multiplo a catene leggere (LCMM) è una neoplasia ematologica caratterizzata dalla proliferazione clonale di plasmacellule a livello del midollo osseo che producono catene leggere libere tipo k o λ. Negli ultimi anni il management di questa malattia è stato modificato dall'introduzione del test di laboratorio Free Light Chains plasmatiche (sFLC) e dall'utilizzo di nuove strategie terapeutiche come l'anticorpo monoclonale Elotuzumab. Evidenze scientifiche mostrano che l'immunoterapia è responsabile di interferenza sui test elettroforesi (sCZE) ed immunofissazione sierica (sIFE) che potrebbe creare problemi nella valutazione della risposta clinica. Metodi: Soggetto di sesso femminile di 72aa, si ricovera presso l'U.O. di Ematologia nel marzo 2016 per anemia e insufficienza renale. Il workup diagnostico è stato orientato alla valutazione del danno renale e alla ricerca della componente monoclonale (CM), con CZE, s-IFE, u-IFE (Sebia) e sFLC (The Binding Site). Risultati: La CZE presentava una CM in zona gamma non quantificabile; la s-IFE e la u-IFE presentavano entrambe catene leggere libere monoclonali kappa, FLCk erano 6812 mg/L, FLCλ 6,8 mg/L, ratio k/λ 990. Gli esami ematochimici: eGFR 34mL/min, Proteinuria Bence Jones 5400 mg/24h, Hb: 9,5 g/dL. La biopsia osteomidollare mostrava un infiltrato plasmacellulare del 60% con clonalità kappa: la diagnosi era LCMM kappa. Essendoci stata "Minimal Responce" alla terapia di prima linea (Melfalan, Prednisone e Bortezomib) secondo i criteri dell'International Myeloma Working Group (IMWG), si intraprese trattamento con: Elotuzumab, Lenalidomide e Desametasone. Il follow-up evidenziò una CM IgG kappa sia alla CZE che alla s-IFE Hight Resolution impedendo una corretta valutazione della risposta clinica. Conclusioni: Il test sFLC non sembra essere influenzato dalle terapie con anticorpo monoclonale; nel caso specifico, ha permesso la valutazione di "Partial Responce". La misura delle sFLC sembra essere anche un indicatore più sensibile di malattia rispetto alla u-CZE e u-IFE, in quanto non risente della fase preanalitica.

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P057

UNSTABLE HEMOGLOBIN RUSH [BETA 101(G3) GLU>GLN, HBB:C.304G>C]: THE FIRST EUROPEAN FAMILY WITH UNRECOGNIZED MODERATE HEMOLYTIC ANEMIA

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Hemoglobin Rush is an unstable variant generated by a mutation of the β-globin gene which causes amino acid replacement Glu>Gln in the core of the hemoglobin molecule. We describe the case of a two Caucasian brothers, 72-year and 64 years old, who were referred during different period to our laboratory for assessment of glycated hemoglobin with high-performance liquid chromatography (HPLC) (G8, Tosoh Bioscience, Rivoli, Italy). During the analysis, the hemoglobin profile revealed the presence of a hemoglobin variant. Subsequent hemoglobin analysis using cation-exchange HPLC system (HLC-G8), confirmed the presence of a hemoglobin variant, with a retention time partially overlapping with that of HbD. Hemoglobin electrophoresis was then performed on agarose gel at both alkaline and acid pH ,using Hydrasis semi-automated agarose gel electrophoresis system (Sebia, Norcross, GA, USA). The hemoglobin variant was then identified as being Hb Rush by molecular analysis. The patients underwent further laboratory investigations, showing reticulocytosis ($201x10^9$/L and $203 x10^9$/L), mild hypochromic normocytic anaemia (hemoglobin 89 g/L and 108 g/L, mean corpuscular volume 99 fL and 86 fL, mean corpuscular hemoglobin 31pg/L and 27 pg/L, mean corpuscular hemoglobin concentration 311 g/L and 317 g/L), hyperferritinemia (1100 mg/L and 650 mg/L) (Blood counts were performed by Advia 2120; Siemens Healthcare Diagnostics, Tarrytown, NY, USA). Low levels of haptoglobin were also observed in one brother (0.17 g/L). HPLC and complete blood count were performed in all family members (2 sisters, 1 brothers and two daughters), but no other subjects were found to carry the hemoglobin variant. To our knowledge, this is the first description of family carriers of Hb Rush in Europe, which was first reported in 1974 in a 43 years old black woman in north America. The second case was described in Brasil, from a descendent Italian family. The description of these cases is useful since it emphasizes the importance of an accurate diagnostic process and collecting medical records of proband and family. The diagnosis of unstable hemoglobin variants is necessary since these conditions may be associated with hemolytic anemia of mild to moderate degree, especially in older patients.

P058

ANOMALIE DELLA DISTRIBUZIONE DEI CLUSTER CELLULARI NEL CITOGRAMMA DEL CONTEGGIO DIFFERENZIALE LEUCOCITARIO DI XN INDICATIVE DELLA PRESENZA DI PROERITROBLASTI

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L'eritroleucemia è una particolare forma di leucemia acuta con proliferazione neoplastica di cellule immature della linea eritroide e mieloide; può presentarsi de novo oppure evolvere da un quadro di mielodisplasia (MDS) o, meno comunemente, da neoplasie mieloproliferative croniche. Si descrivono i casi di due pazienti, il primo con mielofibrosi e il secondo con MDS. In entrambi i casi la formula leucocitaria eseguita in automazione con il contaglobuli XN (Sysmex) mostra alterazioni simili nel rispettivo citogramma (WDF-scattergram) e presenza di blasti con la morfologia tipica dei proeritroblasti. Il primo è il caso di un paziente di 69 anni affetto da policitemia vera dal febbraio 2010 ed evoluta in mielofibrosi. L'analisi emocromocitometrica nel WDF-scattergram mostrava una popolazione cellulare anomala caratterizzata da elevata intensità di fluorescenza e complessità rispetto alle normali popolazioni cellulari presenti. La revisione microscopica ha evidenziato la presenza di blasti di grandi dimensioni con citoplasma iperbasofilo suggerendo la presenza di proeritroblasti.
Il secondo è il caso di un paziente di anni 72 affetto da MDS ricoverato in chirurgia. L'emocromo di controllo effettuato a maggio 2018 mostrava, nel canale WDF, cluster di cellule anomale ad alta fluorescenza ed elevata complessità tali da avere lo stesso codice colore dei granulociti immaturi.
La revisione microscopica ha confermato la presenza di neutrofili displastici, mielociti e metamielociti con segni di displasia e di blasti di grandi dimensioni, citoplasma iperbasofilo vacuolato, nucleo regolare leggermente eccentrico con i margini conservati ed evidente nucleolo, morfologicamente riconducibili a proeritroblasti. L'analisi citofluorimetrica effettuata su entrambi i pazienti ha confermato la presenza di cellule positive per la glicoforina con una bassa positività al CD71, pattern tipico dei proeritroblasti.
Ancora una volta l'attenta osservazione ed interpretazione delle informazioni qualitative (citogrammi e allarmi morfologici) possono aumentare l'appropriatezza e l'accuratezza della revisione microscopica garantendo un rapido e appropriato percorso di approfondimento diagnostico di II livello.

P059

EFFETTO DELLA VARIANTE EMOGLOBINICA DI MOZHAISK SUL CONTEGGIO LEUCOCITARIO DIFFERENZIALE DELL'ANALIZZATORE EMATOLOGICO XN SYSMEX

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Nel database HbVar, le varianti riferibili al gene della globina sono più di 1900, di cui circa il 47% presenti sul gene della β-globina. Si descrive il caso di un paziente pediatrico (anni 3) di etnia mista, primogenito di genitori non consanguinei nato da una gravidanza dopo trattamento di fecondazione in vitro. Il bambino giungeva in pronto soccorso con febbre ed astenia. Gli accertamenti effettuati all'accesso mostravano un'alterazione degli indici di emolisi (BilirubinaTot-Diretta 3.6-0.9 mg/dl, LDH 3479 U/L, Aptoglobina <5 mg/dL, PCR 14.5 mg/dL) e dell'esame emocromocitometrico (RBC $2.38*10^{12}$ /L, Hb 62 g/L, HCT 0.220 L/L, MCV 92,4 fL, MCH 26.1 pg, MCHC 282 g/L, RDW 26.7%, WBC $12.77*10^9$/L, PLT $129*10^9$/L, NRBC $5.34*10^9$/L, RET $430*10^9$L). Il citogramma del canale WDF (White Cell Differential) ottenuto con XN module (Sysmex) mostrava una distribuzione anomala delle popolazioni leucocitarie segnalate dagli allarmi morfologici WBC abnormal scattergam e Blast che hanno evidenziato l'impossibilità di identificare correttamente le popolazioni presenti.
Alla revisione microscopica non è emersa nessuna alterazione a carico delle popolazioni leucocitarie ma solamente anomalie morfologiche delle emazie quali spiccata anisopoichilocitosi, punteggiatura basofila e frammenti eritrocitari oltre alla presenza di eritroblasti.
I dati suggerivano una severa anemia emolitica. Nel percorso diagnostico di inquadramento dell'anemia è stata effettuata una valutazione del profilo emoglobinico tramite HPLC (Bioscience) che ha mostrato la presenza di una variante emoglobinica instabile. Il successivo sequenziamento del gene β-globinico ha rivelato la mutazione HBB: c.278A>G nel codone 92 (emoglobina di Mozhaisk) in eterozigosi. La ricerca di tale mutazione nei genitori e nel fratello ha dato esito negativo, suggerendo quindi la presenza di una rara variante de novo nel paziente in esame.
In fase di monitoraggio l'esame emocromocitometrico mostrava sempre le stesse peculiari anomalie del citogramma WDF anche a distanza di un anno.
Interferenze simili sul canale WDF sono state già descritte per campioni con emoglobina di Leiden.
Tale evidenza, se confermata su una più ampia casistica, può essere utile nello screening delle emoglobinopatie da HB instabile.

P060

CONFRONTO DEI VALORI DI INR IN PAZIENTI IN TERAPIA ANTICOAGULANTE ORALE E NON TRATTATI CON L'IMPIEGO DI DUE DIFFERENTI TROMBOPLASTINE SU SISTEMI STA-R

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La misura del Tempo di Protrombina (PT) consente di valutare se l'insieme dei fattori (I, II, V, VII e X), coinvolti nelle vie estrinseca e comune della cascata coagulativa, funzioni adeguatamente e il coagulo si formi in un tempo adeguato. Poiché il PT è strettamente dipendente dai reagenti utilizzati, il confronto diretto dei risultati ottenuti in differenti laboratori può essere fatto attraverso l'International Normalized Ratio (INR). L'INR è il risultato di un calcolo che normalizza il valore del PT con l'uso dell'International Sensitivity Index (ISI), un indice che quantifica la relazione tra le caratteristiche della tromboplastina presente nei reagenti in con quelle di una preparazione di riferimento. In questo studio sono stati confrontati i valori di INR ottenuti, sullo stesso analizzatore (STA-R, Stago France), con due differenti preparazioni di tromboplastina: STA-Neoplastine CI plus e STANeoPTimal, entrambe ottenute per estrazione da cervello di coniglio ma con valore differente (1,26 la prima e 1,01 la seconda). La preparazione STA-Neoplastine CI è attualmente impiegata nel nostro laboratorio per le determinazioni di routine e verrà sostituita con STANeoPTimal, per questo motivo sono stati confrontati i valori ottenuti per l'INR, sugli stessi campioni, usando entrambi i reagenti e con particolare attenzione sulle differenti modifiche della terapia anticoagulante orale eventualmente indotte dall'interpretazione degli stessi. Sono stati analizzati 160 campioni di pazienti, dei quali 106 in corso di terapia anticoagulante orale per differenti condizioni cliniche e 54 non in terapia. Il confronto dei valori di INR ottenuti per tutti i campioni, considerando la STA-Neoplastine CI come riferimento, ha mostrato la seguente retta di regressione secondo Passing e Bablok y= 1,2x – 0,2 con intervalli di confidenza al 95%: per la pendenza da 1,18 a 1,21 e per l'intercetta da -0,14 a 0,14; il coefficiente di correlazione è risultato r = 0,996 (IC 95%: da 0,994 a 0,997) con P < 0,0001. Se si analizza il grafico di Bland e Altmann si evidenzia che le differenze tra i due metodi sono maggiori in proporzione al valore di INR. Infatti se si analizzano separatamente le correlazioni tra i due gruppi di pazienti si ottengono le seguenti rette di regressione: y = 1,0X – 0,01 (IC 95%: da 0,84 a 1,11 pendenza e da -0,11 a 0,16 intercetta) per i soggetti non in terapia e y = 1,19X – 0,19 (IC 95%: da 1,17 a 1,22 pendenza e da -0,26 a -0,13 intercetta) per quelli in TAO. L'interpretazione clinica dei valori di INR, ottenuti con le due differenti tromboplastine, non induce modifiche all'eventuale trattamento in corso anche nel caso dei pazienti in cui la differenza di valore di INR risulta superiore al 15%.

P061

ANTICORPI ANTI-FOSFATIDILSERINA/ PROTROMBINA: UN POSSIBILE MARKER DI SINDROME DA ANTICORPI ANTIFOSFOLIPIDI

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La Sindrome da anticorpi antifosfolipidi (APS) è un disordine autoimmune acquisito di origine ignota, caratterizzato da trombosi e/o poliabortività, associate alla presenza persistente nel sangue degli anticorpi antifosfolipidi (aPL), un'ampia famiglia di immunoglobuline di tipo IgG, IgM o, meno frequentemente IgA, diretti contro proteine plasmatiche con affinità per le superfici a carica negativa. Può essere primitiva o secondaria ad una malattia autoimmune sistemica, prevalentemente il Lupus Eritematoso Sistemico, e la diagnosi prevede la contemporanea presenza di almeno un criterio clinico insieme a una positività per almeno uno degli aPL indicati dalle linee guida: IgG/IgM anticardiolipina (aCL), IgG/IgM anti-Beta2glicoproteina I (aβ2GPI), lupus anticoagulant (LAC). Tuttavia alcuni pazienti pur manifestando le caratteristiche cliniche della APS risultano negativi per tali anticorpi (APS sieronegativa). Recenti studi sulla correlazione fra manifestazioni cliniche di APS e aPL non presenti fra i criteri classificativi, definiti "non criterio", hanno evidenziato una notevole rilevanza degli anticorpi diretti contro il complesso fosfatidilserina/protrombina (aPS/PT), sia come marker diagnostico in pazienti sieronegativi, che per identificare i casi di APS sieropositivi a rischio di complicanze severe.

Abbiamo analizzato la presenza di aPS/PT IgG e IgM (DSXSystem,Technogenetics) in 62 pazienti con clinica suggestiva per APS, ma negativi per ACA IgG/IgM, aβ2GPI IgG/IgM (Bioflash,Werfen) e LAC (ACLTOP500,Werfen).

Sono risultati positivi per aPS/PT 18 casi su 62, di cui 10 con poliabortività, 4 con trombosi arteriosa e 4 con trombosi venosa profonda. Abbiamo osservato una prevalenza di aPS/PT IgG più elevata nei pazienti con trombosi e di aPS/PT IgM nelle pazienti con poliabortività, in accordo con i dati della letteratura.

La APS è di difficile riconoscimento e sotto-diagnosticata e può avere conseguenze devastanti se non trattata, tra cui gravi eventi trombotici, severa morbidità materna e perinatale, fino all'exitus.

Nella nostra casistica, pur trattandosi di uno studio preliminare, si rileva un'alta percentuale di positività per aPS/PT, la cui ricerca dovrebbe essere quindi considerata nei casi sieronegativi in cui sussiste un forte sospetto per APS.

P062
COST COMPARISON: STRATEGIES OF PATIENTS MANAGEMENT IN ORAL ANTICOAGULANT THERAPY

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Objectives: Vitamin K antagonists (VKA) Oral Anticoagulant Therapy (OAT) is a highly effective therapy for primary and secondary prevention of thromboembolic disorders and vascular disease in general. OAT requires continuous clinical and laboratory monitoring in order to optimize the treatment and achieve the best risk/benefit ratio. Point-of-care devices (POC) can safely and easily monitor VKA efficacy through International Normalized Ratio (INR) detection by portable coagulometers. The aim of this analysis was to compare the “centralized” with the “decentralized” management model.
Methods: This analysis was conducted in the Regional Healthcare System (RHS) perspective, on the cohort of patients who are taken in charge by the General Practitioners (GP) “COOP Medici 2000” in Siena, Tuscany Italy. We evaluated aggregated economic and epidemiological data related to the management of OAT patients and direct costs related to the monitoring and follow up. Data were collected through an aggregated form with questionnaires directed to General Practitioners (GPs), focusing on
patients managed in 2016.
Results: Data were collected by a pool of 753 patients in treatment with Oral Anticoagulants taken in charge by GPs Cooperative in the whole 2016. The implementation of decentralized management model in patient management in OAT generated a reduction in the expenditure charged on Regional Healthcare System. The decentralized management model was a cost-saving choice (€ 289.246).
Conclusions: These results represent the first comparative analysis of the decentralized organizational model implemented by MMG “COOP Medici 2000” in Siena and may represent a more efficient patient management strategy.

P063
INDICE DI EMOLISI SU STA R MAX2: APPLICAZIONE PRATICA SU D-DIMERO

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Introduzione: il coagulometro STA R Max2 presenta il modulo esperto EPC (Expert Pre-Check Module) che consente di evidenziare la presenza di possibili sostanze interferenti nel campione (emolisi, torbidità e ittero). Per ciascun test è possibile impostare delle soglie in modo da poter informare l’operatore della presenza di una sostanza interferente nel campione o anche di bloccare direttamente l’esecuzione del test. Scopo del nostro studio è stato quello di verificare l’interferenza dell’emolisi provocata sul dosaggio del D-Dimero (LIATEST D-DI Plus). Materiali e metodi: il modulo EPC legge la concentrazione di emoglobina del campione tramite 2 diverse lunghezze d’onda 415 e 582 nm e distingue poi 6 diverse classi di emolisi. La metodica del D-Dimero in uso nel nostro laboratorio riporta una interferenza per emoglobine superiori a 2 g/L, equivalente ad un indice di emolisi pari a 4 su STA R MAX2. Su 15 campioni della routine distribuiti in ampio range di concentrazione (219-18408 ug/L FEU) è stata indotta manualmente l’emolisi in modo crescente grazie ad una multipla aspirazione con siringa. I campioni dopo ogni trattamento meccanico venivano centrifugati come da routine e veniva dosato il D-Dimero e verificato l’indice di emolisi. Accanto ai campioni trattati vi era un campione di controllo non emolizzato. Il procedimento è stato ripetuto più volte per raggiungere emolisi 2, 3 e 4. Risultati: il test di Wilcoxon per dati appaiati non ha mostrato differenze statisticamente significative tra i campioni privi di emolisi e con emolisi di grado 3 ($p=0,12$) mentre la differenza è statisticamente significativa quando i campioni presentavano una emolisi di grado 4 ($p<0,01$). Con l’analisi di Bland Altman si nota che il bias è pari a -17,9% ma si tratta di un bias inversamente proporzionale: i campioni al di sotto degli 800 ug/L FEU presentano una differenza statisticamente significativa prima e dopo emolisi, mentre invece non vi sono differenza significative inducendo l’emolisi nei campioni a concentrazione maggiore di D-Dimero. Conclusioni: il modulo EPC presente su STA R MAX2 consente di allarmare o bloccare l’esecuzione di un test in base a specifica soglia di emolisi. Abbiamo quindi implementato la regola che dispone di bloccare il test del D-Dimero nei campioni con emolisi pari o superiore a 4 . Infatti anche se per campioni con concentrazioni di D-Dimero elevate l’interferenza legata ad emolisi 4 non è significativa, tuttavia la valutazione va fatta in sede di pre-analisi e quindi sembra più utile nell’organizzazione di laboratorio adottare una procedura più standardizzata.

P064

UTILIZZO DI ADP/TRAP E ASPI/TRAP RATIO PER VALUTARE L'ATTIVITÀ PIASTRINICA RESIDUA (ARP) SU MULTIPLATE: ANALISI DOPO 16 MESI DI ESPERIENZA

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Premessa Il Multiplate è un aggregometro che, mediante gli agonisti ADP (ADPtest) e Acido Arachidonico (ASPItest), quantifica, l'attività piastrinica residua (APR) in corso di terapia con Clopidogrel (CLOP) e/o ASA. Il TRAPtest (agonista TRAP-6) non risente delle terapie e riflette la reattività piastrinica basale; pertanto le ratio ADP/TRAP e ASPI/TRAP "normalizzano" i risultati. Nel 2016 abbiamo stabilito i range di riferimento delle ratio in volontari sani e calcolato i cut off che distinguono i soggetti rispondenti (R) alla terapia dai rispondenti in modo incompleto (IncR) e non rispondenti (NoR). Da gennaio 2017 i test ARP sono refertati in base alle ratio ADP/TRAP e ASPI/TRAP invece che ai cut off di ADPtest (46 U) e ASPI test (40 U) proposti dal produttore. Scopo del lavoro: Valutare le conseguenze della interpretazione con ADP/TRAP e ASPI/TRAP ratio dei test di ARP. Materiali e metodi: ADPtest, ASPItest e TRAPtest su Multiplate; 1055 pazienti CLOP, 860 ante 2017 e 195 dal 2017; 2048 pazienti ASA, 1530 ante 2017 e 518 dal 2017. Pazienti CLOP: ante 2017 R se ADPtest <=46 U e non R se ADP>=57 U (modo A); da 2017 R se ADP/TRAP <=0.4 e NoR se ADP/TRAP >=0.5 (modo B). IncR intermedi. Pazienti ASA: ante 2017 R se ASPItest <=40 U e NoR se ASPI >=71 (modo A) ; da 2017 R se ASPI/TRAP <=0.4 e NoR se ASPItest>=0,7 (modo B). IncR intermedi. Risultati La differenza di R a CLOP, 70% ante 2017 e 65% dal 2017, non raggiunge la significatività statistica (p 0.20); la % di R ad ASA è identica (90%). L'analisi della casistica post 2017 (R, IncR e NoR) mostra che per ASA la diversa refertazione non comporta modifiche interpretative maggiori: nessun R con modo "B" sarebbe NoR con modo "A" o viceversa. Per CLOP, (195 casi) un R con modo "B" (ratio <4,0) sarebbe NoR con modo "A" (ADP>46U) e 6 NoR con modo "B" (ratio >0,5) sarebbero R con modo "A" (ADP<46U). Per entrambe le terapie aumenta la % di IncR. Conclusioni La valutazione della ARP secondo "ratio cut off", tenendo conto della reattività piastrinica basale espressa dal TRAPtest, elimina il bias implicito della diversa reattività individuale e determina modifiche interpretative maggiori in alcuni pazienti in terapia con CLOP (3,5% dei casi). Bibliografia doi.org/10.1016/j.thromres.2016.12.004.

P065

VALUTAZIONE DELLE PERFORMANCE DELL'ANALIZZATORE DI COAGULAZIONE BIOLABO SOLEA 100 E CONFRONTO CON STAGO STA-R

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Introduzione: Biolabo Solea 100 è un analizzatore di coagulazione che utilizza un sistema ottico per la rilevazione della formazione del coagulo. L'analizzatore può eseguire test cronometrici, cromogenici ed immunologici in quanto le misurazioni vengono eseguite a due lunghezze d'onda (405 nm e 620 nm). Possiede 8 canali di lettura ed una velocità di analisi del pannello PT, APTT, TT, FIB di 100 test/ora ed è destinato a laboratori di medie e piccole dimensioni.

Scopo: lo scopo di questo lavoro è stato quello di valutare le performance per la determinazione dei parametri di coagulazione eseguiti di routine (PT, APTT e FIB), dell'analizzatore di coagulazione Biolabo Solea 100 e dei relativi reagenti.

Protocollo di valutazione: L'imprecisione e l'accuratezza sono state valutate in accordo con le linee guida CLSI EP05-A2 utilizzando plasmi di controllo su tre livelli decisionali (normale, medio ed elevato) per ognuno dei parametri di coagulazione.

Il processo di comparazione tra strumenti, in accordo con il CLSI EP09-A3, è stato eseguito comparando i risultati di campioni clinici (103 per PT, 184 per APTT e 174 per FIB), nel più ampio intervallo possibile, con quelli ottenuti dall'analizzatore meccanico STAGO STA-R. I risultati sono stati analizzati con il metodo grafico di Bland e Altman e scatter plot, con successiva regressione lineare perpendicolare (metodo di Passing e Bablok). Inoltre i valori di riferimento per ognuno dei due analizzatori, sono stati determinati in accordo con il CLSI C28-A3. Le interferenze, in accordo con il CLSI EP07-A2, sono state valutate per la presenza di trigliceridi, bilirubina, emoglobina ed eparina.

Risultati: Ripetibilità, riproducibilità, accuratezza ed errore totale determinati sono tutti nei limiti di accettabilità. Di particolare rilievo è l'elevato livello di concordanza tra Solea 100 e STA-R per quanto riguarda il PT (espresso in secondi, % ed INR) i cui valori correlano perfettamente (y=0+1.00x). Non si osservano interferenze negli intervalli suggeriti dal CLSI EP07-A2.

Conclusioni: In conclusione le performance dell'analizzatore ottico Solea 100 sono più che adeguate per la determinazione dei parametri routinari di coagulazione e perfettamente comparabili con sistemi meccanici come STA-R.

P066

EGINA: AN INNOVATIVE EXCELLENCE MODEL FOR THE MANAGEMENT OF ANTICOAGULATION WITH DOACs

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Background: In Italy the oral anticoagulant therapy has been managed in patients with atrial fibrillation and venous thromboembolism by a network of Anticoagulation Centers (ACs). From 2013 onwards a new approach to anticoagulation has been made available with the introduction of DOACs and the resulting transformation of ACS Centers into Thrombosis Centers (TC). These centers may be crucial in selecting both the most appropriate diagnostic approach and the best therapeutic option. Methods: We have created a new model named EGINA (Excellence model for the Integrated Management of New Anticoagulants) to be used by TC for the management of patients treated with DOACs. This model includes an innovative follow-up system which is able to achieve the ISO9001 certification, necessary for the accreditation to the network, attested by the "Bureau Veritas" company. This agency is involved in the evaluation of TC through different elements, such as the quality of "clinical audit", clinical indicators and the adherence to international guidelines and countable parameters like the amount of outpatient visits and instrumental tools examinations. The evaluation includes 19 TC in 7 different regions in Southern Italy. Results: Preliminary data came from 12 out of 19 ACs, which completed one year of observation, showing good clinical practice, patient compliance and satisfaction and adherence to guidelines. The whole group achieved both the ISO9001 certification and the award of excellence given by "Bureau Veritas". The last 7 ACs of the first set will be examined in the upcoming months and other centers will be added soon. Conclusion: The EGINA model for the management of oral anticoagulation meets the need to set an TC standard for ensuring company organization, appropriateness in medical decisions and cost optimization, defining a new way to treat and, at the same time, to take care of anticoagulated patients with DOACs - who need to feel well and comforted -, ensuring the users best compliance. Concurrently the EGINA model follows the modern trend of scientifically measuring the crucial parameters of a health care establishment with the purpose of improving the quality and ensuring the international standards and compliance.

P067

NEED FOR IMPLEMENTING CONTROL AND VALIDATION PROCEDURES FOR THE TUBES USED FOR THE DETERMINATION OF COAGULATION PARAMETERS

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Our laboratory receives samples of patients from laboratories located in other regions of our country, with requests for specialist tests (PC and PS in particular). Some of these samples have recently given particularly low PC values in the absence of other laboratory or clinical abnormalities. The hypothesis that the phenomenon was due to wrong withdrawals (serum or other anticoagulants) has been excluded and it was hypothesized the use in the peripheral laboratories of test tubes different from those used in our laboratory. In vitro diagnostic devices, including blood collection tubes, are usually introduced into the laboratory routine without any validation. To verify the correct anticoagulant action on all the parameters performed in the coagulation laboratory, we compared the effect of two different tubes (from BD and APTACA) on the results of PT, aPTT, fibrinogen, antithrombin, D-dimer, PC, PS and dRVVT. Blood samples were taken from 5 apparently health people. The differences of the results on blood by BD and APTACA tubes ranged as follow: for the PT from 10.8 to 12.1 sec. (BD tubes) and from 12.9 to 15.8 sec. (APTACA tubes = A); for the aPTT from 23.3 to 25.6 sec. (BD) and from 23.8 to 29.4 sec (A); for fibrinogen from 195 to 293 mg/dL (BD) and from 179 to 323 mg/dL (A); for antithrombin from 98.8 to 123, 9% (BD) and 96.3 to 117.6% (A); for D-dimer from 169 to 995 μg /L (BD) and from 169 to 1020 μg /L (A), for PC from 81.7 to 112.3% (BD) and from 16.5 to 42.2% (A), for the PS from 62.5 to 109.8% (BD) and from 59.7 to 104.3 % (A), for the dRVVT from 30.3 to 37.9 sec. (BD) and from 52.2 to 74.3 sec.(A). The most significant differences were found on the PT (> 30%), on the dVVVT (> 50%) and on the PC (> 50%). Conclusions: The most likely hypothesis is that within the tubes of the APTACA company there have been alterations on the anticoagulant (contaminants? pH changes?) able to express a different action on the parameters investigated. The need therefore arises to verify the correct anticoagulant action in the test tubes used for the coagulation tests, not only when choosing the supplier, but also when new batches of the same company are provided.

P068

STABILITY OF SAMPLES FOR COAGULATION ASSAYS AND GENDER ANALYSIS AT DIFFERENT TIME: A SINGLE CENTRE STUDY

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Introduction: The hub and spoke laboratory organization is a challenge for the management of laboratory. A special issue is represented by the delay between blood collection and coagulation testing. Our study aimed to evaluate plasma stability and gender differences for routine coagulation tests, PC, PS and aPCR, of samples stored as centrifuged. Methods: Plasma of patients (pts) collected in spinner treated polymer tubes, containing 0.109 mol/L tri-Na citrate was stored at room temperature (20°C) and tested unopened at 4, 6, 8 and 24 hours (h). The reagents/instrument Werfen® system were: ACLTOP 750 analyzer equipped with borer and assay (PT: Recombiplastin 2G; aPTT, Synthasil; Fibrinogen F: QFA Thrombin; antithrombin AT: Liquid Antithrombin; D-Dimer DD: D-Dimer HS; Free Protein S PS: Protein S; protein C PC: Protein C; activated PC resistance aPCR: Factor V Leiden APC Resistance V). Results: In 50 random enrolled pts (M 31, F 19) with normal and pathological values no significant differences were found for PT ratio, DD, AT% at 4 6, 8 and 24 h. For aPTT ratio and F, significant difference was detected between 4h and 24h. PC and PS showed significant differences for all time of testing and aPCR between 4h and 8h. The separated analysis of the women's group, as for the men's group, showed the same behavior of the overall analysis for PT, DD, AT, aPTT ratio and aPCR. However, men showed no differences of F between 4h and 24h instead of women that showed a significant difference. For PS, women showed no differences between 4h and 6h, instead of men that showed a significant difference. Conclusion: In our study we stated the stability of PT ratio, DD, AT% assayed before 24h, aPTT and F before 8h, aPCR before 6h, PS and PC before 4h. Analyzing the parameters by separated gender groups we found a substantial coherent trend with the total group stability at different times. In men F showed more stability than in women and in the total group. Otherwise in women, PS resulted more stable than in men and in the total group. These differences should be confirmed in other studies and deeply analyzed for a more careful management of coagulation samples.

P069

MULTICENTRIC EVALUATION OF STABILITY OF FIVE ROUTINE COAGULATION PARAMETERS IN SAMPLE PLASMA STORED AT ROOM TEMPERATURE

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Introduction: Haemostasis testing is influenced by many pre-analytical variables, such as storage time and temperature, which can affect the stability of coagulation factors and influence the results of coagulation assays. The CLSI Document H21-A5 state that whole blood (WB) samples, stored at RT, for routine haemostasis tests should be analysed within 4 h after sample collection, with exception of PT (up to 24 h). However, for many coagulation parameters, acceptance of a longer storage time at RT is described in literature. This information can be interesting when additional coagulation tests are requested or when laboratories have to outsource coagulation tests to a laboratory at distance from the place of sample collection. Aim: to assess the stability of 5 hemostasis screening tests (PT, aPTT, Fibrinogen, Antithrombin and D-dimer) measured at different time points (4h=baseline, 6h, 8h and 24h) on WB stored at RT. This multicentric study was carried out on 280 unselected patients in 6 Italian Hospital Laboratories, all using the same reagents of Werfen for PT (Recombiplastin 2G), aPTT (SynthASIL), Fib (QFA Thrombin), AT (Liquid Anthitrombin) and DD (D-Dimer HS). Statistical analysis was performed according to literature [1]. Differences between baseline and time points were performed by Friedman test for repeated measures and, when significant, paired differences were evaluated by Wilcoxon test with Bonferroni correction. Moreover, in order to evaluate the clinical relevance of a statistically significant variation, the % change between baseline and time points were calculated and the 99%CI was compared with a 10% threshold. Results. A statistically significant change was observed at any time points for PT, aPTT, Fib, DD and after 24 hours for AT. However, at any time points these changes compared to baseline values were less than 10%, according to the 99%CI. Indeed, mean % change (99%CI) at 24h were -0.5% (-1.2 to 0.1) for PT, 5.7% (4.7 to 6.7) for aPTT, -1.4% (-2.7 to -0.1) for Fib, -1.0% (-2.2 to 0.1) for AT and -2.8% (-5.1 to -0.5) for DD. Conclusion. Our results show that for routine coagulation parameters samples are stable at least up to 24h without clinical relevant changes. References. [1] Linskens EA and Devreese KMJ. Int J Lab Hem 2018;40:292-303

P070

MULTICENTTRIC EVALUATION OF STABILITY OF THREE SPECIALIZED HEMOSTASIS ASSAYS IN SAMPLE PLASMA STORED AT ROOM TEMPERATURE

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The reorganization of the labs in HUB and SPOKE led to the extension of the area of the sampling centers belonging to the central lab with possible delays in execution of the tests and hence with possible compromise of the stability of the samples. Document CLSI (H21-A5) recommends that tests should be performed within 4 h of collection, except for the PT (up to 24 h), on samples stored at RT. This means that usually the central lab must performs the specialized coagulation tests sent by the peripheral labs on aliquots prepared elsewhere by the primary tube, therefore ignoring any problems connected to the matrix, under filling or clots. Hence the importance to carry out the tests on the primary tube instead of aliquots prepared in the spoke labs, compatible with the stability of the parameters investigated. Materials and Methods. This multicentric study was carried out in 4 Italian Hospital Laboratories, using the same reagents of Werfen for Protein S (PS), Protein C (PC) and for activated Protein C Resistance (aPCR). These parameters were measured at different time points (4h=baseline, 6h, 8h and 24h) on plasma, stored at RT, of 95 unselected patients. Statistical analysis was performed according to literature [1]. Differences between baseline and time points were performed by Friedman test for repeated measures and, when significant, paired differences were evaluated by Wilcoxon test with Bonferroni correction. Moreover, in order to evaluate the clinical relevance of a statistically significant variation, the % change between baseline and time points were calculated and the 99%CI was compared with a 10% threshold. Results. A statistically significant change was observed at any time points for PC, PS and aPCR. For PC at any time point these changes compared to baseline values were less than 10%, according to the 99%CI, with mean %change (99%CI) at 24 h of -8.2% (-9.9 to -6.4). For PS and aPCR clinically relevant changes were instead evident at 24h with respectively -12.9% (99%CI -14.9 to -10.8) and -11.4% (99%CI -13.9 to -9.0). Conclusion. Our results show that for PS, PC, aPCR samples are stable at least up to 8 (PS and aPCR) or 24h (PC) without clinical relevant changes. References. [1] Linskens EA and Devreese KMJ. Int J Lab Hem 2018;40:292-303

P071
DOSAGGIO DELLA FOSFATASI ALCALINA OSSO SPECIFICA (BAP): CONFRONTO TRA DUE METODI

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L'introduzione nel laboratorio clinico di un nuovo metodo analitico richiede la verifica delle prestazioni del metodo in prova e l'entità dell'errore sistematico eventualmente introdotto, come previsto dallo standard ISO 15189. Lo scopo di questo studio è stato il confronto di due metodi analitici per il dosaggio della BAP nel siero, il metodo colorimetrico in uso, OSTASE BAP EIA (Immunodiagnostic Systems,IDS) e il metodo in chemiluminescenza in prova LIAISON BAP OSTASE (DiaSorin). Il confronto è stato effettuato seguendo le procedure operative suggerite dal documento SIBioC del gruppo di studio Statistica (Vidali M. et al. Biochimica clinica, 2016) e utilizzando, per i calcoli statistici, il modulo software allegato. Il metodo in prova è stato sottoposto a verifica della ripetibilità dosando 3 aliquote di 2 pool di sieri a 2 diversi livelli di concentrazione (6.0 e 19.1 ng/mL) per 5 giorni (protocollo 3x5). I risultati hanno confermato la ripetibilità totale dichiarata dal produttore. Per l'analisi della correlazione, 40 campioni di siero con concentrazioni di BAP comprese tra 4,5 e 31,2 ng/mL, sono stati dosati con entrambi i metodi. L'analisi della regressione di Passing-Bablok ha evidenziato l'assenza di errore sistematico costante e proporzionale. Il grafico di Bland-Altman ha indicato che le differenze delle misure dei due metodi non dipendono dalla concentrazione dell'analita, dimostrando una ripetibilità costante lungo l'intervallo di concentrazione analizzato. La media delle differenze ha mostrato la presenza di un bias significativo di 2.2 (95% IC: 1.5 - 2.9, non comprende lo zero). Infine, abbiamo valutato l'accettabilità delle prestazioni del metodo in prova con due diversi approcci. Il primo, basato sulla imprecisione combinata dei 2 metodi (CV% = 25.5), ha mostrato una percentuale di differenze (5%) al di sotto del valore teorico della percentuale attesa. Il secondo, basato sull'errore massimo ammissibile (14.5%, Ottomano C. et al. Biochimica Clinica 2008) e sul diagramma MEDx Chart, ha evidenziato che i due metodi non sono identici entro la qualità analitica prefissata. Questi risultati indicano la non piena commutabilità dei due metodi e suggeriscono, nel caso di adozione del nuovo metodo, la definizione dei nuovi intervalli di riferimento.

P072
EMOGASANALISI: RISULTATI DI 8 ANNI DI VALUTAZIONE ESTERNA DI QUALITÀ (VEQ).

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Il Centro di Ricerca Biomedica dal 2010 gestisce un Programma di VEQ per Emogasanalisi al quale nel 2018 aderiscono 87 laboratori, 220 POCT. Lo schema prevede 4 esercizi di due campioni ciascuno. Scopo: Valutare la variabilità analitica interlaboratorio ed il livello di armonizzazione dei diversi sistemi diagnostici. Materiali e Metodi: Sono stati analizzati i dati relativi a 62 campioni di controllo, calcolando il CV% medio di tutti i risultati ed il bias% medio di ciascun sistema rispetto al valore di consenso (media delle mediane dei sistemi diagnostici con n. $\geq$ 7). Risultati: CV% medio e (range): pH= 0,38 (0,10-0,80), pO_2= 7,67 (3,13-22,7), pCO_2= 5,31 (2,54-9,16), tCO_2= 8,19 (3,60-13,1), Na^+= 1,16 (0,55-2,61), K^+= 2,11 (0,27-6,25), Cl^-= 2,31 (0,63-6,34), Ca^{++}= 4,26 (1,29-14,5), Glucosio= 4,39 (1,82-14,2), Lattato= 6,84 (3,96-18,8). Bias%: Radiometer, ABL 700-800 (n=15): pH= -0,22; pO_2= 0,42; pCO_2= -1,98; tCO_2= -6,78, Na^+= 0,82; K^+= 2,23; Cl^-= -1,69; Ca^{++}= 6,52; Glucosio= 0,27; Lattato= -4,81. ABL 80-90 (n=10): pH= -0,14; pO_2= -6,39; pCO_2= -0,97; tCO_2= -4,03, Na^+= 2,09; K^+= 2,11; Cl^-= -2,51; Ca^{++}= 4,03; Glucosio= 2,18. Siemens, Rapidlab (n=10): pH= 0,15; pO_2= -4,56; pCO_2= -3,02; tCO_2= -2,19, Na^+= -0,79; K = -0,35; Cl^-= -0,30; Ca^{++}= -0,26; Glucosio= -0,95; Lattato= 5,19. RapidPoint (n=55): pH= -0,24; pO_2= 1,86; pCO_2= 1,74; tCO_2= -2,95, Na^+= -0,70; K^+= -0,82; Cl^-= -1,03; Ca^{++}= -0,94; Glucosio= 1,64; Lattato= 3,80. Werfen, GP 3000-3500 (n=25): pH= 0,25; pO_2= -1,32; pCO_2= 2,09; tCO_2= 6,74, Na^+= 0,16; K^+= -3,07; Ca^{++}= -3.02; Glucosio= -1,68; Lattato= -0,53. GP 4000-5000 (n=70): pH= 0,21; pO_2= -0,64; pCO_2= -0,10; tCO_2= 3,93, Na^+= -0,14; K^+= -0,80; Cl^-= 3,26; Ca^{++}= -2,20; Glucosio= 0,81; Lattato= -1,39. Discussione e Conclusioni: La variabilità analitica è risultata contenuta, ad eccezione di tCO_2, pO_2 e Lattato con CV medi >6,5%. I bias più elevati sono stati riscontrati per pO_2 con ABL 80-90 e Rapidlab; per tCO_2 con ABL e GP; per Ca^{++} con ABL e per Lattato con ABL 700-800, Rapidlab e RapidPoint. Il Programma di VEQ permette di definire lo stato dell'arte delle prestazioni dei sistemi diagnostici ed è uno strumento indispensabile al Laboratorio per la "gestione centralizzata" degli emogasanalizzatori decentrati.

P073

EXTERNAL QUALITY ASSESSMENT FOR BACTERIAL IDENTIFICATION: A 4-YEAR MULTICENTRE IMPLEMENTATION STUDY

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Aim of the Study: The accurate identification of bacterial species isolated from biological sources is of pivotal importance in the diagnosis and management of bacterial infections and, consequently, in preventing increased morbidity and healthcare costs. Regular participation to External Quality Assessment (EQA) schemes plays an essential role in ensuring accurate bacterial identification through monitoring aimed at improving labs' performance. The aim of this study was to analyze the results collected for Bacterial Identification EQA in a systematic manner and based on a yearly schedule and to identify potential benefits of participation to an EQA scheme as well as the common issues encountered by the participants.
Methods: The EQA scheme for Bacterial Identification was designed and implemented by Oneworld Accuracy, renowned EQA Provider, to test laboratories' proficiency. This study's focus was on 8 EQA test events that were conducted between 2014 – 2017 across Italian laboratories. In each EQA test event, five samples consisting of inoculated loops or KWIK-STIK™ Ampoule/ Swab of various matrixes including blood, urine, sputum, swab, wound were provided to labs. Samples were challenged using both manual and automated methods. Data collected over the 4 years of EQA test events have been analyzed and segregated based on the evaluation's outcome. Samples have then been grouped according to source material and compared amongst each other. The established criterion was the identification of bacteria at the species' level. Labs that identified the bacterial species were thus employed to establish the overall performance.
Results: A total of 110 laboratories, with participation to all 8 EQA test events, were included in this study. Although participation rate varied based on sample matrixes, the average participation rate was 84% across the study arc. Overall, laboratories demonstrated good proficiency proving their ability to identify bacteria at the species' level in urine, sputum, swab and wound with high accuracy. Lower accuracy was shown when identifying bacteria from blood matrixes and in cases when anaerobic cultures were required. Other issues encountered by laboratories, preventing them from meeting the study's criterion, included sample contamination, misidentification, wrong culture media employed, missed correlation between source material and clinical history.
Conclusions: Our data show that systematic participation to EQA schemes can be instrumental to improve labs' performance and detection of common issues so that corrective actions can be promptly identified and taken in a timely manner to restore high quality services.

P074

RISULTATI DEL PROGRAMMA DI VALUTAZIONE ESTERNA DI QUALITÀ (VEQ) PER OSSIMETRIA DEL CENTRO DI RICERCA BIOMEDICA.

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Il Laboratorio è responsabile dell'attività decentrata delle analisi e garante della sua affidabilità mediante la verifica dei dati derivanti dal Controllo di Qualità. L'introduzione di uno schema di VEQ dedicato all'ossimetria è pertanto di fondamentale importanza. A questo scopo, il Centro di Ricerca Biomedica (CRB) nel 2016 ha implementato un programma al quale attualmente aderiscono 40 laboratori, per un totale di 100 POCT. Lo schema prevede 4 esercizi di due campioni ciascuno. Scopo del lavoro: Valutare la variabilità analitica ed il grado di armonizzazione dei diversi sistemi diagnostici. Materiali e Metodi: Sono stati analizzati i dati relativi alla concentrazione totale di emoglobina (tHb) e alle frazioni di Carbossiemoglobina (COHb), Ossiemoglobina (O_2Hb) e Metaemoglobina (MetHb) di 16 campioni di controllo, calcolando il CV % medio ed il bias% medio di ciascun sistema rispetto al valore di consenso (media delle mediane dei sistemi diagnostici con n. $\geq$ 7). Risultati: CV% e (range): Radiometer ABL (n=12): tHb = 2,82 (1,65-4,59), COHb = 6,21 (0,91-24,3), O_2Hb = 1,43 (0,26-3,17), MetHb = 2,33 (0,29-5,09); Siemens, RapidPoint (n=35): tHb = 1,16 (0,48-1,74), COHb = 2,86 (1,01-7,41), O_2Hb = 0,85 (0,29-2,09), MetHb = 2,97 (0,65-9,92); Werfen, Gem Premier (n=30): tHb = 1,81 (0,95-3,68), COHb = 4,07 (0,71-17,4), O_2Hb = 0,95 (0,37-2,31), MetHb = 2,78 (1,42-5,46). Bias% e (range): ABL: tHb = -5,01 (-10,1-0,13), COHb = -3,36 (-32,4-5,30), O_2Hb = 0,30 (-8,35-2,25), MetHb = -3,03 (-10,3-0,29); RapidPoint: tHb = 8,87 (4,84-15,2), COHb = -1,41 (-8,11-12,7), O_2Hb = -1,32 (-9,66 — -0,15), MetHb = -1,23 (-2,97-1,66); Gem Premier: tHb = -5,72 (-9,15 — -3,98), COHb = 1,84 (-4,23-4,73), O_2Hb = -0,82 (-9,50-1,10), MetHb = 3,75 (0,91-6,02). Discussione e Conclusioni: La variabilità analitica è risultata contenuta, ad eccezione di COHb per ABL e Gem Premier con CV medi >4% (i CV% più elevati erano associati a valori di COHb = 1,8%). Si è riscontrato un sufficiente grado di armonizzazione tra sistemi diagnostici per COHb [1,8-27%], O_2Hb [47-88%] e MetHb [9,6-28%] con bias medi <3,7% (per COHb, il campione a più bassa concentrazione presentava i bias più rilevanti). Per tHb invece si è evidenziata una minore armonizzazione, con ABL e Gem Premier che hanno presentato un bias positivo e RapidPoint un bias negativo in tutto l'intervallo di concentrazione [91-170 g/ L]. Il Programma di VEQ del CRB permette di definire lo stato dell'arte delle prestazioni dei sistemi diagnostici ed è uno strumento indispensabile al Laboratorio per la "gestione centralizzata" degli emogasanalizzatori/ ossimetri decentrati.

P075
WORKFLOW ANALYSIS ON INTERNAL QUALITY CONTROL MANAGEMENT IN A CLINICAL CHEMISTRY CORE LABORATORY

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Introduction: Workflow analysisis is applied in clinical laboratories to increase efficiency and effectiveness of processes and, hence, improve the quality of results.
Objectives: Aim of this study was the assessment of workflow analysis effects in Quality Control (QC) management in a clinical chemistry Core Laboratory.
Materials and methods: Workflow analysis was carried out with 2 Cobas 8000 analyzers (C1, C2 - Roche Diagnostics, Switzerland) on: 1) QC compartment map; 2) QC scheduling; 3) clinical risk for creatinine, alanine-aminotransferase (ALT), thyroid-stimulating hormone (TSH), prostate specific antigen (PSA), digoxin, and valproic acid. The Mission Control™ (MC) software (Biorad Laboratories Inc., USA), based on laboratory QC rules, analytical goals and instrument performance, was used to create a QC plan consistent with CLSI EP23-A, ISO 14971 and ISO 15189. QC data from a 3-month period were used to determine: the expected number of unreliable results (E_{NUF}) due to any system failure, mitigation strategies, and the derived maximum expected number of unreliable results ($MaxE_{NUF}$) based on new analytical goals.
Results: 1) Control aliquots and, consequently, manual steps were reduced by 21.1% (71→56) and 66%, respectively (6→2); 2) 3-QC-run schedule at 5am, 8am, and 2pm (63% tubes being processed from 9.30am to 3.30pm) was changed to 2 QC runs at 5am and 11.30am, improving laboratory personnel workload and efficiency; 3) E_{NUF}, mitigation strategies and $MaxE_{NUF}$ values (C1/C2) were:

	E_{NUF}	mitigation strategy	$MaxE_{NUF}$
creatinine	12.5/5.2	1-3s→1-2s	2.2/1.0
ALT	1.7/<1	1-3s→1-2,5s	0.7/<0.1
TSH	0.0/0.0	1-3s→1-4s	<0.1/<0.1
PSA	0.0/0.0	1-3s→1-4s	<0.1/<0.1
digoxin	1.1/3.4	1-3s→1-2s	0.7/0.9
valproic acid	2.9/3.0	1-3s→1-2s	0.8/0.8

Mitigation strategies lowered the risk to acceptable values for creatinine, digoxin and valproic acid, and allowed the use of less strict QC rules in low risk cases.
Conclusion: Workflow analysis improved QC management in a clinical chemistry Core Laboratory. The MC software would represent an innovative tool for patient risk evaluation. The effects of these combined strategies are still under investigation; preliminary data show they could help improve the total quality of results in such environments.

Supported by Biorad, Italy

P076
ANALYSIS OF NON COMPLIANCES IN A DEPARTMENT OF LABORATORY MEDICINE

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Aim: Purpose of this work is the analysis of Non Compliances (NC) of MultiMedica Group (Milano) regarding the whole process of laboratory in 2016.
Results: NC, which have been gathered in a punctual and systematic way, have been analyzed. 5473 NC have been collected, with an index of NC of 0,24% related to the number of tests performed. This value results slightly higher than in 2015, but significantly lower than in 2014.

Year	Number of NC	Number of tests	Index
2014	8209	2548033	0,32 %
2015	4989	2396936	0,21 %
2016	5473	2300783	0,24 %

In particular 51 % of NC of 2016 regards the preanalytical phase and 44 % the analytical phase. In the pre-pre-analytical phase (with a very low number of NC, 26), the most finded out is NC 47, “Other”. We suggest to re-examin the form “Legenda of NC” taking into account the opportunity to explicit the item “Other”. In the preanalytical phase (2794 NC), NC mostly finded out concern “uncorrect filling in of the delivery note”, “temperature unsuitable for the preservation of samples during the transport”, “insufficient volume of sample”, “inadequate sample/additive ratio”, “sample not conforming to the prescribed modalities of sampling”, “sample not arrived” and “sample haemolized”. Considering that NC of the preanalytical phase are the most numerous ones, we suggest to inform the wards about the criticalities finded out and to make aware the personnel about a higher attention for modalities of sampling and preparation of samples. In the analytical phase (2403 NC) the most relevant NC is NC 28, pertinent to the IQC out of the ranges of acceptability, with a percentage of 93%. We suggest to perform a more detailed analysis about the equipments for which this problem occurs more frequently to evaluate possible solutions. In the post-analytical phase (250 NC) the most relevant NC are “Delay for diagnostic deepenings”, “Delay of Service Laboratory” and “Delay for lack of material”. For all this cases it is important to determine their causes to intervene with possible corrective actions focused on the problem.
Conclusions: The salient aspects to be considered, to intercept and to solve the most number of NC, are the following ones: problems of sampling, preservation and transport of the samples, making aware the wards for the punctual observance of the information provided; and # problems related to the repetition of the IQC, deepening equipments involved to identify causes and possible solutions.

P077

DEFINITION AND APPLICATION OF STATE-OF-THE-ART ANALYTICAL PERFORMANCE SPECIFICATIONS DERIVED FROM AN EXTERNAL QUALITY ASSESSMENT (EQA) PROGRAM: FINAL RESULTS OF A COLLABORATIVE STUDY BETWEEN AN INSTITUTION AND A SCIENTIFIC SOCIETY

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Introduction: State-of-the-art analytical quality specifications are one of three models proposed in the 1st EFLM Strategic Conference held in 2014 in Milan. Since the EQA programmes can contribute to their definition, to continue the collaboration between the Centro di Riferimento Regionale per la Qualità dei SMeL of the Lombardy Region and the SIBioC "Analytical quality" WG, we extended to 4 years (cycles 2014-2017) the analysis of data submitted by 285 Lombard and Tuscan Laboratories participating in the EQA program "Hormones and tumor markers", to obtain and evaluate targets of maximum allowable error.
Methods: 131431 results for 24 measurands produced in 48 exercises were elaborated as follows: subdivision by exercise and by peer groups (at least 7 users of the same method); calculation of the % deviation of each result from the expected value (robust average of the peer group, after outliers removal according to Huber-Hampel approach); calculation of the 95th percentile of deviations for each exercise (taken as a goal based on state-of-the-art); correlation of the goals identified in each exercise with the corresponding mean concentration of the samples; identification, for each measurand, of a unique or a concentration specific goal; application of goals to the results and calculation, for each individual Laboratory, of the % of Non Conformity (NC) and of the corresponding long term Sigma value of the process.
Results: For each measurand, 17 unique and 14 concentration dependent analytical performance goals were established. Applying these goals to results submitted by 220 laboratories with more than 100 determinations, the percentage of participants showing a number of NC corresponding to a Sigma value > 3 represent 44% of the total. No trend was demonstrated by dividing the NC by year, while significative differences were found between the different analytical platforms ($p < 0,0001$; Chi Square test).
Conclusions: Despite the possible non-commutability of the materials used, the EQA programmes can be a productive source of state-of-the-art analytical performance limits.

P078

INTERPRETAZIONE DEGLI INTERVALLI DI RIFERIMENTO DELLA FOSFATASI ALCALINA IN ETÀ PEDIATRICA

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Gli intervalli di riferimento (IR) sono particolarmente importanti perché rappresentano lo strumento più largamente utilizzato nelle decisioni mediche. Il metodo per calcolarli è dettagliatamente descritto nella teoria, purtroppo però, non si può dire la stessa cosa della pratica. Le linee guida non risolvono, infatti, completamente i problemi pratici che i laboratoristi incontrano nel tentativo di determinare gli IR per ciascun analita. Teoria, pratica e armonizzazione nella costruzione degli IR sono state e sono tuttora oggetto di studio, dibattito e raccomandazioni (1).
Le linee guida suggeriscono l'applicazione di un metodo diretto, anche se in letteratura negli ultimi anni sono apparsi lavori scientifici che descrivono metodi indiretti per determinare gli IR. Il nostro gruppo di ricerca è impegnato nella stima degli IR con il metodo indiretto, sfruttando l'algoritmo Expectation Maximization (EM) (2).
Questo lavoro, in continuità con gli scorsi anni (3, 4), propone una interpretazione della fluttuazione dei valori della fosfatasi alcalina (ALP), su dati pluriennali di pazienti in età pediatrica, nelle fasce di età da 10 a 14 anni per i maschi e da 9 a 13 anni per le femmine. In particolare i dati ALP sono interpretati in riferimento agli IR sia stimati con l'algoritmo EM sia attualmente utilizzati nel Laboratorio di Patologia Clinica dell'AOUS. Le stime degli IR con algoritmo EM, dipendenti da età e sesso, forniscono una nuova e diversa chiave interpretativa delle fluttuazioni dei valori ALP.

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P079

QUATTRO ANNI DI VERIFICA ESTERNA DI QUALITA' IN IMMUNOEMATOLOGIA: BENEFICI DEL PROGRAMMA NEL MIGLIORAMENTO DEI PERCORSI DIAGNOSTICI

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Introduzione: I servizi di Medicina trasfusionale devono partecipare sistematicamente a programmi di valutazione esterna della qualità (VEQ), definiti anche come Proficency Testing (PT) al fine di valutare le perfomance dei sistemi e dei processi analitici gestiti. Il Servizio Trasfusionale dell'ASL Caserta dal 2014 ha aderito al programma One World Accuracy per l'immunoematologia eritrocitaria al fine di garantire una maggior sicurezza per la validazione immunoematologia degli emocomponenti e per la sicurezza dei riceventi. Scopo di questo lavoro è verificare l'accuratezza dei nostri percorsi diagnostici.
Materiali e metodi: Il programma di VEQ prevede 4 invii annuali di campioni incogniti. Ogni invio contiene: Campione A (sospensione di emazie al 8%) Campione B (siero) analizzati come se fossero i campioni di un paziente; Campione C (sospensione di emazie al 8%) Campione D (sospensione di emazie all'8%): usati come le emazie di donatori . Sul campione A è stato eseguito: Gruppo ABO diretto, fenotipo Rh e Test di Coombs diretto con metodica in micro colonna (Ortho Autovue, Johnson -Johnson Company). Sul campione B è stato eseguito: Gruppo ABO indiretto, ricerca anticorpi irregolari,identificazione anticorpi irregolari ,e prove di compatibilità con il campione C e D con metodica in micro colonna.
Risultati: Dall' analisi dei report è emerso che: nell' anno 2014 la percentuale di errore scostamento in test VEQ è stata del 3%, nell' anno 2015 del 2%, nell' anno 2016 del 1% mentre nell' anno 2017 l' accuratezza dei test diagnostici è risultata essere del 100%
Discussioni e conclusioni: Dai risultati si evince che i nostri percorsi diagnostici risultano essere accurati; la percentuale di errore è stata riscontrata nell'identificazione anticorpale soprattutto nel primo anno di partecipazione al programma. Uno dei principali obiettivi della VEQ è di intraprendere azioni correttive. Infatti per ridurre al minimo il rischio di errori abbiamo adottato misure di miglioramento con l'introduzione della tipizzazione del fenotipo eritrocitario esteso in sierologia e in biologia molecolare che ha supportato la diagnostica infatti dal 2015 in poi la percentuale di errore si è ridotta al 1% nel 2016.

P080

UTILIZZO DEL SOFTWARE MISSION CONTROL2 PER LA VALUTAZIONE DELLE PERFORMANCE ANALITICHE DI PIÙ ANALIZZATORI IN UN IMPIANTO DI ALTA AUTOMAZIONE

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Per molti anni la gestione statistica del controllo di qualità (QC) ha permesso ai laboratori clinici di pianificare la migliore strategia scegliendo regole e frequenze per poter determinare la massima capacità di individuare l'errore (Ped>90% e Pfr<5%). Con la richiesta della diminuzione dei tempi di refertazione e l'analisi continua su strumentazione ad alta produttività è necessario mutuare l'approccio e la gestione della qualità basandosi su procedure di risk management, come da linea guida CLSI EP23A e ISO 15189. Scopo del lavoro è l'utilizzo del software Mission:Control[2] di Bio-rad Laboratories per il calcolo dell'indice di rischio (RMI). Abbiamo considerato l'ETa, numero di campioni giornaliero, regole di Westgard e frequenza di QC di 2 test di chimica clinica, Potassio e Glucosio (Glu), dosati su 4 Advia Chemistry XPT, e 2 test immunometrici Troponina I (TnI) e TSH dosati su 3 Advia Centaur XPT (Siemens). Abbiamo assegnato agli analiti una categoria di severità in base alla probabilità accettabile di danno al paziente: Critico per K e TnI, Severo per Glu e TSH. I valori di RMI ottenuti per K sui 4 strumenti sono rispettivamente: 0.177, 0.006, 0.336, 1.19; per il Glu 0.007, 0.062, <0.001, 0.001. Per il TSH l'RMI è risultato di 0.058, 0.396, 1.12 sui 3 strumenti; mentre per la TnI 0.45 e 8.8 sul II strumento. Considerando che il rischio risulta controllato quando l'RMI<1, il software suggerisce nuove strategie di controllo per quei test con l'RMI>1. Per il K, sul IV strumento, l'aggiunta della regola 2-2s ha permesso di ottenere un RMI 0.48 lasciando immutata la frequenza di QC. Per il TSH ha suggerito di aumentare la frequenza di QC 2 volte al giorno per ottenere un RMI di 0.867. Infine per la TnI, che ha mostrato l'RMI più alto e differente tra i 2 strumenti, il software ha proposto la regola 2-2s per diminuire a 7.4 l'RMI, non riuscendo a trovare una strategia per ottenere un RMI<1. L'utilizzo del software ha permesso di evidenziare le diverse performance dei test, di cambiare le specifiche analitiche in base al rischio accettabile per il paziente implementando o riducendo il numero dei QC giornalieri e impostando regole più o meno stringenti; ottenendo così una nuova visione del QC incentrata sul paziente.

P081

VERIFICATION OF PEDIATRIC REFERENCE INTERVALS FOR SERUM ALKALINE PHOSPHATASE USING A LABORATORY DATABASE

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Background: Recently, we partecipated in a multicenter study of the Italian Society of Clinical Biochemistry (SIBioC) aimed to define common pediatric RIs for serum alkaline phosphatase (ALP) measured with automated methods traceable to the IFCC reference method (1). The study gathered a total of 4824 outpatient ALP results and our centre contributed with data recorded for 20 weeks during which our analytical performance was monitored by means of a reference material. Before the implementation in our laboratory of the proposed RIs, we decided to verify them using an indirect method based on data mining.
Method: We extracted from our database all ALP results requested in pediatric outpatients (between 0 and 20 years of age) from January 2010 to May 2018, excluding all individuals with repeated testing. ALP serum activity was measured on Beckman AU680 using the Beckman-Coulter assay. Results were partitioned in sex and age classes as recommended by SIBioC and analyzed with MEDCalc software based on CLSI document EP28-A3 for the non-parametrical calculation of the reference limits (2.5th and 97.5th percentiles), after exclusion of outliers (robust method was used for groups with less than 120 data) (2).
Results: We obtained from our database 6988 results. We obtained reference limits and related 90% confidence intervals (CI) for appropriate sex and age subclasses for ALP serum concentrations in pediatric patients. All 90% CI derived from our study comprised the reference limits proposed by SIBioC.
Conclusions: As mentioned in CLSI guidelines, indirect methods based on data mining of large laboratory databases are suitable to verify external RIs before their implementation (2). In our case, since all 90% CI derived from our study comprised the reference limits proposed by SIBioC, we eventually decided to adopt them.
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P082

CRYOGLOBULIN EVALUATION: ANALYSIS OF INTRA-LABORATORY AND INTER-LABORATORY VARIABILITY

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Background: Cryoglobulins (CRG) are immunoglobulins that precipitate in serum at temperatures below 37°C and resolubilize upon warming. The main reasons of interest in the study of cryoglobulinemia are the peculiarities of physiopathological mechanism and clinical consequences. Authors showed that only 36% of laboratories used standard procedures of analysis and highlighted the relevance of maintenance of the sample at a stable temperature of 37°C; centrifugation; cryoprecipitate quantification; cryoprecipitate washing techniques; immunocharacterization of cryoprecipitates through immunofixation techniques.
Objectives: To verify and assess the variability of laboratory processes of CRG.
Methods: We checked laboratory databases of Hospital and University (Lab A and B) of Modena with long tradition in the cryoglobulin analysis. 734 patient samples were studied in both laboratories. We compared our results according to Brouet classification into subgroups: type I, II and III. We evaluated intra-laboratory variability, compared to previous or more frequent results and we studied inter-laboratory variability based on non-concordant laboratory reports.
Results: The following data represented the comparison between labs about the same patient cohort in 734 patient samples (χ2, Chi-square test):

I type: Lab A=21, Lab B=42 (χ2, P=0.0016); II type: Lab A=242, Lab B=270 (χ2, P=0.0004); III type: Lab A=108, Lab B=108 (χ2, P=ns)

Intra-laboratory variability: 14% Lab A, 16% Lab B (ns). Inter-laboratory variability: non-concordance in 25% of cases, considering 133 patients studied in both laboratories.
Conclusions: No data about variability in CRG analysis are reported in literature. National and international guidelines are not explicative enough. Our experience is unique but limited in two laboratories. Given the variability of testing conditions used in different laboratories and the lack of test standards and reference values, we confirm the need of further investigations into standardization of CRG testing. New guidelines are fundamental, in order to optimize all phases of CRG research (pre and post analysis) and to ensure correct diagnosis and adequate treatments of the associated diseases.

P083

SALIVARY CORTISOL AND CORTISONE BY LC-MS/MS IN A ISO15189 ACCREDITED LABORATORY: WHAT HAPPENS WHEN CHANGING THE CALIBRATORS?

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Introduction: Salivary cortisol (sF) and cortisone (sE) are measured in routine practice by an home brew LC-MS/MS method, with home-made calibrators and internal quality controls [1,2]. The clinicians can request the salivary cortisol and cortisone ratio (sFEr): in the report, only sF and sFEr results are described, with the appropriate reference intervals (RI). sF is an examination procedure (EP) accredited according to the ISO15189:2012 since 2016 [3]. According to the requirement 5.5.1.3, this method was validated [4], producing a validation certificate. sFEr was not accredited since external quality control is not available for sE. Recently CE-IVD calibrators for both cortisol and cortisone were available.
Aim. The aim of this work was to substitute the home-made calibrators with the commercial ones, since a higher standardization is guaranteed.
Methods: Saliva samples from routine analysis (n=160) were used to compare home-made calibrators with CE-IVD calibrators. Saliva samples from healthy volunteers (n=20) were collected to verify the RI, according to CLSI EP28 [5].
Results: No significant bias was observed for sF. A significant bias resulted for sFEr (Bland - Altman -33,6%, Passing Bablok regression y=0,71x+0,01). The RI for sF was not changed, while those for sFEr were recalculated according to the obtained regression. In the verification of the new RI, only 2 saliva samples were outside from the obtained RI.
Conclusions: The new RI are appropriate for the intended use. When a modification is made on a ISO 15189 accredited procedure, the change has to be considered and the impact on the performances has to be evaluated. Moreover, all the data obtained have to be recorded and a new validation certificate has to be released.
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P084

COMPARISON BETWEEN THE RESULTS OF AN EXTERNAL QUALITY ASSESSMENT (EQA) PROGRAM FOR HORMONES AND TUMOR MARKERS OBTAINED USING SAMPLES OF HUMAN ORIGIN NOT TREATED (“FRESH”) AND TREATED (“SYNTHETIC”) OF HOMOGENEOUS CONCENTRATION

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Introduction: One of the EQA's aim is to provide information on the harmonization of results produced by different analytical systems; avoiding the matrix effect by using commutable materials is one of the main requirements to establish if the differences between the instruments are real, eg attributable to a faulty standardization process by the manufacturers. Within of a collaboration between the Centro di Riferimento Regionale per la Qualità dei SMeL (a Lombardy Region institution) and the SIBioC "Analytical Quality" WG, we compared the results obtained from 46 laboratories measuring 19 hormones and tumor markers on two “fresh” and on “synthetic” samples of similar concentration.
Methods: For each analyte and material, after grouping the results into instrument peer groups, the difference between the means of all possible pairs was analyzed, both statistically (ANOVA and Tukey-Kramer test for multiple comparisons), and comparing the mean difference ± its 95% confidence interval (CI) with the bias goal based on biological variation.
Results: Using "fresh” samples the differences between the peer group averages are statistically significant in 68% of cases and in 49% of cases they are also higher than the "clinical" maximum allowable bias. As expected, using "synthetic” samples the percentages of misalignment are higher (84% and 60% respectively); however, the difference across the materials is significant (p=0.0002; Fischer's exact test) only when the instruments alignment is assessed applying statistical and not "clinical" criteria (p=0.058). Moreover, for each measurand the degree of misalignment found with “fresh” samples is not correlated with the demonstrable one using “synthetic” materials (Spearman’s rs=0.16; 95% CI = -0.33 to 0.58; p=0.51). Finally, using “fresh” materials, no instrument shows a greater tendency to produce results misaligned, neither statistically (p=0.48, Chi square test), nor "clinically" (p=0.15).
Conclusions: Although the effect is more evident by using statistical criteria, different analytical systems appear better aligned when the differences are evaluated using samples of human origin not treated, thus confirming the presence of confounding factors when trying to obtain these informations using treated materials.

P085
EVALUATION OF CRYOGLOBULINS: ANALYSIS OF VARIABILITY INTER AND INTRA OPERATOR

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Introduction: Cryoglobulins (CRG) are mono and / or polyclonal immunoglobulins (Ig) that precipitate reversibly at temperatures below 37 ° C. For the correct diagnostic classification and for the clinical monitoring of the patients affected by cryoglobulinemia, on the cryoprecipitate dissolved an immunofixation (IFE) is carried out for the identification of the CRG according to the classification of Brouet in types I, II and III.Preanalytic variables (sampling, transport, preservation), analytical (centrifugation, washing, seeding of antisera) and post-analytic (reading of the IFE) can lead to results subject to a strong operator-dependent variability.The aim of our work is to evaluate the intra and inter-operator variability considering only the reading of the IFEs, omitting all the other aspects. Materials and Methods: We selected 15 of the IFE Lab A and 15 B of the reported laboratory of two of their laboratories operators (A and B). Operator A has reread the IFE of Lab A, so operator B has reread the IFEs of laboratory B. The IFEs have then been exchanged between the 2 operators of the 2 laboratories.Results: the operator A rereading the IFEs of Laboratory A diagnosed in the same previous way 10 (67%) samples out of 15; while the operator B re-reading the IFEs of laboratory B diagnosed in the same previous way 11 (73%) samples.In the exchange of IFEs, operator A reported 5 (33%) samples differently than operator B; the operator B reported 4 (26%) samples differently than the operator A, with a statistically significant difference if the chi-square test was applied (P <0.006). Conclusions: Although our series is very limited, it is clear that the harmonization in this area of important diagnostic importance is a priority. The search for CRG is conditioned by many variables, including the interpretation of the IFEs. In order to standardize the reading of the CRG training courses shared between operators of several centers are necessary and it is essential to expand as much as possible the audience of laboratories adhering to foreign quality controls such as the VEQ experimental program started in 2017 promoted by UKNEQAS.

P086
HB MELUSINE: VARIANTE POTENZIALMENTE SOTTO RIPORTATA NELLA POPOLAZIONE ITALIANA

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Tre pazienti di origini italiane sono giunti alla nostra osservazione per la determinazione dell'emoglobina glicata (HbA1c). La quantificazione di HbA1c è stata inizialmente effettuata mediante elettroforesi capillare CE utilizzando l'analizzatore Capillarys 3 Tera (Sebia) con kit Capi 3 HbA1c. I tre campioni sono stati classificati dal software come "Atipici" a causa di un lieve sdoppiamento delle frazioni HbA2, HbA0 e HbA1c indicativo della presenza di una variante emoglobinica delle catene alfa che ha impedito la quantificazione accurata di HbA1c. Gli stessi sono stati analizzati con un metodo per HbA1c in HPLC (D-100 BioRad) ma i tracciati non hanno evidenziato alcuna frazione addizionale riconducibile a variante Hb. Dato che le possibili interferenze legate alle varianti emoglobiniche devono essere tenute in attenta considerazione da parte del laboratorista per fornire un risultato attendibile e un'interpretazione non ambigua[1], al fine del corretto inquadramento diagnostico dei pazienti si è proceduto ad ulteriori approfondimenti eseguendo l'emocromo e la valutazione degli assetti con programmi specifici. I tre pazienti non presentavano implicazioni cliniche significative ed i parametri emocromocitometrici sono risultati nella norma. L'ispezione visiva dei tracciati eseguiti in CE con il kit Capi 3 Hemoglobin(e) e in HPLC con lo strumento Variant II e "HbA2/HbA1c Dual program" non ha consentito di evidenziare alcuna anomalia riconducibile a variante emoglobinica. I campioni sono stati inviati in genotipizzazione. L'analisi molecolare è stata eseguita mediante sequenziamento diretto del DNA estratto da leucociti da sangue periferico; in particolare le regioni analizzate sono state: la sequenza nucleotidica -100 al +20 3' UTR del gene α2 e la sequenza -140 al +20 3' UTR del gene α1. L'indagine molecolare ha confermato in tutti e tre i casi la presenza di una mutazione in eterozigosi c.343C>T sui geni α2 (Hb Melusine). Non sono state riscontrate invece mutazioni a carico dei geni α1. L'elevato potere risolutivo del metodo per HbA1c in CE ha consentito, nei tre casi, l'individuazione di una variante emoglobinica (Hb Melusine) sicuramente sottoriportata nella popolazione italiana poichè non riscontrabile con i metodi cromatografici fino ad oggi utilizzati.

[1] Refertazione dell'emoglobina glicata in presenza di varianti emoglobiniche M. Carta, R. Paleari, A. Caldini, A. Terreni, A. Mosca per il Gruppo di Studio Intersocietario SIBioC-SIMeL Diabete Mellito. Biochimica clinica, 2011, vol. 35, n. 1

P087
L'ELETTROFORESI SIERO-PROTEICA E L'ALBUMINA GLICATA

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L'albumina glicata (AG) è un marcatore del controllo glicemico del breve-medio periodo nei pazienti diabetici. La determinazione può rivelarsi utile per la valutazione, il follow-up dei pazienti con diabete in gravidanza, nelle anemia e nefropatie. Il diabete poco trattato può portare a complicanze quali: retinopatia, malattie cardiovascolari e neuropatie, più un aumentato rischio di insorgenza di malattie tumorali. Lo scopo dello studio è di valutare sul tracciato elettroforetico capillare la presenza o assenza dell'AG. Ciò si ottiene dall'osservazione, nei diabetici non compensati o scarsamente compensati, di una alterata curva dell'albumina per la presenza di AG. Per valutare ciò si sono esaminati i tracciati elettroforetici di 90 pazienti, correlando l'emoglobina glicata (HbA1c) con l'alterazione dell'ampiezza della banda dell'Albumina del tracciato elettroforetico capillare. I 90 pazienti sono stati divisi in tre gruppi secondo i valori dell'HbA1c. Il gruppo A comprendeva 30 pazienti con diabete poco controllato, il gruppo B 30 pazienti con diabete ben controllato o con condizione di pre-diabete e il gruppo C includeva 30 pazienti non diabetici. La glicemia viene dosata con il metodo enzimatico dell'esochinasi, l'HbA1c con cromatografia liquida ad alta prestazione (HPLC), e l'AG è stata determinata su uno strumento di elettroforesi capillare (Helena V8, Medical Systems Italia). L'ampiezza della base dell'albumina dei 30 pazienti diabetici del gruppo A è risultata più ampia rispetto ai 30 pazienti del gruppo B e del gruppo C e correla con i valori dell'HbA1c. L'ampiezza della base dell'Albumina vs l'HbA1c è, rispettivamente, 39,15+/- 3,72 vs 10,27 +/- 1,88 nel gruppo A; 33,93 +/- 3,69 vs 6,55 +/- 0,64 nel gruppo B; e 31,61+/- 1,92 vs 5,36 +/- 0,20 nel gruppo C. Il test T di Student ha dimostrato che tra i gruppi A vs B, B vs C e A vs C esiste una differenza statisticamente significativa. I risultati di questo studio indicano che l'ampiezza della base dell'albumina è riuscita a distinguere se un paziente aveva valori alterati di HbA1c o meglio se apparteneva ai tre gruppi differenti di pazienti. Sono necessarie ulteriori ricerche per sviluppare un programma informatico che calcoli l'area dell'AG sul tracciato elettroforetico capillare.

P088
DETERMINAZIONE DELLA STABILITA' DEL GLUCOSIO IN PROVETTE DA SIERO E PLASMA CON GEL SEPARATORE

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La determinazione del glucosio riveste un ruolo essenziale nella diagnosi di diabete, malgrado la ben nota instabilità nelle provette. Scopo del lavoro è stato quello di verificare la stabilità del glucosio in provette di siero e plasma litio-eparina con gel separatore (Vacuette®, Greiner Bio-One, Kremsmünster, Austria). 60 coppie di provette di provette di siero e litio-eparina sono state selezionate e poste in ghiaccio subito dopo il prelievo, immediatamente trasportate al laboratorio, centrifugate e analizzate mediante su Beckman Coulter AU480 (metodo esochinasi, Beckman Coulter Inc., Brea, CA, USA). I campioni sono poi stati suddivisi e conservati a temperatura ambiente o a 4°C. La determinazione del glucosio è stata quindi ripetuta dopo 3-6-24-48-72 e 96 ore. Per tutti i campioni è stata anche effettuata un esame emocromocitometrico, prima e dopo centrifugazione, utilizzando un XN 2000 (Sysmex,Inc.Kobe,Japan). I campioni di plasma hanno rivelato una conta residua apprezzabile di leucociti e piastrine, mentre nei campioni di siero la conta cellulare era insignificante. La concentrazione di glucosio nel plasma eparinato ha mostrato un decremento sin dalle 3 ore dopo centrifugazione, e la riduzione si è protratta per tutto il periodo dello studio, sia a 4°C e sia a temperatura ambiente. La riduzione della glicemia non è apparsa significativamente associata alle conte cellulari, prima e dopo centrifugazione. I campioni di siero hanno dimostrato stabilità prolungata del glucosio fino a 96 ore, sia in quelli conservati a temperatura ambiente che a 4°C. L'ispezione visiva ha rivelato come nelle provette di litio eparina il gel avesse intrappolato numerosi globuli rossi, mentre i gel delle provette di siero si sono rivelati liberi da cellule. Questo studio dimostra come la determinazione del glucosio in provette di siero con gel separatore centrifugate possa essere clinicamente valida fino a 96 ore, mentre nelle provette di litio eparina con gel separatore centrifugate diventi inattendibile già dopo 3 ore. Montagnana M, Lippi G. Overcoming preanalytical issues for diagnosing diabetes with fasting plasma glucose. Ann Transl Med 2017;5:257.

P089

RISCONTRO OCCASIONALE DO VARIANTI EMOGLOBINICHE IN CORSO DI DETERMINAZIONE DI HBA1C: ESPERIENZA DI 18 MESI CON CAPILLARYS 3 TERA SEBIA

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L'elettroforesi capillare consente di visualizzare, segnalando il profilo come "atipico" la presenza di varianti emoglobiniche durante l'esecuzione di HbA1c. Le varianti più comuni (HbC, D, S, E) non interferiscono con la determinazione di HbA1c mentre altre varianti possono provocare una sovra o sottostima (es. Hb Camperdown). In altri casi lo strumento non è in grado di quantificare il dato relativo ad HbA1c. Nella nostra esperienza in 18 mesi di utilizzo dell'elettroforesi capillare abbiamo riscontrato 200 pazienti con varianti emoglobiniche che sono state approfondite con corsa dedicata alle varianti in elettroforesi capillare e, nei casi dubbi, con elettroforesi a ph alcalino/acido. Alcuni casi sono stati approfonditi con biologia molecolare in altra sede. Sono state identificate: 28 HbD, 25 HbC, 14 HbE, 96 HbS trait (HbA1c refertata con segnalazione della presenza della variante; 4 Hb Camperdown (HbA1c sottostimata ma può essere ricalcolata); 3 casi di doppia eterozigosi/ omozigosi a carico della catena beta globinica in cui non e' possibile quantificare HbA1c; 2 Hb Lepore e 4 casi con HbF elevata (oltre il 13%, possibile interferenza); 24 casi con varianti non identificabili con le indagini di primo e secondo livello che sono stati refertati fornendo il dato relativo alla glicata (se presente) ma allarmando relativamente alla possibilità che il dato fosse interferito. Analizzando questi casi si è notato che 8 pazienti (italiani) presentavano lo stesso tipo di grafico con una HbA0 non ben definita. Lo strumento in tutti questi casi non forniva alcun dato di HbA1c. Tale variante è stata approfondita con elettroforesi e biologia molecolare e si è dimostrata essere una Hb Ozieri. Altri 6 pazienti (anche questi italiani) presentavano invece un'altra tipologia comune di grafico ovvero la presenza di un picco pari a circa il 26% che con il kit CAPI3-Nemoglobin SEBIA migrava in zona Z13. Questa variante è risultata essere una HbJ Rovigo (possibile sovrastima). Conclusioni: il riscontro occasionale di varianti emoglobiniche non è infrequente (0.5% dei pazienti della nostra casistica) e può provocare sovra o sottostime dell'emoglobina glicata. I grafici vanno quindi valutati con attenzione e commentati ove opportuno.

P090

STUDY OF CORRELATION BETWEEN BMI AND METABOLIC AND INFLAMMATORY BIOMARKERS IN OBESE PATIENTS

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In a population of 204 obese patients (151 females, 53 males, 13-72 years), selected for BMI (3 WHO classes: class 1-<34.9 Kg/m2 class 2-35-39.9 Kg/m2 class 3->40 Kg/m2), clinically monitored at the AOP and whose samples were sent over a 3 month period (November 2017-February 2018) to UOC Laboratory Medicine, we evaluated the correlation between BMI/inflammatory biomarkers (Neutrophils 10^9/L, PCRus mg/L, TNFα ng/L, IL-6 ng/L) and BMI/metabolic biomarkers (Insulin mU/L, C-peptide ug/L, Leptin ug/L, Glycemia mmol/L, Vitamin D nmol/L). The statistical analysis were performed using Mann-Whitney and Pearson tests (significance $p < 0.05$). We observed statistically significant differences between 2^{nd}vs 3^{nd} classes (numerically most represented) for: IL6 ng/L (Median class 2: 2.45 vs class 3: 3.40; p=0.005; Insulin (Median class 2: 10.85 vs class 3:15.10 p=0.01); Leptin (Median class 2:28.5 vs class 3: 38.0 p=0.05) PCRus (Median class 1: 2.0 vs class 2: 4.1 p=0.0006, Median class 1 vs class 3: 4.7 p=0.0009) The comparison between some biomarkers of particular clinical interest (PCRus and Vitamin D), demonstrated (Pearson analysis) a significant positive correlation between PCRus and Neutrophils p<0.0001; PCRus and C-peptide p=0.0021 PCRus and IL6 p<0.0001 and a significant negative correlation between Vitamin D and Glycemia p<0.0001; Vitamin D and C-Peptide p=0.0021; Vitamin D and Insulin p=0.001; Vitamin D and Leptin p<0.0001; Vitamin D and PCRus p<0.0001; Vitamin D and IL6 p=0.0376. The obtained data confirm the close relationship between the considered inflammatory/metabolic biomarkers and the increasing BMI values especially in the obese patients pre and post sleeve gastrectomy, in which a chronic inflammation is evident. The present results will be further investigated searching for new markers with particular attention to the patterns of inflammatory cytokines, that, integrated with clinical data, will allow to assess their pathophysiological significance and clinical impact.

P091

A COMPARISON BETWEEN TWO HPLC-BASED METHODS FOR EVALUATING OF HUMAN HEMOGLOBINOPATHIES

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The term hemoglobinopathy includes all genetic hemoglobin disorders which can be divided into two main groups: thalassemia syndromes and structural hemoglobin variants, the latter called abnormal hemoglobins. Both are caused by mutations and/ or deletions in the α- or β-globin genes. In brief, when gene mutations cause Hb synthesis disorders, hemoglobin structure is normal giving rise to thalassemia. On the contrary, mutations causing changes in Hb structure give rise to abnormal hemoglobin. Many mixed forms combine features of both groups, e.g. β0 /β+ - thalassemias, HbSC disease and HbE α-thalassemias. Hemoglobinopathies and thalassemias are among the commonest inherited disorders in humans and require a wide range of diagnostic and therapeutic measures. We conducted a comparison study on a group of 83 patients affected by different inherited defects of Hb synthesis or structure, treated at the Hematology Center of Sant'Eugenio Hospital in Rome. Two HPLC analyzers from two different companies were compared in order to distinguish inherited disorders affecting either the structure of hemoglobin (such as sickle cell disease (SCD)) or the levels and balance of globin chain production (thalassemias). We also included variants that cause hereditary persistence of fetal hemoglobin (HPFH), a condition associated with increased production of γ-globin which ameliorates the clinical endpoints of SCD and β-thalassemia. Data here reported define acceptability criteria discussing sample measurement, data analysis and evaluation.

P092

COMPARISON OF SEBIA CAPILLARYS FLEX HB A1C KIT CAPILLARY ELECTROPHORESIS AND BIORAD VARIANT II HPLC FOR THE QUANTIFICATION AND IDENTIFICATION OF HEMOGLOBINE VARIANTS

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Introduction: Haemoglobinopathies constitute the commonest recessive monogenic disorders worldwide affecting approximately 7% of the world population. Many heterozigous hemoglobin variants are a fortuitous finding as the result of Hb A1c analysis or of hemoglobin tests during pregnancy. HPLC systems and Capillary zone electrophoresis (CE) are the two presumptive methods recommended for first level hemoglobinopathies detection, since these allow a precise quantification of Hb A2, Hb F and abnormal Hb fractions.
Methods: The objective osisf this study was to to evaluate the analytical performance in Hb variant detection of a capillary electrophoresis instrument (Capillarys 3 Flex piercing, Sebia) equipped with HbA1c kit, in use in our laboratory for HbA1c measurement, in comparison with BioRad Variant II HPLC system, beta-Thalassemia Short Program kit, in use for Hemoglobinopathies screening. The concordance of hemoglobin variant identification on the two systems was evaluated by analyzing samples on both instruments in presence of a chromatogram suspect for hemoglobinopathy at HbA1c or HbA2 investigation. The percentage Hemoglobin variant obtained was compared using Passing Bablok regression.
Results: 185 samples were analyzed on both systems during 12 months (103 detected at HbA2 screening, 82 at HbA1c measurement). The percentage hemoglobin variant results were similar for two methods were similar for Hb S (Pearson's r = 0.987), Hb C (r = 0.957), Hb Hasharon (r = 0.997), and for Hb Lepore (Hb Lepore (r = 0.996). The concordance of Hemoglobin was lower for Hb D (r = 0.694) and Hb E (r = 0.281). The overall concordance, for all hemogoblin variants detected, was high (r = 0.975). Conclusion: HPLC and capillary electrophoresis present a good concordance, also for rare hemoglobins, and the two
method can be used in parallel to confirm each other.

P093

INTERAZIONE δ E β TALASSEMIA IN UNA RAGAZZA DI 15 ANNI: LO STUDIO FAMILIARE EVITA LA MISDIAGNOSI

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Le talassemie sono malattie ereditarie determinate da difetti quantitativi della sintesi emoglobinica e più esattamente da una ridotta o assente produzione di un tipo di catena globinica con manifestazioni cliniche, ematologiche ed emoglobiniche di varia intensità. A seconda del tipo di catena coinvolta nel difetto genetico si distinguono talassemie di tipo α, β, δ, δβ, γ. La δ talassemia è caratterizzata dalla produzione ridotta (δ^+ tal) o assente (δ^0 tal) di catene globiniche di tipo δ; queste, insieme alle α, costituiscono l'HbA2, frazione dell'emoglobina umana poco espressa (circa il 3% Hb totale) motivo per il quale la δ talassemia è clinicamente asintomatica, ha un quadro ematologico normale e l'HbA2 è ridotta o assente. La β talassemia, invece, clinicamente grave allo stato omozigote, è da prevenire identificando i portatori sani (eterozigoti) mediante specifici test di laboratorio (esame emocromocitometrico, determinazione quali-quantitativa delle frazioni emoglobiniche mediante HPLC,….). Il valore aumentato dell'HbA2, patognomonico di β talassemia può, però risultare falsamente normale o al limite della norma nella doppia eterozigosi per β/δ talassemia nello stesso individuo. La δ talassemia non è patologica, ma può determinare misdiagnosi quando è presente nei portatori di β talassemia, pertanto essa assume importanza diagnostica negli studi di prevenzione delle forme gravi. Gli autori presentano il caso di una quindicenne con microcitosi, ipocromia eritrocitaria e HbA2 lievemente aumentata. Lo studio familiare e molecolare ha rilevato la presenza della β talassemia (β IVS-I-110 G>A) e della δ talassemia (HbA2 Yialousa) in doppia eterozigosi nel probando e nel fratello del probando. I genitori sono uno eterozigote per la β talassemia e l'altro eterozigote per la δ talassemia. Le mutazioni β IVS-I-110 G>A e HbA2 Yialousa sono state ricercate con amplificazione allele-specifica (ARMS).

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P094

PREVENIRE L'EMOGLOBINOPATIA DA HBS ALLA LUCE DEI NUOVI FLUSSI MIGRATORI

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L'emoglobina S [HbS, β(A3)6Glu→Val] è la variante emoglobinica più diffusa al mondo: l'Africa sub-sahariana, l'India ed il Medio Oriente sono aree ad alta prevalenza, ma le migrazioni sempre più frequenti da questi territori verso l'Europa, ne hanno determinato un drammatico aumento anche nel nostro Paese. In condizioni di carenza di ossigeno, l'alterata interazione tra i tetrameri dell'emoglobina, dovuta alla variante, determina la formazione di eritrociti a falce, responsabili delle crisi dolorose, degli stroke e dell'emolisi cronica. L'HbS si può riscontrare in eterozigosi (A/S), in omozigosi (S/S) e in eterozigosi composta (S/S, S/C, S/D, S/β…). Le ultime due forme sono associate a quadro clinico grave (SCD), ed anche l'eterozigosi, che si riteneva fosse una condizione benigna, si può accompagnare a complicanze quali ipostenuria, ematuria, carcinoma renale, necrosi papillare renale, glaucoma per occlusione dei vasi retinici, tromboembolismo venoso ed embolia polmonare, osteonecrosi, rabdomiolisi, morte intrauterina, complicanze post stress fisico negli atleti fino a morte improvvisa. Gli autori considerano l'importanza di identificare i portatori di questa variante per la prevenzione delle patologie ad essa associate e per la prevenzione del rischio di coppia. Nel nostro laboratorio di 1° livello dal 2005 al 2008 sono stati riscontrati 7 casi di HbS, di cui uno in associazione con l'α talassemia e dal 2015 al 2018 sono stati individuati 20 portatori così suddivisi: 16 HbS trait, 3 HbS+ α talassemia e 1 HbS + δ talassemia. L'aumentato numero di portatori di HbS, essenzialmente di nazionalità straniera indica una nuova emergenza e la necessità di una maggiore attenzione da parte dei laboratori specialistici di 1° livello.

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P095
VALUTAZIONE DEL PATRIMONIO MARZIALE IN GIOVANI DONNE DEL DISTRETTO OVEST DELL'ULSS 8 BERICA

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Il Distretto Ovest dell'ULSS 8 Berica rispecchia quella società multietnica che sta interessando tutto il nostro Paese, infatti il 13.47% dei residenti provengono dal Sud-est asiatico e regione sub-Sahariane e in questo microcosmo gli aspetti socioeconomici, culturali e religiosi si trasformano in "culture alimentari" in grado di incidere sulla salute. L'anemia è un sintomo di varie malattie e spesso può essere sottostimata come indice di disordini funzionali eritropoietici dove spesso gli "stili e le scelte di vita" possono essere le concause. Scopo di questo lavoro è stato quello di valutare lo stato marziale delle ragazze adolescenti (tra i 14 e i 25 anni) mediante l'analisi statistica dei valori di Emoglobina (Hb), Ferro(Fe) e Ferritina (Ferr). Materiale e metodi: sono stati confrontati i valori di Hb, Fe e Ferr di 1989 ragazze nate tra il 1993 e il 2004 (79% adolescenti Italiane, 21% adolescenti Multietniche) che in 12 mesi si sono rivolte al nostro Laboratorio per esami di routine e suddivisi in 2 gruppi (adolescenti Italiane: ad ITvsadolescenti Multietniche: ad M.Et). I dati sono stati estrapolati dalla funzione MDQ del programma TDSynergy-Siemens. La determinazione quantitativa della Ferr e del Fe è stata effettuata sul sistema Cobas C 702 ROCHE/HITACHI mediante test ECLIA e colorimetrico; Hb è stata determinata nello strumento XE 2100 DASIT con principio di rilevazione fotometrica a 555 nm. Risultati:ad ITHb: mediana135gL; Hb IQ: 128-141;min/max 86-167; % al di sotto dei 120 gL = 10 %; ad M.EtHb: mediana 125gL; Hb IQ: 114-134; min/max 63-157; % al di sotto dei 120 gL = 37 %;ad IT Ferr: mediana 31 µg/L; IQ: 18-52;min/max 2-365; ad M.EtFerr: mediana 21 µg/L; IQ: 12-34;min/max 3-140;ad IT Fe: mediana 85 µg/dl; Hb IQ: 60-113;min/max 18-204; ad M.EtFe: mediana 72 µg/dl; IQ: 40-99;min/max. 12-231. Discussione: la mediana dei parametri rimaneentro i valori di normalità per età e per sesso, tuttavia nel gruppo multietnico tutti i valori sono sensibilmente più bassi e il 37 % delle ragazzepresenta un medio-grave stato anemico. È nostra intenzione estendere lo studio anche all'età infantile e di approntare un'algoritmo informativo per segnalare al medico curante la necessità di approfondimento diagnostico per un potenziale stato anemico.

P096
DIAGNOSI EMOMETRICA DI LINFOMA

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Introduzione: Lo scopo di questo lavoro è porre l'attenzione sull'importante ruolo che ad oggi riveste la medicina di laboratorio nella valutazione di patologie di natura ematologica. E' emersa l'utilità del confronto tra laboratorista e clinico per poter inquadrare in tempi brevi patologie complesse e specialistiche.
Materiali e Metodi: Nel gennaio 2018 Una paziente donna (aa 76) esegue un emocromo presso il laboratorio specialistico di Ematologia dopo essere stata valutata in struttura convenzionata esterna. Arriva alla nostra attenzione con un sospetto di disordine mieloproliferativo. L'attenta lettura dei dati, corredata dall'osservazione della disposizione grafica pone il sospetto di un probabile disordine linfoproliferativo.
Risultati: L'ulteriore valutazione morfologica conferma il sospetto di disordine linfoproliferativo: presenza di cellule linfomatose;cromatina compatta con nucleoli di grandi dimensioni; citoplasma delimitato ma dai contorni indistinti e lievemente basofilo.
Il caso viene posto all'attenzione dei colleghi del laboratorio di Citofluorimetria che così conclude: si conferma il sospetto diagnostico: quadro immunofenotipico compatibile con disordine linfoproliferativo B tipo LNH CD19 + CD20+ (a) FMC7+ CD5- CD200- CD23- CD10- CD103- e restrizione clonale per le catene leggere sK delle Ig.Conclusioni: L' utilizzo di analizzatori di nuova generazione consente ad un esperto laboratorista di fornire ai clinici strumenti adeguati per formulare diagnosi anche per patologie complesse e specialistiche. Utilizzando le più avanzate ed innovative tecnologie di analisi ad oggi disponibili in campo ematologico, si assiste ad un continuo progredire della ricerca e dello sviluppo garantendo così la scelta dei metodi più idonei in grado di assicurare analisi accurate ed informazioni utili nella diagnostica ematologica.

P097

UNO STRANO CASO DI ANEMIA, PIASTRINOPENIA CANDIDOSI OROFARINGEA IN PAZIENTE CON FIBRILLAZIONE ATRIALE IN NAO: A CASE REPORT

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Introduzione: L'esordio della sindrome mielodisplatica (SMD) nell'anziano può manifestarsi con anemia, piastrinopenia, leucopenia. Inoltre le infezioni da Candida sono di frequente riscontro in pazienti con SMD. Tuttavia secondo recenti report i pazienti in trattamento con i nuovi anticoagulanti orali (NAO), possono sviluppare piastrinopenia indotta (dabigatran,apixaban,etc.).
Riportiamo il caso di un uomo di 88 anni con SMD, infezione da Candida, Fibrillazione atriale (F.A.) in terapia con NAO.
Metodi: Giunge in data 16-03-2018, presso la nostra U.O. di Subacuti di Calcinate (BG), paziente di 88 anni con la seguente storia: Ipertensione arteriosa; FAP; TURP per npl; stenosi aortica moderata (09/2017),pregressa TVP in terapia con Dabigatran da Ottobre 2017. All'ingresso in reparto paziente eupnoico,con faringodinia ed esami ematochimici nella norma, tranne riscontro di lieve leucopenia all'emocromo.
Eseguiti durante degenza tamponi faringei per ricerca batteri e miceti, risultati positivi a Candida Albicans, per cui impostata terapia con fluconazolo per alcuni giorni.
In data 30-03-2018 a seguito di anemia severa con valori di Hb 8.2 g/dl si eseguiva trasfusione di 1 sacca di GRC.
In data 01-04-2018 episodio di tachipnea con tachiaritmia da F.A. e dispnea acuta, per cui impostata terapia diuretica e corticosteroidea con lieve beneficio; tuttavia venivano eseguiti esami ematochimici e strumentali in urgenza, visto il rapido peggioramento del quadro clinico. Un Rx torace evidenziava: congestione polmonare. ipoventilazione dei lobi inferiori; ingrandimento dell'immagine cardiaca. L'emocromo mostrava:WBC 2.65 x 10^9 /L; Hb 9.6 g/dl, PLT 68 x 10^9/L sideremia 8 mcvg/dL; Na 136
mEq/L; K 4.8 mEq/L. PCR 10.57 mg/dL, per cui visto il quadro di citopenia trilineare con anemia, piastrinopenia e leucopenia, furono eseguite emotrasfusioni d'urgenza e terapia corticosteroidea ad alto dosaggio.
Risultati: Uno striscio di sangue periferico ha mostrato linfociti atipici ed aggregati piastrinici. Veniva eseguito aspirato midollare ed esame citofluorimetrico su sangue periferico e sangue midollare; si concludeva per SMD e veniva dunque consigliata soltanto terapia di supporto. Con l'inizio di terapia con digitale ed isoptin si è avuto miglioramento della sintomatologia e ripristino del ritmo sinusale. Fu sospesa inoltre terapia con dabigatran nel sospetto di piastrinopenia da NAO ed iniziata terapia con EBPM.
Conclusioni: La diagnosi di SMD nell'anziano è complessa e prevede esami ematologici specifici, tuttavia per la presenza di comorbidità diventa sempre più complessa la gestione ed il management di questi pazienti, sopratutto in soggetti con FA in NAO.

P098

A RARE CASE OF LYMPROLIPHERATION ADENOPATHY MIMIC ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA

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Angioimmunoblastic T-cell lymphoma (AITL) is a rare hematologic neoplasm, typically presents with B symptoms. Its overall prognosis is poor, with a 5- year survival rate of 30%.
We report a case of a 53 years old black man who developed progressive diffuse lymphadenopathy with associated fever, hacking cough, fatigue and loss of dynamic. Total body CT scan showed numerose nodular denser areas with irregular margins at inferior and medium lobes lung and multiple air boil also in apical bilateral and multiple lymphadenopathy upper diaphragm and under diapraghm subcentimetric lymphadenopathy. HCV total ab, BK research, HIV ab, HbsAg, Toxoplasma gondii IgM, EBV IgM were negatives. EBV ab IgG anti capsidis were 34.8 UI/mL (n.v.<1), Toxoplasma gondii IgG were 243UI/mL (n.v.<4). Uricaemia and LDH levels were within normal levels while reactive C protein was increased. Hypergammaglobulinaemia IgG 3126 mg/dl (700-1600) and moderate normocytic anemia (HB 9.8 g/dl), were seen. Total excissional biopsy of integral inguinal sx lymphonode di 4.5cmx2.5x1.5 was performed showing a conservative lymphonode architecture, paracortical zone expanded with prominent vascular proliferation, residual germinative centres seemed “burn out” likely, with lax aggregate of dendritic cells CD21+ and histiocytes and lymphocytes B CD20+, bcl-2 negative; Ki67 resulted in a reactive pattern suggestinga “picture of reactive lymphadenitis, with reminiscent aspects of angoimmunoblastic lymphadenopathy.”Medullar agoapirate didn’t show morphologically lymphocyte proliferation. At medullar biopsy lymphocytes B CD20+ were 5% and lymphocytes T CD3+ were 6% at random interstitial distribution without any clonal lymphocytes prolipheration.
He went into spontaneous remission of clinical symptoms after antibiotic therapy and he was in good healthy at 2 months follow-up.
AITL spontaneous remission is very uncommon occurrence. WHO revision acknowleged the complexity of AITL diagnosis additioning other AITL-like subsets. The case reported diffuse lymphadenopathy likely benign unidentified virus-associated process which mimic AITL morphologically at excisional biopsy, emphasizing need of close specialysts communication for interpretation of clinical and laboratory findings.

P099
AMILOIDOSI AL: PRESENZA DI CM DI ISOTIPO DIVERSO DALLA CATENA AMILOIDOGENICA RISCONTRATA IN CORSO DI FOLLOW-UP

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I criteri di risposta ematologica, stabiliti dall'International Amyloidosis Society, in pazienti affetti da Amiloidosi AL (AL) in trattamento, sono: Risposta Completa (CR), rapporto catene leggere libere sieriche (rFLC) normale e immunofissazione siero e urine (IFEs/u) negativa; Risposta Parziale Molto Buona (VGPR), differenza tra catena leggera libera amiloidogenica e non (dFLC) < 40 mg/L; Risposta Parziale (PR), riduzione dFLC ≥ 50%; Non Risposta (NR) tutti gli altri.
Presso il D.A.I di Medicina di Laboratorio dell'AOU Federico II di Napoli viene eseguito il follow-up dei pazienti affetti da AL impiegando: elettroforesi capillare zonale proteine sieriche (CZE, Capillarys2, Sebia); IFEs/u e elettroforesi proteine urinarie (Hydrasys2-Sebia); FLC (N-Latex, Siemens, V.N. rFLC = 0,31-1,56).
Dall'analisi di 30 pazienti affetti da AL, seguiti presso l'Ematologia dal 2014 al 2018 in follow-up, trattati con terapia di prima linea con Bortezomib e Desametasone + Melphalan o Ciclofosfamide, è stata osservata in 3/30 (10%) all'IFEs, e in 1 dei 3 anche all'IFEu, la comparsa di CM costituita da catena leggera diversa da quella amiloidogenica. Pazienti: 1 (F, 51a) amiloidosi k, localizzazione cardiaca (CM IgA k→IgG λ); 2 (M, 63a) amiloidosi λ, localizzazione cardiaca, renale, intestinale (CM λ → IgM k e BJ λ→ k); 3 (F, 52a) amiloidosi λ, localizzazione renale (CM IgG λ→IgG k).
In tutti e 3 i pazienti l'isotipo dell'amiloide alla diagnosi è stato confermato mediante microscopia elettronica su grasso periombelicale; il cambiamento della CM è stato rilevato in presenza di rFLC normale e in corso di miglioramento degli indici biochimici di danno d'organo (BNP, troponina cardiaca, creatinina, ALP).
Il fenomeno osservato è in linea con quanto riportato in letteratura, anche da noi precedentemente, in pazienti affetti da MM, dove la presenza in corso di follow-up di CM di isotipo diverso dalla CM riscontrata a diagnosi è associata a una prognosi migliore e a una buona risposta alla terapia. Ci sembra importante evidenziare come in corso di AL la positività all'IFEs/u per CM con catena leggera diversa dalla proteina amiloidogenica possa essere comunque considerata una CR; resta da verificare se nel tempo tale CM possa divenire anch'essa amiloidogenica.

P100
EVALUATION OF SYSMEX XN-9000 FOR DETECTION OF MALIGNANT CELL IN PLEURAL AND ASCITIC FLUID

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Differentiation of nucleated cells including malignant cells in various body fluid (BF) samples is an essential technique to determine the clinical treatment strategies. Many of the automated hematology analyzers nowadays available offer an integrated platform specifically designed to give the advantages of both rapidity and standardization of BF analysis. The BF Mode of the Sysmex XN-9000 hematology analyzer differentiates white blood cells and other than blood cells such as high-fluorescent cells (HFC). HFC can represent malignant cells, as well mesothelial cells or macrophage. In this case microscopic review is necessary for discrimination. The purpose of this study was to evaluate the performance of the HFC count on Sysmex XN-9000 BF-Mode in cytometric analysis for detecting malignant cells in pleural fluids (PF), according to the International Cut-offs recommended by CLSI H56-A guideline(1), i.e. Nucleated Cells (NC) ≥1000*10#cells/L with Polymorphonuclear >50% or Lymphocytes cell count >50%. A total of 94 consecutive fresh samples of pleural fluids collected in K_3EDTA tubes and with total cellularity range between 22to 45770*10#cells/L were analyzed up to 2 h without pre-treatment using the XN-9000 BF-Mode and then were microscopically screened on cytospin slides for the presence of malignant cells. The laboratory analysis included automated total and differential cell counts and manual WBC total and differential counts (Nageotte improved chamber and cytocentrifuged air-dried hematological staining of May-Grunwald Giemsa). Several validation rules have been established for the analysis of body fluid. Samples with HFC> 68 x 10^6cells/µL(cut-off established) were blocked for microscopic review.42 body fluids had a number of HFC>68. On the cytospin smears 35 samples were characterized of mesothelial cells and 7 were really characterized of malignant cells.The correlation between the above mentioned methods was assessed by Pearson's coefficient, Passing-Bablok regression and Bland-Altman bias. Diagnostic accuracy was determined with ROC curve analysis. The statistical analysis was carried out with Analyse-it Software. According to the results obtained, HFC count can be useful tool to select samples for microscopic review.

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IDENTIFICAZIONE DI UN CASO DI LINFOCITOSI B MONOCLONALE (MBL): SUPPORTO DIAGNOSTICO DELL'ANALIZZATORE BECKMAN DXH800

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Scopo: presentare il caso di un paziente di 87 anni ricoverato per riacutizzazione di BPCO, che una settimana dopo la sospensione del cortisonico, manifesta nel sangue periferico la presenza di rari linfociti atipici, in assenza di linfocitosi assoluta e di allarmi strumentali per linfociti atipici. L'esame obiettivo non rileva organomegalie o linfoadenomegalie; l'eco addome non evidenzia lesioni; la TAC del torace presenta una pseudonodularità sub-mantellare al lobo polmonare inferiore destro. Materiali e metodi: è stato eseguito l'emocromo (Unicel DxH800, Beckman Coulter) con striscio di sangue periferico e l'immunofenotipo di membrana dei linfociti su sangue periferico (FACSCanto, Becton Dickinson). Risultati: al momento della diagnosi, i linfociti risultano pari a $1.12x10^3$/uL. Il vetrino viene esaminato per pseudopiastrinopenia da aggregazione EDTA-indotta (piastrine pari a $59x10^3$/uL, sottostimate dalla presenza di aggregati). Dall'analisi del citogramma strumentale WBC dell'emocromo, risulta presente una popolazione di linfociti distinta sulla base del parametro dimensionale con una deviazione standard (@SD-V-LY) aumentata rispetto ad un cut-off di normalità. Al vetrino, questi linfociti appaiono di dimensioni aumentate, talora nucleolo visibile, ampio citoplasma, a volte con estroflessioni villose. Dalla valutazione citofluorimetrica, emerge che il 42% dei linfociti totali è costituito da linfociti B con restrizione clonale per le catene leggere lambda ed il fenotipo CD5-, CD10-, CD23-, FMC7+, CD103-, CD25-, CD200+, CD11c-, CD20+ brillante, CD22+ brillante, CD43-. Discussioni e conclusioni: sulla base della valutazione morfologica e degli accertamenti eseguiti, questo caso può essere classificato come linfocitosi B monoclonale (MBL). A fronte di una obiettività clinica e di esami strumentali negativi, la diagnosi è stata condotta sulla base della valutazione del citogramma WBC, sul parametro posizionale @SD-V-LY e sull'esame dello striscio di sangue periferico, in assenza di linfocitosi assoluta e di allarme per linfociti atipici; pertanto la creazione di una opportuna regola strumentale, basata sui Cell Population Data, è in grado di fornire un valido aiuto nell'identificazione dell'anomalia linfocitaria per un più rapido orientamento diagnostico.

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IL DOSAGGIO DELLA GLUCOSIO 6 FOSFATO DEIDROGENASI (G6PD) NEI PAZIENTI ETEROZIGOTI: CONFRONTO TRA METODI

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Introduzione: la G6PD carenza è causata da mutazioni selezionate dall'infezione del parassita malarico, per la protezione conferita dalla carenza enzimatica nei confronti della malattia. Sia allo stato di omozigosi che di eterozigosi, la carenza di G6PD causa gravi crisi emolitiche dopo ingestione di fave o di farmaci pro-ossidanti. L'identificazione degli omozigoti non presenta particolari difficoltà diagnostiche. Al contrario, la diagnosi di eterozigosi è difficile per una serie di fattori quali la variabilità individuale dei livelli enzimatici, la variabilità dovuta alla misura additiva della 6PGD, la variabilità del sistema di riferimento se il dosaggio viene rapportato ai valori di Hb, l'instabilità dell'enzima, la contaminazione leucocitaria. Metodi: 256 campioni (> 1 mese: 116 F e 108 M - < 1 mese: 12 F e 20 M) sono stati testati sia con metodica Sentinel® che con metodica Nurex®. Con metodica Sentinel® il risultato si può esprimere in attività enzimatica della G6PD come mU/106 eritrociti o come mU/g di Hb. In entrambi i casi è necessario eseguire l'esame emocromocitometrico. Con la metodica Nurex® il valore di G6PD può essere espresso come rapporto G6PD/6PGD ove la misura quantitativa della G6PD viene normalizzata rispetto ai valori di 6PGD evitando l'interferenza dovuta alle variazioni individuali di Hb, reticolociti e leucociti o come percentuale di attività enzimatica che conserva i vantaggi del rapporto G6PD/6PGD. Entrambi i metodi sono validati su numerose strumentazioni analitiche di Chimica Clinica e nel nostro Laboratorio sono stati implementati su ADVIA2400-Siemens. Risultati: i due metodi correlano sempre nei 14 pazienti con carenza totale di G6PD e in tutti i pazienti normali. 21 casi di non correlazione sono stati riscontrati in pazienti microcitemici e con anemia sideropenica. Conclusioni: l'introduzione del dosaggio di G6PD con metodo Nurex® ha permesso la diagnosi di eterozigosi sia in neonati che in pazienti con crisi emolitica evitando l'esecuzione dell'esame emocromocitometrico in quanto il test è indipendente dal dosaggio di Hb: pertanto non risente della variabilità individuale nei pazienti talassemici, microcitemici e nei neonati. Ulteriore conferma potrà essere consolidata dall'approfondimento in biologia molecolare.

P103
L'UTILITÀ DEL SOFTWARE SYSMEX NETWORK COMUNICATION SYSTEM (SNCS) NELLA VALUTAZIONE DI QUALITÀ DEI NUOVI PARAMETRI EMATOLOGICI

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Scopo: Gli emocitometri di ultima generazione hanno reso disponibili nuovi parametri quantitativi e qualitativi utili per la caratterizzazione delle cellule del sangue periferico. L'obiettivo di questo lavoro è di illustrare i nuovi parametri della strumentazione Sysmex XN (Sysmex Corp., Japan) ed i vantaggi della valutazione di qualità mediante software SNCS (Sysmex).
Materiali e metodi: L'analizzatore Sysmex XN fornisce oltre ai 21 parametri standard i seguenti parametri: granulociti immaturi (IG); frazione di piastrine immature (IPF); contenuto emoglobinico reticolocitario medio (RETHe). Sono stati analizzati i 3 livelli di CQI (XN-CHECK basso, normale e alto) di questi parametri mediante software SNCS. Il nuovo software SNCS permette di gestire a livello multicentrico i dati di laboratorio. I Coefficienti di Variazione (CV) dei CQI dei parametri IPF, RET-He, IG di un mese di osservazione (31 determinazioni) (CV-LAB), sono stati confrontati con i CV del gruppo omogeneo (CV-O) elaborati dal software SNCS.
Risultati: Nel mese di osservazione sono stati ottenuti i seguenti risultati: per il CQI livello basso, IPF(%) CV-LAB di 1.84% vs CV-O del 1.98%; RET-He(pg) CV-LAB di 0.99% vs CV-O del 2.32%; IG (10^9/L) CV-LAB di 3.67% vs CV-O del 3.90%. CQI livello medio: IPF(%) CV-LAB di 2.77% vs CV-O del 2.53%; RET-He (pg) CV-LAB di 1.07% vs CV-O del 2.30%; IG (10^9/L) CV-LAB di 3.11% vs CV-O del 3.12%. CQI livello alto: IPF (%) CV-LAB di 1.97% vs CV-O del 2.23%; RET-He (pg) CV-LAB di 1.06% vs CV-O del 3.82%; IG (10^9/L) CV-LAB di 3.47% vs CV-O del 3.35%. Dall'analisi dei dati si può osservare come i CV del laboratorio risultano essere perfettamente allineati con i CV dei controlli del gruppo omogeneo.
Conclusioni: Alcuni dei nuovi parametri descritti sono strumento specifici e non esistono materiali di controllo di terza parte. Tali parametri andrebbero utilizzati con cautela in quanto viene a mancare la dimostrazione (seppur indiretta) della validità della misura. SNCS permette di confrontare i valori del laboratorio con quelli del gruppo omogeneo, consentendo una gestione più puntuale dei nuovi parametri ematologici, nonostante la mancanza di una valutazione mediata da controlli di qualità di terza parte.
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P104
NEPHELOMETRIC ASSAY OF URINE FREE LIGHT CHAINS: AN ALTERNATIVE AND EARLY CLINICAL TEST FOR BENCE JONES PROTEIN QUANTIFICATION

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Background: Free Light Chains (FLCs) are an important diagnostic marker for monoclonal gammopathy (MG) and the presence of Bence Jones Protein (BJP) in the urine has been the key indicator of immunoglobulins (Ig) production. They are useful to perform the diagnosis of plasma cells dyscrasias, especially for those oligo-secretory, to follow up therapy in Multiple Mieloma (MM) and they may be an early indicator of renal damage. BJP has been the key indicator of light chain immunoglobulins presence and is used to monitor response to therapy. We have highlighted in a previous paper the 34 positive patients for nephelometry and negative for immunofixation electrophoresis (IFE). The work aim was to follow these patients to see if the urine FLCs (u-FLCS) could predict a subsequent IFE positivity. Materials and methods: We collected samples from the second morning void and the urine were not concentred. We performed FLCs measurement using N Latex FLC kit based on a mixture of monoclonal antibodies for use on the BN ProSpec® System analyzer. Patients came from Haematology Department and with diagnosis of MM, non-Hodgkin lymphoma, MG and MG of undetermined significance (MGUS) with periodic controls of BJP approximately every 1, 3 or 6 months depending on the severity of the diagnosis. Results: 31 patients on 34 became positive for BJP in a range time from 1 to 48 months. Many patients were positive for BJP within two years (23/31 about 75%) while one part was positive after two years and up to four years (8/31 about 25%), the latency time for BJP presence was not related to the pathologies. Conclusion: These results suggested that nephelometric assays provide an alternative clinical test for BJP quantification and the u-FLCs early detection might be important in clinical diagnosis, therapy and follow up.

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RISK ESTIMATE AND FEATURES OF INFECTIOUS EVENTS IN SUBJECTS WITH NEUTROPENIA

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Neutropenia is usually diagnosed when the absolute neutrophil count (ANC) is less than 1,500 cells/µL. Individuals with reduction of this blood cells number are predisposed to bacterial and viral infections. Often, neutropenic subjects present mild or even absent clinical manifestations of infectious events, thus delaying diagnosis, initial treatment and follow-up. It was demonstrated that the duration and severity of neutropenia was directly related to the risk of infection. This work aims to analyze the relationship between the degree of neutropenia and the occurrence of infectious events as well as their features (site of infection and type of microorganism). Laboratory data was collected from a sample of subjects consecutively assessed from 1990 to 2016 in the Italian Hospital of Desio, Lombardy. A total of 708 subjects met the study criteria. ANC, white blood cells count, and neutrophil-to-lymphocyte ratio present similar power for predicting the outcome of infectious episodes. Logistic regression analysis indicated a progressive increment of risk of infection with the decrease of ANC levels: adjusted odds ratios [95 % confidence interval (CI)] were 3.23 (2.00-5.23), 2.37 (1.21-4.64), and 6.14 (3.03-12.41) respectively for mild (1,000-1,500 cells/µL), moderate (500-1,000 cells/µL), and severe (< 500 cells/ µL) neutropenic subjects (p trend: < 0.0001). Likewise, a higher risk of involvement of respiratory tract [adjusted odds ratio 14.18 (5.36-37.50)] and systemic infections [adjusted odds ratio 14.94 (3.77-59.16)] was observed in severe neutropenic individuals (p < 0.0001). In mild-moderate neutropenic subjects a significant higher risk, even if minor, was only observed in respiratory tract [adjusted odds ratio 4.44 (2.08-9.48)]. Moreover, a major risk to Gram-positive infections was observed in severe neutropenia [adjusted OR 3.03 (1.09-8.40)]. Our work urges to bring attention to the degree of neutropenia often related to different features of infection.

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IDENTIFICATION IN SOUTHERN ITALY OF -α3.7 THALASSEMIA ASSOCIATED WITH THE DELETION OF AC AT POSITION -2 AND -3 PRECEDING THE AUG CODON

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α-thalassaemia may be caused by large deletions of the α-globin gene(s), or rarely, non-deletional mutations. Both types of mutations may co-exist, and if located on the same allele can cause an α^0-thalassemia phenotype, producing a reproductive risk of hydrops fetalis. In a study on the molecular epidemiology of α-thalassemia in Southern Italy we found that the most frequent mutant is the $-\alpha^{3.7}$ deletion with a relative frequencies of 58%. Some asymptomatic patients with $-\alpha^{3.7}$ thalassaemia trait and normal iron status were noted to have significant microcytosis that was insufficiently explained by a single $-\alpha^{3.7}$ deletion.
Molecular characterization revealed the deletions of AC at the dinucleotides preceding the AUG codon, associated to the $-\alpha^{3.7}$ allele[1]. An ARMS protocol was set up for the direct identification of the $-\alpha^{3.7\text{-}AC}$ and used for the wide analysis of about 700 $-\alpha^{3.7}$ alleles[2]. The analysis of the data indicated the presence of the –AC microdeletion in 5% of the $-\alpha^{3.7}$ carriers, in families coming from different Italian regions. T-Test analysis revealed that the MCV and MCH value of 35 $-\alpha^{3.7\text{-}AC}$ carriers was intermediate and with difference statistically significant respect either to α^+thal and α^0thal carriers. We observed also complex genotype: in 2 unrelated families, with high level of Hb A2 (5.8-6.1), the patients were double heterozygotes for $-\alpha^{3.7\text{-}AC}$ and β-thalassemia; 2 members of 1 family were compound heterozygotes for the $-\alpha^{3.7\text{-}AC}/\alpha^{PolyA\ SA}\alpha$; 1 patient was homozygotes for the $-\alpha^{3.7\text{-}AC}$. The last two class of patients are very interesting to highlights the weak contribution of the $-\alpha^{3.7\text{-}AC}$ allele to the synthesis of α-globin gene as indicated by the severe phenotype with Hb value respectively of 10.4 and 7.2 (compound heterozygotes) and 8.4 (homozygotes). The homozygote $-\alpha^{3.7\text{-}AC}$ patients showed a typical HbH disease phenotype with the presence of 7.7% of HbH.

These data indicated that the $-\alpha^{3.7\text{-}AC}$ show a severe phenotype and is present in Italy: its identification through accurate genotyping of α-globin determinant is absolutely required considering that could give rise to a reproductive risk for Hb Bart's hydrops fetalis if in association with a α^0 thal.

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SIX NEW ALPHA-THALASSEMIA MUTANTS IDENTIFIED IN SOUTHERN ITALY

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α-thalassemia is a hereditary microcytic anemia caused by structural defects involving one or both of the duplicated 5'-3' α2 and α1 globin genes, on chromosome 16. The hematologic diagnosis is hindered by the absence of markers. Achieving a molecular diagnosis is relevant to the prevention of severe α- and β-thalassemia.

In an epidemiological study of molecular basis of α-thalassemia in Southern Italy were selected families showing microcytosis with normal level of HbA2 and iron. Molecular screening -through DGGE, gap-PCR, ARMS, MLPA and sequencing- brought us to identify the molecular basis in about 1300 chromosomes. Single point mutations account for the phenotype in 28% of carriers and among them, 4 mutations (α2IVS-I–5nt; α2AATAAA>AATAAG, $-\alpha^{3.7\text{-}AC}$, α2ATG>ACG)[1] have the higher relative frequency, but there are an increasing number of new pointform mutations. We report below 6 new alleles: Hb Bernalda (α1 cod119 CCT>TCT), Hb Policoro (α2 cod124 TCC>CCC)[2], Hb Sciacca (α1 cod109 CTG>-TG), HbA1 cod23 GAG>TAG Glu>Stop[3], Hb Rogliano (α1 cod108 ACC>AAC)[2], HbA1 cod22 GGC>GGT[3]. Hb Bernalda, Hb Policoro, Hb Sciacca, Hb Rogliano are unstable variants with α-thal phenotype, cod23 G>T generates a premature stop, cod22 C>T an alternative splicing site.

Hb Bernalda is the 5th most frequent (5%), while Hb Sciacca, Hb Policoro and cod22 shown a frequencies comparable with Hb Constant Spring and Hb Icaria (2%), moreover they are found in several Italian regions. Cod22 and Hb Rogliano are identified respectively in 1 and 2 families. We set up an ARMS protocol for the genotyping of 5/6 of these new alleles.

Misdiagnosis of α-thal leads to repeated and unnecessary haematological analysis to assess the presence of microcytosis. α-thal molecular screening usually consists in the analysis of a panel of the most common mutations widespread in the world. We identified 6 new α-thal mutants in several families of different Italian regions. As the 6 new alleles have a total frequency of 10%, some

showing a geographic prevalence, we suggest to test for these mutants the samples negative at the analysis for the most frequent α-thal mutations.

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THE USEFULNESS OF LYMPHOCYTE CELL POPULATION DATA IN DIFFERENTIAL DIAGNOSIS OF LYMPHOCYTOSIS

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Introduction: The development of a new generation of hematological analyzers can identify cellular abnormalities with high efficiency. The cell population data (CPD) can be used for the screening of several hematological and non-hematological disorders. The Sysmex XN-9000 (XN-module) besides the conventional hematologic parameters, generate 18 CPD parameter of neutrophils, lymphocytes (LY) and monocytes. Their assessment may be useful in the diagnosis of myelodysplastic syndromes and sepsis, but there are few studies about the application of LY CPD parameters in differential diagnosis of lymphocytosis (i.e., viral diseases from lymphoproliferative disorder). The aim of this study was to evaluate the usefulness of the LY CPD parameters to differentiate among acute lymphoblastic leukemia (ALL), other lymphoproliferative disorders (abn-LY), non-neoplastic lymphocytosis (reac-LY), acute myeloid leukemia (AML) and other hematological disease (oth-HD).

Methods: The study population included 742 patients (16 with ALL, 150 with abn-LY, 34 with AML, 59 with reac-LY and 488 with oth-HD). We evaluated the following LY CPD parameters obtained by XN-module: LY-X, LY-Y, LY-Z, LY-WX, LY-WY and LY-WZ. The diagnostic performance was evaluated by ROC curve analysis.

Results. LY-WX LY-WY and LY-WZ showed an area under the ROC curve (AUC) of 0.86, 0.85 and 0.75 respectively, in the discrimination between reac-LY and other groups; LY-X and LY-Z showed an AUC of 0.60 and 0.69 respectively, in the discrimination between abn-LY and other groups; LY-X showed an AUC of 0.82 in the discrimination between ALL and other groups. The combination of LY-WX, WY and WZ parameters in LY–react-factor showed an AUC of 0.87, differentiating react-LY from other diagnostic groups.

Conclusion: The lymphocytes positional parameters provided by XN-module are useful to differentiate react-LY (LY – React-factor) and ALL (LY-X) from other diagnostic groups. These parameters are very useful for detecting changes in the lymphocyte population; in fact, the identification of these alterations can induce the need to carry out a blood smear review in samples without morphological flagging.

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UPDATING PROCEDURES IN HEREDITARY HEMOLYTIC ANEMIAS: TGA/CHEMOMETRICS AS A NEW PROMISING TOOL

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Thermogravimetry coupled with chemometrics proved to be a rapid and cost effective diagnostic tool for β-thalassemia screening. This model, consisting of Partial Least Square-Discriminant Analysis (PLS-DA), permitted the discrimination of thalassemic patients and healthy individuals, using thermogravimetric curves of blood samples [1, 2]. In addition, TGA/Chemometrics method permitted to differentiate thalassemic patients according to the severity of anaemia while the evaluation of the indices and the CBC are not able to identify TI-TD, TI-NTD and TM-TD patients at first level test [3]. In this study, the capability of thermogravimetry in conjuction with a multivariate statistical analysis, was investigated for the screening of hereditary haemolytic anemias due to different erythrocyte defects. Two groups of anemias were considered: the hemoglobinopathies (sickle cells anemia and thalassemia) and erythrocyte membrane defects (hereditary elliptocytosis and hereditary spherocytosis). Whole blood samples from patients with congenital defects were analyzed by the TG7 thermobalance (Perkin Elmer) without any pretreament and the resulting curves were compared to those typical of healthy individuals. The TG and DTG curves of blood samples from anemic patients were clearly distinct from those of healthy individuals as result of the different amounts of water content and corpuscular fraction. The chemometric approach based on Principal Components Analysis (PCA) allowed a quick identification of differences between healthy and anemic patients in order to point out a model of prediction in patients with heterogeneous congenital hematological disorders. The model was validated and used to perform prediction of unknown anemias with 100% of correct classification rate. Results permit to consider the coupling TGA/Chemometrics as a promising diagnostic approach to provide a high-throughput and sensitive tool to obtain an early detection of hereditary hemolytic anemia.

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ESPERIENZA NELL' APPLICAZIONE QUOTIDIANA DI UN PROGRAMMA DI QUALITÀ INTER LABORATORIO TRAMITE IL NUOVO APPLICATIVO SYSMEX NETWORK COMUNICATION SYSTEM (SNCS)

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Scopo: Il controllo di qualità interno (CQI) è fondamentale nel monitoraggio della precisione e accuratezza del metodo analitico in uso. La valutazione quotidiana del CQI consente di mettere in atto azioni correttive al fine di garantire prestazioni adeguate[1]. Il software SNCS (accreditato ISO/IEC 17043)[2] (Sysmex, Kobe, Japan) permette di confrontare in tempo reale i dati del CQI con un gruppo di consenso. Lo scopo è valutare come questo programma permetta di migliorare la gestione del CQI garantendo un mantenimento delle performance analitiche in emocitometria.
Materiali e metodi: È stato valutato l'andamento mensile di 3 livelli di CQI (basso, normale e alto) processati quotidianamente su cinque analizzatori Sysmex XN per un totale di 597 determinazioni. I risultati ottenuti, media e deviazione standard (DS), sono stati valutati quotidianamente e contemporaneamente mediante il sistema SNCS e l'applicativo dei singoli emocitometri (carta Levey-Jennings)
Risultati: Per ciascun parametro di ciascun livello di CQI è stato individuato almeno un punto che nonostante fosse all'interno delle ±2DS nella carta di Levey-Jennings su singolo emocitometro, risultava superiore alle ±2DS nel sistema SNCS. In particolare su SNCS i reticolociti in valore assoluto si collocavano al di sotto del valore medio e nel 77% dei casi erano fuori di -2DS pur mostrando un buon livello di precisione (CV di 4.4%). Anche per l'ematocrito si osservava una lieve sovrastima in uno degli strumenti. Per il livello basso, 11 valori su 32 si collocavano al di fuori della 2DS superiore in SNCS. Lo stesso accadeva per 15 su 32 punti di controllo normale e per 10 su 32 controlli alti. Alcuni parametri (monociti e granulociti immaturi) mostravano lieve scostamento rispetto al gruppo di consenso solo per alcuni dei livelli di CQI. Tutte queste violazioni non erano evidenti sull'applicativo dei singoli emocitometri.
Conclusioni: L'uso di SNCS nella pratica di routine permette di individuare errori di accuratezza altrimenti non identificabili. In tutti i casi non conformi con SNCS sull'applicativo strumentale si osservava un buon livello di precisione (CV mai superiore a 4,6%) con nessuna violazione.
Queste evidenze confermano l'utilità di SNCS nel monitoraggio della qualità analitica. La valutazione di precisione ed accuratezza consente la rapida implementazione di eventuali misure correttive necessarie e garantisce una riduzione del rischio di errore.

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LA VALUTAZIONE PERIODICA DEL CONTROLLO DI QUALITA' CON APPLICATIVO SYSMEX NETWORK COMUNICATION SYSTEM (SNCS)

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Scopo: Il Controllo di Qualità Interno (CQI) valuta la Precisione e l'Accuratezza del proprio metodo analitico per garantire una migliore prestazione e prevenire derive (1). L'obiettivo è quello di illustrare l'utilità del nuovo software Sysmex Network Comunication System (SNCS) per il miglioramento delle procedure di controllo di qualità e quindi delle prestazioni analitiche del laboratorio.
Materiali e metodi: E' stata effettuata una valutazione comparativa tra i report dell'applicativo IPU presente su singolo emocitometro XN-Modulo (Sysmex, Kobe, Japan) ed i report mensili del sistema SNCS. La valutazione è stata eseguita su un solo lotto di controllo per i tre livelli XN CHECK (Sysmex) del Mese di Marzo (lotto: 803711) processato quotidianamente sui cinque emocitometri, per un totale di 597 determinazioni.
Risultati: Con l'applicativo IPU non si dispone di un vero e proprio report, ma solo dei dati di media, imprecisione e DS del parametro. Il report mensile SNCS accreditato ISO/IEC 17043 (2) fornisce il calcolo di MEDIA, DS e CV del laboratorio; permette di visualizzare graficamente l'andamento di esattezza e precisione dei parametri confrontandoli con quelli del gruppo omogeneo. L'esattezza viene indicata in termini di Indice di Deviazione Standard (SDI) del laboratorio e riportata in un range compreso tra -3 e +3. La Precisione è rappresentata dal relativo Indice (PI) posizionato in un range da 0 a +3. I dati sono anche rappresentati in grafici twin plot. La visualizzazione grafica è resa più agevole dalla presenza di uno spazio dedicato all'indicazione dell'eventuale allarme, se presente.
Conclusioni: Il report del CQI ottenuto con SNCS, comparato con le informazioni disponibili mensilmente su IPU, permette una migliore valutazione delle prestazioni perché mostra anche i dati comparati per gruppo omogeno di strumentazione. Il gruppo di consenso ha parametri di precisione e accuratezza più ristretti di quelli indicati nei documenti di controllo, permettendo così una gestione più puntuale della strumentazione in dotazione. Infine nel report SNCS, la visualizzazione in tabella o in grafico dell' andamento del CQI, permette una più semplice, veloce ed armonica valutazione dei risultati permettendo al nostro laboratorio il perseguimento del miglioramento continuo.
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HbS/Hb NOUAKCHOTT: DOPPIA ETEROZIGOSI, OSSERVATA PER LA PRIMA VOLTA IN ITALIA, VISIBILE SOLO IN ELETTROFORESI CAPILLARE

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Una donna di origini marocchine di 64 anni è giunta alla nostra osservazione per la determinazione dell'emoglobinica glicata. Il test è stato eseguito mediante HPLC utilizzando l'analizzatore Tosoh HLC-723G8 nella modalità "HbA1c Variant analysis mode" e in elettroforesi capillare (CE) con lo strumento Capillarys 2 Flex Piercing (Sebia) con il kit Capillarys HbA1c. Il valore di HbA1c in HPLC è risultato essere 68 mmol/mol con rilevazione di una variante emoglobinica del 33%, identificata come frazione H-V1 al tempo di ritenzione di 1.17 min. In CE il campione ha mostrato un profilo "Atipico" per presenza di frazioni anomale riconducibili ad una α variante e ad una β variante. Si è proceduto ad ulteriori approfondimenti eseguendo l'emocromo, la valutazione del bilancio marziale e l'assetto emoglobinico con programmi dedicati ("β-thalassaemia Analysis mode" per G8 e Capillarys Hemoglobin(e) su Capillarys 2 Flex Piercing (CAP 2FP)). I parametri ematochimici non hanno mostrato alterazioni significative mentre il cromatogramma ha confermato la presenza di una frazione aggiuntiva identificata come S+ del 33.1%, presumibilmente HbS in eterozigosi. L'elettroferogramma ha mostrato invece la presenza di 3 frazioni aggiuntive confermando l'ipotesi iniziale della presenza di una α variante (7.3%), di una β variante (32.4%) del loro ibrido $\alpha_2^*\beta_2^*$ (4.7%). Il campione è stato inviato ad un laboratorio di genetica per la genotipizzazione. L'analisi è stata condotta mediante sequenziamento diretto del DNA estratto da leucociti da sangue periferico, in particolare le regioni analizzate sono state: la sequenza nucleotidica -110 del 5' UTR all'IVS II-90 del gene β, la sequenza -100 al +20 3' UTR del gene α2 e la sequenza -140 al +20 3' UTR del gene α1. L'approfondimento diagnostico molecolare ha confermato la presenza della mutazione c.20 A > T sul gene β globinico (HbS) e della mutazione c.344C > T sul gene α2 (Hb Nouakchott). Mentre non sono stati riscontrati difetti a carico del gene α1. L'elevato potere risolutivo della CE e la chiarezza del profilo elettroforetico hanno quindi consentito l'identificazione di un doppio difetto in eterozigosi di tipo HbS/HbNouakchott visibile solo in CE, consentendo il corretto inquadramento diagnostico della paziente.

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VALUTAZIONE DELLE PRESTAZIONI DIAGNOSTICHE DEGLI ALLARMI “BLASTS?” E “ABN LYMPH?” DEL SISTEMA EMATOLOGICO SYSMEX XN

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L’emocromo è il test di screening fondamentale per l’identificazione e il monitoraggio di patologie ematologiche. I moderni analizzatori forniscono allarmi di presenza di blasti e linfociti atipici sospetti neoplastici (Linfo Atipici) la cui accuratezza diagnostica è legata ai metodi di analisi utilizzati. Il sistema Sysmex XN (Sysmex Corp., Giappone) oltre al canale di formula leucocitaria ha un canale opzionale dedicato (WPC, White Pathological Cells) per la rilevazione più specifica di queste cellule. Agli allarmi è associato un valore da 0 a 300 unità arbitrarie (Q-flag) che se uguale o maggiore a un valore soglia (100 nelle impostazioni originali) produce il rispettivo flag “Blasts?” o “Abn Lymph?”. Scopo del lavoro: valutare la concordanza dei flag Blasts e Abn Lymph di Sysmex XN rispetto all’esito della revisione microscopica (MO) su 84 campioni random destinati all’approfondimento morfologico. Materiali e metodi: Gli strisci di sangue periferico preparati con sistema Sysmex SP-10 sono stati esaminati in microscopia automatizzata (Sysmex DI-60) da un operatore esperto valutando, per ciascun campione, almeno 200 immagini di cellule leucocitarie. Risultati: dei 32 positivi a MO per Blasti (17) o Linfo Atipici (15) 29 avevano flag Blasts (19 campioni: 16 con Blasti e 3 con Linfociti Atipici al (MO) o linfociti neoplastici (10 campioni: 9 con Linfociti Atipici, 1 con Blasti a MO). Dei 3 campioni senza flag (positivi a MO per Linfociti Atipici): 2 avevano conteggio di 39 e 5 NRBC/100 WBC confermati a MO, 1 linfocitosi di 5.32x10^9/L e flag “WBC Abn Scattergram”. Dei 52 campioni senza Blasti o Linfo Atipici a MO: nessuno aveva flag Blasts e 22 avevano flag Abn Lymph. Di questi, in 16 si evidenziava a MO presenza di grandi linfociti granulati in percentuale inferiore a 5, per 13 dei quali il flag Abn Lymph aveva valore Q-flag=100. Conclusioni: i risultati evidenziano eccellente accuratezza diagnostica di Sysmex XN per la flag Blasts (Sensibilità, SE 94.1% - Specificità, SP 94.5%) e discreta per la flag Abn Lymph (SE 60.6% - SP 66.7%). La specificità della flag Abn Lymph migliora (91.3%) a valore di Q-flag pari a 110. Considerando globalmente le due flag e i risultati strumentali nessuno dei 32 positivi a MO sarebbe stato valutato negativo da Sysmex XN.

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SHORT-TERM ANALYSIS OF MGUS PATIENTS REFERRING TO THE LABORATORY OF MODENA (ITALY)

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Background: In the elderly population, Monoclonal Gammopathy of Undetermined Significance (MGUS) is a common condition associated with a significant risk of evolution to overt neoplastic diseases, i.e. Multiple Myeloma (MM) and lymphoplasmocytoid lymphomas (B-NHLs). Recently, Kyle et al. [NEJM 2018] updated the follow-up of their historical cohort of 1,384 MGUS patients, enrolled at the Mayo Clinic (Minnesota, USA) in the period from 1960 to 1994, thus providing further insights into MGUS natural history and confirming the effectiveness of the current risk-stratification model to predict long-term MGUS progression to MM/B-NHLs. Objectives: We believe that long-term cohort studies could usefully be complemented by short time-frame surveys on larger MGUS populations, which may serve as “real-life” validation set, as well as may help to describe the actual impact of MGUS clinical-laboratory management, both on patients’ outcome and on health care system. METHODS: For such purpose, we investigated our laboratory database (PAGODA) to outline the picture of total 10,656 MGUS cases referring to our assistential service for MGUS patients (which is based on integrated laboratory monitoring and clinico-hematologic evaluations for gammopathy diagnosis and follow-up), who underwent blood analyses for MGUS assessment during the past 18 months (2016-2018), accounting for total 22,308 routine laboratory accesses (median 2/patient). Results: Overall MGUS progression rate was 0.33% (35 patients) and, for IgG-IgA-IgM subtypes, prevalence/progression rates were 68%/0.30%, 12%/0.42% and 20%/0.33%, respectively. According to the number of MGUS risk factors (RFs), we obtained the following data, for each patients’ group: 34%/0% for no RFs, 45%/0.06% for 1 RFs, 17.5%/1.13% for 2 RFs, 3.5%/3.04% for 3 RFs. Conclusions: Thus, prevalence data and progression rates we observed in our short-term analysis of a large MGUS cohort (>10.000 patients) appear quite well in line with long-term data reported by Kyle et al. [NEJM 2018]. Moreover, time-to-progression after the acquisition of a risk factor could usefully be investigated. Further studies are needed to improve the real-world figure of the MGUS entity and advise on optimal patients’ management and local resource allocation.

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PRELIMINARY DATA FROM A STATISTICAL MODEL AIMED TO VERIFY THE ALIGNMENT OF HEMATOLOGICAL ANALYZERS USING FRESH BLOOD SAMPLE AND CONTROL MATERIAL AFTER COMMUTABILITY VERIFICATION: RED BLOOD CELL INDICES AND RETICULOCYTE COUNT

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Introduction: In the Hub and Spoke laboratory network organization, the number of hematology analyzers (HAs) within each Hub has increased, and the control of HAs alignment is becoming necessary requirement to ensure the quality of the analytical process. The aim of the study was to verify the applicability of a protocol for the instrument alignment of HAs.

Materials and Methods: The alignment of 5 modules of XN-9000 (Sysmex, Kobe, Japan) was evaluated by two materials: 1) peripheral blood sample (PB) collected in K_3EDTA (20 repl/module); 2) quality control (XN-CHECK level 2) (QC) (43 repl/module, Feb-Mar 2018), after the verification of commutability according to the IFCC protocol [1], with maximum bias derived from biological variation (VB) data recently published [2]. The parameters included in the verification of all 5 XN-modules were red blood cells (RBC), hemoglobin (Hb), hematocrit (Ht) and mean globular volume (MCV). HAs 4 and 5 were also compared for the reticulocyte (RET#). The alignment was verified by comparing the 95% confidence intervals of the differences between instrumental averages, calculated using a critical t from the Student's t distribution (for K=2 instruments) or a critical q from the Tukey's distribution (for K=5 instruments), with the relative desirable bias obtained from the VB data [2]. The presence of outliers was verified by Grubbs' test.

Results: The QC was commutable for RBC, Ht, Hb, MCV and RET. No outliers among PB or QC replicates were found. Using the PB, HAs alignment was confirmed for RBC, Hb and RET, while a partial misalignment was found for Ht (1 vs 3; 2 vs 3 instruments) and MCV (2 vs 3; 2 vs 4.5). The use of QC confirms the alignment for RBC, Hb, RET and a partial misalignment for Ht (3 vs 4) and MCV (1 vs 2.3.4; 2 vs 3.5; 3 vs 4; 4 vs 5).

Conclusion: The two materials used in this study have provided comparable results, highlighting a complete alignment for RBC, Hb, RET and a partial misalignment for Ht, MCV, which seems to be attributable to HAs 2 and 3. The slight discrepancies between PB and QC could be likely related to the different number of replicates (20 vs 43) and then to the different statistical power.

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ESAME EMOCROMOCITOMETRICO PRE-DONAZIONE: UTILE NELLA DIAGNOSI PRECOCE DELLA POLICITEMIA VERA?

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Introduzione: Secondo il D.M. del 03/11/2015 il donatore di sangue e di emocomponenti deve essere adeguatamente valutato prima di ogni donazione, a tutela della salute del donatore stesso e a protezione della salute e sicurezza dei pazienti riceventi. Una delle cause più frequenti di esclusione, in particolare nelle donne è il valore basso di Hb ed Hct pre-donazione; nonostante questo però non è raro riscontrare soggetti con valore di Hb o Hct al di sopra del limite superiore.Tali valori possono essere studiati per escludere o confermare presenza di patologie secondarie come eritrocitosi mieloproliferativa primaria e policitemia vera.(Tagariello et al Blood Trasfus.2009)Scopo: Scopo del nostro studio è stato analizzare gli esami emocromocitometrici dei donatori periodici afferenti al Servizio Trasfusionale dell'ASL Caserta al fine di evidenziare soggetti con valori di Hb e Hct al di sopra al limite superiore e di indirizzarli, ove necessario, a maggiori accertamenti clinici.Materiali e Metodi: Da 03/2018 a 06/2018 i donatori con valore di Hct>50% e Hb>15 g/dl sono stati segnalati e richiamati per la ripetizione del prelievo. In contemporanea sono stati valutati anche gli emocromi delle precedenti donazioni per una valutazione clinica globale dello stato di salute del donatore.Infine i donatori che hanno confermato questi valori sono stati indirizzati presso il Servizio di Ematologia del P.O. Moscati dell'ASL Caserta per uno studio più approfondito.Risultati: Sono stati analizzati circa 3000 donatori periodici (70%uomini 30%donne). Di questi n.150 (5%) sono risultati con valore di Hb superiore a ±15.4 g/dl a e Ht superiore a ±51.2%Tutti sono stati indirizzati presso il servizio di Ematologia.Il 2% sono risultati essere affetti da policitemia vera jak 2 positivo.Conclusioni: Dal lavoro si evince che è stata di fondamentale importanza l'anamnesi e la valutazione medica pre-donazione per tutelare la salute dei paziente e dei donatori e garantire la qualità degli emocomponenti. La tutela della salute dei donatori è, infatti, uno degli scopi importanti di un Servizio Trasfusionale.Il monitoraggio di una popolazione sana è utile affinchè patologie secondarie come la policitemia vera possano essere identificate in tempo e seguite nella maniera corretta.

P117
METHOD EVALUATION OF ACTIVE VITAMIN B12 (HOLO-TC) MEASUREMENT IN SEMINAL FLUID

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Introduction: Active vitamin B12 (AB12) is the biologically active part of vitamin b12 (VB12) or cobalamin, which binds to transcobalamin II to form the olotranscobalamine complex (Holo-TC), which promotes its permeability to cells and tissues due to the presence of specific ubiquitous receptors. A reduced intake of VB12 with diet and/or intestinal malabsorption can lead to a negative vitamin balance and a severe deficiency, revealed with hyperhomocysteinemia, increased blood concentrations of methylmalonic acid (MMA), megaloblastic anemia and neutrophil hypersegmented presence. HoloTC is the better indicator of VB12 status than total cobalamine in serum. Lately various studies have revealed the positive effects of VB12, on semen quality and sperm physiology. The aim of this study was to evaluate the analytical performance of the Advia Centaur AB12 assays on seminal fluid.
Materials and methods - In a pool of seminal fluid samples of fertile men was diluted 1:3 and 1:5 and then centrifuged, Advia Centaur AB12 assay and Advia Centaur Total VB12 measurements were repeated 5 times for 5 days respectively and intrassay and interassay precision was evaluated.
Results: The results show that CV Interassay = 4.35% and CV Intrassay = 3.11% for AB12 assay, while CV Interassay = 24% and CV Intrassay = 76% for VB12.
Conclusion: The evaluation of the analytical performance of the AB12 assay and VT12 assay in seminal fluid shows that AB12 assay can be proposed as a routine test because precision values was very good, while VT12 assay can not be proposed as a routine test because the inaccuracy values are not acceptable. The evident differences between assays were imputable to the matrix and probably to the protein binding B12 Vitamin in the VT12 measurement.

P118
DOSAGGIO DELLE METANEFRINE URINARIE IN HPLC : METODO DI IERI E METODO DI OGGI A CONFRONTO

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Il dosaggio delle metanefrine urinarie rappresenta un test per lo screening di feocromocitoma, tumore che provoca una ipersecrezione di catecolamine plasmatiche e urinarie, e dei rispettivi metaboliti (acido vanilmandelico e metanefrine). Studi recenti mostrano che la misura delle metanefrine sia la più specifica (99,7%) per la diagnosi del feocromocitoma: queste vengono secrete dal tumore in modo continuo con meccanismo autonomo rispetto a quello delle catecolamine che è spesso intermittente o in quantità limitate. Valori normali di metanefrine, normetanefrine e 3-metossitiramina nelle urine escludono la presenza del feocromocitoma o paraganglioma secernente. L'esame può essere anche prescritto per monitorare le recidive e l'efficacia del trattamento del feocromocitoma. Il vecchio metodo utilizzato prevedeva l'impiego di Kit per HPLC EurekaLab. Dopo l'idrolisi, l'estrazione su colonnine CBA e derivatizzazione, i campioni erano iniettati in colonna Poroshell EC C18 5 cm e letti in fluorescenza (λex 275nm; λem=325nm), corsa 40 min, tempi di ritenzione: 5.4 min SI, 7.3 min normetanefrina, 9 min metanefrina e 25 min 3-metossitiramina. Recentemente la ditta EurekaLab ha apportato delle modifiche nel kit aumentando la risoluzione dei picchi in termini di efficienza, selettività e fattore di ritenzione. Il nuovo kit ha una colonna analitica Infinity Lab Poroshell 120 PfP 4,6x100mm, 2,7µm, termostatata a 45° che riduce i tempi di ritenzione a 6,5 min per normeta; 7 per metanefrine; 12 per 3-metossi, riducendo la corsa a 20 min. La calibrazione del nuovo metodo è lineare per i 3 parametri con R2=0.98 per la normetafrina, 0.99 per la metanefrina e 0.98 per la metossitiramina. Il CV è variabile tra il 12.7 e il 13% per QC1 e tra 0.8 e 4.4% per QC2. L'accuratezza ha un bias compreso tra l'86 e il 110%. 20 campioni urinari sono stati misurati con entrambi i metodi per il confronto. La correlazione con il metodo di riferimento ha fornito un bias del 3% per la normetanefrina, del -3% per la metanefrina e del -2% per la 3-metossitiramina. Quindi il nuovo metodo da noi provato rientra perfettamente nei valori statistici attesi per la comparazione tra metodi, come riportato nelle linee guida SIBIOC. Lenders J, Eisenhofer G,et al. Phaeochromocytoma. Lancet, 2005,366:665-75.

P119
ESTABLISHMENT OF PTH NORMAL REFERENCE LEVELS BY USING BIG DATA

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Backgrounds and Aims: Normal reference levels (RL) for parathyroid hormone (PTH) is important for metabolism disorders. Being PTH finely regulated by calcium, vitamin D and the renal function, the recruitment of reference populations may represent a challenge. Evidences suggested also that PTH RL should be stratified by age. The aim of this study was to establish normal RL for PTH by data mining.
Methods: For a period between January 2016 and June 2018, all PTH measurement from the LIS of the Department of Laboratory Medicine, University-Hospital of Padova were obtained. Only records with Calcium between 2.1 and 2.6 mmol/L, Vitamin D between 75 and 150 nmol/L, and Creatinine between 15 and 110 umol/L were considered. Hoffmann, Bhattacharya and gaussian mixed model (GMM) methods were used to obtain RL by using R software v 3.5.0.
Results: A total of 19,018 PTH measurements (Diasorin Liaison 1-84 PTH Assay) were obtained matching the defined criteria. Overall, RL were 12.2-55.0 (Hoffman), 11.3-37.9 (Bhattacharya) and 12.8-52.5 ng/L (GMM). Older age was associated with higher PTH concentrations ($p<0.001$). Considering the youngest (age 1-30), RL were 8.5-44.0 (Hoffman), 9.1-25.2 (Bhattacharya) and 8.45-49.1 ng/L (GMM). In the age range 31-60 yrs, RL were 11.7-51.1 (Hoffman), 14.7-50.4 (Bhattacharya) and 12.2-49.5 ng/L (GMM), while for age > 60 yrs RL were 13.4-56.3 (Hoffman), 17.2-50.4 (Bhattacharya) and 14.0-53.9 ng/L (GMM).
Conclusions: Results support the usage of age stratified RL for PTH. The Hoffman and GMM methods allowed to obtain similar results with respect to Bhattacharya's method, despite being more computational intensive.

P120
INDIRECT ESTIMATION OF REFERENCE INTERVALS OF THYROTROPIN IN AN AREA OF SOUTHERN ITALY

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Background: The serum concentrations of thyrotropin (TSH) reflect the functions of thyroid gland and represent first-line test in diagnostic algorithms. The estimation of TSH reference intervals (RIs) is still a matter of debate for difficult to define subjects with "healthy thyroid", because of the high prevalence of subclinical disease. Furthermore, many studies showed higher TSH levels with ageing and gender. The aim of the present study was to investigate the TSH levels, through indirect estimation, inindividuals undergoing routine thyroid function testing and determine the effects of age and gender on the TSH RIs. Methods: TSH data were collected between July 2012 and April 2018 at the C.O.U. of Laboratory Medicine, Department of Laboratory Medicine, University-Hospital, Palermo. Serum TSH, free thyroxine (fT4), free triiodothyronine (fT3) and anti-thyroid-peroxidase antibodies (anti-TPO) were measured using Cobas e801 analyzer (Roche Diagnostics GmbH,Germany) and stored in the Laboratory Information System. Among stored data (n=21088) we excluded patients younger than 15 years, with more than one TSH test performed in the period or with either fT4, fT3 or anti-TPO outside the reference range, TSH data (n=10512) were then subjected to Box-Cox transformation and association with age (5-years groups) and gender was evaluated by General Linear Model (GLM). Results: Among remaining 10512 cases, median TSH (IQR, min-max) was 1.49 mIU/L (0.94-2.20, 0.16-5.03), median age 56ys (41-71, 15-100), with 6457 (61.4%) males and 4055 (38.6%) females.TSH slightly decreased from median 1.76 mIU/L in the (15-20ys) subgroup to 1.26 mIU/L in the (85-105ys) group.A GLM showed a statistically significant interaction between gender and age groups ($p=0.019$), suggesting that the effect of age on TSH is different among genders. Whereas age was found associated to TSH levels ($p<0.0001$), no differences between genders were instead observed ($p=0.867$). Conclusions: This study indicates, unlike other studies on different ethnic groups, a decrease in TSH levels with ageing differently between males and females. These results suggest that ethnicity can play a key role in TSH RIs and in clinical approach to thyroid dysfunction.
Reference: Wang Y. J Clin Lab Anal 31;2017.

P121
SERUM INSULIN AND HAEMOLYSIS, A COMPLEX RELATIONSHIP

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Background: Insulin is a polypeptide hormone, secreted from pancreatic β-cell, involved in the regulation of glucose and lipid metabolism. Insulin assay is an useful tool to identify some clinical conditions, such as fasting hypoglycemia, some forms of diabetes, the presence of insulin resistance. An important pre-analytical aspect that influences the determination of insulin levels is the presence of haemolysis. In fact, it is well known that insulin is degraded by a protease released by red blood cell after haemolysis. The aim of the study was to evaluate the interference of haemolysis on insulin measurements performed by chemiluminescence method (Roche COBAS e602).
Materials and methods: To study the effect of haemolysis on insulin degradation we added increasing concentrations of red blood cell haemolysate (reaching haemolysis from 0.05 to 0.2 g/L) to a serum pool without haemolysis, known for insulin value. A change of more than 10% of insulin value was considered statistically relevant, highlighting a significant interference of haemolysis on insulin results. Firstly, we evaluated the influence of several degrees of haemolysis on insulin assay and in relation to the incubation time. Secondly, we performed the same experiment both storing the samples with different degrees of haemolysis at room temperature and at low temperature keeping the tubes in ice and water until the assay.
Results and discussion: The reduction of insulin levels was affected by the degree of haemolysis, by the time elapsed before assay and by the temperature of samples storage. Our results showed that even small degrees of haemolysis (0.06 g/L) reduce significantly the insulin results if samples are stored for 3 hours at room temperature. On the contrary insulin values did not decrease significantly for an haemolysis up to 0.2 g/L of haemoglobin if the samples are kept immediately in ice and water until assessment.

P122
CAMPAGNE DI PREVENZIONE ENDOCRINOLOGICA NEI DONATORI DI SANGUE DELL'ASL DI CASERTA

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Introduzione: Nelle ultime decadi l'epidemiologia della patologia nodulare tiroidea si è modificata, con un aumento progressivo di noduli tiroidei nella popolazione adulta. Nel territorio italiano la prevalenza dei noduli tiroidei è del 50% con picchi fino al 75% in alcune regioni. A fronte di tale incremento di prevalenza si riscontra un'analoga tendenza alla crescita dell'incidenza dei tumori della tiroide. Per tali ragione il SIT Aversa in collaborazione con le associazioni di donatori ha promosso campagne di prevenzione per il tumore alla tiroide. Lo scopo del nostro lavoro è stato il valutare l'utilità diagnostica e predittiva degli esami effettuati. Materiali e metodi: Da Gennaio 2016 ad Aprile 2018 i donatori di sangue intero, che hanno aderito alla campagna di prevenzione, al momento della donazione sono stati sottoposti ad esame obiettivo della tiroide e nei casi dove questo è risultato positivo sono stati effettuati ecografia tiroidea e prelievo per il dosaggio di TSH. I donatori, che hanno evidenziato alcuni dei parametri analizzati alterati, sono stati richiamati ed indirizzati ad ulteriori approfondimenti diagnostici tra cui, agoaspirsato, anti tpo anti tg. Risultati: Sono stati screenati 3600 donatori di sangue intero, che hanno aderito alla campagna di prevenzione, di cui 2500 donne e 1100 uomini di questi il 7% delle donne ed il 4,1% degli uomini ha presentato alterazione dei parametri analizzati. Discussioni e Conclusioni: La campagna di prevenzione ha contribuito alla diagnosi della patologia tiroidea in soggetti potenzialmente sani come il donator di sangue. Ciò che la nostra esperienza ha messo in evidenza è che sicuramente il singolo esame non può essere predittivo ma l'insieme degli esami strumentali, laboratoristici e delle varie professionalità ha contribuito all'ottimo risultato raggiunto.

P123

DETERMINAZIONE SPETTROFOTOMETRICA DELLA CONCENTRAZIONE URINARIA DI IODIO: VALUTAZIONE DI UN CIRCUITO INTERLABORATORIO E CONFRONTO CON LA SPETTROMETRIA DI MASSA AL PLASMA

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Introduzione: La determinazione del valore mediano della concentrazione urinaria di iodio (UIC), misurata in campioni rappresentativi della popolazione scolare residenti in una certa area geografica, viene indicata dal WHO come metodologia indispensabile per valutare l'assunzione di iodio nella popolazione residente in quell'area e per monitorare i programmi di iodoprofilassi nei vari Paesi. La misurazione della UIC può essere effettuata con una variante del metodo spettrofotometrico di Sandell-Kolthoff (SK) e con la spettrometria di massa al plasma (ICPMS), considerata il metodo analitico più accurato per il dosaggio di questo microelemento. Obiettivi: 1) Valutare il circuito costituito dai 4 laboratori italiani che in passato hanno determinato UIC con SK e che hanno generato i dati per il monitoraggio della iodoprofilassi in Italia negli anni 2007-2012; 2) confrontare il metodo SK con la ICPMS, utilizzata per generare i dati più recenti di monitoraggio (2015-2018).

Materiali e metodi: Ogni laboratorio ha ricevuto 75 campioni spot di urine a partire da una collezione disponibile dell'Università di Genova (22 con UIC <50µg/L; 21 con UIC=50-99; 19 con UIC=100-199; 13 con UIC>200). I campioni sono stati analizzati con SK dai laboratori del circuito e mediante ICPMS dal Laboratorio dell'Istituto Superiore di Sanità. Il parametro utilizzato per la valutazione del circuito e per il confronto tra SK e ICPMS è lo z-score (z). Valori assoluti di $z \leq 2$, $2 < z \leq 3$ e $z > 3$ sono stati ritenuti rispettivamente soddisfacenti, accettabili e non accettabili. Valori di UIC compresi tra 100 e 200 µg/L indicano un adeguato apporto nutrizionale di iodio.

Risultati. I risultati ottenuti dal circuito evidenziano un sostanziale accordo tra i 4 laboratori soprattutto per UIC comprese tra 100 e 200 µg/L (range -1,7–2,1); in un solo caso è stato rilevato un valore non ottimale (z=2,1). Maggiori deviazioni da valori di z ottimali sono stati osservati per UIC<100 (2,9%) e >200 µg/L (5,8%). Anche il confronto SK vs ICPMS ha mostrato valori soddisfacenti di z per UIC comprese tra 100 e 200 µg/L (range -1,8 – 2,2).

Conclusioni: La valutazione del circuito ha evidenziato un soddisfacente accordo tra i laboratori ed un idoneo grado di accuratezza delle misure ottenute con SK rispetto all' ICPMS.

P124
FREE TESTOSTERONE ELISA COMPARED TO CALCULATED FREE TESTOSTERONE BY VERMEULEN'S FORMULA

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Background: Testosterone (T) circulates in plasma specifically bound to SHBG, non specifically bound to Albumin and in a small percentage unbound (FT). Calculated values for FT are recommended as the most useful estimate for determining androgen status in men and women. Analytical performance of direct immunoassay for FT is generally quite poor, results tend to be an order of magnitude lower than equilibrium dialysis or ultrafiltration, which are still considered the gold standard methods. Objective: To assess a direct competitive immunoenzymatic colorimetric assay for measurement of free testosterone with respect to calculated FT. Methods: FT was quantified in 144 male serum samples by two different methods: a direct immunoenzymatic assay (d-FT) by DRG Instruments GmbH, Germany, and applying the Vermeulen's formula (c-FT), which required the measurement of total T and SHBG (Architect Immunoassay Analyzer Abbott Laboratories USA). The relation between the assays was assessed by regression analysis and the correlation coefficient; statistical significance was set at $p < 0.05$. Results. The d-FT values resulted 10 fold lower compared to c-FT, but the regression analysis showed a good agreement and a statistically significant linear relation between methods; $y = 0.0932 + 0.08943x$; the intercept 95%CI was -0.01 - 0.196; the slope 95%CI was: 0.081- 0.0978. The correlation coefficient was 0.87. The regression between TT and c-FT was linear ($p<0.001$): $y = 2.8232 + 0.001438x$; the intercept 95%CI was 1.398 - 4.258; the slope 95%CI was: 0.012 - 0.0167. The correlation coefficient was 0.722. The regression between TT and d-FT was linear ($p<0.001$): $y = 0.1527 + 0.001662x$; the intercept 95%CI was 0.03 - 0.275; the slope 95%CI was: 0.0015 - 0.0019. The correlation coefficient was 0.814. Conclusions: This study confirms literature evidences reporting FT generated from IA correlate better with total T than to c-FT, perhaps due to antibody binding of protein-bound T. Nevertheless data are one order of magnitude lower than c-FT, as consequence values for healthy men are necessitating method-specific reference intervals.

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P125
LA PREVALENZA DI MACRO-TSH IN UNA POPOLAZIONE AMBULATORIALE: RISULTATI PRELIMINARI

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L'ipotiroidismo subclinico è una condizione caratterizzata da concentrazioni aumentate di tireotropina (TSH) sierica, in presenza di concentrazioni degli ormoni tiroidei (fT3 e fT4) entro i limiti di riferimento.
Questa situazione può talvolta essere mimata dalla presenza di macro-TSH. Si tratta di una forma di TSH ad alto peso molecolare, costituita dall'ormone complessato con immunoglobuline. Il complesso ha scarsa attività biologica, ma le sue grandi dimensioni ne rallentano la clearance, provocando un aumento delle concentrazioni sieriche di TSH.
Ad oggi nessun metodo analitico è in grado di discriminare la forma macro dalla forma attiva dell'ormone: la presenza di macro-TSH è verificabile solo misurando il TSH dopo precipitazione del campione con polietilenglicole (PEG).
Soltanto pochi studi, condotti soprattutto su popolazioni asiatiche, hanno indagato la prevalenza di macro-TSH. Abbiamo perciò voluto valutarla in una popolazione ambulatoriale di 500 pazienti di età ≥18 anni, con un quadro di ipotiroidismo subclinico (TSH >5 mUI/L con fT4 e/o fT3 nella norma).
I campioni di siero sono stati sottoposti a precipitazione con PEG 6000 (125 g/L), quindi è stato misurato il TSH sul surnatante (Cobas e602/e801, Roche).
Sono stati considerati positivi per macro-TSH i campioni che presentavano un'attività precipitabile >75%, in accordo con quanto proposto in letteratura.
Ad oggi abbiamo studiato 415 pazienti (300 femmine e 115 maschi), di età compresa fra 18 e 97 anni, afferenti a diversi ambulatori della Fondazione (298 centro prelievi, 68 endocrinologia, 34 medicina del lavoro e 15 ambulatorio donatori).
I soggetti presentano un ampio ambito di valori di TSH (5,01-223,4 mUI/L; attività precipitabile: 16-76%); soltanto per due pazienti abbiamo ottenuto risultati indicativi della presenza di macro-TSH (attività precipitabile uguale al 76% in entrambi i casi).
I risultati preliminari del nostro studio indicano pertanto, nella popolazione studiata, una prevalenza di macro-TSH pari allo 0,48%. Tale dato risulta inferiore a quanto riscontrato da altri autori, che riportano valori tra 0,6% e 1.62%.

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P126
LOW FT3, FRAILTY AND MORTALITY IN THE ELDERLY

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Introduction: Non-Thyroidal Illness Syndrome (NTIS) is frequently observed in the elderly. The main alteration found in NTIS is the reduction of serum concentrations of FT3, generally in association with normal or reduced concentrations of FT4, normal levels of TSH and increased levels of reverse T3 (rT3).
Aim: We report the relationship between low FT3, frailty and mortality in order to evaluate the potential predictive role of FT3 as a marker of frailty and mortality in the elderly.
Methods: An observational-perspective study was carried out at University Hospital of “Tor Vergata” in Rome, among outpatients and hospitalized patients. The study population consisted of 144 elderly subjects aged between 65 and 102 years. At baseline (T0) all subjects enrolled in the study underwent a clinical examination and received a multidimensional geriatric evaluation. The degree of frailty was also assessed. TSH, FT3 and FT4 were measured to evaluate thyroid status. FT3/FT4 ratio was used as a proxy of rT3 levels and NTIS. After a two-year follow-up (T1), all subjects enrolled in the study received an outpatient geriatric evaluation. Data on mortality were also collected at T1.
Results: At baseline, frail subjects had significantly lower levels of FT3 than those observed in pre-frail and not frail (ANOVA $p<0.05$), but the three groups did not differ in terms of mean values of FT4 and TSH. Furthermore, the FT3/FT4 ratio was significantly lower in frail elderly patients (ANOVA $p<0.01$). A linear regression analysis showed that the degree of frailty was negatively correlated with FT3 ($r=-0.442$; $p<0.0001$) and with FT3/FT4 ratio ($r=-0.307$; $p<0.005$). Subjects who died during the follow-up had lower levels of FT3 and of FT3/FT4 ratio, and a higher degree of frailty at T0.
Conclusions: In the elderly the presence of NTIS is associated with a higher degree of frailty. Frailty is a biologic syndrome characterized by reduced resistance to stressful events, derived from a functional decline of multiple physiological systems and associated with an increased risk of death. Measuring FT3 and, possibly, rT3 can be useful to identify frailty and, in frail elderly subjects it can also have a predictive role of worse prognosis.

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STABILITY OF PLASMA FREE METANEPHRINES IN WHOLE BLOOD

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Background: Evaluation of plasma metanephrine and normetanephrine (MN and NMN) by liquid chromatography tandem mass spectrometry (LC-MS/MS) is currently considered the gold standard for biochemical diagnosis of pheochromocytomas and paragangliomas. Since MNs are stable methylated metabolites of their parent chatecholamines, stringent preanalytical measures have generally been regarded as less important. However, the stability of MNs in whole blood has been systematically evaluated only once in literature, and without using the reference method.
Aim. To analyze with LC-MS/MS the short-term stability of plasma free MNs in whole blood.
Methods: Whole blood from 10 healthy volunteers was collected in sitting position into 8 EDTA tubes. Two tubes were centrifuged within 15 minutes from collection at room temperature (RT) or at 4°C. The other tubes were kept for 1, 2 and 3 hours (h) either at RT or at 4 °C before centrifugation. The ClinMass Complete kit (Recipe, Munchen, Germany) was used for MNs quantification in LC-MS/MS (Nexera X2 UHPLC-4500MD Sciex). Repeated measures one-way ANOVA and Students' paired T test were used for statistical analyses.
Results: At RT, MN concentrations slightly increased within the first hour ($p=0.039$) than rapidly decreased until reaching values approximately half of the baseline within 3h after venipuncture ($p<0.0001$). NMN increased rapidly within the first hour ($p=0.0002$) and then returned to the baseline values after 3h. At 4°C, differences between time points were statistically significant for NMN ($P=0.028$) but not for MN ($p=0.22$). NMN increased up to 6% within 3 h even in samples kept at controlled temperature.
Conclusion: MN and NMN show different patterns of stability before centrifugation. Spuriously increased values of NMNs (up to 47%) were found 1h after collection. Such increase, likely attributable to metabolism of sympathetic-derived norepinephrine by catechol-O-methyltransferase present in red blood cells, might lead to a significant dilution of adrenal medulla-derived NMN, thus potentially impairing the diagnostic efficiency of the test.
Reference: Danese et al. Oncotarget. 2018 Feb 26;9(21):15650-15657

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FARMACORESISTENZA AGLI ANTIEPILETTICI E MDR1: UNO STUDIO SU DI UNA POPOLAZIONE ITALIANA

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La terapia dell'epilessia impiega farmaci stabilizzanti le proprietà elettriche della membrana delle cellule nervose. Il 30% circa dei pazienti affetti continuano a manifestare crisi malgrado la terapia con i farmaci antiepilettici (FAE). Il susseguirsi delle crisi può essere associato ad un trattamento non adeguato nella scelta o nel dosaggio dei farmaci ma una parte consistente di pazienti con epilessia non risponde in maniera soddisfacente a molteplici FAE assunti singolarmente o in combinazione alle massime dosi tollerate. Questa osservazione induce a ipotizzare che esistano dei fattori congeniti e/o acquisiti in grado di influenzare la risposta a diversi FAE, indipendentemente dal loro meccanismo di azione. Diversi studi si sono concentrati su proteine della famiglia “ATP-binding cassette” trasportatori attivi dei farmaci, soprattutto su MDR1. Il polimorfismo C3435T nel gene codificante la proteina MDR1 è stato associato a farmacoresistenza in soggetti con epilessia refrattaria. In questo studio ci siamo proposti di valutare la correlazione tra alcuni SNP di MDR1 e la farmacoresistenza nell’epilessia. Mediante RFLP-PCR abbiamo tipizzato per 7 diversi SNP 25 pazienti farmacoresistenti, 27 pazienti farmacosensibili e 99 volontari sani. Quattro SNP (A-41aG, T-129C, A2956G e G4030C) non hanno mostrato alcuna differenza di distribuzione tra le tre popolazioni in oggetto. Il polimorfismo C3435T, che in letteratura è stato associato in maniera discordante alla farmacoresistenza nell’epilessia non è risultato correlare significativamente ($p>0.05$) nei pazienti presi in esame in questo studio. Diversamente due dei polimorfismi studiati T1236C e G2677T/A correlano significativamente con la farmacoresistenza (p rispettivamente di 0,020565 e 0,034753). Inoltre considerando gli aplotipi presenti nella popolazione abbiamo riscontrato una correlazione significativa (p=0,036578) tra farmacoresistenza e presenza di una condizione di eterozigosi in associazione tra i due polimorfismi T1236C e G2677T/A essendo l’aplotipo Het/Het presente nel 56% dei pazienti farmacoresistenti, contro il 29,6% presente in quelli sensibili. Questo studio supporta quindi l’ipotesi di una associazione tra alcuni polimorfismi del gene MDR1 e la farmacoresistenza nella popolazione italiana.

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ETILGLUCURONIDE URINARIA A CONFRONTO CON QUATTRO QUESTIONARI DI SCREENING: QUAL È IL MIGLIOR INDICATORE DI CONSUMO ALCOLICO IN GRAVIDANZA?

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L'etilglucuronide (EtG) è un metabolita dell’etanolo e viene comunemente utilizzata come biomarcatore di consumo alcolico. L'EtG può essere rilevata nel sangue e in diverse matrici biologiche tra cui urina, capelli e unghie. Il consumo di alcol durante la gravidanza è un importante fattore di rischio per la salute del feto, quindi, negli ultimi anni, sono state applicate diverse strategie per rivelarne l'uso, come la somministrazione alle gestanti di questionari di screening quali AUDIT-C, T-ACE e TWEAK. Il nostro studio si propone di indagare, su una popolazione di donne in gravidanza, la specificità e il valore predittivo dei questionari AUDIT-C, T-ACE, TWEAK e di un “diario alimentare” in uso presso l’AOU Policlinico Umberto I, confrontandoli con i risultati della misurazione dell'EtG in campioni di urine spot random. Sono state arruolate ed esaminate 269 donne in gravidanza. I campioni di urina sono stati raccolti immediatamente dopo la somministrazione dei questionari. Le determinazioni dell’EtG sono state eseguite mediante dosaggio immunoenzimatico, il valore soglia per la positività è stato fissato a 100 ng/mL. I dati mostrano che il 18,2% delle donne esaminate ha superato il valore di cut-off stabilito. Non è stata trovata alcuna correlazione diretta tra i dati EtG e gli score complessivi ottenuti dai questionari (che definiscono categorie a rischio/non a rischio) che mostrano mediamente livelli più bassi di consumo alcolico. Sebbene il questionario T-ACE abbia rivelato la stessa percentuale di rischio, non è stata tuttavia osservata una concordanza significativa con i dati del dosaggio dell’EtG urinaria. Questo studio fornisce prove oggettive che la diagnosi di consumo materno di alcol durante la gravidanza, basata solo su metodi indiretti come questionari e diario alimentare, può significativamente sottostimare l'uso di alcol.

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IL LABORATORIO DI TOSSICOLOGIA QUALE OSSERVATORIO EPIDEMIOLOGICO DELL'ETILISMO ACUTO

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Il consumo di alcol mostra un nuovo profilo. Lo illustra la Relazione al Parlamento del Ministro della Salute in materia di alcol e problemi correlati, di marzo 2018 con dati epidemiologici del 2016 che descrive le azioni di prevenzione del 2017. E' ridotto il consumo di vino durante i pasti ma è in costante aumento il consumo occasionale di bevande alcoliche al di fuori dei pasti, specie nel fine settimana (c.d."movida"). I dati del 2016 mostrano una diminuzione dei consumatori giornalieri e l'aumento dei consumatori occasionali (dal 42,2% del 2015 al 43,3% del 2016) e di coloro che bevono alcolici fuori dai pasti (nel 2014 erano il 26,9%,nel 2015 il 27,9%,nel 2016 risultano il 29,2%). La prevalenza dei consumatori a rischio è stata nel 2016 del 23,2% per gli uomini e del 9,1% per le donne di età superiore a 11 anni, per un totale di circa 8.600.000 individui (M=6.100.000, F=2.500.000). Le fasce di popolazione più a rischio sono quella dei 16-17enni (M=49,3%, F=40,0%) e quella dei 65-75 anni. Circa 800.000 minorenni e 2.700.000 ultra sessantacinquenni sono consumatori a rischio per problematiche alcol-correlate. Nella fascia giovanile il binge drinking (assunzione di numerose unità alcoliche a digiuno e in breve tempo)rappresenta l'abitudine più diffusa e consolidata. Nel 2015 il fenomeno riguardava il 15,6% dei giovani tra 18 e 24 anni, di cui il 22,2% maschi e il 8,6% femmine. Nel 2016 il fenomeno riguarda il 17% dei giovani tra i 18 e 24 anni, di cui il 21,8% maschi e l'11,7% femmine. Si conferma la tendenza degli ultimi 10 anni che vede una progressiva riduzione della quota di consumatori che bevono solo vino e birra,soprattutto fra i più giovani e le donne, mentre aumenta la quota di chi consuma, oltre a vino e birra, anche aperitivi,amari e superalcolici, specie nei giovani e giovanissimi, ma in misura percentuale maggiore negli adulti oltre i 44 anni e negli anziani.Nel periodo 2005-2017 abbiamo analizzato n.2268 pazienti provenienti dai PS ospedalieri aziendali (n.1028 positivi e n. 1240 negativi) sottoposti a controllo delle forze dell'ordine ai sensi degli artt.186 e 187 del nuovo c.d.s. Abbiamo documentato n. 147 positivi al solo etanolo e n. 253 positivi a miscela di alcol e sostanze di abuso. La maggiore distribuzione è nella fascia di età tra 19 e 34 anni.

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UNINTERPRETABLE CARBOHYDRATE-DEFICIENT TRANSFERRIN (CDT) SAMPLES IN A CLINICAL SETTING

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Carbohydrate-Deficient Transferrin (CDT) is defined as the percentage of disialo-transferrin on the sum of all the sialoforms (from disialo-transferrin to pentansialo-transferrin). CDT is widely recognized as the most reliable biomarker of chronic alcohol abuse. Uninterpretable CDT patterns have been shown using both capillary electrophoresis (CE) or HPLC as measuring technique. The aim of this study was to compare the performances of CE and HPLC on some common uninterpretable sample patterns; in particular samples presenting: low transferrin concentration (LT), di-trisialotransferrin bridging (D-TB) or abnormal peak profile (APP). From September 2015 to August 2017 9120 routine serum samples were tested, out of them, 123 (1.35%) resulted uninterpretable by CE due to: LT (n=42, 0.46%), presence of D-TB (n=63, 0.69%) or APP (n=18, 0.20%). These uninterpretable samples were retested by HPLC. The HPLC method was able to quantify 58 of the 123 (47%) not interpretable samples by CE, in particular 21 out of 42 (50%) with LT, 27 out of 63 (43%) with D-TB and 10 out of 18 (55%) with APP pattern. Despite the improvement of CDT determination demonstrated by HPLC, in our routine, HPLC resulted in a overall 0.63% increase of quantifiable samples in comparison to CE alone. The employment of HPLC to minimize uninterpretable samples have to be considered in the context of each laboratory, considering the specific laboratory referring population, the percentage of uninterpretable samples and the costs of the implementation of an additional method.

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ESPERIENZA DI APPLICANZIONE DI UN PROTOCOLLO PER COMMISSIONE MEDICO LOCALE PER ACCERTAMENTO DEL CONSUMO DI COCAINA NEI CONDUCENTI SANZIONATI PER GUIDA IN STATO DI EBREZZA.

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Scopi ed obiettivi: Lo stato derivante dalla dipendenza da alcool e droghe compromette i requisiti fisici e psichici richiesti nella valutazione d'idoneità alla guida.
"La Commissione Medica Locale deve accertare l'idoneità alla guida e a tal fine: può avvalersi di singoli consulenti oppure di istituti medici specialistici appartenenti a strutture pubbliche, con onere a carico del soggetto esaminato (D.P.R. 495/92, art. 330, c.6)".
Per tali accertamenti è stato predisposto dal nostro Laboratorio un protocollo che vede oltre alla tradizionale quantificazione della CDT su siero, quella dell'Etilglucuronide (ETG) e della Coca-etilene su matrice cheratinica.
A tal fine, tramite uno studio retrospettivo andremo a dimostrare come l'utilizzo del nostro Protocollo, sia uno strumento efficace nel monitoraggio per l'idoneità alla guida, e come questo ci abbia permesso di evidenziare un fenomeno misconosciuto quale l'uso di sostanze stupefacenti nei conducenti sanzionati per guida in stato di ebrezza.
Materiali e metodo: Le indagini su matrice cheratinica (ETG,COCAETILENE) sono state eseguite in LC-MS/MS:
• Agilent Infinity 1260 LC-MS/MS 6470 Triple Quadrupole
La determinazione della Transferrina Carboidrato Carente (CDT) è eseguita con:
• Ultimate 3000 Thermo Scientific HPLC UV/VIS.
Risultati e Conclusioni: Dall'osservazione dei nostri dati, emerge che su 674 utenti monitorati nel 2017 per guida in stato di ebrezza a cui è stato applicato il nostro protocollo, circa il 9% presentava una positività alla Coca-etilene, mentre solo lo 0,7% di questi presentava una concomitante positività all'ETG.
Confrontando la positività al marker ematico tradizionale di abuso alcolico CDT (2.2%) con quella dell'ETG (7.9%) su matrice cheratinica, abbiamo riscontrato un aumento di casi di inidoneità alla guida del 5.7 % , a questo dato va aggiunto l' 8.3% di positività alla Coca-etilene.
Possiamo concludere che l'introduzione del nostro Protocollo può sicuramente essere di ausilio alle Commissioni Medico Locali fornendo nuovi elementi valutativi per l'idoneità alla guida.

P133
EVALUATION OF A HOMOGENOUS ENZYME IMMUNOASSAY FOR HAIR DRUG SCREENING AND COMPARISON WITH UPLC-MS/MS

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Background: Hair testing for drugs of abuse is a developing technology, which offers the possibility of longer detection times in respect of urine analysis. This method is becoming an increasingly basic tool in clinical practice and laboratory medicine.
Aim: The aim of the present study was: to evaluate the analytical performances and the diagnostic efficiency of HEIA™ immunoassays (Immunalysis) using hair specimens after extraction with Comedical M3 Extraction Reagent; to confirm all obtained results by UPLC-MS/MS method accredited according to the International Standard ISO15189; to optimize the sample purification procedure for confirmatory analysis.
Materials and methods: Assessment of precision was carried out according to the reduced (n=10) CLSI-EP5A2 protocol using positive QC (TricoCheck®Screening, Comedical), whereas negative QC was used to determine the Limit of Blank (LoB). To evaluate the diagnostic efficiency calculating the relative sensitivity (RSn), relative specificity (RSp) and relative accuracy (RAc) for HEIA™ immunoassays in comparison to UPLC-MS/MS, 34 real negative samples and 25 real positive samples for each class of Amphetamines, Methamphetamine, Cocaine, Methadone, Opiates, Cannabinoid, Buprenorphine, EDDP were tested. In order to optimize the sample purification step 15 positive samples were prepared using two purification protocols after sample digestion: solid phase extraction (SPE) using Oasis HLB 1 cc Vac Cartridge, 30 mg, 30 µm Particle Size (Waters) and liquid/liquid extraction with Solvent M3 S1 and S2 (Comedical).
Results: Within-assay reproducibility (CV%, n=40) of positive QCs is <15% for all analytical classes. LoBs for all analytes were below cut-off limits defined by the SoHT. RSn, RSp and RAc were 100% for all classes except Opiates (RSp 95%, RAc 96%), Cocaine (RSp 88%, RAc 92%) and Amphetamine (RSp 89%, RAc 92%). No false negative results were detected. The comparison of sample purification procedures using SPE and liquid/liquid extraction did not present statistically significant bias.
Conclusion: The HEIA™ immunoassays exhibit good diagnostic efficiency for drug screening of hair as it does not present any false negative results. From clinical point of view, reporting a false negative results is a severe oversight.

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IL LABORATORIO DI TOSSICOLOGIA E LE DIPENDENZE DA SOSTANZE D'ABUSO NELLA PROVINCIA METROPOLITANA DI NAPOLI

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Agli 11 Ser. D. di Napoli afferiscono 800 pazienti (12 mila nella regione Campania).La dipendenza da sostanze non può prescindere da un intervento sanitario, sia per le implicazioni medico-farmacologiche che per curare i danni prodotti sul cervello, che per le patologie correlate.Negli ultimi 5 anni è costante l'arruolamento di giovani di età compresa tra i 14 e i 24 anni. Questa utenza assume nuovi comportamenti di consumo, specie per il trattamento per abuso di eroina e cocaina. Con l'unità di strada la Asl Napoli 1 Centro ha compiuto 800 interventi per overdose nell'ultimo biennio. Nel periodo 2005-2016 abbiamo esaminato 55572 campioni provenienti dagli undici Ser.D. aziendali (47781 positivi,7791 negativi), 2074 dai PS ospedalieri (941 positivi,1133 negativi), 14214 dalla Commissione Medica Locale (782 positivi, 13432 negativi), 15.018 dalle tre strutture carcerarie metropolitane e dall'ambulatorio forense (11982 postivi, 3036 negativi).Mentre l'eroina è consumata maggiormente da migranti e senza dimora a più alto rischio overdose e infezioni; la cocaina, prima considerata la droga dei ricchi,è utilizzata in quartieri come Chiaia, Posillipo e Napoli Centro da studenti, professionisti, abitanti della notte. Il suo uso è più tollerato,salvo superare un limite che mette a rischio la stessa quotidianità.L'uso di stupefacenti è diffuso ovunque: l'eroina iniettiva è più diffusa nella zona della ferrovia e di Scampia ed è l'eroina tagliata male o l'abuso di alcol ad essere fatali in contesti così precari; a consumare cocaina invece sono soggetti più integrati,che tendono a fare un uso più sporadico della sostanza, o chi lavora di notte o molte ore al giorno.Molto diffuso tra i giovani è l'uso combinato di cannabis e alcol, così come il policonsumo di coca, alcol, pasticche nei contesti di divertimento notturni o feste private in cui alcune sostanze disinibitorie e stimolanti sono collegate alla fruizione di un certo tipo di musica e alla ricerca di una "tribalità" collettiva. Lo spaccio è molto articolato,in tutti i quartieri c'è un pusher che si adatta alle esigenze dei consumatori, portando anche a casa la droga. Si tratta di strategie di mercato, non è un caso che quando il mercato dell'eroina è saturo si attuano strategie per diffondere maggiormente l'uso della cocaina".

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NANOTECNOLOGIE E MONITORAGGIO TERAPEUTICO DEI FARMACI: IMPIEGO DELLA TECNOLOGIA SERS (SURFACE ENANCHED RAMAN SPECTROSCOPY) PER IL MONITORAGGIO DELLE CONCENTRAZIONI PLASMATICHE DI CARBAMAZEPINA E APOMORFINA

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Questo studio presenta per la prima volta lo sviluppo di un sensore a nanotecnologie per il rilevamento dell'apomorfina e della carbamazepina, finalizzato all'applicazione clinica per il monitoraggio del livello di farmaci nei pazienti affetti da morbo di Parkinson e da epilessia. Il sensore è composto da un sottilissimo strato di particelle di oro o argento disposto su di un supporto inerte. Mediante la Spettroscopia Raman applicata ad una superficie (Surface Enhanced Raman Spectroscopy SERS) è stata testata la possibilità di dosare entrambi i farmaci in una ampio range di concentrazione, compreso nel range terapeutico. La selezione di specifici picchi SERS, assegnati a specifiche modalità vibrazionali delle molecole del farmaco, ha permesso di valutare il range dinamico delle concentrazioni del sensore. La preparazione minima dei campioni e la capacità di operare in un ambiente acquoso rendono la rilevazione di farmaci tramite questa tecnica trasferibile alla sua rilevazione in fluidi biologici. Esperimenti condotti su plasma sanguigno non filtrato con diverse concentrazioni di farmaci hanno dimostrato l'applicabilità del metodo proposto con una buona sensibilità e riproducibilità dei segnali SERS.

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STUDIO RETROSPETTIVO SULLE INDAGINI DI LABORATORIO PER L'ASSOLVIMENTO DEGLI OBBLIGHI DI LEGGE DERIVANTI DALL'APPLICAZIONE DEL CODICE DELLA STRADA (Art.187) RELATIVAMENTE AI CUT-OFF APPLICATI

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Scopi ed obiettivi: Ai fini dell'accertamento del reato ex art. 187 del C.d.S. è necessario un accertamento tecnico-biologico, per provare lo stato di alterazione psico-fisica del soggetto alla guida. I Protocolli Operativi per gli accertamenti delle sostanze d'abuso non riportano allo stato attuale i valori relativi ai cut-off delle singole sostanze al fine di poter dichiarare inequivocabilmente e con un dato che sia difendibile in sede legale, tale stato di alterazione. L'applicazione di valori soglia diversi, può portare a giudizi finali diversi e questo rappresenta una grossa criticità e fonte di forte disomogeneità territoriale, collegata proprio al vuoto legislativo in materia. A tal fine, andremo a valutare uno studio retrospettivo che va a dimostrare come gli aspetti formali del "consenso informato" e della "catena di custodia" non sono sufficienti a produrre un referto che definisca lo stato di "alterazione psico-fisica per uso di sostanze stupefacenti" perché applicando cut-off diversi cambiano il numero di positività e negatività e quindi i relativi giudizi finali.
Materiali e metodo: Il sangue è stato direttamente analizzato con metodologia di conferma LC-MS/MS per le droghe utilizzando la strumentazione:
• Agilent LC-MS/MS (per conferma urine e analisi sangue intero).
Risultati e Conclusioni: Dall'osservazione dei nostri dati, emerge la necessità di rendere omogenei e corretti i percorsi operativi che la Legge ci impone, arrivando a fornire all'autorità giudicante le prove di condotta di guida illecita ed al contempo garantire la non punibilità per i conducenti che non siano sotto l'effetto di sostanze stupefacenti o d'abuso; questo assume ancor più valore alla luce dell'entrata in vigore della Legge che prevede il reato di Omicidio stradale (Legge 23/3/2016 n°41). In altri termini ogni singolo Laboratorio che svolga indagini tossicologiche, è tenuto a implementare un iter procedurale che sia aderente alla normativa vigente, ma emerge la necessità di arrivare a determinare i valori soglia per le diverse sostanze psicotrope da correlare con la disabilità alla guida, definendo in maniera convenzionale la negatività o la positività di un campione di sangue con cut-off di Legge.

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DEVELOPMENT AND VALIDATION OF A FAST UHPLC-MS/MS METHOD FOR THE QUANTIFICATION OF FOURTEEN ANTIHYPERTENSIVE DRUGS AND TWO MAJOR METABOLITES IN PLASMA OF PATIENTS AFFECTED BY RESISTANT HYPERTENSION: AN UPDATE FOR ADHERENCE TESTING

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The management of resistant hypertension (RH), consisting in high arterial pressure (BP >140/90 mmHg) despite the administration of 3 or more antihypertensive drugs (including a diuretic) is difficult due to the high prevalence of poor therapeutic adherence (TA). The adoption of Therapeutic Drug Monitoring (TDM) of antihypertensive drugs could be a useful tool for TA testing, especially before adopting hypertension-related surgery. Recently, our group published a UHPLC-MS/MS method for adherence testing in suspected RH patients, capable of testing 10 different drugs. Nevertheless, several other antihypertensive drugs are largely used in this context, so a wider multiplexed method was needed. We hence updated that method for the quantification of 4 more drugs and 2 active metabolites in plasma samples. This method has been validated according to FDA and EMA guidelines. 200µL of plasma sample, calibration standard and quality control were added with 40µL of internal standard working solution (IS, 6,7-dimethyl-2,3-di(2-pyridyl)quinoxaline + $^{2}H_{6}$-Atenolol,^{13}C-$^{2}H_{2}$-Telmisartan and $^{2}H_{6}$-Nifedipine) and 1mL of acetonitrile in amber PTFE tubes (to limit nifedipine photodegradation), vortex mixed for 5 sec and centrifuged at 20000 x g for 10 minutes. After a drying step in vacuum centrifuge (about 1.5h) all samples were reconstituted with 200 µL of water:acetonitrile 90:10(vol:vol): 5 µL were injected in a Perkin-Elmer UHPLC system, coupled with a Q-Sight 220 tandem mass detector. Chromatographic separation was performed on a Acquity ® HSS T3 1.8µm2.1x150 mm column (Waters, Milan, Italy), with a gradient of two mobile phases, water and acetonitrile, both added with 0.05% of formic acid.
Method performances fitted FDA and EMA guidelines for all analytes. All drugs were successfully separated, except for ramipril, amlodipine and nebivolol. No interfering peaks were detected in our conditions. Recoveries and matrix effects resulted consistent, contained and reproducible. Sensitivity was high enough to successfully quantifying expected trough concentration of all the considered drugs and metabolites (up to 19.7 pg/mL for nebivolol).This method was tested on real samples from patients with apparent TRH, upon informed consent and successfully revealed some cases of poor TA.

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DOSAGGIO DI UN PANNELLO DI OTTO METABOLITI DELLE CATECOLAMINE URINARIE MEDIANTE CROMATOGRAFIA LIQUIDA ACCOPPIATA ALLA SPETTROMETRIA DI MASSA TANDEM (LC-MS/MS) IN NEUROBLASTOMA ALL'ESORDIO

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Le catecolamine urinarie e i loro metaboliti, in particolare l'acido vanilmandelico (VMA) e l'acido omovanillico (HVA) sono comunemente dosati nelle urine di pazienti con neuroblastoma (NB) all'esordio. E' già stato dimostrato che la loro accuratezza diagnostica è molto elevata in tutte le fasi, in particolare negli stadi 4 e 4S. È stato inoltre dimostrato che il rapporto VMA / HVA è un fattore prognostico indipendente in pazienti con diagnosi di malattia localizzata senza amplificazione di MYCN.

Altri gruppi hanno dimostrato che l'analisi di un più ampio pannello di metaboliti delle catecolamine urinarie può migliorare la loro accuratezza diagnostica. Tuttavia, pochi dati sono attualmente presenti sul loro significato prognostico. Recentemente è stato dimostrato che la 3-metossitiramina (3-MT) è un biomarcatore prognostico indipendente associato ad NB alto rischio e a prognosi infausta.

Il metodo standard per la misurazione è la cromatografia liquida con rivelatore elettrochimico (HPLC-EC) che fornisce un'alta sensibilità ma lunghi tempi di analisi. L'uso della cromatografia liquida associata alla spettrometria di massa garantirebbe un'analisi molto rapida e specifica da volumi di campione limitati e potrebbe essere utile nella valutazione di un pannello più ampio di metaboliti. In questo lavoro abbiamo applicato un kit commerciale (Chromsystems) al nostro sistema HPLC-MS/MS (TSQ Quantiva ™ UHPLC Ultimate 3000, Thermofisher Scientific). Abbiamo analizzato un profilo mirato di 6 metaboliti delle catecolamine urinarie (epinefrina [E], norepinefrina [NE], dopamina, metanefrina [MN], normetanefrina [NMA] e 3-metossitiramina[3-MT]), in aggiunta a HVA e VMA misurati mediante HPLC-EC, in campioni di urina di 79 pazienti con NB all'esordio (età 2 mesi-10 anni) in stadi diversi (17 stadio 1-2, 12 stadio 3, 34 stadio 4 e 16 stadio 4S) utilizzando campioni della biobanca dell'Istituto Giannina Gaslini, centro di riferimento italiano per la biochimica del NB.

L'uso combinato dell'analisi statistica multivariata e della cluster analisi consente di distinguere le diverse fasi in un ristretto pannello di metaboliti.

Un'associazione statisticamente significativa potrebbe essere trovata solo per NMN ($p = 0,01$), MN ($p = 0,05$) e VMA / HVA ($p = 0,0009$). I livelli di NMN e MN erano più alti nei pazienti con malattia stabile o in remissione completa se confrontati con quelli in pazienti con malattia attiva o con paziente deceduto.

NMN e MN sono sottoprodotti della stessa via metabolica a partire da NE e hanno come prodotto finale VMA. Questo risultato conferma l'ipotesi che una via noradrenergica potrebbe essere altamente espressa nei tumori con prognosi favorevole.

P139
NEW PROSPECTS IN BLOOD ALCOHOL CONCENTRATION (BAC) DETECTION IN CASE OF DRUNK DRIVING-RELATED CRIMES ACCORDING TO ITALIAN LAW 41/2016 AND HIGHWAY CODE: A POTENTIAL APPLICATION IN FORENSIC CASES

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Law 41/2016 was introduced in Italy to discipline drunk driving related crimes such as homicide and serious injuries. As a result, Region Lazio issued the decree D.C.A. 288 which established a list of regional health structures committed to do toxicological analysis. The decree states that analysis must be done in the process of chain of custody and results given in a few hours in relation to ethanol kinetic. The art. 186 of Italian Highway Code establishes fines and apprehension in relation to the subject BAC. Blood samples were collected in Emergency Room (ER) on average 1 or 2 hours after the accident. However, the BAC should be evaluated at the time of driving. For this reason, the aim of this study is to investigate the post-absorptive phase of Widmark curve in order to perform the retrograde extrapolation of BAC. We performed three real life situations in which volunteers drunk different alcohol amounts in fed condition. Volunteers stopped drinking at 90 minutes after the start. We collected blood samples 15 minutes apart, 30 minutes apart and 60 minutes apart. In addition, we analyzed BAC of 97 ER patients with two consecutive blood specimens sampled at least 30 minutes apart from each other. Ethanol assay was performed on ADVIA Chemistry 1800 Siemens. For each volunteer BAC analysis and linear regression was performed. Alcohol elimination rate for volunteers was on average 0,14 g/l/h whereas for the 97 ER patients was on average 0,24 g/l/h. Unlikely other studies dealing with this topic, we simulated real life situations. We confirmed that elimination of ethanol is still linear. Therefore, we can make a linear regression analysis for estimating BAC at the time of driving. These considerations could potentially be used in forensic drunk driving cases according to the Italian Laws.

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NUOVO METODO UHPLC-MS/MS PER LA QUANTIFICAZIONE DI QUATTORDICI ANTIBIOTICI E SUA APPLICAZIONE PER IL TDM

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L'incidenza crescente di infezioni dovute a batteri Gram-positivi e Gram-negativi antibiotico-resistenti sta diventando un grosso problema per il sistema sanitario, sia in pediatria sia negli adulti. I problemi causati dalla selezione di agenti patogeni resistenti sono particolarmente preoccupanti se consideriamo la completa assenza di nuovi farmaci, almeno per le infezioni da Gram-negativi, con l'esigenza di somministrare farmaci vecchi e meno studiati in combinazioni complesse, spesso gravato da effetti collaterali e minori possibilità di raggiungere l'esposizione PK / PD ottimale. Negli ultimi anni c'è stato un interesse crescente nel collegare l'uccisione batterica nel sito di infezione, farmacodinamica (PD), con la misura dell'esposizione all'antibiotico, farmacocinetica (PK / PD), normalizzata alla minima concentrazione inibitoria (MIC) del patogeno.
Attualmente strategie basate su dati PK / PD e loro rapporti con la MIC batterica potrebbero probabilmente rappresentare l'unico trattamento di successo per queste infezioni, con un rischio ridotto di ulteriore resistenza. Considerando queste premesse è cruciale che il laboratorio possa disporre di metodi analitici robusti per misurare le concentrazioni ematiche degli antibiotici maggiormente utilizzati, soprattutto nei pazienti critici (terapie intensive, emato-oncologia, neonati di basso peso). In questo lavoro mostriamo una nuova piattaforma, basata sulla spettrometria di massa (UHPLC-MS/MS) per la determinazione di 14 antibiotici (amoxicillina, ceftazidime, ciprofloxacina, daptomicina, linezolid, meropenem, piperacillina, vancomicina, tigeciclina, colistina, amikacina, gentamicina, tobramicina, teicoplanina) che prevede l'utilizzo di due differenti colonne UHPLC con differenti caratteristiche e fasi mobili comuni. Il campione di plasma (50 µL) viene estratto mediante una semplice e rapida precipitazione proteica e, dopo l'aggiunta degli specifici standard interni deuterati, viene processato in doppio con due differenti separazioni cromatografiche in automatico mediante un sistema di divert valves. Il metodo è stato sviluppato su due differenti sistemi UHPLC-MS/MS (Thermofisher Quantiva e Endura, entrambi accoppiati a UHPLC Ultimate 3000) validato secondo le linee guida per la validazione dei metodi bioanalitici e i risultati mostrano un'elevata accuratezza e riproducibilità in un ampio range di concentrazioni (variabile a seconda dell'antibiotico) utili per la loro determinazione a scopo di TDM. Questo metodo è stato inoltre testato su campioni reali di pazienti in trattamento con uno o più antibiotici. In conclusione riteniamo che questo lavoro possa essere utile per introdurre in routine il dosaggio degli antibiotici a scopo di TDM per mettere in correlazione le concentrazioni

ematiche con la MIC dei germi per una corretta gestione delle antibiotico-resistenze nei pazienti critici.

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RUOLO DIAGNOSTICO DELLE METANEFRINE PLASMATICHE LIBERE (IN PARTICOLARE DELLA 3-METOSSITIRAMINA) NEL NEUROBLASTOMA

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Le metanefrine plasmatiche libere (PFM) sono il parametro più specifico per la diagnosi del feocromocitoma. La loro quantificazione è difficile a causa della loro natura polare e la concentrazione fisiologica molto bassa nel plasma umano. In questo lavoro abbiamo applicato un kit commerciale (Recipe, Munich, Germany) sul nostro sistema LC-MS/MS (Thermofisher TSQ Quantiva Ultimate 3000) per valutare l'utilità diagnostica in pazienti con neuroblastoma (NB) all'esordio. Il NB è il più comune tumore solido extra-cranico in pediatria e la determinazione delle catecolamine urinarie rappresenta lo standard diagnostico di primo livello, seguito dalla diagnostica per immagini (TC, RM e MIBG) e dalla conferma isto-patologica. Il ruolo diagnostico delle PFM nel NB non è mai stato valutato su una casistica numerosa. Abbiamo analizzato 54 campioni di plasma di pazienti con NB all'esordio (4 stadio 1, 16 stadio 3, 24 stadio 4 e 10 stadio 4S) e 49 controlli (età 0-5 anni). I campioni di pazienti con NB sono stati collezionati tra il 2012 e il 2016 e appartengono alla biobanca NB dell'Istituto Gaslini, centro di riferimento nazionale per la biochimica del NB. Nei controlli range di riferimento sono stati ottenuti mediante test non parametrico (CLSI 28-A3, distribuzione non normale) e i risultati sono i seguenti: 3MT: 0.2-9.3; M: 1.1-75.4; NM: 11.4-169.6 ng/L. I risultati sono stati comparati con i dati della letteratura. La 3-MT e la NM hanno mostrato sensibilità e specificità molto elevate (3MT: 90.5 e 100% con AUC=0.939 e NM: 79.555 e 95.7% con AUC=0.893 rispettivamente) e una differenza molto significativa tra NB e controlli (test di Mann Whitney $p<0.001$). La MN, al contrario, ha dato risultati deludenti e questo è spiegabile con le differenti vie metaboliche cui appartengono. La concentrazione della 3-MT aumenta con l'aumentare dello stadio. Recentemente abbiamo dimostrato sia noi che un altro gruppo che il marker diagnostico su urine con migliore performance è la NMN. In questo lavoro abbiamo analizzato inoltre il valore aggiunto di questi biomarkers per specifici sottogruppi di NB (HVA e VMA urinari negativi e MIBG negativi) che potrebbero beneficiare maggiormente del dosaggio delle PFM. L'eventuale ruolo di questi biomarkers nell'identificare pazienti con outcome sfavorevole alla diagnosi va indagato in una coorte più ampia di pazienti. In conclusione questo test ha un'ottima performance per la diagnosi del NB e può essere molto utile in aggiunta o in alternativa al campione di urine, la cui raccolta potrebbe essere problematica.

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VALIDAZIONE DI UN NUOVO METODO UHPLC-MS/MS PER LA QUANTIFICAZIONE DI THC E CBD SU PLASMA E DECOTTI PER IL MONITORAGGIO TERAPEUTICO DELLA CANNABIS MEDICA

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La cannabis medica è sempre più utilizzata in diverse condizioni. Il monitoraggio dei livelli ematici di Δ9-tetraidrocannabinolo (THC) e cannabidiolo (CBD) è necessario per valutare i parametri farmacocinetici (PK) al fine di ottimizzare la somministrazione del farmaco. In questo articolo descriviamo la validazione di un nuovo metodo UHPLC-MS/MS per quantificare il THC e il CBD da campioni di plasma e decotto utilizzando un semplice protocollo di preparazione del campione e la sua possibile applicazione per il monitoraggio terapeutico dei farmaci.

Campioni di plasma (100 μl) e decotti sono stati estratti con una precipitazione proteica semplice e rapida dopo l'aggiunta di standard interni deuterati (IS). L'analisi UHPLC-MS / MS è stata eseguita utilizzando un triplo quadrupolo TSQ Quantiva ™ accoppiato a un sistema UHPLC Ultimate 3000 utilizzando la ionizzazione chimica a pressione atmosferica (APCI). La quantificazione è stata effettuata mediante il monitoraggi odi specifiche transizioni ioniche (SRM). Per la validazione del metodo sono state applicate le linee guida EMA. Il metodo è stato poi utilizzato per misurare i livelli di THC e CBD in decotti e campioni di plasma di dieci pazienti a cui è stata somministrata cannabis medica.

Il metodo è lineare su un'ampia gamma di concentrazioni in plasma (0,16-10 ng / ml) e in decotti (4.6-500 ng/ mL) sia per il THC che per il CBD. Il metodo è stato validato seguendo le linee guida EMA per la validazione dei metodi bioanalitici. E' stata studiata la decarbossilazione in sorgente dei precursori acidi THC-A e CBD-A ed è stato dimostrato che non influenza l'accuratezza delle misurazioni. I dati hanno mostrato una considerevole variazione interindividuale delle concentrazioni plasmatiche di THC e CBD e quindi nei parametri PK che riflettono la variabilità delle concentrazioni riscontrate nei decotti e nell'adsorbimento dei pazienti.

Il presente documento è il primo che descrive un metodo UHPLC-MS / MS semplice per la quantificazione simultanea di THC e CBD nel plasma umano e nel decotto, convalidato per scopi di TDM. Dato l'imprevedibile comportamento PK di THC e CBD nei pazienti, il monitoraggio delle concentrazioni plasmatiche è fortemente raccomandato per i pazienti sottoposti a trattamento con cannabis medica.

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A VALIDATED UHPLC METHOD FOR THE DETERMINATION OF MEROPENEM AFTER EXTRACORPOREAL MEMBRANE OXYGENATION CIRCUIT CHANGE IN CHILDREN

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Antibiotics are commonly prescribed for children in hospital, but few data are available to inform optimalduration of therapy. The Therapeutic Drug Monitoring (TDM) represents a unique opportunity to improve the management of many antibiotics, especially in the pediatric hospital settings, where patients often exhibit pharmacokinetic changes (PK). Critically ill patients constitute an extremely challenging objective, due to the sudden and unpredictable changes of PK parameters they can show in dependence of age, status of disease and their reciprocal interaction. The use of extracorporeal membrane oxygenation (ECMO) is associated with major PK changes. Meropenem is a useful and frequent antibiotic choice for treating severe infections in critically ill patients in the intensive care unit (ICU). Since Meropenem action is time-dependent, a careful administration and dosing plan should be made in order to maintain concentrations above the minimum inhibitory concentration. Pediatric data regarding the PK of Meropenem in ECMO are few and reported an increase in Clearance (Cl). The recommended dose for Meropenem should be higher than the standard one when the patient is on ECMO. The use of TDM coupled to MIC will allow clinicians to titrate the best dose for each patient. We describe the TDM for Meropenem in children undergoing ECMO because of severe respiratory failure due to Klebsiella pneumoniae infection, using a ultra-high performance liquid –chromatography (UHPLC) method with Diode Array Detector. Sample clean-up included a protein precipitation followed by chromatographic separation on a C18 reverse phase UHPLC column within 8#min, using a gradient. The method was validated according to the protocol from the European Medicines Agency and was thoroughly evaluated for interferences and quantification linearity. Linear relationships between peak area responses and drug concentrations were obtained in the range of 1-50#mg/L with an $R^2 > 0.999$. Imprecision and inaccuracy values (intra- and inter-assay) were ≤ 2.5% and ≤ 3% for Meropenem in quality control samples, respectively. We will show the importance of TDM after the circuit exchange that may lead to suboptimal antimicrobial therapy and potential increase of antibiotic resistances.

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DRIED BLOOD SPOT SAMPLING COUPLED WITH TANDEM MASS SPECTROMETRY FOR NEONATAL THERAPEUTIC DRUG MONITORING

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Therapeutic Drug Monitoring (TDM) aims at integrating the results of drug measurement into clinical decision making, to define the optimal individual therapy, to improve efficacy and minimize risks and toxicities. This is particularly important in pediatric practice as major differences in drugs absorption, distribution, metabolism, and clearance have been observed not only between adults and children, but also between newborn and pre-pubertal children. Moreover, newborns body weight can change significantly in a few days, as well as the development of some of their organs, that are not yet fully developed at birth. Antibiotics, such as beta-lactams or aminoglycosides, are the most commonly prescribed drugs in neonatal intensive care units (NICUs) as an empiric antimicrobial therapy. Other drugs widely used in infants are antimycotics, caffeine for apnea of prematury, and phenobarbital in the treatment of partial and generalized tonic-clonic seizures. In this framework, the use of TDM is mandatory. In our laboratory, we have developed a fast and robust method for the determination of various drugs used in pediatric applications, involving dried blood spot (DBS) sampling and ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) system. DBS is a very advantageous minimally invasive micro-sampling method that utilizes an ultra-low blood volume, obtained from a finger-or heel-stick, with minimal requirement for training, therefore facilitating serial sampling. In addition, it is characterized by ease and low cost of sample collection, transport, and storage. Our methods have been validated following international guidelines and European Bioanalysis Forum recommendations. Workflows, which include rehydration of the DBS and a simple early extraction with organic solvent, have allowed us to overcome the influence of hematocrit. The methods were sensitive and reproducible, allowing the identification and quantification of very low drug concentrations in blood samples.

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LIVER AND RENAL DYSFUNCTION AFFECT THE PHARMACOKINETIC OF NEW DIRECT-ACTING ANTIVIRAL DRUGS IN HIV/HCV CO-INFECTED PATIENTS

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Background: Sofosbuvir (SOF) plus daclatasvir (DAC) is one of the direct-acting antivirals (DAA) recommended options for HCV treatment. This regimen achieved high rates of sustained virologic response (SVR) with no difference according to HIV serostatus. Only limited information is available on the pharmacokinetics variability of SOF and DAC in HIV/HCV co-infected patients. Aim was to evaluate the association of plasma drug concentrations of SOF and of DAC with patient-, treatment-, and disease-related factors in HIV/HCV persons.

Method: HIV/HCV co-infected patients, undergoing SOF plus DAC treatment, were prospectively enrolled. At baseline, week 4 (W4), end of treatment (EOT), and after EOT, biochemical and viro-immunological parameters were assessed. Fibrosis 4 (FIB-4) score and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation were used for estimation of liver disease severity and of glomerular filtration rate (eGFR), respectively. SVR was defined as HCV RNA <12 UI/mL at 12 weeks after EOT.

SOF, SOF metabolite (GS-331007), and DAC plasma concentrations were measured, using a validated UPLC-MS/MS method. Venous blood samples for Ctrough levels (24 h post-dose) were assessed at W4 and W8, during DAA treatment and the mean value between the two time-points (mean-Ctrough) was calculated. Mann-Whitney as well as Spearman rank tests were used for statistical comparisons.

Results: Thirty-five patients were included, 32 had an available HCV-RNA at least 12 weeks after EOT, SVR12 achieved in 93.8% of subjects. As expected, SOF was undetectable in all samples. No statistically significant association were found between GS-331007 or DAC mean-Ctrough and most the analyzed variables. Only renal (CKD-EPI) and liver function (FIB-4), significantly correlated with, GS-331007 and DAC Ctrough values, respectively. Increasing GS-331007 mean-Ctrough significantly correlated with decreasing eGFR at W4 (rho=-0.36; p=0.04) and at EOT (rho=-0.34; p=0.048). This association was not found with eGFR at baseline (rho=-0.27; p=0.11) and lost after EOT (rho=0.01; p=0.97). Between DAC plasmatic exposure and FIB-4 a statistically significant correlation was observed at all time-points: baseline (rho=-0.35, p=0.03), W4 (rho=-0.44, p=0.008), EOT (rho=-0.40; p=0.02), after EOT (rho=-0.39; p=0.03).

Conclusion: In HIV/HCV patients receiving SOF plus DAC, plasma drug concentrations are associated with renal dysfunction for SOF metabolite and with liver impairment for DAC. In order to prevent drug failure and toxicity, therapeutic drug monitoring of DAA could be useful in

difficult-to-treat patients, as cirrhotic and renal impaired subjects.

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ON-SITE DETECTION OF COCAINE IN ORAL FLUIDS BY MICRONIR/CHEMOMETRICS: A NEW ANALYTICAL PLATFORM

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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. 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.Spectroscopic techniques have great potential for improving the discrimination of materials that show similar instrumental responses [1]. Among these techniques, near-infrared (NIR) spectroscopy is gaining global attention in field of forensics as an analytical technique able to give qualitative as well as quantitative information about complex samples [2]. To this end, coupling NIR spectroscopy with chemometric techniques may provide more useful information, as the results could be easily interpreted, and the effects of any kind of interference on the spectral signal may be evaluated [3].The smallest portable spectrometer operating in the Near Infrared region is the MicroNIR, a very compact and portable device (45 mm in diameter and 42 mm in height) that weighs about 60 g, and is entirely powered (5 V) and controlled via the USB port of a portable computer [4]. As a consequence, after the development of the model and the optimization of all of the statistical treatments, the approach is easily performed by an unskilled person. In this work, a novel on-site MicroNIR/Chemometris platform was developed to perform a real-time prediction of cocaine and its metabolites in oral fluids. The procedure was developed and validated by comparing results with the official method. The preliminary outcomes demonstrated the feasibility of the miniaturized approach to provide a correct identification of cocaine intake and to propose the MicroNIR as innovative personal screening system to prevent accidents and intoxications.

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THERAPEUTIC DRUG MONITORING OF TYROSINE KINESE INHIBITORS: A NOVEL RP-HPLC FL METHOD TO QUANTIFY RUXOLITINIB IN PLASMA SAMPLES

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Therapeutic Drug Monitoring (TDM) plays a pivotal role in assisting the clinicians in the decision-making process of adjusting the drug dosage of a current treatment, or changing the type of drug administered to the patient. This is particularly critical in case of treatment failure, severe side effects or suspected drug interactions.
Ruxolitinib is a JAK 1/2 inhibitor, approved in 2011 by FDA for the treatment of patients with intermediate or high-risk myelofibrosis (MF). Currently, side effects related to the use of Ruxolitinib are managed through therapeutic dose adjustment and blood count monitoring. However, the evaluation of plasma concentration of this compound and of other Tyrosine Kinase Inhibitors (TKIs) is becoming of increasing importance; the therapeutic efficacy of this class of drugs is indeed characterized by a marked variability caused by the presence of genetic polymorphisms, drug-drug interactions, and potential poor patient adherence. TDM of these compounds is thus required to ensure both an optimal response and the reduction of potential adverse effects. In addition, attention has been recently devoted to a possible topical use of JAK inhibitors in dermatological therapies; when such therapy is carried out it will be important to dose this compound in biological matrices to evaluate its systemic absorption, given the lack of literature data regarding the plasma concentration of Ruxolitinib.
We have developed and validated a novel RP-HPLC method for the quantification of Ruxolitinib in plasma using a HPLC system equipped with a fluorometric detector. Lower limit of detection (LLOD) for Ruxolitinib in plasma was of 0.05 ng/mL, whereas the lower limit of quantification (LLOQ) was 0.1 ng/mL. The linearity of the response provided by our method was verified over a concentration range going from 0.2 ng/mL to 500 ng/mL. Our methodology, based on a simple extraction procedure and on a cheap and fast chromatographic analysis, involves the use of an internal standard, thus partially overcoming intrinsic measurement errors related to the precipitation in organic solvents. The methodology has been validated according to the EMA guidelines, and can be considered as alternative and complementary to other previously proposed approaches.

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UTILIZZO DELLA CLEARANCE PLASMATICA DELLO IOEXOLO PER LA DETERMINAZIONE DELLA VELOCITÀ DI FILTRAZIONE GLOMERULARE (GFR)

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INTRODUZIONE: la GFR è misurabile in vari modi basati sia sulla capacità del rene di eliminare alcune sostanze endogene (es. Clcrea /CLcistaC) che di sostanze esogene somministrate al paziente (es. Cr-EDTA, inulina, ioexolo) o su equazioni che stimano la GFR (eGFR CKD-EPI) sulla base del sesso, età, razza del paziente. L'utilizzo di equazioni o della clearance della creatinina/cistatina C non sono però spesso in grado di fornire misure accurate della GFR, in quando sottostimano o sovrastimano la GFR a seconda che vengano applicate in pazienti sani o malati. E' utile disporre di un metodo accurato e praticabile in una routine clinica per la determinazione della GFR
OBIETTIVO: scopo del nostro studio è stato validare la determinazione dello ioexolo plasmatico da utilizzare presso l'ambulatorio prelievi per la valutazione della GFR in pazienti selezionati. MATERIALI E METODI: lo ioexolo è stato determinato con metodica HPLC UV detetector (Shimadzu) colonna (GENESIS C18 4µ) fase mobile: acqua con 5% di acetonitrile a pH 3.04; SI è ISO-VMA ricostituito in ac.perclorico ad una concentrazione finale di 100 mg/L. 6 soluzioni standard sono ottenute con diluizioni scalari di Omnipaque 300™. Previa acquisizione del consenso ai pazienti sono stati somministrati e.v. di 5 ml di ioexolo e sono stati eseguiti 8 prelievi seriali ad intervalli di 1 ora fino a 480 min. post infusione. La valutazione della GFR viene effettuata attraverso uno specifico sw. Sono stati indagati 26 pazienti (22 M/4F) età compresa tra 30 e 87 anni (media 67) con compromissione funzionalità renale (S-Crea media 1.67 mg/dl). RISULTATI: il metodo analitico ha dimostrato nelle nostre mani) a 3 differenti concentrazioni 35, 93 e 132 µg/ml una precisione nella serie (CV di 1.5, 0.7 e 0.2). CV interserie di 2.7, 0.5 e 0.51%; sensibilità 16.1µg/mL; linearità fino a 647 µg/ml. Il recupero 96.% al 101.1%. Il carry over è stato valutato dopo 10 iniezioni del calibratore 161 µg/ml e risulta assente. Non si osserva effetto matrice essendo la curva di calibrazione preparata su plasma che risulta sovrapponibile a quella in acqua. 5 replicati di un campione (45 µg/ml) preparato in acqua e plasma hanno un CV di 5.6 e 3.9%. Attraverso la clearance plasmatica dello ioexolo siamo riusciti a calcolare la VFG in numerosi pazienti con difficoltà ad effettuare raccolte temporizzate o impossibilità a calcolare la VGF con formule matematiche. Nessuno dei pazienti ha presentato effetti collaterali.

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APPLICAZIONE DEL METODO FMECA PER LA GESTIONE DEGLI ESAMI URGENTI

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Introduzione: Oltre due decisioni terapeutiche su tre si basano sui risultati degli esami di laboratorio e un errore anche minimo nel trattamento dei campioni, dal prelievo allo scambio di provette, può avere effetti gravissimi sulla diagnosi e di conseguenza sulla cura del paziente. Per prevenire varie tipologie di errore, il Laboratorio di Analisi Chimico-Cliniche con sede presso il P.O. Bassini, ha applicato il metodo Fmeca (Failure Mode and Critical Effect Analysis) per analizzare il processo della gestione degli esami urgenti provenienti dai 3 Pronto Soccorso e dai reparti dell'azienda ASST Nord Milano, che inizia con la raccolta dei campioni e termina con la produzione di un referto di laboratorio.
Metodi: Il processo è stato scomposto in 10 attività cui sono state associate 25 modalità di errore, e per ciascuna i potenziali effetti sul paziente, le misure di controllo e le cause. Ogni tipologia di errore è stata quindi valutata per gravità (G), probabilità dell'evento (PB) e rilevabilità (R) ed è stata classificata con un indice di priorità di rischio (IPR). Le modalità di errore con IPR $\geq$ 30 sono considerate ad alto rischio, con necessità di un'azione correttiva immediata.
Risultati: Molte delle modalità di errore, compresa quella con il più alto IPR (ritardo di consegna del campione) si è verificato nella fase pre-analitica, mentre nessuna modalità di errore ad alto rischio è stata trovata durante la fase analitica e post-analitica. Rischi ad alta priorità sono stati "ritardo di consegna del campione" (IPR, 48), "condizioni pre-analitiche non conformi" (IPR, 32), "gestione dei reagenti" (IPR, 24) ed "errore identità del paziente" (IPR, 20).
Conclusioni: In base all'analisi delle criticità riscontrate, è stata modificata l'attività del laboratorio introducendo moduli di rilevazione per il monitoraggio dell'arrivo dei campioni, per il monitoraggio delle non conformità e un nuovo programma di gestione magazzino.
Il processo verrà analizzato nuovamente a distanza di un anno per valutare se le azioni correttive avranno permesso una diminuzione dell'IPR, specialmente per i rischi ad alta priorità.

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BUDGET IMPACT ANALYSIS OF THE SUBSTITUTION OF NON-PROFESSIONAL-BLOOD GLUCOSE METER BY PROFESSIONAL BLOOD-GLUCOSE METER

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Background: The events of hyperglycemia or hypoglycemia in hospital setting are common, 30% to 40% of inpatients are diagnosed with glycemic crisis during hospitalization. It is already consolidated among medical societies the relevance of glycemic monitoring system since the concentration of plasma glucose higher than 140 mg/dL or lower than 54 mg/dL is strongly associated with mortality, mobility, and length of stay (LOS). There are two different scenarios of glycemic monitoring: 1) characterized by the Non-Professional Blood Glucose Meter(NP-BGM) usage, which leads to 1,8% of post-analytic errors on patient's electronic records; 2) characterized by Professional Blood Glucose Meter (P-BGM) usage, which is connected to online management system avoiding the manual reporting and post-analytic errors.
Aim: To measure the economic impact from the hospital payer perspective of the substitution of NP-BGM by P-BGM with higher accuracy and potential reduction of post-analytic errors, taking into consideration the impact on the reduction of length of stay, the reduction on nurse's labor time.
Method: We used a decision tree model to project health and economic outcomes over a 5 years' time horizon. The model takes into account an event of hypoglycemia increase LOS by 2.5 days and for hyperglycemia up to 1.5 days, the substitution of 80 NP-BGM by 12 P-BGM, and a population of 16 patients undergoing glycemic testing per day. The following costs were included in the model: 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 introduction instead of NP-BGM represents a positive budget impact of 33% decrease on total cost, and significant decreasing of 67% in nurse time required to manage glucose tests. The correct glycemic events identification ensures fast time to treatment, reducing patient LOS and increasing hospital bed availability of 3.24 patient days.
Conclusions: Innovations in technology could improve patient's outcome and at the same time efficiency in hospital settings, the accuracy and practicability of diagnostic test enhance the quality of work and reduce costs of adverse events.

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IMPLEMENTAZIONE DI INTERVALLI DI RILASCIO AUTOMATICO COME BASE PER LO SVILUPPO DI REGOLE DI AUTOVALIDAZIONE IN BIOCHIMICA CLINICA

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Introduzione: L'implementare nel Laboratorio di Biochimica Clinica un sistema di regole di autovalidazione che favoriscano il rilascio automatico di risultati dalle linee analitiche, bloccando quei campioni che potrebbero rivelare l'esordio di una patologia e/o la riacutizzazione di un evento avverso, permette di migliorare l'organizzazione dell'attività del laboratorio stesso e di standardizzare le procedure di refertazione.
Scopo: Lo studio è stato avviato attraverso l'elaborazione di Intervalli di Rilascio Automatico (I.R.A.) che definiscono gli estremi entro cui i risultati vengono rilasciati e validati automaticamente dal LIS.
Materiali e metodi: Sono stati calcolati i Limiti di R.A. di 30 analiti di biochimica attraverso la formula:
Punto centrale range di riferimento (R.R)=(Limite basso R.R+limite alto R.R)/2
Limite basso R.A. =[(Punto centrale-limite basso linearità)/2]+limite basso linearità
Limite alto R.A.=[(Limite alto linearità-punto centrale)/2]+punto centrale (1)
Gli I.R.A., perfezionati attraverso ricerche in letteratura sulla base di sesso ed età del soggetto, sono stati impostati nel middleware (cITm, Roche Diagnostics, Mannheim, Germany). L'attendibilità del dato è garantita dalla precedente valutazione di controllo di qualità interno, indici del siero ed eventuali allarmi strumentali/analitici. Sono stati raccolti una media di 2832 test/die per 10 giorni sia da pazienti ricoverati presso l'Ospedale di Desio, sia da utenti esterni.
Risultati e conclusioni: Applicando gli I.R.A., lo 0,95% delle alanina aminotransferasi e l'1% delle aspartato aminotransferasi analizzate giornalmente viene fermato dalle regole, è pertanto immediato constatare che gli I.R.A agevolino in questo caso l'attività di validazione. Al contrario, creatinine e glicemie (bloccate rispettivamente il 18% e il 4%), suggeriscono la necessità di un ulteriore perfezionamento del filtro attraverso una distinzione tra i reparti richiedenti e costruendo curve di distribuzione che permettano di valutare e consolidare l'utilizzo degli I.R.A. nella validazione dei campioni di routine.
(1) M.S. Feitosa et al. Implementation of criteria for automatic release of clinical chemistry test results in a laboratory at an academic public hospital, 2016. J Bras Patol Med Lab, v. 52, n. 3, p. 149-156

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LA GESTIONE DEL RISCHIO IN UN LABORATORIO ANALISI AUTOMATIZZATO

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Scopo del lavoro: La nuova norma UNI EN ISO 9001/2015 supera l'attuale concetto di azione correttiva/preventiva ed orienta la gestione del rischio verso un approccio proattivo. Abbiamo, pertanto, esaminato alcuni dei nostri sub-processi per attribuire uno score di priorità alle varie fasi, identificando e quantificando le potenziali fonti di rischio.
Materiali e metodi: Abbiamo scelto il metoto FMECA (Failure Mode Effects and Critically Analysis) come approccio multidisciplinare pro-attivo che, partendo dallo studio del processo nella sua globalità, individua e classifica (attraverso un prodotto di pesi) i fattori di rischio e facilita le relative azioni preventive. Il nostro ospedale dispone di un laboratorio ad alta automazione "multitasking" con le classiche fasi del processo (pre-analitica, analitica, post analitica) che impattano con una criticità diversa sul flusso di lavoro: la fase più salvaguardata è proprio quella analitica "core-lab" gestita da sistemi robotici. Le criticità vanno, pertanto, ricercate e prevenute nella gestione delle fasi pre analitica e post analitica, anche perché parzialmente espletate in un contesto esterno al laboratorio centrale.
Risultati: Abbiamo applicato il metodo FMECA al sub-processo "gestione dei campioni urgenti o prioritari" La codifica delle criticità in termini di IPR ha dato luogo ad una scala ponderale di valori che, una volta inseriti in una matrice gravità/probabilità, tracciano le priorità di interventi al fine di prevenire o limitare l'impatto di un sempre possibile evento avverso. Sono emerse alcune aree di criticità, che hanno totalizzato gli scores più elevati: a) Corretta interpretazione della richiesta medica (score 27); b) Corretta identificazione del paziente (score 27); c) Tecnica della venipuntura (score 16); d) Trasmissione/ consegna del referto di laboratorio (score 18).
Discussione e conclusioni: L'utilizzo del metodo proattivo FMECA ha consentito evidenziare, anche quantitativamente, che le fasi pre e post analitica rimangono, anche in una realtà ad elevata automazione, momenti insidiosi nel processo di laboratorio. Nel nostro caso i correttivi che abbiamo proposto sono: 1) centralità della figura del Medico in ambulatorio ed in sala prelievi; 2) corretta identificazione del paziente, nel rispetto della privacy, tramite un percorso "barcodato"; 3) facilità di accesso, da parte dei prescrittori, al repertorio degli esami di laboratorio con relative codifiche; 4) formazione del personale sanitario secondo le necessità emerse dall'analisi; 5) rivoluzione telematica nella trasmissione e consegna del referto. Sarebbe, infine, utile rivalutare gli indicatori utilizzati (pesi e scores) dopo un congruo periodo dall'implementazione dei correttivi individuati.

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IMPLEMENTATION OF POCT BLOOD GAS ANALYZERS NETWORK AT POLICLINICO GEMELLI FOUNDATION: OUR EXPERIENCE

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Background: According to ISO 15189, it is mandatory that any POCT network is under the laboratory control, to ensure results in accordance with the required quality standards and correlatable with those obtained by the laboratory.
Aim of the work. In our hospital (FPG), are currently present 25 blood gas analyzers (BGAs) which do not meet ISO 15189 requirements, so FPG has announced a commercial competition to replace them and to satisfy the in demand requisite of regional accreditation and guidelines.
Materials and methods: Three BGA apparatus (cassette system) were tested: GEM 5000 (ILWerfen), ABL90Flex (Radiometer), and Rapid Point 500 (Siemens), while the Phox Ultra (Nova Biomedical) served as reference tool.
One hundred whole blood samples from critically ill patients were measured with all the 4 instruments alternately, in order to minimize the variability of exposure at room air. Moreover, 10 samples were analyzed consecutively in triplicate to evaluate the instrumental reproducibility, and the intra and inter series imprecision has been assessed with a third party quality control (three levels).
For each sample, were measured the following parameters: pH, pCO2, pO2, Na+, K+, Ca++, Hb, Glucose and Lactate. The obtained results were analyzed with linear regression analysis, Bland-Altman plot and Student's T-test ($p<0.05$).
Results and discussion: All the BGAs considered produce clinically acceptable and interchangeable results. The total bias of the differences expressed as mean ±2 S.D. is very low for all tests, confirming the good accordance existing between the four instruments. However, statistical significance differences ($p<0.05$) were found between the BGAs relatively to the determination of pCO2, Na+, Ca++, Hb and Lactate, due to different analytical methods.
Since these technical differences are unavoidable, the final choice of BGA instruments will be performed depending on the health staff impact and feeling, the analytical characteristics and the best interoperability with LIS/HIS of FPG.

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THE ORGANIZATION OF THE PROTEIN UNIT: A BENEFICIAL EXAMPLE FOUNDED ON EVIDENCE BASED LABORATORY MEDICINE CRITERIA AND ON THE APPROPRIATE USE OF THE AVAILABLE RESOURCES

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Introduction: The sustainability of the National Health Service is a current subject of debate due to the pressure that the changes in our societies are having on health systems. Reviewing diagnostic processes is increasingly urgent to contain costs and to maintain the quality of the health services provided.
The Evidence Based Laboratory Medicine (EBLM) approach allows to identify and eliminate obsolete methods, replacing them with more adequate ones. The EBLM can also provide higher diagnostic accuracy, a rationalisation of diagnostic pathways, a reduction of turnaround-time and a decrease of costs. To reach these objectives, a careful analysis of production processes and assessment of the costs are both necessary. Methods: The EBLM approach has been applied at the Protein Unit of Laboratory of Modena. We used the available data from Laboratory Informatic System to kwon the number of the analyses and the stock reports for the reagents costs. The reorganization began in the 2015 so we take data of the 2014, as reference year, and we compared them with the data of 2015, 2016, 2017. A study of the processes of each single analytical method was carried out highlighting the critical issue. Results: The change in the workflow of the Bence Jones protein determination and the consolidation of the measurement of a number of serum proteins on clinical chemistry analysers allowed a better diagnostic accuracy coupled to important economical savings. The savings made it possible to extend the availability of the serum free light chains measurements, that was originally restricted to patients admitted to the haematology department, to all patients of the Province of Modena. Conclusion: The EBLM approach is the most effectives way to reach the such objectives: really, providing better quality performance does not necessarily correspond to an increase of costs.
In addition to developing an adequate level of scientific expertise, the laboratory professional must acquire managerial skills to introduce up-to-date diagnostic methods and to optimize the use of assigned resources, in all areas of the laboratory medicine.

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SUPERSATURAZIONE RENALE RELATIVA DI ACIDO URICO DETERMINATA IN EXCEL

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Premessa: Pazienti affetti da calcolosi renale sono generalmente monitorati servendosi dello studio metabolico urinario praticato su campioni delle 24 ore. Il nostro lavoro è riferito a pazienti con calcolosi urica. Al fine di valutarne l'efficacia delle scelte terapeutiche potrebbe essere utile eseguire rapidamente, su urina estemporanea, la determinazione del pH, dell'acido urico, degli elettroliti urinari (sodio e potassio) e la relativa estrapolazione della supersaturazione renale relativa (SRR UA) calcolata in Excel.
Materiali e metodi: I dosaggi urinari di acido urico (UA), sodio (Na) e potassio (K), espressi rispettivamente in mg/dL ed in mmol/L, si effettuano su COBAS 6000 della ditta Roche Diagnostics. Il pH viene determinato con pHmetro portatile Checker della ditta Hanna. Gli analiti sopra citati e il pH urinario consentono di calcolare la SRR UA con un nostro programma costruito in Excel e riferito al software Litho Risk della ditta BioHealth Italia.
Risultati: Con le relazioni 1, 2 e 3 si calcola SSR UA riferita alla concentrazione di 35 mg/dL di UA.

Relazione 1 : $m = -14{,}365.pH^5 + 406{,}49.pH^4 - 4545{,}9.pH^3 + 25077.pH^2 - 68148.pH + 72964$;

Relazione 2 : $n = -0{,}0806.pH^4 + 2{,}0766.pH^3 - 19{,}301.pH^2 + 75{,}697.pH - 102{,}76$;

Relazione 3 : $SSR\ UA_{\ a\ 35\ mg/dL} = [Na+K].m.10^{-5} + n$

La relazione 4 determina la SSR UA finale in funzione della effettiva concentrazione di uricuria:

Relazione 4 : $SSR\ UA\ finale = SSR\ UA_{\ a\ 35\ mg/dL} . [UA]/35$.

I dati riscontrati sono ben correlati con quelli ottenuti con il software Litho Risk ($SSR\ UA_{\ Litho\ Risk} = 0{,}9995.SSR_{\ UAproposta} - 0{,}0214$; $r^2 = 0{,}998$). Nella estrapolazione della $SSR\ UA_{\ Litho\ Risk}$ è necessario inserire il volume equivalente ad 1 litro, la concentrazione di uricuria trasformata in mg/L e tutti gli altri parametri urinari con valore di 0,001.
Conclusioni: La verifica delle scelte terapeutiche in pazienti affetti da calcolosi urica può effettuarsi su urine estemporanee con semplici e rapide determinazioni di pH, di acido urico, di Na e K, e con la relativa estrapolazione della SRR UA in Excel da noi proposta, evitando così il meno agevole e più articolato studio metabolico delle 24 ore.
Letteratura: Marangella M, Petrarulo M, Daniele PG et al. LithoRisk: software per il calcolo e la visualizzazione dei profili di rischio di calcolosi renale. Giornale Italiano di Nefrologia 2002;6:693-8.

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TOWARDS AN EVIDENCE-BASED PRICING FOR DIAGNOSTIC LABORATORY TESTING

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Background: Despite pressures to increase efficiency and reducing cost per test in clinical laboratories, few data are available in the literature on costing analysis. The evaluation of the relationship between volumes and costs, highlights the evidence that is not linear and numerous variables should be taken into consideration. Material and methods. 1575 different tests performed by UOC Medicina di Laboratorio - Azienda Ospedaliera-Padova in 2013 (total volume 9.350.000 tests) have been evaluated for establishing the operational costs considering all total testing process (pre-analytical, analytical and post-analytical, reporting, steps). All laboratory cost items (reagents, disposables, maintenance, service, cost of the entire personnel) have been considered and referred to each laboratory test. Direct and indirect costs have been calculated. Results: A wide range of costs have been found, ranging from 0.21 Euro for commonly clinical biochemistry measurands (glucose, creatinine,) to 37.0 for metanephrins: higher costs affect molecular biology investigations and tests of autoimmunity and allergy. In a benchmark with other two huge laboratories, the costs for clinical biochemistry measurands appear to be similar. Lower costs have been found for some clinically relevant tests: for TSH, 1.09 Euro in Padova, 1.62 and 2.19 in the other two laboratories; for FSH 2.43 Euro in Padova, vs 2.37 and 3.23 respectively; for CEA, 2.10 in Padova and 2.41 and 3.30 in the other laboratories. Subdividing all tests in three different categories according to their complexity (Regional Hub, Hub, and Spokes), and comparing costs to regional prices (reimbursement), the marginality was found very different being higher for spoke tests (1.04) and lower for more complex tests, Hub Regional in particular, with a ratio costs/reimbursement of 4.87.Conclusions: This activity-based cost analysis of a huge number of laboratory tests emphasizes the complex relationship between variables affecting the cost of individual laboratory tests. Much complex a test much more difficult the analysis of individual variables, and much more costly the test. Further studies are needed to provide a benchmark between different size laboratories and different testing complexity.

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COMPARISON BETWEEN IMMAGE 800 AND OPTILITE TO DETECT SERUM FREE LIGHT CHAINS

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Introduction: Serum Free light chains (sFLC) analysis is a crucial assay to evaluate patients affected by monoclonal gammopathy. FLC analysis has a high risk of incurring in the antigen excess, which requires expert operators to manage it.
Turbidimeter Optilite (Binding Site Birmingham, UK), equipped with software capable of identifying "high responsiveness" samples, was recently introduced to our laboratory. Thanks to a kinetic measurement of the antigen-antibody reaction, it allows to detect antigen excess. The aim of this study was to compare the analytical performance of Optilite with nephelometer Immage 800 (Beckman Coulter, CA, USA) currently used in our Laboratory.
Methods: We tested 87 consecutive sera from patients with multiple myeloma with both Immage 800 and Optilite using FreeliteTM FLC kit reagent (Binding Site Birmingham, UK).
To assess the agreement between the 2 instruments, Spearman's correlation coefficient (rs) was applied with 95% confidence interval.
Results: Due to the high reactivity of the samples tested with Immage 800, it was necessary to manually dilute 24 samples 1:250 (19 κ-FLC and 5 λ-FLC); 7 samples required additional dilution 1:1000 of which 3 up to 1:5000. Five samples gave no results because of concentration lower than the detection limit of the instrument.
All 87 samples gave results with Optilite, 26 were automatically diluted with serial logarithmic dilutions up to 1:1000 by Optilite, 2 of which recognized as "high responsiveness" specimens due to the antigen excess.
Four samples gave results >5000 with Immage 800, but Optilite provided 30% lower values possibly due to automatic dilutions avoiding operator errors.
Eighty-two out of 87 were the specimens analysed with statistic test. rs analysis gave the following results: κ-FLC rs=0.97 (0.95-0.98); λ-FLC rs=0.96 (0.93-0.97); rFLC (ratio κ/λ FLC) rs= 0.95 (0.93–0.98) and P<0.0001.
Conclusion: Excluding the outlier data, the results show an excellent agreement between the 2 instruments. However, the highest detection range, especially for low values, the automatic sample dilution, the ability to recognize and manage the antigen excess make Optilite a high-performance instrument which saves operators' time, standardizes procedures and provides high quality outcomes.

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G26 EASYFIX INTERLAB, COMPARISON WITH HYDRASYS SEBIA AND PRELIMINARY ASSESSMENT ON THE DETECTION OF BENCE JONES PROTEIN

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Introduction: In the management of monoclonal plasma cells dyscrasia, the evaluation of the monoclonal protein (M-protein) is crucial. The diagnosis of M-protein occurs only through immunofixation, a high time-consuming and operator-dependent method. G26 EasyFis Interlab (G26), marketed by Sebia (France), is a new fully automated instrument capable of performing serum and urinary immunofixations.
The aim of this study was to evaluate performance of G26 in comparison with Hydrasys (Sebia, France) currently used in our laboratory for Bence Jones protein (BJP) detection.
Methods: We selected 2 urine samples with characterized BJP (BJP-κ and BJP-λ) from patients without renal damage. By subtracting the concentration of physiological urine albumin (uALB) from the concentration of total urinary proteins (uPT) measured with AU680 chemical instrument (Beckman Coulter, Italy), we approximately obtained the concentration of BJP. Further, we performed serial dilutions of the urine samples which we analysed with both G26 and Hydrasys, using agarose gel (AG) and antisera of both Interlab and Sebia in different combinations. The antisera consist of a mix of total κ+λ antisera in a 1:1 ratio, it is prepared in-house for Sebia (Sebia-mix) and ready to use for Interlab (Bivalent).
Results: The sensitivity of Hydrasys with AG Sebia and Sebia-mix antiserum in detection of BJP was 3-5 mg/L; in the case of G26 with AG Interlab and Sebia-mix antiserum it was 5-7 mg/L; in the case of Hydrasys with AG Sebia and Bivalent antiserum it was 6-8 mg/L; in the case of G26 with Interlab AG and Bivalent antiserum it was 9-10 mg/L.
Conclusion: The results show that both AG and antisera by Interlab are less performant than AG and, especially, antisera by Sebia. The Sebia antisera do not have conformity certificate to be used by Interlab instruments and the two AG are not interchangeable due to their different sizes. The only option for G26 is to work with Interlab AG and antisera. With a sensitivity just slightly lower than 10 mg/L, this combination meets the recommendations of the guidelines indicating 10 mg/L as detection limit. However, G26 has sure advantages regarding automation, data traceability, archiving of AG images, standardizing procedures and lastly, optimizing work and saving operator-time.

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A COMPUTER MODEL FOR THE PROFESSIONAL COMPETENCES ASSESSMENT ACCORDING TO ISO 15189 STANDARD ACCREDITATION

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As defined by ISO 15189 competence is the "demonstrated ability to apply knowledge and skills" thus its assessment is fundamental for ensuring the operational quality in order to reduce the risk for the patient.
To this aim we have developed a functional software for the measurement of professional competences, suitable for the Laboratory in accordance with the accreditation requirements, which standardises the procedure and collects all the data in a single platform, avoiding redundancy and dispersion. Our model objectively assess the skills, as they become measurable and comparable with appropriate standards and involves both managers and operators, to increase their active engagement and to exploit their contribution. The assessment concerns everyone, but the standards to be met can vary according to the responsibilities. For each role for each target competences numerical values are assigned as standards to achieve.
Several subjective and objective criteria are evaluated: each parameter can contribute in a variable proportion to the total skills measured according to the needs of the organization.
The subjective criteria include a self-evaluation expressed by the operator combined with an evaluation made by the managers.
The objective criteria represent the evidence of the continuing professional development and consider all the education, training and work activities that can be objectively quantified on the basis of the hours worked, the certificates of attendance and the publications.
The data are automatically analyzed and can be easily monitored in real time in the form of indicators, thanks to dashboards. The comparison between the skills required (personalized target) and those owned (measured) allows to highlight the gap useful for planning personalized training paths.
Our tool is reliable and highly adaptable to laboratories about competences to track, criteria, standards and monitored indicators.
The computerized management is a strategic action as it fulfills the requirements of registration, traceability, communication, data analysis and indicators development, which are the tenets of the continuous improvement, and allows the plannings to be made on the basis of the actual training needs, translating the Plan Do Check Act (PDCA) model into practice.

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E' POSSIBILE L'UTILIZZO DI UNA MATRICE PLASMATICA SU PIATTAFORME CORELAB?

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Introduzione: l'uso del plasma offre numerosi vantaggi vs.siero - non richiede coagulazione, permette di ottenere un maggior volume di campione dopo centrifugazione; minimizza la formazione di reti di fibrina e permette una riduzione del TAT, ma in moltissimi laboratori anche Corelab è il siero la matrice maggiormente utilizzata Obiettivo: valutare la trasferibilità dei risultati ottenuti su una provetta di siero BD Vacutainer SST™ II Advance (3.5 ml) vs.2 diverse provette di plasma eparinato (BD Vacutainer LH PST™ II e BD Vacutainer Barricor™ LH Plasma) 3.0 ml, per al fine di verificarne la possibilità di introdurre la matrice plasmatica su una piattaforma Corelab che produce circa 5.5 milioni di analisi/anno.
Materiali e metodi: 70 volontari, 29 maschi e 41 femmine età compresa fra 18 e 65 anni, a digiuno da 12 ore. Le provette raccolte sono state centrifugate entro un'ora dal prelievo ed analizzate entro 4 ore su piattaforma Cobas 8000 (Roche Diagnostics). Sono stati esaminati 84 analiti (chimica clinica, immunochimica e sierologia). Per ogni analita è stato calcolato il bias con la formula e comparato con le specifiche desiderabili presenti nel database sulla variabilità biologica di Ricos. È stata eseguita l'analisi di Bland-Altman Risultati.
L'utilizzo di una matrice plasmatica ha evidenziato una migliore qualità del plasma vs. siero (Index serum SST è 1.5 volte > a PST e > 2 volte Barricor); la quantità di materiale ottenuto con PST e Barricor ha permesso di eseguire la quasi totalità degli analiti indagati. Il Bland-Altman plot ha evidenziato bias significativi per il $K^{+;}$ LDH , proteine totali, fosforo e glucosio. Non si sono evidenziate differenze per analiti di immunochimica e di sierologia.
Conclusioni: la buona correlazione fra siero e plasma evidenziata per gli analiti indagati permette di ipotizzare, anche in su piattaforme ad elevata automazione, l'utilizzo del plasma migliorando la qualità del materiale analitico particolarmente evidente con Barricor che di ridurre problemi di inadeguatezza dei campioni per incompleta coagulazione. L'utilizzo del plasma comporta la ridefinizione degli intervalli di riferimento per quegli analiti che hanno evidenziato significative differenze tar le 2 matrice quali di K^+, LDH, Ptota, Phos e Glu.

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UTILIZZO DI UN SOFTWARE HOME MADE PER IL MONITORAGGIO DEL PROCESSO DI COMUNICAZIONE DEI RISULTATI CRITICI: DUE ANNI DI ESPERIENZA IN UN LABORATORIO DI UN GRANDE OSPEDALE METROPOLITANO

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Introduzione: Uno dei processi che più impattano sulla sicurezza del Paziente è la comunicazione dei risultati critici. Per questo motivo, il documento SIBioC di riferimento (Bioch Clin 2018;42:167-179) raccomanda di "valutare le prestazioni della gestione del processo" mediante indicatori. Convinti che questa esigenza possa essere soddisfatta più agevolmente mediante strumenti informatici dedicati, per tracciare e analizzare i dati abbiamo creato e utilizzato un software in Microsoft Access.
METODI: 12510 comunicazioni registrate fra luglio 2016 e giugno 2018 sono state analizzate (complessivamente e su base semestrale) in termini di tempi di notifica (rispetto alla disponibilità del dato (T1) e rispetto all'arrivo del campione in Laboratorio (T2)), percentuale di notifiche entro il tempo stabilito (1h), frequenza di notifiche per analita; l'analisi è stata effettuata sui dati complessivi e dopo stratificazione per tipologia di richieste (U=urgenti, V=refertazione "rapida" (entro 2h) e R=routine), provenienza, operatori comunicanti e riceventi.
Risultati: Il 51% dei risultati comunicati era riferito a richieste U, il 13% a V e il 36% a R. T1 medio é stato rispettivamente di 5 (95% CI: 4,7÷5,3), 8 (6,9÷9,1) e 15 (13,8÷16,2) min; i dati comunicati oltre i tempi stabiliti sono stati rispettivamente 0,7%, 2,2% e 4,9%. T2 medio: 59, 80 e 188 min. Nel 75,6% dei casi il dato è stato comunicato ad un infermiere, nel 23% ad un medico (del reparto, specializzando, di medicina generale o pediatra di base), nel 1,4% direttamente al Paziente. Il parametro più comunicato è risultato Emoglobina per U (14%), Potassio per V (26%) e Fosforo per R (15%). Per alcuni analiti (es. CK) si sono riscontrate differenze significative fra i diversi operatori nella frequenza di comunicazione, probabilmente attribuibili alla diversa competenza nel giudicare la rilevanza clinica del dato rispetto al contesto clinico o al reparto di provenienza; ciò suggerisce di affinare le modalità di "warning" del dato da parte del sistema informatico del Laboratorio.
Cconclusioni: L'utilizzo di un software progettato da professionisti di Laboratorio può agevolare il monitoraggio dei parametri utili a definire l'efficacia e l'efficienza di un processo e ad apportare eventuali miglioramenti in tempo reale.

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GESTIONE AUTOMATIZZATA DI ANALISI SIEROLOGICHE SU UNA PIATTAFORMA CORELAB ROCHE

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Introduzione: la disponibilità di una piattaforma Corelab in grado di eseguire oltre che analisi di chimica clinica/ immunochimica anche indagini sierologiche pemette di semplificare significativamente il work flow recuperando risorse tecniche e introducendo sistemi di reflex testing con riduzione dei TAT. Obiettivo: scopo delllo studio è stato valutare su una piattaforma Corelab Roche (Cobas 8000 e C801) la qualità dei reagenti per indagini sierologiche (HIV, HCV, HBV, sifilide e complesso TORCH) e l'applicazione di sistemi automatici di approfondimento diagnostico per una riduzione dei tempi di risposta. Materiali e metodi: abbiamo utilizzato 3 pannelli di sieroconversione per HIV-1, HCV e HBV. Sono stati analizzati circa 100 sieri di pazienti precedentemente testati con altro sistema (Abbott). I campioni positivi sono stati analizzati con metodo di riferimento (Western Blot) e si è proceduto ad approfondimenti clinico diagnostici. I risultati ottenuti con i 2 metodi sono stati confrontati e valutati in termini di concordanza dei positivi/negativi e totale e nei confronti del metodo di riferimento. Risultati: rispetto al metodo in uso abbiamo riscontrato una piena concordanza tra i 2 sistemi per HBsAg, HBeAg, Ac.anti HBc totali, Ac.HAV totali, Lue e Ac.anti CMV IgG. I restanti test hanno mostrato differenti percentuali di concordanza sia per i negativi che per i positivi: HIV neg.(100%) pos.(91.7%); HCV neg.(87.7%) pos. (94.6%); Ac.anti HBsAg: neg.(93.8%) pos.(89.2%); Ac. Anti HBeAg.: neg.(95.8%) pos.(100.0%); Ac.anti HBcIgM: neg.(100.0%) pos.(75.0%); Ac. Anti Toxo IgG: neg. (100.0%) pos. (93.3%); Ac. Anti Toxo IgM: neg.(100.0%) pos.(62.5%); Ac. Anti Rubeo IgG: neg.(100.0%) pos. (62.5%); Ac. Anti Rubeo IgM: neg.(97.9%) pos.(55.5%); Ac. Anti CMV IgM: neg.(100.0%) pos.(28.6%).I pannelli di sieroconversione per HIV e HCV hanno evidenziato una più rapida positivizzazione per i test Roche. Non si sono evidenziate differenze per HBV. I i campioni risultati positivi per HIV sono stati confermati con metodo di riferimento; 3 campioni positivi per HCV con di metodo di routine ma negativi con il metodo Roche sono risultati negativi al test di conferma. Conclusioni: nella nostra esperienza è possibile consolidare su una piattaforma Corelab Roche anche analisi sierologiche. La qualità dei risultati ottenuti appare elevata con evidenti vantaggi quali la riduzione del numero di campioni falsamente positivi per HCV e la possibilità di implementare logiche di approfondimento automatico per CMV e Toxo (avidy) che riducono significativamente i tempi di risposta.

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ASSESSING THE LABORATORY WORKFLOW IN THERAPEUTIC DRUG MONITORING BY LCMS

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Therapeutic drug monitoring (TDM) requires accurate and timely results to avoid severe side effects or treatment failure. The objective of this work was to evaluate the new approach to TDM assays in “Baldi e Riberi” Laboratory using traditional and innovative indicators as well as to compare previous and new performances. In the previous laboratory equipment (PLE) immunosuppressant drugs (ISD) and other TDM were tested using: two immunoassay instruments, two HPLC and two LCMS. The new laboratory equipment (NLE), instead, consists of: one automatic sample-preparation system (HAM) and three LCMS. Two LCMS are used for immunosuppressant drugs (ISD) analysis (ISD-MS) and one for all the remaining assays (TDM-MS). Automated sample preparation is used for ISD and for a few other TDM, the remaining samples being processed manually. To assess Overall Equipment Effectiveness (OEE) for NLE a performance indicator was applied to calculate instrument capacity utilization, based on three categories: Availability (A), Performance (P) and Quality (Q), where A=Run Time/Planned Production time, P=Net Run Time/ Run Time and Q=Good count/Total count. ISD tests were further investigated by assessing Total Testing Cycle Time (TTCT), Labor Time (LT) and Cost of testing (CT) for one representative batch (51 samples +4 QC). CV% and Tour Around Time (TAT) were assessed for both the PLE and the NLE. Overall 47000 ISD and 10100 TDM tests are annually performed on a seven-day a week basis. Mean value for A was quite good for both HAM (0.92) and ISD-MS (0.87), worst for TDM-MS(0.69), whereas P was better for TDM-MS (0.78) than for HAM (0.67) and ISD-MS (0.47); Q was > 0.94 for all the instruments. Moreover ISD TTCT was 3.55 h, LT 0.98 h and CT 6.6 Euros. CV% was <15% for all the tests in the PLE as well in the NLE. The TAT for ISD was on average increased from 2.6h to 3.6h with the NLE. Our findings showed that P of HAM and ISD-MS, working on a batch-based flow, was mainly affected by the timing of sample arrival, while the lower A value for TDM-MS was due to the daily multiple set-up procedures needed to switch among analytical methods. In conclusion OEE resulted a suitable indicator for workflow monitoring and the NLE provided reliable performances, even if TAT should be improved.

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EVALUATION OF EFFECTIVENESS OF A NEW PNEUMATIC TUBE SYSTEM FROM THE EMERGENCY ROOM

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Therapeutic turnaround time (TAT) and process traceability are critical issues for First Aid laboratory tests. There is a wide variability in pre-analytical activities from blood sampling to laboratory arrival. These phases account for over 20% of the total TAT. Aim of this work is to evaluate the improvement of TAT after activation of the Tempus 600 system and its impact on the organization of emergency room and on the integrity of blood samples. Methods: until December 2017 the pre-analytical flow at the Hospital dell'Angelo emergency room included 1) blood collection, 2) registration of the requests, 3) application of identification labels, 4) storage of samples in the sending area, 5) manual transportation every 20 minutes by an operator, a path of 150 meters and 1 floor of stairs to the laboratory. The TEMPUS600® system (produced by Timedico A/S, Bording, Denmark and enhanced by EOS srl, Padova, Italy), is an innovative pneumatic tube system that allows to send blood samples immediately to the Laboratory, by tubular path (internal diameter 2.5 cm) with compressed air system. Containers are not needed. The new system allowed the suppression of phases 4 and 5 with a reduction of FTe (full-time equivalent) of 1,24. In addition, each tube is scanned and registered at departure and upon arrival in the laboratory. The integrity of the samples was considered 130 pairs of sample with the manual handling considering the Total Error Allowable (ETa) of several biochemical variables. Results: During 1 month of comparison the improvement of total TAT was of 17 minutes, both as median and as a 90th percentile. The time from the decision of testing and the arrival to the laboratory was reduced from 45 minutes with couriering to 28 minutes. Regarding stability, K can be considered within the limits of the ETa but a little higher than the analytical variability, while for LDH the 14.6% of cases exceeds these limits with a mean overestimation (+4%). No other analyte has shown significant variations. The complaints of delays to the laboratory were reduced. Conclusion: In this case, the installation of TEMPUS600® has significantly reduced the TAT and has brought great benefits in the management field. The integrity of the samples should be further checked on other analytes.

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CYSTATINE C AND BETA-TRACE PROTEIN TO ASSESS KIDNEY FUNCTION IN PRETERM NEWBORNS <32 WEEKS OF GESTATIONAL AGE.

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Background: Nephrogenesis is active until 36 weeks' gestation and may be negatively affected by kidney-detrimental events. Little is known about biomarkers other than serum creatinine (sCr), such as serum cystatine C (CysC) or beta-trace protein (BTP), in preterm newborns. Aims:to evaluate CysC and BTP levels in subjects born at a gestational age (GA)<32 weeks, and to assess the impact of pre-natal and post-natal kidney-detrimental factors on kidney function.
Methods: newborns with GA<32 weeks were enrolled. Blood samples for sCr, CysC, BTP and urea measurement were obtained on the 3rd day of life (T0) and at a GA of 36 weeks (T36). eGFR was calculated according to 9 existing formulas (Schwartz 2009, Brion, Schwartz 2012, Zappitelli, Filler, Dorum, Trieber, Zappitelli-combined) at T36. Pre-natal and post-natal kidney injury risk scores were calculated by the number of potentially kidney-detrimental factors.
Results:We enrolled 71 newborns at T0 (31 with GA#28 weeks) and 53 subjects at T36 (25 with GA#28 weeks). At T0, newborns with GA#28 weeks had higher sCr levels than those with GA>28 weeks (p=0.016). At T0, sCr was negatively correlated with GA (R= -0.315, p=0.009),whereas CysC and BTP were not influenced by GA. At T36, newborns with GA#28 weeks had lower sCr, BTP and higher urea levels (p=0.007, p=0.005 and p=0.029, respectively). At T36 eGFR values calculated by the four formulas using only CysC were not different in newborns with GA#28 and >28 weeks. Conversely, eGFR values by other formulas were higher in subjects born at a lower GA. The pre-natal score did not correlate with eGFR. We found a direct correlation only between the post-natal score and eGFR according to Schwartz 2009 (R=0.345, p=0.027) and Brion's formulas (R=0.312, p=0.044), using sCr. However, these correlations did not persist when adjusted for urea levels at T36 and GA.
Conclusions:CysC-based eGFR are not influenced by GA. Post-natal score shows a direct correlation with eGFR according to sCr-based formulas, not persisting after adjustment for GA and urea levels, suggesting that the confounder may be the nutritional status of preterm newborns. Indeed, more premature subjects receive a more aggressive nutritional regimen, as suggested by higher T36 urea in newborns with GA<=28 weeks.

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ANALISI EPIDEMIOLOGICA SULL'UTILIZZO DEL QUANTIFERON TEST PRESSO IL PRESIDIO OSPEDALIERO "F. SPAZIANI" DI FROSINONE

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L'infezione tubercolare latente è una patologia asintomatica non trasmissibile, che in alcuni soggetti può persistere anche per mesi o anni prima che la malattia diventi conclamata. Il test Quantiferon rileva, nel sangue dei soggetti infetti, la risposta immune cellulo-mediate agli antigeni peptidici che simulano le proteine micobatteriche. Queste proteine ESAT-6, CFP-10 stimolano la risposta all'IFN-γ nelle cellule T dei soggetti contagiati da M. tuberculosis ma non in quelle di soggetti non infetti o vaccinati con BCG. Il crescente interesse per i test che misura la produzione di interferone-γ prodotto dai Linfociti T attraverso stimolazione ha fatto iniziare una valutazione su pazienti afferenti pressso codesta azienda ospedaliera. Lo studio è stato eseguito su un numero di soggetti pari a 925, italiani e stranieri di varie etnie. Di questi 198 sono risultati positivi, 39 indeterminati. L'arco temporale dello studio si estende dal 01/03/2015 al 31/03/2017. Sono stati studiati tutti i pazienti risultati positivi e indeterminati, verificando le altre indagini eseguite: Ricerca M. tubercolare in PCR ed attraverso esame batterioscopico. Per ogni paziente risultato positivo sono stati raccolti dati di carattere demografico, epidemiologico, clinico, stato sierologico per infezione da HIV, microbiologico e molecolare. La determinazione legata alla ricerca del quantiFERON test in un arco temporale di due anni ha evidenziato innanzitutto un incremento di richieste e un incremento di positività. Tutto questo porta a concludere che i fattori di incremento della richiesta di esecuzione del QFT siano legati all'allarmante recrudescenza della TB nel territorio provinciale preso in esame, in rapporto a quello regionale e inevitabilmente nazionale: Tasso di incidenza t. polmonare nella popolazione residente (2014): FR 3,6 - Media Lazio 7,63- % di esami colturali per diagnosi di t. polmonare (2014): FR 50% - Media Lazio 79,68%. Conferme colturali diagnosi di t. polmonare (2014): FR 27,78 - Media Lazio 60,73. Le cause sono l'aumento dei flussi migratori che hanno interessato Frosinone e la sua provincia prima del 2015.Nella seconda metà del 2016 il territorio frusinate è stato interessato dall'apertura di campi accoglienza profughi provenienti dall'Africa. L'importanza di questa indagine epidemiologica si evidenzia soprattutto in campo clinico, in particolare per quanto riguarda la diagnosi di infezione latente in soggetti asintomatici venuti a contatto con M. Tubercolosis. Nei pazienti immunodepressi il test Quantiferon rappresenta una soluzione ideale rispetto al tradizionale test Mantoux poiché in caso risultasse negativo potrebbe essere legato all'anergia e quindi un falso negativo.Il test Quantiferon molto sensibile e specifico rappresenta un ottimo ausilio diagnostico nei programmi di controllo della tubercolosi.Fonte m&s.

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BIOMARKER PANEL IN HCV-RELATED MIXED CRYOGLOBULINEMIA SYNDROME

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Background: Hepatitis C virus (HCV) infects both hepatocytes and B-lymphocytes, provokes cellular dysfunction and causes B-lymphocyte expansion-related diseases such as mixed cryoglobulinemia (MC) that is considered a crossroad of autoimmunity and cancer, evolving into B cell Non-Hodgkin Lymphoma (B-NHL). Differences in serum IgG subclasses distribution between patients with autoimmune diseases have been described, with distinct patterns in different conditions. Production of free light chains (FLC) of immunoglobulins is abnormally high in several autoimmune disorders and reflects B cell activation. Angiogenesis plays an important role in the progression of systemic autoimmune disease and vascular endothelial growth factor (VEGF) high levels correlate with disease activity in patients with rheumatoid arthritis (RA). Our aim was to evaluate serological profile of IgG subclasses, FLCs and VEGF in HCV-related MC patients.
Materials and Methods: Ninety-three patients with chronic diseases were retrospectively enrolled, including 53 with HCV-related MC, and 40 HCV-negative patients with RA characterized by absence of radiologically visible lesions, not receiving therapy and 30 healthy blood donors. In these patients the four IgG subclasses, FLCs and VEGF serum concentrations were measured.
Results: We found a statistically significant increase in IgG3 subclass only in HCV-related MC patients with an increase of FLCs levels in patients groups respect to healthy subjects. While IgG2 and IgG4 were lower in HCV patients than to controls. Serological determination of VEGF levels showed a significantly increased in HCV-MC.
Conclusion: The different distribution of IgG subclasses that we found between HCV-MC and RA patient groups, could reflect an immune response depending on the type of the autoantigen and duration of antigen exposure and it could show that IgG subclasses levels are correlate with in driving the disease process. Our results confirm that HCV-related MC is a lymphoproliferative disorder strongly associated with high levels of FLCs comparable to RA. The biomarker panel we analyzed appear to be a useful tool to monitor B cell and angiogenesis activation. Further investigations are necessary to exploit its potential of reliability for disease activity and progression.

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I BIOMARCATORI NELLA GUIDA ALLA TERAPIA ANTIBIOTICA NEI PAZIENTI CON SEPSI: QUALE RUOLO PER LE MOLECOLE ST2 (STIMULATION EXPRESSED GENE 2) E SCD 14 (PRESEPSINA)?

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L'incidenza della sepsi è in incremento con una mortalità legata alle complicanze che si aggira intorno al 30-50%. Una diagnosi tempestiva è fondamentale per un rapido intervento terapeutico. Il gold standard per la diagnosi è l'esame colturale, che però richiede 24 ore o più per avere un risultato positivo, per cui l'antibioticoterapia è empiricamente avviata prima che siano disponibili i risultati. Emerge quindi la necessità di individuare strumenti in grado di valutare tempestivamente la risposta del paziente alla terapia antibiotica, col duplice scopo di ridurre il rischio di evoluzione verso lo shock settico e di prevenire la sovraesposizione agli antibiotici, causa delle resistenze. La proteina ST2 appartiene alla famiglia dei recettori dell'interleuchina-1(IL1RL1); la sua forma solubile si lega alla IL-33 bloccandone l'effetto antinfiammatorio. Il CD14 è una glicoproteina espressa sulla membrane di monociti/macrofagi che, a seguito di fagocitosi, lega il complesso LPS e LPB che attiva il toll-like receptor 4 (TLR4), dando inzio alla cascata infiammatoria. Scopo dello studio: valutare se in pazienti con emocoltura positiva è possibile predire il successo della terapia antibiotica monitorando le concentrazioni di ST2 e Presepsina.
Materiali e metodi: i biomarcatori ST2 e Presepsina sono stati determinati al momento del riconoscimento della sepsi e per i successivi due giorni, in 35 pazienti con emocolture positive:14 maschi (età mediana 74) e 21femmine (età mediana 69). Le concentrazioni plasmatiche della Presepsina e quelle sieriche dell'ST2 sono state misurate rispettivamente in chemiluminescenza mediante analizzatore PathFast (Mitsubishi) ed in ELISA con analizzatore DSXSystem(Technogenetics). Risultati. Concentrazioni medie dei biomarcatori al baseline e nelle successive 24e48h: Presepsina=2.924,1.984,1.150 pg/L; ST2=142,99,72 ng/mL. Decremento 48h vs baseline:sCD14=60%; ST2=49%. Lo studio delle curve ROC evidenzia: AUC per la sCD14 pari a 0.97 (CI al 95% da 0.95 a 0.99), con una sensibilità ed una specificità del 99% per valori di sCD14 >299 pg/L, per ST2 AUC è pari a 0.98 (CI al 95% da 0.96 a 0.99) con una sensibilità ed una specificità del 99% per valori di ST2>38ng/ml. Conclusioni: I nostri dati preliminari confermano quanto riportato in letteratura sulla attività dell'ST2 durante processi infiammatori acuiti e nei pazienti con sepsi. Un decremento della sCD14 accompagnata ad una riduzione della proteina ST2, che si nota soprattutto in terza giornata, potrebbe aumentare il potere predittivo positivo del trattamento farmacologico e del decorso

clinico, garantendo un aumento della sopravvivenza e un notevole risparmio in tema di spesa sanitaria.

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REATTIVITA' ASPECIFICA PER LA LUE

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Premessa: Ad ogni donazione la sicurezza trasfusionale viene assicurata da esami eseguiti in sierologia e biologia molecolare. Tra gli esami di screening è obbligatoria la ricerca di anticorpi anti Treponema p., batterio responsabile della LUE per il quale però non è previsto un esame in biologia molecolare. Spesso la presenza di anticorpi aspecifici del donatore diretti verso costituenti dei kit commerciali sono responsabili di reattività aspecifiche. Ciò comporta l'eliminazione degli emocomponenti e la sospensione temporanea del donatore. Lo scopo del seguente lavoro è stato quello di analizzare risultati con dubbia reattività per il Treponema p. con l'introduzione di una seconda strumentazione e, quindi, la possibile riammissione di donatori in precedenza esclusi.
Materiali e metodi: Presso il Sit Asl CE i test di screening sierologico sono eseguiti con l'apparecchio Architect i2000 (Abbott) e da settembre 2017 quest'ultimo è stato affiancato dallo strumento Vitros 3600 (OrthoClinicalDiagnostics). Entrambi gli apparecchi si basano sulla metodica in chemiluminescenza. I campioni con reattività dubbia (valori di S/CO compresi tra 0.80 a 1.0) ottenuti con lo strumento Architect sono stati ripetuti con Vitros 3600 allo scopo di confermare o meno i risultati dallo score dubbio.
Risultati: Da settembre 2017 a giugno 2018 sono afferiti 9980 donatori di sangue presso il SIT Asl Ce. Lo 0.53% di questi hanno presentato reattività dubbia al test iniziale di screening per la LUE eseguito con lo strumento Architect i2000, con conseguente scarto degli emocomponenti e sospensione temporanea del donatore in attesa del test di conferma. Gli stessi campioni ritestati con Vitros 3600 hanno portato ad un abbassamento delle reattività dubbie del 33 %.
Conclusioni: La discordanza dei risultati è da spiegare con un'elevata sensibilità dello strumento Architect i2000 e quindi una maggiore specificità del Vitros 3600 nello screening per la LUE. L'esecuzione dei test di screening con il nuovo strumento comporta l'abbassamento di reattività aspecifiche e di conseguenza un minor scarto di emocomponenti. Restano, però, problemi per la riammissione dei donatori in precedenza sospesi che potrebbero risolversi con l'introduzione di test di conferma per la ricerca del Treponema p.

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VALORE PROGNOSTICO DI GALECTINA-3, PROCALCITONINA (PCT), PRESEPSINA (SCD14) E PROTEINA C REATTIVA (PCR) NEI PAZIENTE CON SEPSI NELLA NOSTRA ESPERIENZA

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La Galectina-3, fa parte della famiglia delle lectine leganti β-galattosidi, è ampiamente espressa sui macrofagi/ monociti attivati ed risulta implicata in molte risposte infiammatoriei. La PCT può essere prodotta da diversi tipi di componenti cellulari in risposta a stimoli pro-infiammatori, ha mostrato una buona accuratezza diagnostica e prognostica nei pazienti con sepsi. sCD14 è una glicoproteina espressa sulla membrane di monociti/ macrofagi, risulta fortemente aumentata nella sepsi. PCR è una proteina della fase acuta della flogosi, aumentata in corso di sepsi. Scopo di questo studio è stato quello di confrontare, in pazienti con emocolture positive,

l'accuratezza diagnostica tra Galectina 3, sCD14, PCT e PCR. Materiali e metodi: sono stati selezionati 30 pazienti con emocolture positive (14 maschi e 16 femmine con età mediana 82 e 84 rispettivamente) e 30 soggetti apparentemente sani (età mediana 55). I biomarcatori sono stati determinati al momento del riconoscimento della sepsi e per i successivi due giorni. Le concentrazioni plasmatiche (litio-eparina) della Presepsina e quelle sieriche di PCT e Galectina3, sono state misurate rispettivamente in chemiluminescenza mediante analizzatori PathFast (Mitsubishi) e LIAISON, ed in ELFA sull'analizzatore VIDAS (Biomeriux), mentre il dosaggio nefelometrico su siero della PCR è stato eseguito su analizzatore VISTA (Siemens). Risultati. Concentrazioni medie dei biomarcatori al baseline e nelle successive 24 e 48 h: Presepsina= 4390,3702,3011pg/ L; galectina 3= 72,58,50 ng/mL. PCT 33,26,18 ng/dl, PCR=137,105,72 mg/l. Decremento 48h vs baseline: sCD14=32%; Galectina3=31%;PCT=43%, PCR=40% con $p<0.001$. Lo studio delle curve ROC evidenzia: AUC di sCD14 pari a 0.98 (CI al 95% da 0.94 a 0.99), con una sensibilità ed una specificità del 99% per valori di sCD14 >299 pg/L, mentre per galectina-3 AUC è pari a 0.98 (CI al 95% da 0.94 a 0.98) con una sensibilità ed una specificità del 99% per valori di galectina3>16ng/ml; per PCT: AUC è pari a 0.97 (CI al 95% da 0.96 a 0.98), con una sensibilità ed una specificità del 99% per valori di PTC>0.08ng/ml per PCR AUC è pari a 0.95 (CI al 95% da 0.92 a 0.96) con una sensibilità ed una specificità del 99% per valori di PCR>2.9 mg/l. Conclusioni: I nostri dati preliminari indicano che nella gestione clinica della sepsi la galectina-3 potrebbe essere un promettente marcatore di gravità. Un decremento di sCD14, accompagnato da una riduzione di galectina3, PCTePCR potrebbe aiutare nel valutare ildecorso clinico con valore predittivo positivo del trattamento farmacologico.

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THE PAPILLOMAVIRUS (HPV) MIGHT HAVE A KEY ROLE IN BENIGN AND MALIGNANT PROSTATE LESIONS

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Aim of this study: is to show the role of the Papillomavirus (HPV) in benign and malignant prostate lesions, expecially in malignant. HPV is a DNA virus related to cervical carcinoma; a lot of reports, in literature, confirm that there is a relationship between HPV and other cancers: oral cancer, skin cancer, and some types of lung cancer. At the moment men are considered only a healty bearer and only in few cases HPV causes penile cancer. Methods: we examinated 30 patients with pre-cancerous and/or cancerous lesions. DNA extraction was carried out from paraffined tissue of prostatic glands. Subsequently an amplification of a 450 bp, HPV specific L1 gene, was performed with the MY09/11 consensus primers (HPV Screening L1- NanoGen Advanced Diagnostics). The PCR products were revealed on the agarose gel and genotyped with reverse hybridization on nitrocellulose strips. This test for HPV genotypes identification was based on amplification of a part of L1 viral region (450 bp) by polymerase-chain-reaction (PCR) using the primers MY09- MY10, while a shorter sequence (150 bp) was obtained by nested PCR, using the primers GP5 – GP6 and involves genotyping strip of 40 HPV types (ABAnalitica®-Advanced Biomedicine). Results: on 30 patients examinated, we detected the presence of HPV High Risk (HR-HPV) in 14 samples with positive biopsy for malignant cells (Gleason score 2-5), in 3 patients with prostatitis we detected HPV 6 (Low Risk), 4 patients, with a prostatic carcinoma (Gleason score 6-8), were HPV negative, and 9 patients were negative for HPV and histological diagnosis. Gleason system is based glandular architecture of the tumor. They were considered the architectural patterns: primary (predominant) and secondary to which was assigned a score from 1 to 5; the Gleason score is thesum of the two patterns. Conclusions: the results show that HPV infection could play a key role in benign and malignant lesions of the prostate, and not only in cervix cancer; therefore the HPV screening and genotypizzation might be foundamental also in man. Further studies are needed to confirm the results obtained.

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DIAGNOSTICA MOLECOLARE DI C. DIFFICILE INTEGRATA CON UNO STANDARD DI PRODOTTO: INDAGINE RETROSPETTIVA EFFICACE

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Le infezioni da C. difficile (C. difficile infection - CDI) sono causa di diarrea infettiva in ambito ospedaliero. Una diagnostica rapida è cruciale per un rapido trattamento del caso e per limitane la diffusione. I test diagnostici per CDI si basano sulla ricerca di antigeni, tossine o acidi nucleici dalle feci diarroiche del paziente e sulla coltura tossinogenica. I saggi molecolari hanno come target i geni per le tossina A/ tossina B TcdA e/o tcdB. L'alta sensibilità e specificità (95%) e la velocita di esecuzione rendono questi test ideali. Scopo del presente lavoro è proporre un algoritmo diagnostico quale standard di prodotto per una diagnosi rapida, sensibile e specifica di CDI. A partire dal 2014 presso il nostro laboratorio il CDI è stato inserito nel profilo di sorveglianza delle infezioni ospedaliere. Tutti i campioni fecali provenienti da pazienti con un quadro clinico compatibile di CDI sono stati testati con metodica molecolare "Illumigen C. difficile", prefissando un TAT di 1 ora. Tale metodica utilizza la Tecnica LAMP (amplificazione isotermica di DNA loop-mediata) per rilevare il locus di patogenicità PaLoc, codificato da entrambi i geni della ToxA/B. I campioni sono stati refertati come: POSITIVO (presenza di DNA per il locus di patogenicità PzLoc), NEGATIVO (non presenza di DNA per il locus di patogenicità PzLoc). Tutti i campioni POSITIVI sono entrati a far parte della "CATENA DI SORVEGLIANZA AZIENDALE PER I GERMI ALERT", che prevede la stretta collaborazione fra Dirigente di Laboratorio, Dirigente di Reparto, Ufficio Igiene ed Osservatorio Epidemiologico. Si è fatto uso del Software VIGI@ct per la gestione degli alert di isolamento dei germi sentinella e la preparazione dei report epidemiologici. Nei 4 anni presi in esame, sono stati esaminati: 2014: totale 196 di cui 34 positivi (17.34%), 2015: totale 308 di cui 63 positivi (20.45%), 2016: totale 171 di cui 24 positivi (14.03%), 2017: totale 242 di cui 32 positivi (13.22%). Il presente documento propone un algoritmo diagnostico quale standard di prodotto per una diagnosi rapida, sensibile e specifica delle CDI Dall'analisi dei dati degli anni presi in esame, si evince: Maggiore attenzione verso le CDI da parte dei clinici, Riduzione % dei positivi nei 4 anni presi in esame.

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VALORI DI PROCALCITONINA >100 NG/ML PREDITTIVI DI PROGNOSI INFAUSTA

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Introduzione: La procalcitonina (PCT) è prodotta selettivamente in presenza di infezioni batteriche, sepsi e sindrome da disfunzione multiorgano (MODS). L'aumento della sua concentrazione plasmatica è correlata alla reazione infiammatoria; mentre se ne osserva il decrescere nel paziente sotto cura antibiotica. Pertanto la PCT può essere utilizzata come marker per il controllo di focus batterici, per la valutazione della prognosi e per l'efficacia dello schema terapeutico.
Materiali e metodi: Nel periodo 01/01–31/12/2017 sono stati analizzati 11144 campioni di sangue di 4118 pazienti ricoverati presso i reparti di urgenza e terapia intensiva dell'Azienda Ospedali Riuniti di Ancona. La popolazione studiata è stata suddivisa per range di valore e sono stati valutati in dettaglio 85 pazienti (178 campioni) con valori di PCT>100 ng/ml. Il metodo utilizzato è un immunodosaggio automatizzato chemioluminescente (CLIA) con Liaison XL (Diasorin, sensibilità 0,3 ng/ml; Cut-off 0,05 ng/ml). Risultati e conclusioni: Degli 85 pazienti con valori di PCT>100 ng/ml, 65 hanno mostrato infezioni batteriche (80%); mentre i restanti 20 hanno mostrato patologie differenti soprattutto di tipo cardiaco (9 pazienti). Tra i pazienti con infezione 26 (40%) erano causate da batteri multiresistenti che nel 100% dei casi ha portato ad exitus. Nel 90% dei casi i batteri multiresistenti erano E. coli (25) e Klebsiella Pneumoniae (17). Nei restanti 39 pazienti (60%), anche se l'infezione non era sostenuta da batteri multiresistenti (19 Gram negativi, 13 Gram positivi e 7 Miceti), il danno è stato tale da dare exitus nel 95% dei casi. Nel 65% dei casi (42 pazienti) i patogeni sono stati isolati da emocolture. I pazienti sono stati sottoposti a valutazione di diversi parametri quali conta dei bianchi, Proteina C Reattiva e Troponina, ma nessuno di questi ha mostrato migliore indice prognostico. Alla luce dei dati esaminati la PCT è il valore prognostico più indicato per la valutazione delle infezioni e suoi valori elevati sono i migliori indici di prognosi infausta.
Bibliografia: Magrini et all. "Comparison between white blood cell count, procalcitonin and PCR as diagnostic and prognostic biomarkers of infection or sepsis in patients presenting to emergency department." Clin Chem Lab Med.2014Oct.

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PROCALCITONIN REQUESTS AT THE FIDENZA HOSPITAL: A SIX MONTH EXPERIENCE

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Procalcitonin (Pct) is a sepsis biomarker, detectable within 2 h and peaking at 12-24 h after bacterial infections. A latex particle immunoturbidimetric assay (Diazyme) was used on an AU5800 analyzer (6 points calibration; 0.01-58.7 ng/mL; LoB 0.06 ng/mL, LoD 0.16 ng/mL). Pct results <2 ng/mL were considered Negative (N) with low risk of sepsis, >2 ng/mL Positive (P), and suggestive for sepsis or severe bacterial infection. In Fidenza Hospital, 1523 tests were ordered for 792 patients (6 months period). Requests were from Emergency Dep (24%), Intensive Care Unit (27%), Internal Medicine (18%), the remaining 31% were from other units.
1079 (70,7%) tests had Pct <2 ng/mL, 221 (14,4%) between 2-10 ng/mL, and 226 (14,9%) >10 ng/mL. 495 patients (62,5%) were prescribed with a single test, 158 (19.9%) with two, 58 (7,3%) with three, 38 (4,8%) with four, 14 (1,8%) with five, 9 (1,1%) with six, and 20 (2,3%) between 7 to 19. 54/495 patients (10,9%) with 1 test had Pct >2 ng/mL, 33/158 patients (20,8%) with 2 tests
had at least 1 value >2 ng/mL, 28/58 patients (48,2%) with 3 tests had at least 1 value >2 ng/mL. Patients with 3 or more tests at different time points were analysed on follow-up. Patients were classified N when all values were < 2 ng/mL, Decreasing (D) when all values had a decreasing trend starting from a baseline >2 ng/ml value, Increasing (I) when all values had an increasing trend with at least 1 value >2 ng/ml, and Uncertain (U) when values fluctuated over and below 2 ng/ml. Three tests were performed in 58 patients (30 N, 23 D, 2 I, 3 U), 4 tests in 38 patients (15 N, 14 D, 2 I, 7 U), 5 tests in 14 patients (1 N, 8 D, 1 I, 4 U), 6 tests in 9 patients (0 N, 3 D, 0 I, 6 were U), >6 tests in 20 patients (1 N, 6 D, 0 I, 13 U). Emergency Department used Pct to evaluate if a patient needed hospitalization for severe infection. With Pct < 0,5 ng/mL, the decision to rule-in the patient was taken according to other diagnostic investigations. In patients with >2 Pct tests, the trend depended on response to therapy. Patients with >6 assays had long periods of hospitalization and the Pct trend was undetermined.

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USEFULNESS OF PROCALCITONIN IN CEREBROSPINAL FLUID TO ESTABLISH THE EFFECTIVENESS OF EMPIRICAL ANTIBIOTIC THERAPY IN NEUROSURGERY-ASSOCIATED BACTERIAL MENINGITIS: PRELIMINARY DATA

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Procalcitonin (PcT), prohormone of calcitonin, is released by several cell types in response to pro-infiammatory stimuli, mostly during bacterial inflammation.
In the central nervous system PcT is produced by trigeminal glia cells.
Since serum-PcT assay is controversial as marker for bacterial infection diagnosis and for monitoring empirical antibiotic therapy, we aimed to establish the effectiveness of a treatment strategy based on PcT levels in cerebrospinal fluid (CSF) to reduce patients' exposure to antibiotics in neurosurgery-associated bacterial meningitis (NABM).
To date, 9 patients who underwent neurosurgery or ventricular shunt device positioning and presenting with fever and clinical features suggestive of central nervous system infection have been enrolled. CSF and blood samples were collected on the day of fever onset, and after 2 and 5 days of empirical antibiotics therapy.
CSF samples were centrifuged at 2500 rpm for 10 minutes; CSF and serum PcT were analysed on Siemens ADVIA Centaur® BRAHMS Immunoassay Systems. The functional sensitivity of the PcT assay is <0,05 ng/mL, with an analytical sensitivity of 0.02 ng/mL.
Reported reference serum PcT concentration in healthy adults is <0.05ng/ml. Reference values for PcT in CSF are unknown.
In patients with positive CSF culture confirming NABM, mean CSF-PcT was 1.86 ng/ml+-0.65 at fever onset; 0.3 ng/ml+-0.15 after 48 hours of antibiotics; and 0.1 ng/ml+-0.03 after 5 days, proving treatment effectiveness. Median of the ratio between CSF and serum PcT (CSF-PcT/serum-PcT) was 0.76(IQR 0.56-3.17), 0.45(IQR 0.29-1.15) and 1.67(IQR1.14-2.33) respectively.
In patients with NABM associated bacteremia and in those with other source of infection and associated bacteremia, CSF-PcT/serum-PcT was significantly lower ($p<0.05$). Patients in whom infection was excluded both CSF-PcT and serum-PcT were negative. Patients with a rapid and significant CSF-PcT clearance and a lower CSF-PcT/serum-PcT had a good outcome ($p<0.05$), whilst, a slower CSF-PcT clearance and a higher CSF-PcT/serum-PcT were correlated with a worse outcome ($p<0.05$).
CSF-PcT and CSF-PcT/serum-PcT may be valuable tools in diagnosing NABM being effective especially

in guiding antibiotic treatment and, finally, improving patients' outcome.

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EVIDENCE FOR THE USE OF BLOOD BIOMARKERS IN THE DIAGNOSIS OF SEPSIS

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Background: Many biomarkers have been studied to determine their utility for early detection of sepsis which would be necessary to initiate prompt antimicrobial treatment. They need validation by diagnostic test accuracy (DTA) studies. This work assesses the weight and quality of the evidence of primary DTA studies on sepsis biomarkers.
Methods: Literature search was performed to identify all potentially relevant DTA studies relating to procalcitonin (PCT), c-reactive protein (CRP) and prosepsin compared to blood pathogen identification. The reporting and methodology were evaluated using the Standards for Reporting of Diagnostic Accuracy (STARD-30 items) and Quality Assessment of Diagnostic Accuracy Studies (QUADAS-14 items) assessment tools, respectively. For each article, we analyzed the compliance to checklists calculating the median number of items reported by articles included, and the proportion of articles adhering to each specific item. Finally, we classified the reporting as poor (less than 10 items reported), acceptable or optimal (more than 20 items reported).
Results: In the preliminary results, we identified 7 DTA studies published in 2018, 4 on CRP, 5 on PCT and only 1 on prosepsin. We evaluated data about 1460 patients (653 neonates and 807 adults) with sepsis confirmed by blood culture. There was a considerable variation across studies in terms of diagnostic accuracy: Sensitivity (Se) for CRP ranged from 67% to 84% and specificity (Sp) from 51% to 93%, for PCR Se ranged from 35% to 97% and Sp from 72% to 100%. Overall, the median number of items reported was 7 (range: 3-8) for QUADAS, and 15 (range 13-21) for STARD. The most frequent reported items were the description of the index test and methods for estimating DTA measures. Papers often lacked an accurate description of the population enrolled and the execution of the reference standard. All studies evaluated show the characteristics of patients table, but none of these reported the flow. None of the studies comply with the entire checklist. Reporting was judged acceptable 6 studies and optimal in only one study.
Conclusions: The body of evidence of sepsis biomarkers showed important methodological shortcomings, highlight the need to improve the rigor of biomarkers reporting and validation.

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THE MOLECULAR LABORATORY IN THE INFECTIOUS DISEASE EMERGENCY: THE MODEL "DIAGNOSI IN BANCHINA"

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The migratory phenomenon that is needed in the Mediterranean by migrants moving from the African continent to Sicily has accentuated the need for proactive surveillance for tuberculosis on the migrant population. The "Diagnosis in Banchina" project is "post-arrival" carried out at the port of disembarkation in Catania to all migrants presenting fever and cough in the years 2016 and 2017. A sample of the migrant's expectorate is transported to the Clinical Pathology Laboratory of Garibaldi Centro Hospital of Catania to be analyzed with the GeneXpert MTB/RIF method that allows the result in two hours.A total of 17.989 migrants have landed in 2016. At clinical screening, 510 (2%) were febrile (>37°C); 191 (37%) presented cough and fever at the same time and therefore were initiated for tuberculosis screening. A total of 19 (0.10%) migrants were positive for the GeneXpert MTB/RIF assay. Instead, in 2017 the project involved 15.600 migrants. At clinical screening 358 (2%) were febrile; 91 (35%) presented cough and fever at the same time and were therefore initiated for tuberculosis screening. In 16 (0.13%) migrants, the GeneXpert MTB/RIF test was positive. The massive wave of migratory landings on Italian coasts, particularly in Sicily, is proposing, with reference to tubercular infection, a series of unique, exceptional and not comparable with other world migratory situations. Therefore, the idea of carrying out a microbiological screening based on classical cultural examinations seems to be unreasonable. The "Diagnosi in banchina" model has documented an active disease burden of between 0.10% and 0.13%. However, this result, if widely applied to all the ports of disembarkation of migrants, would allow to identify several cases and to initiate treatment for these patients, significantly reducing the risk of spreading the tuberculosis disease and improving the clinical conditions of these patients. Early diagnosis of tuberculosis can promptly initiate therapy, preventing the rates of airborne infection of the tuberculosis infection.

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EVALUATION OF A MULTIPLEX IMMUNOCHROMATOGRAPHIC ASSAY FOR THE RAPID DETECTION OF CARBAPENEMASE-PRODUCING ENTEROBACTERIACEAE FROM CULTURE COLONIES

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Background: The increasing worldwide spread of multidrug resistant bacteria, in particular of carbapenemase-producing Enterobacteriaceae (CPE), constitutes a serious clinical and public health concern because these strains are responsible for a large number of hospital-acquired infections that are difficult to treat and have high mortality rates. An accurate and fast detection of infected patients or colonized carriers is mandatory for both therapeutic management and infection control purposes. The aim of this study was to assess the performance of a multiplex immunochromatographic assay (NG-Test CARBA 5, NG Biotech, Guipry, France) for the rapid detection (15 minutes) of KPC, NDM, VIM, IMP and OXA-48 CPE directly from pure colonies.
Methods: A collection of 49 non-replicated Enterobacteriaceae isolates with decreased susceptibility to carbapenems, including 45 Klebsiella pneumoniae, 3 Escherichia coli and 1 Enterobacter cloacae, were analysed with NG-Test CARBA 5. Concurrently combination disk test (CDT) was performed according to EUCAST indications, while confirmation of carbapenemase production was achieved by PCR. ATCC 700603 and NCTC 13438 were used as negative and positive control, respectively.
Results: PCR assay permitted to found 41 CPE strains, including 38 K. pneumoniae (29 producing KPC, 5 NDM, 1 VIM and 3 co-producing NDM and OXA-48) and 3 E. coli (2 NDM+OXA-48 and 1 OXA-48), while 8 isolates were found as non-carbapenemase producing: 6 K. pneumoniae, 1 E. coli, 1 E. cloacae. CDT allowed us to consider those 8 strains as ESBL or AmpC β-lactamase producers. NG-Test CARBA 5 successfully identified 41/41 CPE (100% sensitivity, 100% specificity). In addition we report that, unlike NG-Test CARBA 5, CDT was not able to correctly identify the 5 strains co-producing NDM and OXA-48 carbapenemases.
Conclusion: Our results showed that NG-Test CARBA 5 is a reliable assay that can be useful in contexts requiring a rapid identification of CPE directly from culture colonies. Furthermore this test is an easy-to-use option that permits to avoid misidentification of carbapenemases co-producers strains. Finally it would be important to extend the study to other CPE strains, such as IMP producers, in order to obtain further confirmation of assay efficiency.

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CORRELATION BETWEEN VITAMIN D LEVELS AND SEVERITY OF CORONARY ARTERY DISEASE. STEMI VS NSTEMI

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Background: Vitamin D plays a classical hormonal role in skeletal health by regulating calcium and phosphorus metabolism. Vitamin D metabolites also have physiological functions in nonskeletal tissues, where local synthesis influences regulatory pathways via paracrine and autocrine mechanisms. Recent studies have shown a possible correlation between vitamin D deficiency and cardiovascular disease. however, its role is still unclear. The aim of this study is to evaluate vitamin D in patients with myocardial infarction Methods: We valuated 92 patients with myocardial infarction. 44 with ST-elevation myocardial infarction (STEMI), 48 with non-ST elevation myocardial infarction (NSTEMI) who were admitted to the Cardiology Department of the University Hospital Tor Vergata. Serum samples were analyzed with 25 OH Vitamin D Test, on the Dimension View analyzer from Siemens, Newark, DE 19714, USA. Results: Patients are equally distributed for ages with an average of 68,9 ± 14,1 (Median 70,5) for patients with STEMI and with an average of 67,2 ± 13,6 (Median 70,5). The concentration of vitamin D was significantly lower in STEMI than in NSTEMI, 9,98 ± 4,78 and 17,51 ± 7,20 for STEMI e NSTEMI respectively (Student's t test for independent samples $p<0,001$). Conclusions: Our study demonstrated a correlation between the levels of Vitamin D and the severity of coronary artery disease. Patients with STEMI, in fact, had lower levels of vitamin D. Patients with NSTEMI, who therefore have less severe coronary atherosclerosis, showed higher levels of vitamin D. More studies are needed to evaluate the effect of vitamin D on cardiac function and if vitamin D insufficiency may be a cardiovascular risk factor

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DESMOSOMAL PROTEINS AUTOANTIBODIES IN ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY

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Background: Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) is predominantly a genetically determined heart muscle disorder. Disease expression is variable from palpitations to syncope. If the myocardium becomes severely damaged in the later stages of the disease, it can lead to heart failure. Clinical manifestations vary with age and stage of disease. ARVC/D is considered to be familial with autosomal dominant inheritance, although there are recessive forms that are associated with a cutaneous phenotype. Genetic variations have been found in the desmosomes that are responsible for cell-to-cell binding. Seven genes have been identified that are associated with ARVC/D: plakoglobin (JUP), desmoplakin (DSP), plakophilin-2 (PKP2), desmoglein-2 (DSG2), desmocollin-2 (DSC2), transforming growth factor beta-3 (TGFβ3), and TMEM43 (Marcus FI et al, Circulation 2010). The diagnosis of ARVC has been developed on the basis of the fulfilment of major and minor criteria encompassing structural, histological, electrocardiographic, arrhythmic and genetic features of the disease. Mutations in the genes encoding desmosomal proteins play a key role in the pathogenesis of ARVC. Mutated proteins might act as autoantigens.

Aim: To evaluate the potential role of desmosomal proteins autoantibodies as serum biomarkers in patients with ARVC.

Methods: 50 serum of ARVC patients and 39 serum of ARVC relatives were tested by a Dermatology Mosaic indirect immunofluorescence (IIF) assay provided by Euroimmun (Lubeck, Germany).Dermatology Mosaic contains: transfected cells (Dsg-1, Dsg-3, BP-230, Collagen VII), BP 180-NC16A-4X BIOCHIPs, salt split skin (SSS), monkey oesophagus and bladder mucosa (DSP, PKP and DSC).

Results: Sera from both patients and relatives were antibody-negative for Dsg-1, Dsg-3, BP-180, BP-230, Collagen VII. However sizable proportions of both sera from ARVC patients and relatives tested positive on SSS and on bladder mucosa (45% and 39% of patients, 47% and 35% of relatives respectively).

Conclusion: Our preliminary data suggest that the reactivity of ARVC sera on salt-SSS and bladder mucosa might be related to desmosomal protein antigens, such as DSP, PKP, DSC or other unidentified targets. Further work is warranted.

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HELPER-DEPENDENT ADENOVIRAL VECTOR EXPRESSING mLDLR/mTf FUSION PROTEIN UNDER THE CONTROL OF A MUSCLE SPECIFIC PROMOTER FOR THE TREATMENT OF FH

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Familial hypercholesterolemia (FH) is a genetic Hyperlipidemia most commonly due to a mutation in the Low Density Lipoprotein- Receptor (LDLR) that result in high levels of LDL-cholesterol (LDL-C) with consequent development of xanthomas, atheromas and an increased risk of premature cardiovascular complications. Several therapeutic options are available to tackle the FH. The conventional therapy is based on the use of high-dose statins. When the pharmaceutical approach is not effective, patients often rely on more invasive interventions such as LDL apheresis. The need for effective treatments make gene therapy an attractive approach to reduce LDL-C in FH patients with a single vector administration. Adenoviral vectors (Ad) are highly efficient gene transfer vectors; in particular Helper dependent (HD-Ad) are characterized by long-lasting high level transgene expression without chronic toxicity. In the past, we developed safe and effective gene therapy strategies developing a HD-Ad vector for the expression of the soluble form of a chimeric protein intravenously administered in LDLR-deficient mice and we observed an amelioration of the lipid profile and a reduction of aortic atherosclerosis. To improve the safety profile and increase the therapeutic window leading to a safer clinical application, we have recently generated a HD-Ad vector encoding for a circulating fusion protein composed by the extracellular portion of the murine LDL receptor (mLDLR) fused in frame with the entire murine transferrin (mTf) under the control of a muscle specific promoter (HD-AdmCK-mLDLR/mTf) for intramuscular delivery. The chimeric protein binds and remove circulating LDLs from the bloodstream by receptor-mediated endocytosis through the interaction with the transferrin receptor (TfR). We evaluated the ability of our vector to drives the expression of the fusion protein in C2C12 cell lines and we are characterizing the vector in order to evaluate expression and the activity after intramuscular delivery in LDLR deficient mice.

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INFLUENZA DELLA FUNZIONALITÀ GLOMERULARE SUI VALORI DI TROPONINA CARDIACA T (cTNT) IN PAZIENTI CON SOSPETTA SINDROME CORONARICA ACUTA (SCA)

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Introduzione: Le troponine cardiache sono i biomarcatori d'elezione nella diagnostica delle SCA. La malattia renale cronica di grado severo si associa a valori di Tn aumentati anche in assenza di danno cardiaco acuto.
Scopo del lavoro: Verificare se:
a) il tasso di filtrazione glomerulare (eGFR) influenza i valori di cTnT in pazienti con dolore toracico (DT) non riconducibile a cause cardiache;
b) l'utilizzo della differenza tra determinazioni consecutive di cTnT (Δass) risente della stessa influenza.
Materiali e metodi: La popolazione in studio ha compreso 736 soggetti con età >60a, giunti consecutivamente in PS con DT e in seguito dimessi con diagnosi di DT aspecifico. Per ciascuno erano disponibili determinazioni di hs-cTnT (ng/L) basale (T0) e dopo 3h (T3), ed eGFR (mL/min) secondo equazione CKD-EPI. I pazienti sono stati classificati in otto gruppi: 1) Femmine (F), <75a, eGFR≥60, n=148; 2) F, <75a, eGFR<60, n=33; 3) F, ≥75a, eGFR≥60, n=96; 4) F, ≥75a, eGFR<60, n=73 ; 5) Maschi (M), <75 anni, eGFR≥60, n=191; 6) M, <75a, eGFR<60, n=24; 7) M, ≥75a, eGFR≥60, n=97 ; 8) M, ≥75a, eGFR<60, n=74. Per valutare le differenze tra i gruppi delle distribuzioni di valori di T0, T3 e Δass sono state eseguite elaborazioni di statistica descrittiva e non-parametrica.
Risultati: Nelle F con età inferiore a 75a i valori mediani di T0 e T3, in caso di eGFR≥60, sono: T0,T3=6; con eGFR<60 T0=11, T3=12. Nelle F≥75a ed eGFR≥60 i valori mediani sono T0=12, T3=14; con eGFR<60 T0=18, T3=19 (P<0,001). Nei M con età inferiore a 75a i valori mediani di T0 e T3 in caso di eGFR≥60 sono: T0=8 e T3=9; con eGFR<60 T0=8 e T3=9 (P=0,9). Nei M≥75a ed eGFR≥60 i valori mediani sono T0=14, T3=13;con eGFR<60 T0 e T3=20 (P<0,001). La valutazione di Δass ha indicato che le differenze tra T3 e T0, sia nei maschi che nelle femmine, non variano al variare di eGFR.
Conclusioni: Nelle F con DT aspecifico di età >60a, una riduzione di funzionalità glomerulare si associa ad un aumento dei valori di hs-cTnT sia T0 che T3. Per i M invece la ridotta funzionalità glomerulare sembra influenzare i valori di troponina solo in pazienti al di sopra dei 75a. La valutazione delle Δass è la modalità migliore per inquadrare il sospetto di SCA perché non influenzata da variazioni di funzionalità glomerulare.

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SERUM FREE LIGHT CHAINS IN PATIENTS WITH ST ELEVATION MYOCARDIAL INFARCTION (STEMI). A POSSIBLE BIOMARKER FOR CARDIAC DYSFUNCTION.

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Background: The increase in concentration of monoclonal light chains derives from an excess of production of chains of a single type by a single clone of lymphocytes, which proliferates beyond its normal limits (as in the case of multiple myeloma, of Waldestroem's macroglobulinemia, etc.). Recent studies have shown a possible role of serum Free Light Chains (sFLC) as an inflammatory marker in patients with chronic heart failure (HF). The potential causes of inflammation in patients with HF are numerous, including the activation of innate immune responses following tissue injury, neurohormonal activation, oxidative stress. However, patients with chronic heart failure often have comorbidities that could increase the concentration of sFLC, such as chronic renal failure. Thus the role of sFLC in patients with heart failure is still poorly understood. The aim of our study is to evaluate the concentration of sFLC in patients with ST-elevation myocardial infarction (STEMI), and consequent acute ischemic heart failure. Materials and Methods: We evaluated the FLC in patients with STEMI (n=113), who were treated with primary angioplasty in the Cardiology Department of the University Hospital Tor Vergata. Inclusion criteria: patient with STEMI in the absence of previous cardiovascular diseases. Exclusion criteria: chronic heart failure, diabetes, haematological diseases, renal 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 acute cardiac dysfunction in patients with STEMI. 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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TROPONIN REQUEST IN THE EMERGENCY DEPARTMENT. ALL ACUTE CORONARY SYNDROMES? THE EXPERIENCE OF A NATIONAL REFERENCE CENTER FOR ISCHEMIC HEART DISEASE.

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Background: Cardiac troponins are the most sensitive and specific indicators of heart damage. Troponin dosage is considered the gold standard and the reference point for the diagnosis of acute coronary syndrome. However, an increase in troponin can also occur in other cardiac and extracardiac diseases. The aim of this study is to evaluate the number of acute coronary syndromes and other cardiac diseases among all the troponins required in one year by the emergency department (ED) of one of the national reference centers of ischemic heart disease

Materials and methods: We have analyzed 16064 troponin requests by the Emergency Department of the University Hospital Tor Vergata in Rome from 1 January 2016 to 31 December 2016. Plasma (Lithium-Heparin) samples were analyzed with the Troponin I Test, on the Dimension View analyzer from Siemens, Newark, DE 19714, USA.

Results: Patients with high level of troponin I were 5473 (34.07%). Patients with normal level of troponin I were 10591 (65.93%). 728 (13.30%) of 5473 patients were hospitalized in the Cardiology Department , the remaining 4745 patients (86.7%) were hospitalized in other departments because they did not have cardiac disease. 344 (32.09 %) of the 728 patients with high level of troponin I hospitalized in the Cardiology Department had a diagnosis of acute coronary syndrome, the remaining 384 (67,91) patients had a different heart disease. Of all the patients admitted to the emergency room and with troponin I requests, only 2.1% had a diagnosis of acute coronary syndrome.

Conclusion: Our hospital data show that troponin I is a highly sensitive marker not only of acute coronary syndrome but of cardiac injury in general. On the other hand, the data show that sometimes, following the internal protocols of ED, there is an inappropriate troponin request. This happens because according to the patient's severity (red, yellow or green code) in the ED a panel of laboratory tests is selected including the troponin dosage, even if the patient has an extracardiac disease.

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VALUTAZIONE DELL'INTERFERENZA DA EMOLISI SUL TEST TROPONIN T HS STAT

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L'emolisi è uno dei motivi più frequenti di non conformità di un campione di plasma/siero nella pratica di laboratorio, in grado di influenzare significativamente l'attendibilità e l'interpretazione di molti test. Ad oggi, molti analizzatori automatici sono in grado di quantificare il grado di emolisi (indice di emolisi, HI), fornendo così una misura oggettiva utile per la rilevazione dei campioni emolisati e la stima dell'interferenza sul risultato. Benché le case produttrici forniscono soglie di HI prestabilite per gli analiti adottati, da più parti è sollecitata la definizione locale del grado d'interferenza dell'emolisi sui singoli parametri e l'aggiustamento contestuale delle soglie predefinite. In particolare, ai fini di una definizione più appropriata dei criteri di accettabilità, le linee guida internazionali raccomandano la valutazione in loco del grado d'interferenza ai livelli clinicamente rilevanti.
Obiettivo - Valutare l'effetto di vari gradi di emolisi sul test Troponin T hs STAT (cTNT) a livelli di cTNT clinicamente rilevanti usando l'analizzatore Cobas C6000 (ROCHE).
Disegno e metodi - Sono stati selezionati pazienti con valori di cTNT in un intervallo di 14-30 ng/L. Pool dei campioni e singoli campioni sono stati sottoposti a dosaggio della cTNT e dell'indice HI dopo aggiunta di concentrazioni crescenti di emolisato. L'emolisato è stato preparato secondo la procedura basata sullo shock osmotico descritto dalle linee guida CLSI EP7-A2. È stata calcolata la percentuale di interferenza dell'emolisi e valutata la sua correlazione con l'indice HI. L'analisi della curva ROC è stata utilizzata per valutare l'impatto della percentuale d'interferenza sulle misdiagnosi dell'infarto del miocardio.
Risultati - In accordo con dati già pubblicati, l'emolisi interferisce negativamente sul dosaggio di cTNT, con una riduzione del 7-27 % per HI di 180-410. La percentuale d'interferenza correla positivamente con l'HI. L'analisi della curva ROC delle percentuali d'interferenza dei soggetti correttamente e non-correttamente diagnosticati ha consentito di identificare l'indice di allarme HI intra-laboratorio che ottimizza la sensibilità diagnostica del dosaggio di cTNT nell'intervallo di 14-30 ng/L.

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PERFORMANCE EVALUATION OF ATELLICA IM HIGH-SENSITIVITY TROPONIN I ASSAY IN A CLINICAL CHEMISTRY LABORATORY

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Background: Cardiac troponins have become the preferred biomarker for diagnosis of MI. "High-Sensitivity" troponin assays have increased precision at the lower end shortening time points between serial measurements for early detection of MI. The Atellica® IM High-Sensitivity Troponin I (TnIH) Assay* is an in vitro diagnostic immunoassay for the quantitative determination of cardiac troponin I. The objective of this study was to verify the analytical performance of the Siemens Healthineers Atellica IM TnIH Assay on the Atellica® IM 1600 Analyzer, and perform method comparison with the ADVIA Centaur® High-Sensitivity Troponin I (TNIH) Assay.
Methods: The Atellica IM TnIH Assay is a dual-capture sandwich immunoassay using three monoclonal antibodies. The precision studies were evaluated according to EP05-A3 and EP15-A2 and method comparison to EP09-A3. Precision studies used two lithium heparin sample pools and three levels of controls. One aliquot of each sample pool and each QC material was tested in replicate in two runs per day on each analyzer for a minimum of ten days with one lot of reagent and calibrator. Each run was separated by approximately a two-hour time interval. A total minimum of 40 replicates were generated per sample. Serial measurements were obtained for lithium heparin samples from >50 chest pain Emergency Department patients. Troponin samples at admission and 1, or 2, or 3, or up to 6 h later were analyzed using the Atellica IM TnIH Assay, and the ADVIA Centaur TNIH assay. Siemens Healthineers supported the study by providing systems, reagents, protocols, and data analysis.
Results: Within day CV%(SD)s were 4.7(0.53), 3.8(0.97), 1.7(0.68), 1.6(57.42), 2.3(447.49) for concentrations of 11.2, 25.4, 40.3, 3684.1, 19228.1 ng/L (pg/mL); within lab (total) CV%(SD)s were 6.7(0.75), 3.9(0.99), 2.5(1.02), 1.8(67.2), 3.5(675.30), respectively. Method comparison between the Atellica IM TnIH Assay and ADVIA Centaur® High-Sensitivity Troponin I (TNIH) assay showed a regression slope of 1.045 (95%CI 1.03 to 1.06), intercept of -2.396 pg/mL(95%CI -2.62 to -2.00) (n=77). Serial measurement results demonstrated 100% total agreement for subjects falling above and below the respective assay 99th percentile value, when comparing Atellica IM TnIH Assay with ADVIA Centaur TNIH assay.
Conclusion: The Atellica IM TnIH Assay has demonstrated good precision for detecting low cardiac troponin I concentrations, good correlation and agreement with the Siemens ADVIA Centaur TNIH assay.

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ANALYTICAL PERFORMANCE AND COMPARISON WITH PREVIOUSLY METHOD OF THE NEW CHEMILUMINESCENCE ENZYME ASSAY FOR TROPONIN I

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Background: the study aim was to evaluate the analytical performance of the new chemiluminescence enzyme immunoassay (CLEIA) for troponin I measurement (CL AIA-PACK cTnI TEST), using the automated AIA CL-2400 platform (TOSOH Bioscence, Japan). Furthermore, this method was compared to the previously fluorescent enzymometric (FLUOR) method of the same manufacturer.

Method: limits of blank (LoB), detection (LoD) and quantitation (LoQ) at 10% and 20% CV were evaluated according to international standardized protocols. Heparinized plasma samples were collected from healthy subjects and patients with cardiovascular disease.

Results: LoB, LoD and LoQ at 20% and 10% CV values of the CLEIA method were: 1.1, 2.1, 15.5 and 30.9 ng/L, respectively. The new CLEIA method actually shows significantly better analytical sensitivity parameters than the FLUOR method. A very close linear relationship was found between cTnI values, measured in healthy people (n = 103) and patients with cardiac disease (n = 136) with both assays [Log CLEIA = 0.2959 + 0.9057 Log FLUOR, R = 0.9883]. Moreover, a mean systematic difference of -3.2% ([CLEIA-FLUOR]/FLUOR%; $p < 0.001$ by Wilcoxon test) was found between these two methods. A worse agreement was observed between FLUOR and CLEIA methods, considering only 105 cTnI values with concentration < 60 ng/L (CLEIA = -0.9013 + 0.7552 FLUOR; R = 0.6636).

Conclusion: these preliminary data suggest that new CLEIA method allows the measurement of the 99th percentile (i.e., 31 ng/L suggested by the manufactory) with an imprecision of 10% CV, as requested by quality specifications made by international guidelines. However, these results should be confirmed by studies including large reference populations.

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BIOCHEMICAL ROLE OF LIPOPROTEIN SCREENING IN PATIENTS WITH PREMATURE MYOCARDIAL INFARCTION AND IN ELITE ATHLETES

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Elevated concentrations of lipoprotein(a) [Lp(a)] have been shown to be an independent risk factor for atherosclerotic disease. However, evidence for its clinical role in patients with premature coronary artery disease remains limited. Moreover, physical activity have been shown to improve lipoprotein metabolism reducing the risk of coronary artery disease. We aimed to elucidate the role of the Lp(a) screening in patients with premature myocardial infarction and in elite athletes to assess its impact on the clinical decision-making and patients' management and also to define the biochemical role of this protein and clarify the influence of physical activity on the Lp(a) profile. We prospectively screened for Lp(a) 105 consecutive patients (age <50 years old) admitted to division of Cardiology of University of "Luigi Vanvitelli" for premature myocardial infarction and 30 elite basket athletes (age <28 years old) admitted to division of Sport Medicine of AORN Santobono-Pausilipon. All patients were treated according to European guidelines recommendations. 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 premature myocardial infarction population, Lp(a) resulted elevated (≥30 mg/dL) in the 28.5% (n=30) of all subjects. Moreover, the 12.3% (n=13) of patients had a Lp(a) value ≥70 mg/dL, with a clinical indication for Lp(a)- specific apheresis. All patients with high levels of Lp(a) were on optimal medical therapy and with well-controlled risk factors, according to European guidelines. In our elite athletes, Lp(a) resulted elevated (≥30 mg/dL) in the 23% (n=7) of all subjects and the 13% (n=4) of patients had a Lp(a) value ≥70 mg/dL. Elevated levels of Lp(a) are highly prevalent in young patients presenting with myocardial infarction. In our very preliminary study on elite athletes also we found elevated levels of Lp(a). A systematic screening for Lp(a) might intensify the control of traditional risk factors in young population and in elite athletes.

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NEW ADVIA BNP AND PBNP IMMUNOASSAYS: EVALUATION OF CLINICAL AND ANALYTICAL PERFORMANCES

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Introduction: The study aim was to evaluate and compare analytical performance and clinical results of ADVIA BNP and ADVIA PBNP methods using Centaur XPT platform with those of Access BNP assay, using the DxI platform, and ECLIA NTproBNP method, using the Cobas e411 platform, respectively.
Materials and Methods:The limits of blank (LoB), detection (LoD), and quantitation (LoQ) at 20% and 10% CV were evaluated according to the international standardized CLSI EP17-A and EP5-A2 protocols. For the evaluation of analytical performance and comparison of clinical results EDTA plasma samples, collected from healthy subjects and patients with cardiac diseases.
Results: The LoB, LoD, and LoQ at 20% CV and 10% values of the BNP ADVIA method were 1,0, 1,9, 2,9, and 7,4 ng/L, while those of the ADVIA PBNP method were 1,8, 7,1, 12,2 and 27,9 ng/L, respectively. The imprecision for BNP (about 50 ng/L) and PBNP (about 150 ng/L) values around the decision values was ≤ 5% for both the ADVIA methods. Moreover, BNP concentrations measured with ADVIA BNP method showed very close linear regressions with those measured with Access BNP method (R= 0.9923, N=200, p<0.0001); similar results were also showed between ADVIA PBNP and ECLIA NT-proBNP methods (R=0.9954, N=201, p<0.0001). However, the ADVIA method (mean BNP ±SD: 289,7 ± 499,1 ng/L) on average (-20.9%) measured significantly lower BNP values (n=200, p<0.0001) than the Access method (mean BNP ±SD: 366,1 ± 737,7 ng/L). Moreover, the ADVIA PBNP method (mean PBNP ±SD: 3777,3 ± 13215,0 ng/L) on average (+17,8%) measured significantly higher PBNP concentrations (n=202, p<0.0001) than those measured by the ECLIA method (mean PBNP ±SD: 3105,5 ± 10706,0 ng/L).
Conclusions: The clinical results found with BNP and PBNP methods showed a close linear relationship with those observed with BNP Access and ECLIA NT-proBNP methods. Moreover, the results of this study suggest that the bias between new BNP ADVIA and Access method is significantly decreased (from about 50% to 20%) compared to that reported with the previous BNP ADVIA method evaluated some years ago. Further studies are needed to confirm that there is a trend to a progressive harmonization among the results of most popular BNP immunoassay methods.

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NEW ADVIA IMMUNOASSAY FOR THE MEASUREMENT OF CARDIAC TROPONIN I

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Background: The study aim was to evaluate the analytical performance and clinical results of the ADVIA immunoassay for cardiac troponin I (cTnI), using Ceuntaur XPT platform. Moreover we compared this method with previous ADVIA Centaur ultra-cTnI assay, and also with the high-sensitivity method using ARCHITECT platform, already evaluated in our laboratory.
Methods: Limits of blank (LoB), detection (LoD), and quantitation (LoQ) at 10% and 20% CV were evaluated according to international standardized protocols. For the evaluation of analytical performance and comparison of clinical results, heparinized plasma samples, collected from 105 healthy subjects and 134 patients with cardiac diseases were used.
Results: The LoB, LoD, and LoQ at 20% and 10% CV values of the ADVIA Centaur cTnI method were: 1.0 ng/L, 2.2 ng/L, 3.5 ng/L, and 8.0 ng/L, respectively. The analytical performance of ADVIA Centaur TNIH method was significantly better than those of ADVIA Centaur ultra cTnI method, but similar to those of high-sensitivity immunoassays using ARCHITECT platform. Moreover, the cTnI concentrations measured with the ADVIA Centaur TNIH showed very close linear regression with those of ADVIA Centaur ultra cTnI method
(R=0.9932, N=222) and also with those of high-sensitivity immunoassay using ARCHITECT platform (R=0.9921, N=239). However, a systematic difference was observed between the results of ADVIA Centaur TNIH and ARCHITECT hs-cTnI methods (mean percent difference 28.8%).
Conclusions: The results of the present study suggest that the ADVIA Centaur TNIH method shows analytical performance characteristics of a high sensitive immunoassay for cTnI. However, the results found in the present study should be confirmed by other multicenter studies in order to actually consider the ADVIA Centaur TNIH method a high-sensitivity assay for cTnI.

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COST ANALYSIS OF AVOIDED READMISSION FOR HEART FAILURE ASSOCIATED WITH THE INCLUSION OF NT-proBNP IN THE INTEGRATED HEALTHCARE PATHWAY, DISCHARGE AND FOLLOW-UP OF PATIENTS, FOR THE LIGURIAN REGIONAL HEALTHCARE SYSTEM (RHS)

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Background: Chronic heart failure (CHF) prevalence in Italy is around 2% in the general population, doubling for each age decade reaching >10% after the age of 80. CHF patient population usually has a high number of comorbidities representing a major public health issue due to disease's epidemiological and economical burden on NHS. CHF economic impact is mainly related to multiple hospitalizations and subsequent readmissions. International and National guidelines strongly recommend NT-proBNP as a key tool in diagnosis, discharge risk stratification and patient follow-up. In addition, literature review has shown that NT-proBNP included in an integrated care pathway is statistically associated with 35% reduction in HF readmission. AIM: To estimate the avoided costs associated with the reduction in hospital readmission for CHF patients at regional level, taking into account the impact associated with NT-proBNP inclusion in the integrated diagnostic pathway. Method: The economic analysis considered RHS perspective and local epidemiological data from Liguria Region. PNE (Programma Nazionale Esiti) national source reported a total number of 2835 readmission cases for HF in Liguria. In our analysis we applied the 35% reduction for HF readmission and we considered the estimated direct cost reported in literature of 5,900€ per patient readmission. The median reimbursement tariff for natriuretic peptides is 18.85€/test. Results: We found that a NT-proBNP based approach could reduce HF readmissions in Liguria by 992, with a total cost avoided of 5,854,275.40€. However, considering an ideal NT-proBNP based model including an average of 3 test/patient/year, the total reimbursement cost for hospitalized population in Liguria would be 260,918.21€. A one-way sensitivity analysis was applied. Conclusion: The NT-proBNP based model could represent a potential cost reduction for Italian NHS. This cost analysis represents a first step towards improving with health economic research the clinical outcomes and quality of life providing a more effective integrated care for CHF patient management.

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EVALUATION OF LAST GENERATION HS TROPONIN I IN CLINICAL ROUTINE

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Introduction: From 2015 IFCC recommend to implement high sensitivity TnT or I in clinical practice in respect to gender cut-offs,
99th percentile as upper reference limit (URL), and serial drawn (Apple, Clinical Biochemistry 2015).
AIM Our laboratory has Beckman Coulter AccuTnI+3 (TN3) in routine on UniCel DxI800 platform, and is preparing shift to hsTnI.
We verified hsTnI QC performances, and Emergency Department (ED) and Intensive Care Unit (ICU) patient classification impact.
Methods: Access hsTnI is a non-competitive chemiluminescent immunoassay with 7 points calibration curve assayed in laboratory. Women URL: 11.6 ng/L (CV %: 4.2); men URL: 19.8 ng/L (CV%: 3.6). 5X5 table, 5 replicates/5 days, was performed with Seronorm QCs, target level 1 at 72 and level 2 at 225 ng/L checking outliers with Grubb's test.
84 females (F) and 137 males (M) patients anonymized selected from ED and ICU based on TN3 (URL: 40 ng/L) result were analyzed and concordance was evaluated for each case according to clinical records.
Results: QC performances: no outliers detected. Clinical concordance: patients' results were negative concordant for 23 F and 47 M; positive concordant for 44 F and 73 M. 17 F and 15 M had hsTnI positive and TN3 below cut-off; 2 male TN3 positive hsTnI negative. Non-concordant patients: 4 F and 6M had high TN3 values in the previous days; 2 F and 1 M TN3 became positive the next day. In 19 patients there were non-Acute Coronary Syndrome diseases, as arrhythmia, hypertensive cardiopathy, chronic renal or heart failure, sepsis, often related to old age. One case TN3 positive hsTnI negative had extensive brain hemorrhage, and the other was interfered by antibodies against recombinant alkaline phosphatase, as shown in Beckman Coulter Complaint Investigation Laboratory.
Considering published TN3 99th percentile at 22 ng/L on an Italian population (Moretti, Annals Clinical Biochemistry 2015), 6 F and 10 M
would become positive concordant.
Conclusion: The hsTnI shows better performances compared with the previous one and confirms adherence to recent guidelines.
Increased values above URL in diseases different from Acute Coronary Syndromes underlines the need to look for kinetics with serial drawn; moreover, in the elderly appropriate normal values should be identified.

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GALECTIN-3 AND Lp(a) SERUM LEVELS AND ADVANCED ATHEROSCLEROTIC PLAQUES: CORRELATION WITH PLAQUE PRESENCE AND FEATURES

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Introduction: Atherosclerosis is one of the leading causes of death and morbidity worldwide. lt consists in the development of plaques in the intima media layers of arteries due to lipid accumulation and oxidation causing a massive inflammation. Plaque complication is the most relevant predisposing factor for instability and acute events. We aim to better understand the role of Galectin-3 (Gal-3) and Lipoprotein(a) [Lp(a)] in atherosclerotic process in order to identify possible peripheral markers of plaque development and complication.

Materials and Methods: Advanced human carotid plaques from 99 patients undergoing carotid endarterectomy were classified histologically, according to American Heart Association (AHA) guidelines as type Va (fibroatheroma), type Vb (mainly calcific) and type Vl (complicated lesion).Additional characteristics of plaques related to plaque instability such as percentage of fibrosis, necrosis or calcification were also recorded. Gal-3 and Lp(a) were measured in serum samples from patients and 78 healthy controls by immunometric assays. The quantification of Gal-3 in plaque was performed by immunohistochemistry.

Results: Gal-3 and Lp(a) serum levels showed increased levels in patients compared with controls (19.8±5.8 vs 14.0±3.6 ng/mL,
p <0.0001 and 8.4(4.0-25.1) vs 4.7(2.4-12.7) mg/ dL, p=0.0003, respectively). Analysis of ROC curves confirmed the discriminating power of these markers obtaining an area under the curve of 0.806 (p<0.0001) for Gal-3 and 0.657 (p=0.0001) for Lp(a). At multivariate logistic regression Gal-3 and Lp(a) serum levels are associated with the plaque presence independently from each other and from age, sex and LDL levels with an odd ratio of 1.26 (1.13-1.41, p <0.0001) and 1.06 (1.02-1.11, p=0.004) respectively. No correlations have been observed between Gal-3 serum levels and Gal-3 levels in atherosclerotic plaques. No differences were found between Gal-3 and Lp(a) serum levels among the different plaques types, nor between complicated and uncomplicated plaques.

Conclusion: Our data showed that Gal-3 and Lp(a) are good markers of advanced atherosclerotic plaque. The absence of differences among the different lesion types suggest that the increase of Gal-3 and Lp(a) is independent by the specific plaque features.

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CAN THE NEW HS-TNI IMPROVE DIAGNOSIS IN EMERGENCY DEPARTMENT? THE PINEROLO'S HOSPITAL EXPERIENCE.

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Introduction: The Emergency Department (ED) of Pinerolo Hospital accepts approximately 50000 patients/year, 4% with chest pain and potential acute coronary syndrome (ACS) symptoms. Cardiacc troponins (Tn) are the gold standard biomarkers for aid in ACS diagnosis and their evaluation is used for rule in or rule out; in our Hospital there is 0-6 hours serial drawn protocol. Unforunately, many Tn's are required in the absence of ACS clinical suspicion: inappropriate testing can lead to over diagnosis and unnecessary treatment, increasing risk of patient harm.
Aim: To determine if actual TnI assay replacement with an hsTnI assay would increase positive TnI results patients, and to describe characteristics and outcome of patients group with positive hsTnI and negative TnI results.
Methods: 166 samples of 123 patients (51 females, 72 males) entered ED between March 11and April 20 2018 tested with AccuTnI and new hsTnI on DxI800 analyzer Beckman Coulter (Brea, CA, USA); 18% only had serial determinations at time 0 and 6 hours. McNemar test compared paired samples and Fisher test compared results groups in terms of positives, negatives and discordant. Patients were classified by manufacturer cut-offs at 40 ng/L regardless of sex for AccuTnI; at 11.6 ng/L for females and 19.8 ng/L for males for hsTnI, checking with electronic medical records data if hsTnI could have impacted rule in and rule out.
Results: Residence time in ED: mean 607'; median 268'.
McNemar's test (to compare differences in assays results): difference is very statistically significant with P value equals 0.0095; odds ratio is 5.000 (95% CI: from 1.414 to 26.945).
Groups: NN, both TnI negatives, 111 results; NP, AccuTnI negative and hsTnI positive, 15); PP, both TnI positives, 37.
Fisher's exact test (to compare outcomes between groups): PP vs NN gives significant P value at <0.00001 and NP vs NN gives significant P value=0.0038.
Conclusions: The new hsTnI allows early detection of myocardial involvement (plausible ACS). A positive result with the new method allows a faster assignment of hospitalization, consequently reducing boarding in ED; moreover, introducing 0-3 hours protocol, an improvement in timing of both rule in and rule out can be achieved.
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Pro-GASTRIN-RELEASING PEPTIDE (proGRP) FOR DIAGNOSIS OF SMALL-CELL LUNG CANCER (SCLC)

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Lung cancer is a malignant tumor characterized by the highest rates of morbidity and mortality in the world. There are two main histological subtype of lung cancer that present a wide variability in morphological, biological and clinical features: small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC). Since SCLC has a poorer prognosis, it is important to discriminate between NSCLC and SCLC. Even if the diagnosis of these tumors is only based on histology, the selection of specific biomarkers could be useful, other than for an early diagnosis, also for the monitoring and the evaluation of treatment response. Neuron-specific enolase (NSE) is considered the marker of choice for SCLC diagnosis but it is not pathognomonic. Recently, it has been investigate the utility of circulating Pro-GRP as a putative biomarker for differential diagnosis of NSCLC and SCLC. We evaluated the diagnostic effectiveness of pro-GRP to differentiate patients with NSCLC and SCLC, the usefulness of combined measurement of pro-GRP and NSE for the diagnosis of SCLC, the comparison of diagnostic efficacy of pro-GRP vs a combined panel of tumor markers and, finally, the reference values of pro-GRP in healthy patients. Serum Pro-GRP, NSE, CYFRA 21.1, SCC and CEA were prospectively collected and measured in 77 patients (49 females and 28 male) with a new diagnosis of lung cancer. Moreover, serum Pro-GRP was measured from 50 healthy subjects. In our study, median proGRP levels were significantly higher in patients with SCLC (1484 pg/ml) than in those with NSCLC (45 pg/ml) or healthy subjects (35.3 pg/ml). Serum ProGRP at cut-off level of 100 pg/ml showed a high sensitivity and specificity (82.4% and 93.3%, respectively) in identifying patients with SCLC, with a specificity higher than NSE (difference in proportion of 47.5%). Positive and negative predictive value were 77.8% and 94.9%, respectively. Moreover, responsive patients presented a decrease in proGRP levels. In conclusion, proGRP is an accurate biomarker for diagnosis of SCLC and discriminating SCLC from NSCLC. Further studies should confirm its utility also for treatment monitoring of SCLC patients.

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ROLE OF ALPHA-FETOPROTEIN (AFP) AND PROTEIN INDUCED BY VITAMIN K ABSENCE (PIVKA II) IN PATIENTS WITH HEPATOCELLULAR CARCINOMA (HCC) AND WITH CIRRHOSIS.

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Background and aim: The availability of biomarkers is useful in the surveillance of patients with cancer. The AFP has been widely used in hepatic context for many years. However AFP levels may be elevated in a number of nonspecific conditions such as chronic liver disease and malignancies other than HCC. Aim of this study was to investigate and compare AFP and PIVKA II accuracy in patients with HCC and with cirrhosis. Methods: AFP and PIVKA II were detected in ninety patients consecutively enrolled in Section of Gastroenterology, University Hospital Policlinico of Bari. Group I consisted of 27 patients with cirrhosis (21M/6F, median age 60y), group II included 25 patients with HCC (22M/3F, median age 59y), and group III, as control, consisted of 38 patients underwent liver transplantation (32M/6F, median age 32y). AFP and PIVKA II serum levels were measured with a chemiluminescent immunoassay on Centaur XP-Siemens and Architect 1000-Abbott respectively. Results: Values in Group I; PIVKA II: 20 - 170.256 mAU/mL, AFP: 1.3 - 23.063 ng/mL. Group II; PIVKA II: 9.0 - 37.304 mAU/mL, AFP: 1.3 - 1.679 ng/mL. Group III; PIVKA II: 14 - 49 mAU/mL, AFP: 1.3 – 9.3 ng/mL. ROC curves. Group I. PIVKA II: AUC=0.79 (95%CI: 0.67-0.88), p<0.0001; cutoff >49 mAU/mL (sensitivity=62%, specificity=97%, +PV=94%,-PV=77%). AFP: AUC=0.56 (95%CI: 0.43-0.68); p=0.41; cutoff >8.8 ng/mL (sensitivity =24%, specificity=92%,+PV=70%,-PV=61.4%). Group II. PIVKA II: AUC=0.91(95%CI: 0.81-0.97) p<0.005; cutoff >50mAU/mL (sensitivity =88%, specificity= 97%, +PV=95.7%, -PV=92.5%). AFP: AUC=0.72 (95% CI: 0.59-0.82); p<0.005; cutoff >5.6 ng/mL (sensitivity =68%, specificity=76%, +PV=65.4%, -PV=78.4). Comparison of ROC curves. Difference between areas in group I: 0.231, 95% CI: 0.049-0.414, p<0.012. Difference between areas in group II: 0.197, 95% CI: 0.020-0.374, p=0.02. Conclusions. These preliminary data showed that both biomarkers could be useful in the surveillance of HCC patients. At the optimal cutoff, PIVKA II accuracy was higher than AFP both in HCC patients and in cirrhotic patients suggesting the potential role in the identification of patients at higher risk of HCC development.

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25(OH)VITAMIN D LEVELS AND PSA “GRAY ZONE”

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Prostate cancer (PC) is the most common malignancy among men worldwide. Prostate specific antigen (PSA) is the screening tumor marker for PC. Lately, attention has been focusing on the relationship between 25(OH)vitamin D (25(OH)vit D) and PC. It has been observed that high 25(OH)vit D levels don’t decrease PC risk: conversely, in vitro and in vivo studies demonstrated a link between 25(OH)vit D and PC growth and aggressiveness. Regarding the association between 25(OH)vit D and total PSA serum levels, findings are confusing: some studies have found that administration of 25(OH)vit D can increase PSA levels but it has also been reported that 25(OH)vit D supplementation has no effect of on PSA values. The total PSA range of 4 to 10 ng/ml has been described as a "gray zone" for PC risk. Aim of this study was to evaluate the association between serum total PSA and 25(OH)vit D in a population with total PSA values in the ”gray zone”. 1768 male blood donors (caucasian, aged 34-65 years) from the Transfusion Service of Policlinico Umberto I, Roma were enrolled in the study, from December 2014 to December 2017. Total PSA serum levels were determined using an “Hybritech”calibrated system (Beckman Coulter Access) while 25(OH)vit D was quantified with LUMIPULSE® G1200, an automated assay system based on chemiluminescent enzyme immunoassay (CLEIA) technology. The threshold value, identified by ROC curve analysis, 20.2 ng/ ml (sensitivity 73.3%, specificity 84%) was chosen corresponding to the cut-off for insufficient 25(OH)vit D according to the World Health Organization (WHO). Total PSA levels of 4 to 10 ng/ml was detected in 5.6% (100/1768) of blood donors. We observed that 55% of men with total PSA in ”gray zone” had sufficient levels of 25(OH)vit D, while only 40% of men with total PSA<4 ng/ml had sufficient levels of 25(OH)vit D (p<0,05). This study showed that men with total PSA in ”gray zone” has higher 25(OH)vit D levels than those with total PSA<4 ng/ml: these findings are worth investigating in future prospective studies with a bigger sample size in order to confirm this association and to assess if 25(OH)vit D evaluation could improve the diagnostic and prognostic proficiency of total PSA test for men in the “gray zone”.

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KINETICS OF D-LACTATE IN PATIENTS UNDERGOING HEPATIC SURGERY: PRELIMINARY DATA

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Hyperlactatemia has been demonstrated in ischemic and hypoxic models of acute liver injury, I which may also have prognostic significance. Lactate is often clinically used as a marker of anaerobic metabolism assuming to mirror inadequate tissue perfusion. Lactate exists in nature in two stereoisomeric forms, D and L. Metabolic production of D-lactate in human cells results from methylgloxal pathway, a minor off-shoot pathway of glycolysis, finally generating nanomolar concentration of methylgloxal, a toxic product then converted to D-lactate. This study was aimed to assess the kinetics of D-lactate in patients undergoing hepatic surgery. Materials and Methods. The study population consisted of 22 patients (12 males and 10 females) with benign or malignant liver tumor undergoing liver resection. According to surgeon preference, patients were submitted to intermittent portal triade clumping. Blood samples were collected before (T0) and after (T1) induction of anesthesia, before (T2) and after (T3) arterial and vein clumping, immediately at end of surgery intervention (T4), and then 2 hour (T5), 4 hour (T6), 24 hour (T7), 48 hours (T8), and 72 hours (T9) thereafter. The D-lactate was measured with D-Lactic Acid Assay Kit (Megazyme International, Ireland) adapted for use in the local laboratory. L-lactate was also measured using COBAS 8000 (Roche Diagnostics, Germany). Results. Significant increases were observed for D and L-lactate ($p<0,05$) during and after surgery. The median and interquartile range for D-lactate (nmol/L) and L-lactate (mmol/L) at different time points were: 23 (21-34) at T0, 25 (19-36) at T1, 27(19-34) at T2, 25(18-32) at T3, 29 (23-35) at T4, 32 (25-40) at T5, 41 (21-34) at T6, 36 (26-46) at T7, 37 (29-48) at T8, 33 (24-37) at T9 and 1,08 (0,84-1,33) at T0, 0,95 (0,75-1,25) at T1, 1,18 (1,03 -1,59) at T2, 2,29 (1,2 -3,04) at T3, 1,94 (1,32 -2,32) at T4, 2,51 (1,63 -3,48) at T5, 2,26 (1,77 -2,89) at T6, 1,14 (1,02 -1,36) at T7, 1,34 (1,09 -1,89) at T8, 1,32 (1,15 -1,41) at T9 respectively. Conclusions. A significant increase od D-lactate was observed starting after 24 hours after surgery, which is consistent with prolonged impaired permeability of gastrointestinal barrier.

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USE OF LIQUID BIOPSY IN METASTATIC NON-SMALL CELL LUNG CANCER WITH EGFR MUTATION: A COST CONSEQUENCE ANALYSIS

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Objectives: To develop a cost-consequence analysis (CCA) and compare the adoption of three different diagnostic pathway in the first and second-line treatment of metastatic Non-Small Cell Lung Cancer (mNSCLC) patient: tissue pathway (only tissue biopsy for first and second line); combined pathway (first line: tissue biopsy, if its outcome is unknown proceed with liquid biopsy; second line: liquid biopsy, if its outcome is negative proceed with tissue biopsy); potential pathway (first line: tissue biopsy and liquid biopsy for tissue ineligible patients, if the outcome of tissue biopsy is unknown proceed with liquid biopsy; second line: liquid biopsy, if its outcome is negative proceed with tissue biopsy).
Methods: A decision-analytic model was developed considering the Italian NHS's perspective and diagnostics sensitivity and specificity derived from clinical studies. We only evaluated direct medical costs (tissue biopsy, management of complications associated with tissue and liquid biopsies). The CCA was conducted over a time horizon of 1 year. Key variables were tested in the sensitivity analysis.
Results: The results of the model are shown in: number of correctly identified cases, the total cost of the pathway and average cost per correctly identify cases. Considering both the first and the second line of treatment, the potential pathway constitutes the alternative with a greater number of correctly identified cases and characterized by an average cost per correctly identified case (€ 685) lower than that estimated for the combined pathway(€ 732) or for the tissue pathway(€ 1,004). The potential pathway constitutes the best alternative.
Conclusions: The choice of a correct diagnostic pathway is crucial in order to optimize cancer therapies in the first- and second-line treatment of mNSCLC. The addition to the diagnostic pathway of the liquid biopsy would correctly identify a greater
number of cases, supporting the prescription of the most effective oncological therapy.

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miRNAS AS NOVEL BIOMARKERS IN BREAST CANCER

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Background: The prognostic value of pathological complete response (pCR) on survival is still debated. Previous studies have revealed that breast cancer intrinsic subtypes show different molecular profiles and several miRNAs have important roles to determine and regulate such subtypes. We investigated the potential role of miRNA signature as predictor of outcome in early breast cancer.
Methods: We retrospectively analyzed 12 patients with locally advanced breast cancer who received neoadjuvant chemotherapy (NAD). Purification of miRNA from paraffin-embedded tissue sections was performed by miRNeasy FFPE kit (QIAGEN); miRNA sequencing libraries, prepared with QIAseq miRNA Library Kit (QIAGEN), was sequenced using Illumina NGS system (MiSeq Personal Sequencer). Identification of miRNAs in the samples was performed using software QIAseq miRNA-NGS data analysis. The miRNAs that differed between pre-NAD and post-NAD were taken into consideration. The miRNA targets were predicted by the MiRDB tool (http://www.mirdb.org).
Results: Median follow-up was 61 months (range 43 – 93 months). We analyze miRNA expression profile in patient who did not obtain pCR but experimented a long term disease free survival. We found that miR-510-3p, a miRNA related to angiogenesis, is down-regulated in post-NAD samples, regardless to breast cancer subtypes. miR-191-5p and miR100-5p, that promote proliferation and migration of cancer cells, are down-regulated in HER2 luminal post-NAD samples. miR196a-5p is downexpressed in triple negative (TN) post-NAD samples and is associated with important signalling pathways in cancer, including breast carcinogenesis, progression and drug resistance. On reverse, we found overexpression of miR-143-3p and miR30a-5p in TN and in HER2 non luminal post-NAD samples: both miRNAs are inversely correlate with invasive capacity.
Conclusions: pCR might not be a surrogate prognostic factor of outcome; indeed, modulation of expression of different miRNAs in pre- and post-NAD samples may be better related to breast cancer prognosis in respect of pCR. The profiling of miRNA expression in breast cancer and clarifying molecular mechanisms of breast cancer-specific miRNAs are important future topics for basic and clinical research of breast cancer. Overexpression of above mentioned miRNAs in post-NAC specimens might represent a signature able to identify a population with good prognosis in the group of patients not achieving pCR.

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VITAMIN D LEVELS IN WOMEN AFTER BREAST CANCER SURGERY

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Background: Vitamin D deficiency has been linked to higher risk of breast cancer and preliminary studies suggest that normal-high ranges of serum vitamin D levels improve breast cancer survival. We analyzed blood levels of 25(OH)D at baseline 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. Methods: Eligible women (n=203) 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 levels of 60ng/ml); Group B (standard of care, general advice to follow a traditional Mediterranean diet and exercise + vitamin D to avoid insufficiency or deficiency). Clinic visits for study participants included the evaluation of 25(OH)D levels in blood samples. 25(OH)D levels were analysed on the Liaison XL (Diasorin) according to the manufacturing instruction.Results: the average circulating 25(OH)D was 22.1±11.6 ng/ml in 203 women aged 51.7±9.2 and with body weight of 70.8±14.9 kg (BMI 27.8±5.9 kg/m^2). The mean 25(OH) D in women not previously taking oral vitamin D supplements (n=82) was 16.0±7.3 ng/ml (deficiency 24%, insufficiency 43%, mild insufficiency 27.3%, sufficiency 5.8%). The mean 25(OH) D in women previously taking oral vitamin D supplements (n=121) was 31.2 ±10.9 ng/ml (deficiency 0%, insufficiency 11%, mild insufficiency 41.5%, sufficiency 47.6%). The differences were statistically significant (P<0.0001).Discussion and Conclusion: These results show that vitamin D levels in women previously treated for breast cancer tend to be

low even in a country located in Southern Europe. When supplemented with oral vitamin D there is still a large percentage (more than 50%) who do not reach blood levels of sufficiency. Therefore it is advisable that the oncologists prescribe the 25(OH)D testing to all women with a diagnosis of breast cancer both at diagnosis and during treatment.

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BLOOD CELL COUNT INDEXES OF SYSTEMIC INFLAMMATION AS PREDICTIVE BIOMARKERS OF IMMUNOTHERAPY OUTCOMES IN ADVANCED NON-SMALL-CELL LUNG CANCER.

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Introduction: Several targeted therapies have been recently introduced for the treatment of advanced non-small cell lung cancer (NSCLC). Nivolumab, a monoclonal antibody that targets programmed death -1 protein (PD-1), was the first immune checkpoint inhibitor approved in this setting. The aim of this study was to evaluate a number of blood cell count indexes of systemic inflammation as predictors of response to nivolumab, in order to allow early identification of non-responders. Methods: A retrospective analysis including 78 consecutive advanced NSCLC patients treated with nivolumab at the institutions involved in the study was performed to investigate correlations between blood cell count indexes, progression free survival (PFS) and overall survival (OS). Nivolumab was initially administered at 3 mg/kg intravenously over 60 minutes every 2 weeks, and later at 240 mg. Patients underwent serial clinical evaluations and radiographic imaging every 8 to 12 weeks. Associations of PFS and OS with the following blood cell count indexes before starting treatment and at 6 weeks thereafter were investigated: neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), monocyte to lymphocyte ratio (MLR), systemic inflammation index (SII) and the Aggregate Index of Systemic Inflammation (AISI), calculated as the platelet count multiplied by the monocyte count and by the NLR. Moreover, ROC analysis was carried out to determine cut-offs, sensitivities and specificities of the indexes. Results: The mean (±SD) age of the patients was 67±7 years, and 66 (84.6%) of them were males. The mean follow-up time was 11 months (range 3–23); no patients were lost to follow-up. None of the indexes evaluated at baseline showed any association with the outcomes under investigation, while NLR, PLR and AISI at 6 weeks were significantly associated with both PFS (p=0.003, 0.039, 0.007, respectively) and OS (p=0.007, 0.036, 0.007, respectively). The best AUROC was shown at an AISI cut-off value of 351 (0.732, 95% CI:0.591-0.845), with 69% sensitivity and 82% specificity. Conclusions: Our results suggest that NLR, PLR and especially AISI, might be useful biomarkers in monitoring the outcomes and the prognosis of advanced NSCLC patients treated with anti-PD 1 agents.

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HUMAN MICROBIOME COMPOSITION IN BREAST CANCER TISSUES AS COMPARED TO PAIRED NORMAL TISSUES

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Breast cancer (BC) is the most common malignancy in women. Despite a low fraction of hereditary forms (about 15%), arising in individuals carrying germline predisposing-mutations in the BRCA1 and BRCA2 genes or in other genes with lower incidence, most BCs are considered sporadic (1). A large number of factors, including familiarity, age, hormonal cycles and diet, have been reported to increase BC risk (2). The human microbiota is important for maintenance of a healthy status (3). Indeed, its imbalance, namely microbial dysbiosis, has been associated to various human diseases, including cancer (4). Next-generation sequencing (NGS) and its metagenomic applications lend themselves to the study of the human microbiome (5). These procedures can provide information about the microbial composition in diverse body areas and its association to many diseases, thereby shedding light on its relationships with the human host. The aim of this study was to characterize, using an NGS-based method, the breast tissue microbiome to identify a microbial signature related to BC. To this aim, 34 women affected by BC were enrolled. Tumor tissues and non-tumoral adjacent tissues were collected from each subject, for a total of 68 samples. Sequencing of the variable V4-V6 regions of the 16S rRNA gene was performed with the Illumina MiSeq System. Finally, data were analyzed with the QIIME tool, and to consolidate results and statistics, have been reanalyzed by BioMaS pipeline. Bacterial composition and richness differ significantly between healthy and tumor tissues. Similar results were obtained with the two bioinformatic tools, and confirmed the statistical difference. Moreover, breast tissue microbiome also seems to be related to the exposure to lifestyle factors. Taken together, our findings suggest a link between tissue dysbiosis and BC that could have potential clinical implications. Limitations appear to be only the necessary extension to other cancer centers in order to validate the obtained results independently from a single cohort.

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SERUM PROGRAMMED DEATH-LIGAND 1 (PD-L1) AS BIOMARKER OF RESPONSE FOR ANTI-PD-L1 IMMUNOTHERAPY

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The advent immune checkpoints inhibitors as powerful therapeutics in oncology has been demanding the development and validation of biomarkers of response and disease course in cancer immunotherapy. Tumor PD-L1 expression by IHC has been linked to responses to anti-PD-L1 antibody treatment. Accordingly, the measure PD-L1 expression by IHC assay has been approved by the FDA as companion diagnostic, or as a complementary diagnostic in a variety of cancers. However, IHC may be an insensitive measure of tumor PD-L1 expression, and it is conceivable that incomplete tumor sampling may mischaracterize PD-L1–positive tumors as negative. Moreover, IHC is not suitable for the dynamic evaluation of immune status and response to treatment.
We developed in-house sandwich ELISA for serum PD-L1 (sPD-L1) detection, with a linear range of 0.4–50.0 ng/mL and intra/inter-assay CV% < 10%. Spike recovery and linear dilution analysis revealed a higher accuracy of our in-house ELISA when compared two commercial ELISAs. The mean sPD-L1 level of healthy blood donors was 0.67 ng/ml (range 0.09 – 3.50 ng/ml: 95% CI: 0.39 to 0.45 ng/mL). Predictive value of sPD-L1 was evaluated in a cohort of cancer patients treated with PD-L1 inhibitors (n=20, 7 ADK lung; 4 Mel; 1 Merkel; 8 SQ lung) with respect to Progression-Free Survival (PFS) and Overall Survival (OS). Baseline sPD-L1 levels in cancer patients was 4.83 ng/ml (range 0.76 – 11.30 ng/ml: 95% CI: 3.29 to 6.36 ng/dL), which tested significant as compared to healthy donors group (P <0.0001). Then, we used the median value of sPD-L1 concentrations in the samples of study cohort as a cut-off to divide two groups: low (<4.6 ng/dL) and high (sPD-L1 ≥4.6 ng/dL) sPD-L1. Patients with high sPD-L1 levels (sPD-L1 ≥4.6 ng/mL) showed an improved PFS (HR 0.31) and OS (HR 0.19) as compared to the low sPD-L1 group (sPD-L1 ≥4.6 ng/dmL). Our exploratory analysis supports a promising role for sPD-L1 detection as biomarker of response for anti-PD-L1 immunotherapy. Different ELISAs showed significant analytical bias with possible clinical implications for the interpretation of the sPD-L1 results, which demands for efforts to harmonize the sPD-L1 detection across manufacturers.

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METODI LABORATORISTICI DI SUPPORTO ALLA DIAGNOSI DI SCLEROSI MULTIPLA: ESPERIENZA DELL'U.O. DI PATOLOGIA CLINICA DELL'OSPEDALE DI VAIO, FIDENZA

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Secondo la letteratura nel 95% dei pazienti (pz) affetti da Sclerosi Multipla (SM) è presente Sintesi Intratecale (SI) di IgG nel Sistema Nervoso Centrale (SNC). L'identificazione delle IgG nel CSF è legata a formazione di Bande Oligoclonali (OCB) che migrano nella regione γ in elettroforesi o nella regione catodica nell'Isoelettrofocalizzazione (IF - tecnica elettroforetica ad alta risoluzione) quando sottoposte ad un campo elettrico. L'IF è il metodo più sensibile per la determinazione di IgG di SI nel CSF rispetto ad altri metodi quali-quantitativi. La determinazione qualitativa delle OCB è un'informazione essenziale per confermare o escludere un'attivazione immunitaria del sistema nervoso centrale (SNC) a supporto della diagnosi di SM, nei casi in cui clinica ed esami strumentali non orientino la diagnosi. Riportiamo qui la nostra esperienza, svolta in collaborazione con l'U.O. di Neurologia di Fidenza. Per rilevare qualitativamente e tipizzare le OCB siero/CSF abbiamo utilizzato il kit HIDRAGEL 3 CSF ISF sullo strumento HYDRASYS SEBIA. L'analisi è stata effettuata mediante IF su gel di agarosio e Immunofissazione con antisiero anti-IgG marcato con perossidasi per rivelare le BO di IgG e per dimostrare differenza la similitudine della distribuzione delle IgG nel siero e nel CSF. Abbiamo analizzato campioni accoppiati di CSF e siero di 61 pazienti, sui quali abbiamo svolto analisi chimico fisica dei campioni, IF, esami ematochimici e virologici. I pazienti sono stati selezionati in diagnosi differenziale, essendo stati sottoposti ad analisi di risonanza magnetica nucleare (RMN) e essendone stata verificata l'obiettività neurologica. Dai dati raccolti da Gennaio a Giugno 2017, 36 pazienti sono risultati negativi per OCB e 25 positivi (22 dimessi con diagnosi di SM); per i restanti 36 sono state diagnosticate altre patologie neurologiche. Sia l'età dei pazienti alla diagnosi (20-40 anni) che il sesso (15 F e 9 M; ≈2:1) confermano i dati della letteratura. Anche se solo l'88% di essi è risultato OCB positivo, non proprio corrispondente con la letteratura, se si proiettano i dati raccolti in 6 mesi alla media di 48/50 pazienti/anno con SM degli ultimi 10 anni afferiti all'UO di Neurologia, il riscontro è in linea con la letteratura.

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DETERMINATION OF ANAs POSITIVITY AND PATTERN IN SICILIAN CENTENARIANS

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Previous studies have clearly demonstrated that elderly people show remarkable antinuclear antibodies (ANAs) positivity, although autoimmunity and autoimmune disorders should not be considered clear features of ageing. This phenomenon is probably due to the changes of immune system function of old age. Of note, presence of ANAs significantly increases both in the elderly and centenarians when compared with the prevalence observed in sera from young people. However, fluoroscopic patterns and their relationship with health state of centenarians have never been described in none paper. The present study was aimed to examined ANAs positivity and specific patterns of the positive specimens, and to analyse the relationship between ANAs positivity and healthy status of elderly and centenarians, particularly. To date, we determined ANAs pattern in 11 healthy long-living individuals (5 men, 6 female; age range 95-111), 18 centenarian offspring (8 men and 10 women, age range 63-80) and 40 young subjects (29 men and 27 women, age range 28-45). Screening of IgG ANAs were detected by HEp-2000 indirect immunofluorescence assay (IFA) (ImmunoConcepts, Sacramento, CA, USA) in accordance with the manufacturer's instructions. An ANAs HEp-2000 assay result was considered positive when a clear ANAs pattern was observed at 1:80 dilution by 2 blinded independent observers. ANAs positivity was found in 64% of centenarians versus the 39% of centenarian offspring and the 10% of healthy young people. ANAs titer was higher in elderly and centenarian confirming the increased incidence of these autoantibodies with age. Among ANAs positivity the most frequent patterns were nuclear coarse and fine speckled pattern, which occurred at lower titer in young individuals than in old people. Interestingly, we observed that centenarians showed specific nuclear as well as cytoplasmic patterns like multidots, nucleolar, and anti-Jo-1 known to be frequently associated with systemic autoimmune diseases as systemic sclerosis, primary biliary cholangitis, and polymyositis/dermatomyositis. The present study is the first study reporting the positivity ANAs pattern in centenarian. Information about the pattern types and the health status as well as biochemical parameters will be discussed in the poster.

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FECAL AND SEROLOGIC BIOMARKERS DURING DMARDS THERAPY OF AUTOIMMUNE RHEUMATOID DISEASES IN PATIENTS WITHOUT INTESTINAL SYMPTOMS REPORTED

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Background: A biological marker is a measurable indicator of some biological state or condition. Aim of our study was to evaluate the clinical utility of some fecal and serological biomarkers for the assessment of autoimmune rheumatoid diseases therapy.
Methods: We enrolled eighteen patients, twelve females and six males (mean age of 63.3 ± 9.2), with a new diagnosis of rheumatic arthritis (22%), polymyalgia rheumatica (33%) or psoriatic arthritis (45%) according to 2010 ACR/EULAR Classification Criteria for Rheumatoid Arthritis and not showing gastrointestinal manifestations which started, at recruitment, therapy with at least one of the disease-modifying antirheumatic drugs (DMARDs). We evaluated symptoms and some fecal and serological biomarkers at recruitment and after one, three and six months from the start of treatment. Fecal calprotectin was evaluated on Phadia® 100 with EliA™ Calprotectin (Thermo Scientific) kit and serum Interleukin-6 levels (IL-6) with Cloud-Clone Corp ELISA kit. Statistical significance was calculated with Student's t-test. A p-value of <0.05 was considered significant.
Results: We observed a significant decreased level of fecal calprotectin in patients after three months from the start of treatment ($p=0.003$) but not after six months ($p=0.778$), probably because some patients stopped the DMARDs therapy after the third medical control due to an improvement of painful symptoms. On the other hand, we observed a significant decrease of erythrocyte sedimentation rate (ESR) at third ($p=0.019$) and fourth ($p=0.011$) controls, and a significant decreased level of IL-6 in serum after six months from the start of therapy ($p=0.005$). No significant differences were found during six months of therapy for other biological markers as C-reactive protein (CRP) and leucocyte count.
Conclusion: We suggest that IL-6, ESR and fecal calprotectin, that in a previous study we found to be associated to systemic autoimmune rheumatic diseases independently from the presence of gastrointestinal manifestations, might be used to predict response to DMARDs treatment in patients with systemic autoimmune rheumatic disorders.

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SCREENING PER ANTICORPI ANTI ds-DNA: CHEMILUMINESCENZA O IMMNUOFLUORESCENZA?

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Scopo del lavoro: Valutazione del metodo di screening per Ab anti-dsDNA in Immunofluorescenza Indiretta(IFI) e Chemiluminescenza(CLIA). Materiali e metodi: Sono stati analizzati 126 sieri di pazienti afferenti presso la nostra struttura per lo studio di patologie di tipo autoimmune. È stato utilizzato il QuantaFlash dsDNA CLIA (INOVA Dx) e due linee cellulari di Crithidia Luciliae IFA (BIOSYSTEMS e ALPHADIA). I valori di riferimento CLIA sono: negativo<23 UI/mL,dubbio 24-28 IU/mL, positivi >28 IU/mL. Risultati: 43 pazienti positivi (34,12%): 31 positivi in IFI e in CLIA (25%); 12 debolmente positivi in IFI e positivi in CLIA (10%). 56 pazienti negativi (44.4%): 10 IFI positiva e CLIA negativa (8%) e 46 IFI dubbia e CLIA negativa (37%). Abbiamo classificato come debolmente positivi 27 pazienti (21.4%), 17 come dubbi in IFI e in CLIA (13%) e 10 positivi in IFI e dubbi in CLIA (8%). Le due linee cellulari IFI impiegate hanno confermato gli stessi risultati. Conclusioni: Il test in CLIA, metodo più sensibile e meno specifico rispetto a IFI, può essere impiegato nello screening, per valutare in tempi rapidi i pazienti francamente negativi. Per confermare la positività riscontrata in CLIA o per definire i casi dubbi si impiega IFI. La positività in IFI non riscontrata in CLIA (8% dei debolmente positivi) non confortata da dati clinici e da altri indici biochimici, potrebbe essere attribuibile a cause di tipo infettivo(EBV, CMV, ecc). Nei casi in cui le condizioni cliniche del paziente giustifichino la presenza degli anticorpi anti ds-DNA, la positività in CLIA non confermata in IFI, potrebbe essere indice di ripresa della malattia o di cambiamenti nel percorso terapeutico, essendo il metodo CLIA altamente sensibile anche alle minime variazioni della quantità di anticorpi circolanti. Si conclude considerando il metodo in CLIA adatto allo screening degli anticorpi anti ds-DNA e IFI come metodo di conferma. Si ritiene opportuno impiegare entrambi i metodi per i pazienti che necessitano uno studio più accurato e per i quali i clinici intendano indagare sui molteplici aspetti delle malattie nelle quali questi anticorpi risultano positivi.

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UTILITÀ DIAGNOSTICA DEI TEST SIEROLOGICI NELLA DIAGNOSI DI CELIACHIA E ANALISI EPIDEMIOLOGICA NEL TERRITORIO CASERTANO

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Introduzione: La celiachia è una patologia immunomediata scatenata dall'ingestione di glutine, che si manifesta con un ampio ventaglio di sintomi. L'avvento dei test sierologici ha segnato una svolta, permettendo di identificare soggetti con sospetta celiachia da confermare con l'indagine istologica. Tali test sono gli anticorpi anti endomisio (EMA) ed anti-transglutaminasi tissutale (anti-tTG) che combinati permettono di identificare i casi di celiachia con sensibilità e specificità quasi assoluta.

SCOPO: Scopo del lavoro è stato quello di valutare l'incidenza della celiachia nel territorio casertano mediante l'analisi di pazienti pervenuti presso l'A.O.R.N. di Caserta, con dolori addominali, alterazioni dell'alvo, condizioni di sottopeso, anemie, gonfiore intestinale e perenne astenia.

Materiali e metodi: da gennaio 2013 a maggio 2018 sono stati analizzati 3603 campioni. Gli anticorpi anti-tTG di tipo IgA sono stati dosati mediante test immunoenzimatico automatizzato (Alegria Technogenetics), mentre gli EMA di tipo IgA con metodica IFI (EUROIMMUN).

Risultati e discussioni: Nei campioni esaminati sono state riscontrate complessivamente 181 positività per anticorpi anti-tTG, confermate mediante ricerca degli EMA. Il tasso di positività è stato del 5% circa con una prevalenza del sesso femminile rispetto a quello maschile, in un rapporto di circa 2:1 (113 F e 68 M). Il 64% dei pazienti postivi (116) rientrava nella fascia di età 0-13 anni, il 26% in quella 14-40 anni (47) e il 10% aveva età superiore ai 40 anni. Inoltre, nella fascia di età 0-13 anni circa il 27% dei pazienti aveva età uguale o inferiore a 3 anni, mentre l'età media degli adulti era di 30 anni. Infine, si è evidenziato un incremento delle nuove diagnosi a partire dal 2015.

Conclusioni: l'utilizzo dei test sierologici riveste a tutt'oggi un ruolo fondamentale per la diagnosi di celiachia. Inoltre, nel nostro laboratorio è richiesta sempre più frequentemente l'associazione con i test genetici, cosa che potrebbe sostituire la biopsia come già suggerito dai nuovi protocolli diagnostici in pediatria. I dati dimostrano anche che circa un terzo delle nuove diagnosi avviene in età adulta, ribaltando il concetto che la celiachia sia una malattia esclusivamente infantile.

P210

CYTOPLASMIC FLUORESCENCE PATTERN ON HEp-2: A PROTOCOL FOR DIAGNOSTIC PROFILE EVALUATION

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Purpose: Selection of in-depth tests in case of cytoplasmic fluorescence (Cyt.Fl.) on HEp-2 cells. Sera of 35 patients from Rheumatology and Hepatology with abnormal liver biochemical parameters were tested on HEp-2cells, showed Cyt.Fl. Materials methods: Indirect Immunofluorescence(IFI): HEp-2 (Immunoconcepts), rat liver, kidney, stomach tissues(LKS)(Immunoconcepts);Immunoblotting(IB) Blue Diver LIVER Profile 10 antigens: M2/nPDC,M2/OGDC-E2,M2/BCOADC-E2,M2/PDC-E2,gp210, sp100, LKM1, LC1, SLA, f-Actin(Alphadia). Results: Serum of 35 patients were detected by HEp-2, LKS and IB. All of them showed Cyt.Fl.16 patients were AMA positive on LKS confirmed by IB(anti-M2/nPDC positive); 2 patients were negative on LKS and positive for anti-M2/nPDC (IB). 6 patients showed Nuclear Dots pattern on HEp-2:5 were negative on LKS and anti-sp100 positive (IB),1 was AMA positive on LKS and positive for anti-M2/nPDC and anti-sp100(IB).2 ANA positive patients(Homogeneous pattern)with anti-SMA-V (vessels) on LKS were anti-f-Actin positive (IB). 2 patients with LKM-like pattern on HEp-2, were positive for autoantibodies anti-LKM-1 (IB). 2 patients with ANA positive (Coarse Speckled pattern) and AMA positive on LKS both were anti-M2/nPDC positive, but one anti-SSA associated and other anti-SSA and anti-SM (IB). 3 patients ANA positive (Fine speckled pattern): 2 AMA positive on LKS and 1 negative, all resulted anti-M2/nPDC and anti-gp210 positive (IB). 2 patients negative on LKS were positive for anti-M2/nPDC, M2/OGDC-E2,M2/BCOADC-E2, M2/PDC-E2(IB). Conclusions: about 29% of the examined patients were negative on LKS in IFI. In order to avoid losing fundamental informations for a correct diagnosis, the evidence of the Cyt.Fl. on Hep-2 needs to be carefully investigated by IB. The involvement of one or more liver antigens in the case of Nuclear Dots pattern associated with Cyt.Fl, was explained by IB. In about 6%of cases, Cyt.Fl can be justified by the presence of antibodies directed towards nuclear antigens accounted for associated autoimmune diseases. The evidence of Cyt.Fl. requires a detailed investigation in IFI on HEp-2 and LKS, but also through more specific and sensitive techniques such as IB. Bibliografia: Trivedi PJ, Hirschfield GM. Overlap syndrome and autoimmune liver disease. Aliment Pharmacol Ther 2012.

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DETERMINATIONOF INFLIXIMAB TROUGH LEVELS (IFX-TL) AND ANTIBODIES TO INFLIXIMAB (ATI) IN INFLAMMATORY BOWEL DISEASE (IBD)

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Anti-TNF blocker (Infliximab, IFX) is approved and used in the treatment of Crohn's disease (CD) and ulcerative colitis (UC). Therapeutic algorithms based on drug monitoring combined with anti-drug antibodies detection were proposed. Aim of this study was to compare IFX trough level (IFX-TL) and antibodies to IFX (ATI) with two different commercial kits. Furthermore, we evaluate association between IFX-TL and better disease outcomes and between ATI and loss of response to treatment. Forty-two inflammatory bowel disease (IBD) outpatients on IFX maintenance treatments were enrolled in the study. Serum samples were taken before planned IFX infusion, when blood IFX levels are at nadir. Two different ELISA-sandwich tests were used in order to determine IFX-TL and ATI concentrations: Lisa Tracker duo IFX determination of drug and anti-drug antibodies concentration (Theradiag, Alifax , Padova) and TNF Blocker Monitoring/Antibodies against TNF Blocker (ImmunDiagnostik Bensheim, Germany). Good correlation (Pearson's coefficient ρ=0.893; p< 0.0001; IC 95%= 0.81-0.94) and substantial agreement (y=0.747+1.474x) between IFX-TL methods was found. As regards ATI detection (4/42 samples, 9.5%), both assays showed similar results, correlated with loss of clinical response to treatment. The agreement between absence or presence of antibodies has been evaluated using Cohen's Kappa (Kappa=0.62; % agreement=90.2). The results show similar performance for the two different assays methods. Measurement of IFX and ATI proves to be a useful tool to monitor IFX therapy, trough clinical implications are still matter of debate before these tests can be proposed for routine clinical practice.

P212
Anti-HMGCR ANTIBODIES AS A USEFUL BIOMARKER FOR THE DIAGNOSIS OF IMMUNE-MEDIATED NECROTIZING MYOPATHIES: A CASE REPORT

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We report a case of a 47-years-old woman who, as part of blood routine evaluation, presented a mild alteration of transaminases (AST: 54 U/L,ALT:65 U/L) and increase of CK (1507 U/L). Clinicians decided to monitor the mild hepatopathy and CK levels. Few months later, patient referred the appearance of tremors and was submitted to brain neuroimaging and muscle biopsy respectively concluding for frontal hypodensity, treated with acetylsalicylic acid, and a mild muscle alterations whose cause was unclear. Because of patient's symptoms did not improve and CK was increasing (2793 U/L), clinicians decided to investigate the immunological status, assuming a possible autoimmune condition. Anti-nuclear (ANA) antibody and anti-smooth muscle antibodies (ASMA) were required. The latter, evaluated on rat liver-kidney-stomach (LKS) section, resulted positive with an unusual cytoplasmatic staining of few hepatocytes. This rare pattern, named HALIP, is due to the presence of antibodies against 3-hydroxy-3-methylglutaryl-coenzyme A reductase (anti-HMGCR), a key enzyme in the cholesterol biosynthesis pathway. We confirmed the presence of these antibodies by CLIA method (anti-HMGCR: 178 UA, cut-off >20 UA).
Anti-HMGCR antibodies have been found in a specific kind of myositis classified as a subtype of the immune-mediated necrotising myopathy (IMNM) and characterized by muscle-cell necrosis and macrophage infiltration. It is well known that HMGCR is the target of statins therapy used for lowering cholesterol concentration and statins seems to be a potential trigger for this clinical entity. In fact, most of patients with IMNM and anti-HMGCR positive were or had been treated with statins nevertheless some cases not treated have been described. Our patient was not in therapy with statin but referred to have taken some herbal products of unknown composition. After the diagnosis, a metotrexate therapy was started leading to both the improvement of patient's symptoms and a gradual reduction of CK values (361 U/L).
In conclusion, this is a case of an IMNM positive for anti-HMGCR antibodies in a patient without a history of statin therapy in which the role of laboratory in highlighting these rare antibodies was very important to obtain a clinical diagnosis.

P213
DETERMINAZIONE DEI PEPTIDI IMMUNOGENICI DI GLUTINE NELLE URINE E NELLE FECI DI PAZIENTI CELIACI A DIETA GLUTEN-FREE

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Introduzione: La valutazione dell'aderenza alla dieta priva di glutine (GFD) nei pazienti celiaci risulta attualmente difficoltosa per la mancanza di strumenti di provata efficacia: i criteri istologici, sierologici e clinici, utilizzati per evidenziare il danno causato da glutine, non permettono infatti una valutazione accurata della compliance alla dieta.
Recentemente, è stata proposta la determinazione dei peptidi di glutine nelle feci e nelle urine per rilevare la compliance alla dieta dei soggetti celiaci. Si tratta di peptidi che derivano dalla digestione delle proteine del glutine e che sono correlati al peptide immunotossico di 33 mer dell'alfa gliadina.
In questo studio abbiamo valutato le performance di questi nuovi test per la determinazione dei peptidi immunogenici del glutine (GIP) nelle urine e nelle feci di soggetti celiaci a dieta gluten free.
Materiali e metodi: Sono stati reclutati 15 pazienti celiaci a dieta gluten-free e 21 controlli sani a dieta contenente glutine, istruiti a raccogliere nello stesso giorno un campione di feci e di urine.
0,2-0,5 g di ciascun campione di feci erano trattati con una soluzione di estrazione per rendere accessibili i peptidi tossici del glutine, incubati a 50°C per 60 minuti, centrifugati e analizzati con il test immunoenzimatico iVYLISA GIP-S (Biomedal SL, Spain). 70 µl di ciascun campione di urina erano trattati con una soluzione di condizionamento e analizzati con il test rapido immunocromatografico iVYCHECK GIP-U (Biomedal SL, Spain).
Risultati: Tutti i controlli sono risultati positivi ad entrambi i test. Nel gruppo dei celiaci i GIP erano presenti nelle feci di 2 pazienti alla concentrazione di 210 ng/g e 167 ng/g (cut-off 156 ng/g di feci), mentre le corrispondenti urine erano negative; un altro soggetto presentava GIP rilevabili sia nelle urine che nelle feci.
Conclusioni: La determinazione dei GIP nelle feci con il test iVYLISA GIP-S sembra essere più sensibile rispetto al test effettuato sulle urine e può rappresentare un valido strumento per valutare in maniera oggettiva l'effettiva aderenza alla GFD.

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LABORATORY PHENOTYPES OF ANTIBODY-MEDIATED ENCEPHALITIS: A RETROSPECTIVE ANALYSIS IN SECONDARY AND TERTIARY CARE UNIVERSITY-HOSPITAL LABORATORY

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Antibody-mediated encephalitis is a group of inflammatory brain diseases characterized by neuropsychiatric symptoms, abnormal movements, dysautonomia and a decreased level of consciousness and are associated with antibodies against neuronal cell-surface proteins, ion channels or receptors. Population-based studies of its incidence and prevalence are lacking, however the estimated annual incidence of all types encephalitis is about 5-8/100000 persons (Dalmau et al. NEJM 2018) and a recent study in a limited region of US found a prevalence of the specific diseases with antibodies of 6.5/100000 (Dubey et al. Ann Neurol 2018).
At the end of 2013 we introduced in our Department of Laboratory Medicine of University-Hospital of Padua an indirect immunofluorescence cell based assay (CBA) using human embryonic kidney 293 cells transfected for the detection of antibodies against: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), contactin-associated protein-like 2 (CASPR), GABA γ-aminobutyric acid type B receptor (GABAB), leucine-rich glioma-inactivated protein 1 (LGI1) and N-methyl-D-aspartate receptor (NMDAR) (EUROIMMUN, Lübeck, Germany).
From December 2013 to June 2018 we collected a total of 632 single requests for both serum and CSF, corresponding to 499 single patients. We found 5.2% positive results (26 patients). About 94% of total requests (n 595) were for inpatients coming from both our hospital and hospitals of a wide area of Veneto region. The most common antibody we found was against NMDAR (n 11), followed by LGI1 (n 6) and CASPR (n 6). We found a single GABAB positivity and 2 patients with both LGI1 and CASPR antibodies. 121 total requests (19%) were on CSF samples. 9 patients (35%) were younger than 18 years old and 17 were older than 50 (65%). 8 of the 9 young patients had NMDAR antibodies. Total requests per year rose from 42 in 2014 to 129 in the first 6 months of 2018.
Our findings are consistent with the known clinical phenotypes and age distribution of autoimmune antibody-mediated encephalitis. A reliable CBA assay has now become an essential diagnostic tool for specialized neurological units and central laboratories of secondary and tertiary care hospitals should improve and closely monitor this pivotal test panel.

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NEW OPPORTUNITY FOR THE DIAGNOSIS OF PRIMARY BILIARY CHOLANGITIS (PBC): A MULTICENTER EVALUATION OF A NEW CHEMILUMINESCENCE METHOD FOR DETECTION OF ANTI-MITOCHONDRIAL ANTIBODIES

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Background: Anti-mitochondrial antibodies (AMA) are the main antibodies for PBC, usually detected by indirect immunofluorescence (IIF), despite the well-known limitations of this method. Aim of this multicenter study was to assess QUANTA Flash® M2(MIT3) on the BIO-FLASH® (Inova), in comparison to IIF rodent KSL (ANA KSL®, Inova)using a third method (Euroline Liver Disease®-LD, Euroimmun), as confirmation test.
Methods: 724 serum samples were tested: 153 AMA-IIF positive and 447 AMA-IIF negative, consecutively collected from the routine samples with request of AMA; 124 samples from patients with other autoimmune diseases or acute and chronic infectious hepatitis. All samples AMA positive for IIF or MIT3 were confirmed with LD.
Results: 138/153 (90.1%) samples with AMA-IIF positivity were positive for MIT3 and LD (true positive); 12/153 (7.8%) were negative on MIT3 and positive on LD (false negative of MIT3); 3/153 (1.9%) were true negative of MIT3 since the positivity of AMA-IIF was not confirmed with LD (AMA different from M2).442/447 (98.9%) samples were negative both on IIF and MIT3 (true negative); 5/447 (1.1%) were negative on AMA-IIF but positive both for MIT3 and LD (true positive). 124/124 patients with other autoimmune diseases/ infectious diseases were negative both with IIF and MIT3. Sensitivity and specificity were: 92.0% and 100% for MIT3 at the cut off proposed (20 CU) and 96.6% and 100% at the cut off calculated with ROC curve (10 CU); for IIF, using a diluition 1:40 as cut off, 96.5% and 99.4 respectively.
Conclusion: MIT3 is a very interesting alternative to IIF for detection of AMA.

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ASSOCIAZIONE TRA INSUFFICIENZA EPATICA ACUTA E TROPONINA T: DATI PRELIMINARI

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Introduzione: L'insufficienza epatica acuta (ALF) spesso è una condizione fatale, associata a danno multiorgano. La ricerca di fattori prognostici in grado di predirne l'evoluzione è oggetto di numerosi studi. L'associazione fra elevati valori di Troponina I (TnI) e ALF è controversa: TnI è stata descritta come fattore diagnostico di danno cardiaco precoce e fattore prognostico dell'evoluzione sfavorevole dell'ALF[1] ma anche solo come un generico indicatore di stress metabolico dell'organismo[2].
Scopo. L'obiettivo è indagare statisticamente l'eventuale alterazione della TnT ad alta sensibilità (hs-TnT), in concomitanza di ALF, descrivendo la ricorrenza delle patologie in cui più frequentemente si osserva tale associazione.
Metodi: Una popolazione di 17961 pazienti ricoverati nei reparti e nel Pronto Soccorso dell'Ospedale di Desio dal 2013 ad oggi è stata valutata retrospettivamente: sono stati selezionati soggetti maschi (M) e femmine (F) classificati prima per l'entità dell'alterazione delle transaminasi (ALT e AST comprese tra 5 e 15 volte il limite di riferimento superiore -URL- o superiori a 15x l'URL) e all'interno di questi sottogruppi sono stati individuati soggetti con hs-TnT compresa tra 13 e 50 ng/L o maggiori.
Risultati: Sia in M che F, con valori di ALT superiori a 5x l'URL (41 U/L per F e 59 U/L per M), si nota un aumento della prevalenza di patologie epatiche rispetto a quelle cardiologiche solo nel gruppo dei soggetti con valori di hs-TnT compresi tra 13 e 50 ng/L all'esordio. Per i valori di AST superiori a 5x l'URL (34 U/L), l'aumento di prevalenza delle patologie epatiche avviene solo in F, mentre in M prevalgono nettamente le patologie cardiologiche. Quando hs-TnT supera 50 ng/L, prevalgono le diagnosi di patologie cardiologiche indipendentemente dai valori delle transaminasi. Quando ALT supera 15x URL, la prevalenza di patologie cardiache aumenta sia in F che in M, mentre quando AST supera 15x URL il riscontro di patologie epatiche è più frequente solo in F. In tutti questi casi la prevalenza è intorno al 50%.
Conclusioni: I dati preliminari indicano un'associazione tra livelli elevati di transaminasi e morbidità cardiaca senza poter discriminare se quest'ultima sia danno conseguente o causa.

1 Parekh NH, 2007
2 Audimooolam VK, 2012

P217
RELATIONSHIP BETWEEN 25(OH)VITAMIN D, VITAMIN D BINDING PROTEIN (DPB) AND FREE LIGHT CHAINS (sFLC) LEVELS IN HCV POSITIVE NAÏVE PATIENTS AND HCV SUBJECTS WITH EITHER MIXED CRYOGLOBULINEMIA (MC) OR LIVER FIBROSIS.

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Hepatitis C virus (HCV) is one of the main causes of chronic liver disease worldwide. It can lead to extensive fibrosis, cirrhosis and hepatocellular carcinoma.Mixed cryoglobulinemia (MC) is the most frequent HCV-associated extrahepatic manifestation. Recent studies have found that there is a close relationship between vitamin D and HCV and chronic liver diseases. 25-hydroxyvitamin D3 [25(OH)D], active form of vitamin D, is believed to have beneficial effects on the innate immune system. The quantitative assay of serum free light chains (FLCs), is a useful diagnostic tool in plasma cell dyscrasias. Recently, an altered k /# FLC ratio and FLC patterns were associated with the presence of MC vasculitis and/or B-NHL in HCV-positive patients and suggested that serum FLC ratio could be used as a marker for HCV-related lymphoproliferation after IFN-based antiviral therapy. Aim: to investigate the relationship between 25(OH)D and FLCs levels in HCV positive naïve patients and HCV subjects with either MC or liver fibrosis. Sixty-five untreated patients with chronic HCV infection, 42 HCV-naïve infected individuals without MC and 23 HCV infected individual with symptomatic MC and 21 healthy age-matched and sex-matched individuals (blood donors) were tested for vitamin D, FLCs and cryoglobulins levels. Patients were divided in three subgroups on the basis of cryoglobulin type, without cryoglobulins, type II and type III cryoglobulins. The mean serum FLC levels were significantly higher in HCV-positive patients than in blood donors (p-value <0.001). No difference resulted from the comparison between patients and controls for 25(OH)D. Overall 25(OH)D levels were deficient in 7.7%, insufficient in 40% and normal in 52.3%. Within subgroups: without cryoglobulins five patients had insufficient levels of 25(OH)D and 3 were normal, nobody were deficient; patients with type III eight patients out of 20 cryoglobulins showed normal levels of 25(OH)D, 10 patients had insufficient levels and 2 were deficient; of 34 patients with type II cryoglobulins 23 had normal values,11 were insufficient and 3 deficient, displaying no statistically significant differences between groups (p=0.774).The statistical analysis of the #### and FLC ratio among patients subgroups did not reveal significant difference. The differences between HCV-naïve infected individuals and HCV infected individual with symptomatic

MC in 25(OH)D levels showed no statistically significance (p=0.434). Vitamin D levels cannot be considered a valid task for discriminating different stages of HCV-associated chronic liver diseases.

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A USEFUL BIOMARKER TO DISCRIMINATE METABOLIC FROM VIRAL HEPATOCELLULAR CARCINOMA

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Background: Hepatocellular carcinoma (HCC) is a neoplastic form, which is ranked fifth in the world prevalence of malignant tumors and second in the frequency of related death. Most HCCs develop from a chronic viral infection (hepatitis B or C) and in 90% of cases in the presence of cirrhosis of the liver. Since HCC is often characterized by a silent onset and by a rather aggressive evolution, it is important to be able to detect this neoplasia in the onset phase when any metastases or infiltrations of tumor cells are still in the initial phase, where the use of a biomarker can be complementary to imaging methods (Ultrasound/MRI/CT). The current study aims to evaluate the performance of a recent discovered biomarker, PIVKA-II (Protein Induced by Vitamin K Absence or Antagonist-II), as well as in combination with AFP (α-fetoprotein), in the diagnosis of early stage of HCC in patients with metabolic or viral hepatitis. Materials and Methods: 102 patients with HCC and/or HCV infection were enrolled, including 20 metabolic HCC individuals, 40 with viral HCC and 42 HCV naïve patients. Furthermore, 30 healthy donors were recruited as controls. Serological testing for HCV and the main laboratory tests were performed. AFP and PIVKA-II concentrations were assessed by immunoassays.
Results: From the comparison of PIVKA-II levels between groups a statistically significant difference was observed between metabolic HCC patients and healthy donors (p=0.012), and between metabolic and viral HCC (p=0.007). The correlations between all the variables showed a moderate correlation between AFP and PIVKA-II (r=0.633; p= 0.03), PIVKA-II and ALT (r=0.481; p= 0.037). The diagnostic performance of PIVKA-II and AFP were plotted on a ROC curve showing areas under curve for PIVKA-II and AFP of 0.651 (95%CI: 0.562-0.74) and 0.823 (95%CI: 0.748-0.897) respectively. The following concentrations were selected as best cut-off values: 33 mAU/mL for PIVKA-II (Sensitivity 64%; Specificity 67%), 2.7 ng/mL for AFP (Sensitivity 81%; Specificity 67%).
Discussion: PIVKA-II is an independent predictor of microvascular invasion regarding the invasiveness of HCC. It is also considered for tumor relapse where high levels of PIVKA-II and AFP are linked. From our study emerged that PIVKA-II can also be used to better stratify HCC patients, able to discriminate metabolic from viral carcinoma.

P219
WHOLE-EXOME SEQUENCING AND ASSESSING LYNCH SYNDROME RISK

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Lynch syndrome is a clinical condition characterized by predisposition to develop cancers mainly in colorectal side. The lynch syndrome is associated with germline mutations in MisMatch Repair (MMR) genes. Identifying genetic mutation profiles, may aid treatment selection and decision making for the endoscopic surveillance. However, many case remain without a molecular diagnosis. Indeed, many patients show a like Lynch syndrome phenotype but they aren't carried of pathogenetic mutations in MMR genes. High-throughput sequencing has dramatically improved our ability to determine and diagnose the underlying causes of human disease. The use of whole-genome and whole-exome sequencing has facilitated faster and more cost-effective identification of new genes implicated in Mendelian disease. It has also improved our ability to identify disease-causing mutations for Mendelian diseases whose associated genes are already known. We applied whole-exome sequencing to two patients diagnosed with a clinical suspect of Lynch syndrome. Multiple bioinformatic approaches were applied to these data to assess germline mutation profiles. In addition, we have created an excel worksheet to classify and analyze all genes that could cause a predisposition to develop tumors and thus favor the onset of a Lynch like phenotype. In this study we have identified a large number of variants in MMR genes and in various other genes involved in tumor predisposition. We conclude that the several genetic variants identified in these two patients they could behave as low-risk alleles that contribute to the risk of colon cancer in families with Lynch-like phenotype , cooperating in synergist effect with other low-risk alleles identified in MMR genes.

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DUPLICATION OF D-ASPARTATE OXIDASE GENE IN A GIRL WITH SEVERE INTELLECTUAL DISABILITY

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A genetic diagnosis is of great importance in order to know the cause of mental disabilities providing insight in co-morbidity, associated behavior problems, prognosis and lifespan, and recurrence risk. 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 female child with a severe intellectual disability. High resolution a-CGH analysis was performed on genomic DNA from the patient and their parentsby 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 Feature Extraction software (V11.5.1.1, Agilent); data were visualized and analyzed with the Genomic Work Bench Standard Edition (V7.0.4.0, Agilent). Moreover, DDO sequence analysis was carried out in the trio to verify the presence of single nucleotide variants or small insertions/deletions, independently inherited respect to the identified LGR (large genomic rearrangement), that may be a disease risk factor and contribute to its onset. Direct sequencing was performed with the ABI 3100 capillary sequencer (Applied Biosystems Inc., Foster City, CA, USA), and sequence data analysis was carried out using the SeqMan software). By using a-CGH we detected a duplication of around 127.8 Kb on the 6 chromosome at q21 region in the evaluated patient. The duplication was paternally inherited and includes DDO gene. The above mentioned alteration was confirmed by using Quantitative PCR analysis. Only one variant was identified in the proband by using direct sequencing. This variant, namely c.80+60A>G (rs9384742) is carried by the patients in heterozygous status; even if its significance is still unknown, it is almost frequent and is predicted to be benign by Varsome tool. The same variant was found also in the parents and haplotype reconstruction suggests a maternal inheritance.DDO duplication has never been observed to date in patients affected by neurological diseases. Presence of the same alteration in the father suggest the presence of additional genetic and/or environmental causes. The peculiar neuropsychiatric profile of the patient

with ASD-line behavioral aspects combined with the cognitive disabilities and dysmorphology aspects, suggest a possible role of the duplication of the DDO gene.

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EVALUATION OF CIRCULATING IF1 SERUM CONCENTRATION IN SMITH-LEMLI-OPITZ SYNDROME TWINS: A NEW POTENTIAL BIOCHEMICAL TARGET

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Smith-Lemli-Opitz syndrome (SLOS, OMIM #270400) is an autosomal recessive syndrome characterized by multiple congenital malformation and mental retardation. It is caused by the deficiency of 7-dehydrocholesterol reductase (7-DHCR) and constitutes a clinical and biochemical continuum from mild to severe. At the severe end of the phenotypic spectrum, SLOS is lethal, due to congenital malformation, while at the mild end of the phenotypic spectrum, it features fewer and minor physical abnormalities, mild cognitive and behavioural deficits. The treatment approach is based on dietary cholesterol supplementation, even if mild serum 7DHC persists even after years of therapy. Recently, Inhibitory Factor (IF_1) was reported to be expressed on the surface of many cell types where it acts as a regulator of HDL uptake. Interestingly, IF_1 was found to be present in the human systemic circulation, and to be positively correlated with the expression of serum HDL. Here we evaluated the ATPase IF_1 levels in two identical SLOS twins with a different phenotype to some extent. The patients were born from caesareansection after 36 weeks of pregnancy complicated with gestosis. At birth, both of them presented multiple congenital malformation suggestive of SLOS type 1. The 7-dehydrocholestrol assay was performed showing very high level of this steroid. The further DHCR7 gene sequencing confirmed the diagnosis showing a compound heterozygosis with paternal c.452G>A (p.W151X) mutation and maternal c.278C>T (p.T93M) in the exon 6. Even they were identical twins, the phenotype was different, as one showed more severe mental impairment, larger stunted growth and higher requirement of dietary cholesterol intake. Quantitative measurement of serum IF_1 concentration was performed by an ELISA sandwich assay. Despite the patients were twins, we found high difference in serum IF_1 concentrations, accounting for the different impact of the disease. IF_1 measurement might be proposed as a novel SLOS-related biomarker useful for a better titrationof the dietary treatment.

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GENETIC CHARACTERIZATION AND FREQUENCY OF HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA IN CAMPANIA REGION

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Introduction: Familial Hypercholesterolemia (FH) is characterized by increased LDL-cholesterol levels and increased cardiovascular risk. Autosomal dominant FH is caused by mutations in LDLR, APOB and PCSK9 genes while the very rare recessive form of FH is caused by mutations in LDLRAP1 gene. It is necessary to discriminate between heterozygous FH patients (HeFH), which carry a single mutation in one of the causative genes, and homozygous FH patients (HoFH) which present a double mutation at homozygous or compound heterozygous status. HoFH is a rare disease characterized by plasma cholesterol levels >13 mmol/ L (500 mg/dL), extensive xanthomas and very early atherosclerotic cardiovascular disease. Materials and methods: based on clinical diagnosis, 602 patients (449 unrelated) with a clinical suspect of FH have been enrolled. The coding regions with the flanking intronic regions of LDLR, PCSK9, LDLRAP1 and the exons 26 and 29 of APOB were amplified by PCR and directly sequenced; large rearrangements of LDLR were detected by Multiplex Ligation dependent Probe Amplification (MLPA). Results: among 449 unrelated patients, 347 carried mutations (mutation rate 77.3%); in particular 326 were HeFH, while 21 were HoFH (3 true homozygotes and 18 compound heterozygotes for mutations in LDLR). Total and LDL cholesterol and LDL/HDL ratio were statistically higher in HoFH respect to HeFH patients ($p<0.001$), while HDL cholesterol levels are lower in HoFH respect to HeFH patients ($p=0,002$). No differences were observed between HoFH patients carrying 2 missense mutations (n=15) or a missense and a null mutation (n=6). Considering that 20 HoFH originate from the Campania region that consists of about 6,000,000 of inhabitants the prevalence of HoFH in our region is estimable as at least 1:300,000. Conclusions: our screening revealed a mutation in 77.3% of patients of which 6% are HoFH; this phenotype is severe already in the childhood suggesting an early diagnosis followed by cascade screening to identify HeFH present in families with a mild phenotype.

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MARCATORI LIQUORALI NELLA DIAGNOSI PRECOCE DELLA MALATTIA DI ALZHEIMER: LA NOSTRA ESPERIENZA

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Introduzione: La causa più frequente di demenza è la malattia di Alzheimer (MA) per la quale oggi la diagnosi precoce offre diverse possibilità terapeutiche. È possibile determinare la presenza di marcatori liquorali (β amiloide 1-42 e Tau totale) fin dalle fasi iniziali della malattia. Una tipica riduzione della concentrazione di Aβ42 insieme all'aumento di T-Tau permettono di discriminare forme di demenza di tipo MA da demenze di altra origine. Il loro utilizzo nella pratica clinica è stato fino adesso limitato per mancanza di standardizzazione e armonizzazione nei valori di riferimento. Materiale e metodi: Nella nostra esperienza abbiamo esaminato retrospettivamente 46 liquor raccolti secondo le procedure standard in provette di polipropilene dal 01/09/17 al 20/03/18 di pazienti con neuropatologie di età compresa tra 10 a 83 anni (media 57,1) provenienti dalle Neurologie degli Ospedali Riuniti di Ancona. In tutti i campione di liquor sono state ricercate le bande oligoclonali con isoelettrofocalizzazione su gel di agarosio e Aβ42, T-Tau con metodo chemiluminescente (Lumipulse G600 II Fujirebio Inc, sensibilità Aβ42 7.17 ng/L, T-Tau 141 ng/L). Cut-off utilizzati Aβ42 <500 ng/ L; T-Tau >350 ng/L. 24 campioni di liquor (52.2%) avevano concentrazioni di Aβ42 e T- Tau all'interno del range di riferimento. 2 campioni (4.3%) di pazienti con diagnosi di Alzheimer, presentavano Aβ42 diminuita e T-Tau aumentata. 10 campioni (21.7%) avevano solo T- Tau aumentata di cui 5 (50%) presentano danno di barriera. 11 campioni (21.9%) avevano solo Aβ42 diminuite di cui 6 (54,5%) avevano danno di barriera. Conclusioni: I risultati ottenuti suggeriscono che la combinazione dei marcatori liquorali T-Tau e Aβ42 possa fornire un aiuto al medico per confermare un sospetto clinico di AD o per la sua esclusione. L'introduzione di sistemi automatizzati in chemiluminescenza, che eliminano le differenze in fase analitica, potrebbero più facilmente entrare nella routine diagnostica e aiutare a raggiungere una armonizzazione dei valori di riferimento.
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P224

BRAIN HEMODYNAMIC AS AN INTERMEDIATE PHENOTYPE LINKING VITAMIN B12 BLOOD CONCENTRATIONS TO VISUO-SPATIAL ABILITIES IN HEALTHY AND MILD COGNITIVE IMPAIRED ELDERS

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Low blood levels of vitamin B_{12} and folic acid have been described to affect cognition, mostly in the elderly, as they are implicated in the reconversion of homocysteine (Hcy) to methionine. High levels of homocysteine, in fact, are known to promote pivotal neurodegenerative processes, such as oxidative stress and apoptosis, and to exert neurotoxic effect on neural stem cell proliferation by altering DNA methylation (Sharma et al, 2015). However, scientific findings on the relationship between B vitamins and cognitive decline are conflicting (Ellison et al, 2004; Ariogul et al, 2005; Rodriguez-Oroz et al, 2009). Measuring brain structure and function by in vivo neuroimaging techniques may represent a useful strategy to investigate how blood biochemistry contributes to cerebrovascular health, ultimately affecting cognition. Here we used functional Magnetic Resonance Imaging (fMRI) to measure brain responses to a visuo-spatial attention task in sixty-five healthy or mild cognitive impaired (MCI) elders. We tested whether hemodynamic activity may increase the ability to predict subjects' neuropsychological abilities investigated by a comprehensive battery of 18 tests exploring multiple cognitive domains.We found that the activity of right dorsal anterior cingulate cortex (dACC) predicted visual search and attention abilities ($p=0.002$). Notably, subjects' B_{12} blood levels were positively associated with their dACC hemodynamic ($p <0.05$ corrected), but not directly with their visuo-spatial abilities.For the first time we proved that the endophenotypic approach, usually adopted to explore associations among genes, brain and behaviour, can be extended to clinical biochemical markers too. The relationship between B_{12} and visuo-spatial abilities, in fact, was revealed only when brain activity was added as an intermediate phenotype.

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BIOMARKERS FOR PHYSICAL FRAILTY AND SARCOPENIA: AN EXPLORATORY STUDY

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Chronic inflammation, changes in body composition, muscle loss (sarcopenia) and decreasing homeostatic reserve (frailty) are hallmarks of aging. Several circulating inflammatory markers have been associated with these age-related conditions. However, a gold standard biomarker able to predict functional impairment in older adults is currently unavailable. Muscle is crucial for several metabolic processes, including protein/aminoacid metabolism. Perturbations in protein/ aminoacid metabolism may play also a role in the development of physical frailty and sarcopenia (PF&S). The simultaneous analysis of an array of circulating aminoacid/metabolites and their relationship with inflammatory mediators may help gain insights in the pathophysiology of PF&S. To this aim, we analyzed the profile of circulating inflammatory mediators and amino acids in older people with and without PF&S.
Five hundred persons aged 70+ years were screened. Of these, sixty were diagnosed with PF&S. Thirty non-sarcopenic, nonfrail persons were enrolled in the control group. A panel of 27 cytokines, chemokines and growth factors was analyzed via a multiplex, magnetic bead-based immunoassay on a Bio-Plex® System with Luminex xMap Technology. A panel of 37 serum amino acids and derivatives was assessed by UPLC-MS. Multi-block partial least squares discriminant analysis (PLS-DA) was employed to explore the relationship among inflammatory and amino acid profiles of people with PF&S. Double cross-validation procedures were used to validate the predictive ability of the PLS-DA model. The optimal complexity of the PLS-DA model was found to be three latent variables. The proportion of correct classification was 85.5 ± 4.4 for persons with PF&S and 88.3 ± 3.6 for controls. As for the amino acid profile, people with PF&S showed higher levels of aspartic acid, asparagine, taurine, citrulline, α-aminobutyric acid, methionine, and glutamic acid. Increased levels of interleukin-8, myeloperoxidase, and platelet derived growth factor-BB were also found in people with PF&S.
The dissection of these patterns may provide novel insights into the role played by inflammatory mediators and protein/amino acid perturbations in the disabling cascade associated with PF&S and possible new targets for interventions.

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IS AN INTRATHECAL KAPPA-CHAIN ORIENTED IMMUNE RESPONSE TYPICAL OF MULTIPLE SCLEROSIS?

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Background: The immune intrathecal response in patients with MS has been used for the development of biochemical assays in cerebro-spinal fluid (CSF). They included: Link index (which measures IgG), detection of oligoclonal bands (OCB) and recently the K index (which measures the kappa free light chains). The present study was performed to verify whether a kappa-chain oriented response was typical of MS.
Methods: this preliminary study enrolled 137 patients: 45 with MS (MS), 57 with non-inflammatory neurological disease (NID), 29 with neurological inflammatory disease other than MS(ID). Free light chain kappa (KFLC) and lambda (LFLC) as well as IgG were measured in serum and CSF by nephelometry.
Results: The KFLC/LFLC ratio in serum in MS was 0.98±0.51, not different from NID (0.81±0.29) and ID (1.23±0.66) whereas in CSF in MS was 12.7±21.7 significantly ($p<0.001$) higher than NID (0.84±0.58) and ID (1.65±1.84). The IgG/KFLC ratio in serum in MS was 809±270, not different from NID (687±237) and ID (778±369). The IgG/KFLC ratio in CSF in MS was 23±25 significantly lower ($p<0.001$) than NID (151±92) and ID (128±98). The IgG/LFLC ratio in serum in MS was 737±366, not different from NID (618±235) and ID (708±536). The IgG/LFLC ratio in CSF in MS was 96±108, not different from NID (101±54) and ID (148±123). Finally, whereas the K index (Ki, a ratio between the KFLC quotient and albumin quotient) was markedly ($p<0.0001$) higher in MS (80±96) as compared to NID (4.6±9.0) and ID (13.1±25), the L index (Li, a ratio between the LFLC quotient and albumin quotient) was only slightly higher ($p=0.017$) in MS (16.4±17.7) as compared to NID (3.7±4.04) and not statistically different as compared to ID (6.9±15).
Conclusion: The results reported, including a high KFLC/ LFLC ratio together with a low IgG/KFLC ratio in CSF (but not IgG/LFLC ratio), seem to suggest that a kappa free light chain oriented immune response is occurring intrathecally in MS and supports a more powerful diagnostic value of K index when compared to L index.

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MULTIPLE SCLEROSIS: GENDER RELATED DIFFERENCES IN KFLC INDEX VALUES

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Background: Multiple sclerosis (MS) is a chronic multifactorial inflammatory and neurodegenerative disease of the central nervous system (CNS). The identfication of biomarkers with good diagnostic and prognostic power is of great importance for monitoring and treating MS patients. Materials and Methods: We analyzed serum and cerebrospinal fluid of 228 patients, with different neurological disorders admitted at the Neurology Clinic of the Tor Vergata University Hospital of Rome and at Neuromed Institute in Pozzilli. We analyzed serum and CSF for biochemical routine testing. CSF was collected and stored at #80 °C, following standard pre-analytical procedures; immunoglobulin and albumin concentrations were measured by nephelometry in fresh CSF and serum samples. We performed nephelometric measurement of k and λ FLCs with the N Latex FLC kappa and lambda Kit (Siemens Healthcare Diagnostics) that uses monoclonal antibodies for determination. The aim of this work was to confirm our previous results and determine a possible gender difference of kFLC Index cut-off. Results: The ROC curve analysis, performed on the total number of patients, confirms our previous observations (Pieri, Storto, 2017) for all MS patients (kFLC Index cut-off = 12.3, 93% sensitivity and 100% specificity). Analyzing data respect to gender (ROC curves), we obtained a kFLC Index cut-off of 12.5 (100% specificity and 90.4% sensitivity) for women, while for men a cut-off of 11 (100% specificity and 97.5% sensitivity). We also correlated the kFLC Index values with the age in the females and the males. The Pearson' correlation shows an inversely proportional correlation only in men with a significant value of $p <0.05$. Conclusions: This study reinforces the importance that kFLC Index could have as a diagnostic aid to detect MS. Moreover, our data highlight a difference in the kFLC Index cut-off calculated by gender; male patients with a kFLC Index value greater than 11 are at higher risk to develop MS respect females having the same result.

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SERUM AMINOACID PROFILE IN PATIENTS WITH PARKINSON'S DISEASE

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Introduction: Parkinson's disease (PD) is a progressive neurodegenerative disorder of unknown origin. Several studies demonstrate that mitochondrial dysfunction, oxidative stress and genetic factors contribute to PD development. PD is classified as a movement disorder. The aim of this study was to evaluate the utility of a semi-target metabolomics approach that focuses on a specific class of metabolites (aminoacids) in diagnosing and understanding these diseases, as well as screening and monitoring of patients.
Methods: We measured the aminoacid profile of 37 amino acids and their derivates, in the serum of 20 patients, compared to 30 healthy subjects by Ultra Pressure Liquid Cromatography-Mass Spectrometry (UPLC-MS) 50 µL of sample were mixed with 100 µL 10% (w/v) sulfosalicylic acid containing an internal standard mix of 50 µM and centrifuged at 1000 g for 15 min. 10 µL of the supernatant was transferred into a vial containing 70 µL of borate buffer and then 20 µL AccQ Tag reagents was added, vortexed for 10 sec and heated at 55 °C for 10 min. The chromatographic separation was performed by ACQUITY H-Class using an ACQUITY CORTECS C18 column eluted at a flow rate of 500 L/min with a linear gradient (9 min) from 99 to 1 water 0.1% formic acid in acetonitrile 0.1% formic acid. The mass spectrometry was a ACQUITY QDa single quadrupole equipped with electrospray source operating in positive mode.
Statistical analysis was performed with multi-block partial least squares discriminant analysis (PLS-DA).
Results and Discussion: The patients with PD showed an increased in serum of following aminoacids: fosfoetanolammina, proline, ornithine, cystine, β-aminobutyric acid , β-alanine.
While an increased in the healthy subjects of these aminoacids: citruline, 3-methylhistidine, α-aminobutyric acid, serine, tryptophan.
PLS-DA model permits a correct classification for patients with MP around 99.3% ± 2.5 and for controls around 94.7% ± 3.
The preliminary results of our study show that semi-target metabolomics approach is a successful tool for generating new information on the biochemical in PD pathogenesis.
In summary our data support the strong prediction of PD progression through aminoacid profiling, suggesting a therapeutic strategies for biomarkers in PD pathogenesis.

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VALUTAZIONE DELLE PIASTRINE RETICOLATE IN EMODIALISI

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Introduzione: Le piastrine reticolate (RP) sono una sottopopolazione di piastrine immature considerate sia indice di capacità rigenerativa midollare, che potenziale fattore di rischio cardiovascolare (CV), perché meno responsive alle terapie antiaggreganti (TAA), oltre che marker di infezione batterica e di sepsi.
Abbiamo studiato le RP confrontando soggetti sani (C) e pazienti in emodialisi (HD).
Metodi: Di 42 C (43±13 anni) e 64 pazienti in HD (70±15 anni) dializzati con ritmo bi- o trisettimanale, escludendo pazienti con infezioni recenti, neoplasie o emopatie, sono stati raccolti dati clinici, di laboratorio, livelli di interlukina-6 (IL6) con metodo immunoenzimatico automatizzato (ADVIA Centaur, Siemens) e le RP, con analizzatore BC-6800 PLUS (MINDRAY) espresse come %IPF delle piastrine totali (PLT) e come numero assoluto (AIPN).
Risultati: In preHD vi era un minor numero di PLT e IPF rispetto a C (2,1±1.2 vs 3.8±2.6%, p <0.001), con un valore di AIPN significativamente più basso (4,4±2,7 vs 9,2±5,2 x103/mm3, p <0,001).
Nel postHD, le PLT restavano invariate, mentre vi era un aumento significativo di IPF (Post 2.6±1,5%, p <0,001 vs Pre) e AIPN (Post 5,3±3<103/mm3, p <0,001 vs Pre).
I valori di IL6 in HD erano significativamente maggiori che in C (6,7±5,8 vs 1,2±0,7 pg/ml, p <0,001) e incrementavano ulteriormente nel postHD (Post 7,5±6,4 pg/ml, p=0.05 vs Pre).
In preHD, IPF correlavano positivamente con l'età anagrafica e dialitica e negativamente con i livelli di albumina. Vi era una correlazione diretta tra IPF e livelli di IL6 in PreHD (r=0,26, p=0,04). Tra i pazienti HD in TAA quelli con valori più elevati di RP (indicativi di minor risposta terapeutica) risultavano avere una maggiore età dialitica e una minor fosforemia.
Conclusioni: L'esame delle sottopopolazioni piastriniche rivela che in HD è presente una piastrinopenia iporigenerativa, che può essere di genesi multifattoriale.
In HD si assiste ad un aumento di IL6 e di RP, probabilmente a causa dei meccanismi infiammatori innescati dalla HD stessa e dal consumo di PLT. L'aumento di RP si verifica anche nei pazienti più fragili (anziani, storia dialitica più lunga, malnutriti e infiammati), anche se in TAA. Lo studio delle RP in HD potrebbe migliorare la stratificazione del rischio CV e la gestione della TAA.

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EARLY ASSESSMENT OF NGAL IN ACUTE RENAL FAILURE OF PATIENTS UNDERGOING CARDIAC SURGERY

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Purpose: Acute renal failure (IRA) is associated with a high incidence of mortality and morbidity. Great importance has been given to both urinary and serum biomarkers. Patients at greater risk can be identified early so a personalized medical and dialysis therapy can be undertaken. Our task was to compare the laboratory variations of three renal function biomarkers: creatinine, cystatinemia and urinary NGAL to predict IRA in patients undergoing cardiac surgery. Materials and methods: The study protocol defined the eligibility criteria of the patients and the modalities of sampling the serum and urinary samples. 11 patients (9M, 2F), age range 63-78 years, were admitted to the ICU of Cardiosurgery of our hospital. They were all subjects of complex cardiac surgery (valve replacement, myocardial revascularization, thoracic ascending aortic surgery). The protocol provided for each individual patient to the following times: TO (before surgery or within 12 hours), T1 (after 24 hours), and T2 (after 48 hours). The assessment of creatinine, of cystatin C, urinary NGAL, haemocytometric testing, C-reactive protein, evaluation of the hemodynamic state, volume of diuresis. The dosage of creatinine was performed using Fusion system, cystatin C with nephelometric method on a Dimension Vista instrument, while for NGAL, it was dosed on a Bn2 instrument. Results: of the 11 patients observed only two (G1) had a significant and early increase of urinary NGAL, initiating at 12 hours after surgery (G1: 337 ± 62 ng/ml vs G2 28.5 ± 26.8 ng/ml, p <0.001) and were subjected to continuous dialysis treatment. The increased value of NGAL correlates with the duration of extracorporeal circulation> 3 hours. Conclusions: Creatinine is still the most widely used biomarker despite the severe delay in the diagnosis of IRA. Our preliminary data show that the increase in urinary NGAL was earlier and more significant than changes of creatinine in predicting the onset of IRA. NGAL, used in early identification of IRA and its severity, could initiate new clinical developments and future prospects.

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PIASTRINE RETICOLATE E DISORDINE MINERALE OSSEO NEL TRAPIANTATO DI RENE

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Introduzione: Il disordine minerale osseo, spesso osservato nei trapiantati di rene (RT), è responsabile di elevata morbidità nel medio-lungo termine. Il trattamento dell'osteoporosi (OPN) in RT è complicato dalla presenza di osso adinamico e dovrebbe essere guidato dallo stato di turn-over osseo. Recenti studi hanno evidenziato una stretta relazione tra megacariociti midollari (MKs) e attività osteoblastica/osteoclastica (OBL/OCL). Le piastrine reticolate (RP) sono forme circolanti immature delle piastrine (PLT), riflettenti l'attività megacariocitopoietica con ruolo nel rischio cardiovascolare e predittivo di infezione, ma poco studiate in RT. Scopo del presente lavoro è stato correlare la frazione immatura di PLT in RT con i principali indici di metabolismo osseo.
Metodi: Di 40 soggetti sani (C, 47±10 anni) e 101 RT (50±12 anni) sono state misurate le RP con analizzatore MINDRAY BC-6800 PLUS, espresse come % (IPF) delle PLT totali e sono stati contestualmente raccolti e analizzati dati clinici e bioumorali.
Risultati: RT avevano IPF e PLT significativamente più bassi di C (3,1±0,2; 228,1±6,0; vs 4,7±1,1; 287,4±11,3, p<0,005 p<0,0001). In RT, osservavamo una significativa correlazione diretta tra IPF e Volume Piastrinico Medio (MPV), magnesemia, terapia con inibitori di mTOR (mTORi), warfarin e colecalciferolo; e inversa con reticolociti (RET), steroide, calcitriolo, inibitori di pompa e BMI. Di 38 RT di cui si disponeva di recente t-score femorale, il 67,5% aveva t-score patologico (A) e il 32,5% normale (B). PLT non differiva tra A e B, mentre IPF in B era maggiore rispetto A (3,4± 0,2 vs 2,5± 0,2; p=0,02) e non differiva da C. In A, IPF correlava positivamente con MPV e terapia con statine, negativamente con RET e calcemia; mentre in B, positivamente con livelli di PTH e terapia con mTORi e negativamente con livelli di fosfati, fosfatasi alcalina, terapia con calcitriolo e ciclosporina.
Conclusioni: L'OPN femorale è associata ad un ridotto numero di RP circolanti in RT. Verosimilmente esistono differenze nel microambiente osseo tra sano e OPN in termini di relazione tra MKs e OBL/OCL. Ulteriori studi sono necessari al fine di convalidare questi dati e considerare le RP quali marcatori dello stato di turn-over osseo, per guidare la scelta terapeutica di OPN in RT.

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RIDOTTA PRODUZIONE DI PIASTRINE RETICOLATE NEL TRAPIANTATO DI RENE

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Introduzione: Le piastrine reticolate (RP), forme circolanti immature delle piastrine, sono utilizzate nella diagnostica delle trombocitopenie poiché riflettono l'entità della megacariocitopoiesi. Nella popolazione generale, l'incremento di RP è considerato indice precoce d'infezione e d'inefficacia della terapia antiaggregante (TAA). L'andamento di questo parametro nei trapiantati di rene (RT) è poco investigato.
Scopo: Valutare l'andamento di RP, misurando il numero assoluto delle forme immature (AIPN) e delle forme mature (PLT) in RT e studiare le differenze in rapporto ad infezioni (I) e TAA.
Metodi: AIPN e PLT erano studiate in 157 RT (M/F= 1,5) e 32 soggetti sani (C) (M/F =1,3) mediante analizzatore MINDRAY BC-6800 PLUS.
Risultati: AIPN e PLT erano significativamente più elevati in C rispetto a RT (8,9±0,9; 284,3±12,2 vs 6,3±0,3; 231,2±7,2; p <0,005), non differivano in rapporto al sesso nella popolazione sana mentre in RT il sesso femminile mostrava un maggior numero di PLT e AIPN (7.1 ±0.5; 253.9 ± 14.7 vs 5.0±0.3; 218.6 ±18.5. p <0.05).
AIPN e PLT non differivano tra RT infetti e RT non infetti (5,9±0,5; 227,9±13,8 vs 6,4±0,3; 232,8±8,5), rimanendo inferiori a C (p <0,05), Il trattamento con TAA non modificava il numero di AIPN e PLT in RT (RT trattati con TAA 6,0±0,4; 231,1±9,9 vs RT non trattati 6,6±0,4; 231,2±7,2) rimanendo inferiori a C (p <0,05), In RT, AIPN e PLT non differiva tra i due sessi in rapporto a I e TAA.
Conclusioni: La trombopoiesi in RT è meno efficace che in C; RT hanno una scarsa risposta agli stimoli che inducono trombopoiesi. È verosimile che la terapia immunosoppressiva inibisca l'attività di megacariocitopoiesi oltre alla leucopoiesi. In RT, RP non correla con I in atto né con TAA. Il dato è interessante e merita una più ampia numerosità campionaria.

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NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN (NGAL) IN CRITICALLY ILL PATIENT: SEPSIS OR EARLY ACUTE KIDNEY INJURY (AKI) BIOMARKER?

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Background and aim: Sepsis and septic shock represent a significant healthcare problem in intensive care units, strongly associated with morbidity and mortality. AKI is a frequent (20-50%) pathology sepsis-correlated. Improvements in diagnostic procedures are required for effective management of infectious disease. Biomarkers as presepsin and procalcitonin contribute to monitoring sepsis and therapeutic interventions; creatinine is an indicator of renal function but serum concentration does not change until around 50% of kidney function is lost. NGAL, produced in kidney tubular cells after ischemic or nephrotoxic injury, can be detected in patient with AKI within 2-4 hours. Moreover NGAL exsists as monomer specific to neutrophils and increased in inflammation state. In order to evaluate the contribution of NGAL in septic patients, the urinary NGAL, procalcitonin (S-PCT), presepsin (S-PRE) and eGFR level were observed. Methods. 23 patients (6 with sepsis, 17 with septic shock) admitted to intensive care unit (15 M: median age 48y, 8 F: median age 58y), were recruited according to new criteria of severity and clinical diagnosis recommended by the 2106 Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). Specimens were collected at baseline (T0), after 24h (T1) and 48h (T2). U-NGAL, S-PCT, S-PRE and S-Cre were measured using Architect i1000-Abbott, Liaison-Diasorin, PathFast-Mitsubishi and Vista-Siemens respectively. Results: Mean concentrations of biomarkers at T0, T1 and T2: NGAL=230, 239, 407 ng/mL; PCT: 13, 28, 26 ng/mL; PRE: 1.042, 1.481, 1.886 pg/L; eGFR: 85, 81, 86 mL/min. Mean differences T2 vs T0: NGAL 175 ng/mL (p=0.0001), PCT: 6.5 ng/mL (p<0.0001), PRE: 844 pg/L (p<0.0001), eGFR: 1 ml/min (p=0.0269). Conclusions: These preliminary data showed NGAL, PCT, and PRE values significantly higher in T2 compared to T0; no significantly difference was observed in eGFR. These data confirmed the importance of sepsis multimarker approach to increase the antibiotic therapy success that could reduce the antibiotic resistance trouble. Further studies are needed to assess if NGAL increasing is due to leucocytic sinthesys in response to sepsis or to reduced tubular reabsorption in response to AKI.

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THE URINALYSIS IN EMERGENCY: THE ITALIAN SITUATION

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Introduction: The patients in the Emergency Room (ER) may have a wide spectrum of kidney illness severity from asymptomatic CKD to UTIs (Urinary tract infections) often become acutely symptomatic. The urinalysis consisting of physical-chemical and morphological urine examination (ECMU) can be of great help for ER clinicians, who in a short time must clinically frame the patient and manage it therapeutically. The aim of this study is to show and evaluate the organization of the emergency urinalysis (EU) in Italian laboratories and to know when and how the urinalysis can be useful in emergency through two surveys addressed to the Italian laboratories and to the ER clinicians.
Material and Methods: 294 italian laboratories participated in the investigation by answering a survey of 14 questions available on the website of 2 Scientific Laboratory Societies. In addition, a survey of 16 questions was submitted to 7 ER clinicians. The statistical elaborations were carried out through Office Excell 2013.
Results: The survey shows that the responses came from 42% North, 27% Center and 31% South and Isles of Italy. Only 30% of laboratories is in private structures, the remaining 70% in public hospitals, of which 80% has emergency room. In 89% of participants, the work activity is carried out in routine/emergency regime, in which EU is requested. For 43%, the EU is composed only of chemical-physical exam (dipstick), while for 57% in ECMU. About survey to ER clinicians, the EU is requested for selected patients, where the clinical features are represented by: renal colic, IVU, abdominal pain, fever, ketoacidosis, diabetic coma and hematuria. In the 43%, the EU clarifies the diagnostic question, while in the 57%, further testing laboratory or specialist consulting and/or instrumental examinations are required.
Conclusions: As the preliminary results show, the aims of this study have been achieved, demonstrated by the adequate number of participants, from all parts of Italy, that carries out the EU. From this survey emerges a non-homogeneous behavior for the execution of UE, half performs the chemical-physical test. This is the most important result as it demonstrates that the first goal is to standardize the analytical process to optimize the diagnostic accuracy of the test.

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GLOMERULAR FILTRATION RATE (eGFR) AND ALBUMIN/CREATINE RATIO (ACR): RISK INDEX EVALUATION

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Background: Chronic kidney disease (CKD) is increasing wordwide; in 2012 KDIGO guidelines suggested to stratify patients using eGFR and ACR to predict the prognosis of CKD. Following this suggestion, we report the preliminary data about the evaluation of outpatients that has performed both eGFR and ACR in our laboratory.
Material and methods: 437 consecutive patients (212 male and 225 female, age range 19-92, mean age 58, median age 60) with both eGFR and ACR available , was evaluated for the risk of progression of CKD.
eGFR were calculated using CKD-EPI formula; urine albumine (µALB_2, immunoturbidimetric method, Siemens) and creatinine (ECRE_2, enzimatic method, Siemens) was measured on ADVIA 1800 (Siemens). ACR was expressed as mg/g.
Results: 174/212(82%) male and 198/225(88%) female was classified in the lower risk class (eGFR >90 mL/min/1.73m^2 and ACR < 30 mg/g); 38/212(18%) male and 27/225(12%) female showed a risk class from moderate to very high. In particular, 11.4%,3.7% and 2.9% of male were classified in moderate, high and very high risk class, respectively; the percentage of female in the same risk class were 6.2%,3.3%,and 2.8%.
7.6% male and 1.3% female with moderate risk of progression and 1.4% male and 0.4% female with very high risk of progression has a phisiological value of eGFR. Moreover, 3.8% male and 4.9% female into moderate risk class, 1.4% male and 2.2% female into high risk class and 0.5% male and 0.1% female into very high risk class showed ACR < 30 mg/g.
Conclusion: The simultaneous evaluation of eGFR and ACR allows the early identification of patients with normal eGFR but an incresed risk of CKD progression instead of eGFR alone. Thus, we decide to include in our laboratory report the eGFR/ACR risk classes for all patients.

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DETECTION OF UREMIC AND NORMAL FREE PLASMA CONCENTRATIONS OF INDOXYL SULPHATE AND p-Cresyl SULPHATE. COMPARISON BETWEEN HPLC/FLD AND UPLC/MS METHODS.

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Indoxyl sulphate (IS) and p-cresyl sulphate (pCS) are two uremic retention solutes found in the uremic syndrome. Both IS and pCS are increased in end-stage renal disease and their removal during dialysis is limited, mainly due to protein binding. In dialysis, their clearance is a function of their free concentrations (fIS and fpCS), their removal being just approximately 40%. Long chromatography times, incomplete data on the reference ranges of the free (and total) fractions, further limit their wider clinical use. To evaluate uremic and normal fIS and fpCS, plasma samples of 30 healthy subjects and 24 non hemodialyzed end-stage CKD patients were ultrafiltered by use of Vivaspin ultrafiltration device (cut-off 10 kDa). The high-performance liquid chromatography fluorescence detection (HPLC/FLD) and ultra-performance liquid chromatography (UPLC/MS) tandem mass spectrometric detection methods were optimized and compared. Both methods are rapid: chromatography time was 15 and 5 min, for HPLC/FLD and UPLC/MS methods, respectively, with excellent linearity, precision, and LOQ for both methods. Validation experiments revealed good recovery rate of 102±3.12%, CV 3% for fIS and 94±9.6%, CV 10% for fpCS, good linearity (>0.997) within the established concentration range, and excellent repeatability (<15%) values. Both methods were sensitive: LOQ of the fIS was 3.03±0.15 and 2.06±0.20 nM; LOQ of fpCS was 117±2.51 and 3.2±0.2 nM for HPLC/FLD and HPLC/MS, respectively. In healthy subjects, the fIS (nM, median,range) was 8,5 (<3.03-25), and 9.0 (<2.06-5.0) for HPLC/FLD and UPLC/MS, respectively; the fpCS (nM, median,range) was 180 (120-580) and 185 (21-560), for HPLC/FLD and UPLC/MS. In non hemodialyzed end-stage CKD patients, the fIS (nM, median,range) was 6940 (1000-14100) and 18000 (2600-36700) , for HPLC/FLD and UPLC/MS, respectively; the fpCS (nM, median,range) was 34250 (780-123550) and 17400 (200-35900), for HPLC/FLD and HPLC/FLD. A correlation was obtained between HPLC/FLD e UPLC/MS, with correlation coefficients (Pearson's) higher than 0.898 in healthy controls, but not in uremic patients. However, UPLC/MS proved to be an improved, simple, and fast approach for determining the free plasma content of IS and pCS, endowed with high sensitivity and selectivity.

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PROTEINURIA REFLEX TEST: THE IMPORTANCE OF PROTEINURIA CHARACTERIZATION IN PATIENTS WITH NO HISTORY OF RENAL OR HAEMATOLOGICAL DISEASES

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Background: As suggested by KDIGO guidelines, since April 2016 in our laboratory we had measured albumin to creatinine ratio (ACR) and protein to creatinine ratio (PCR) for all the patients with medical prescription of urinalysis. It is common to find patient with abnormal PCR values without albuminuria. In these cases, the presence of proteinuria could be the sign of renal or haematological diseases that can cause tubular or renal overload proteinuria respectively. The purpose of this study is to characterize the origin of proteinuria.
Materials and methods: Between May and June 2018, we identified 48 patients (33M, 15F; age range 4-87, mean age 54, median age 61) with no history of proteinuria, PCR >200 mg/g, ACR <30 mg/g and no other alteration of urinalysis parameters.). Urinary albumin (μALB_2, Siemens), total protein (UPRO_2, Siemens) and creatinine (ECRE_2 Enzymathic, Siemens) were measured on ADVIA 1800 (Siemens). Proteinuria characterization was conducted with gel agarose electrophoresis and immunofixation using Hydragel Urine Profil(e) (Sebia).
Results: 13/48 (27%) patients had tubular proteinuria with ACR <10 mg/g; 23/48 (48%) patients had mixed proteinuria with ACR between 10 and 30 mg/g; 6/48 (13%) had physiological proteinuria. Surprisingly, in 6/48 (13%) patients, with no history of haematological disease, Bence Jones proteinuria was found.
Conclusion: Our data shows the importance of proteinuria characterization in patients with no history of renal or haematological diseases to make early diagnosis and refer the patients to clinicians in order to start the correct therapies. If our data will be confirmed on a large population, it demonstrates the importance of the creation of a reflex test for proteinuria characterization.

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HEMATOLOGIC CHANGES IN DIABETIC AND NON-DIABETIC SUBJECTS UNDERGOING A HALF-MARATHON RUN

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The aim of this study was the evaluation of acute hematologic changes in diabetic and non-diabetic subjects after a half-marathon run. The study population consisted of 12 healthy amateur runners (12 males; man age, 49±16 years) and 12 physically active diabetics (9 males and 3 females; 6 diabetes type 1 and 6 diabetes type 2; mean age, 52±14 years), who were engaged in a 21.1 km (half-marathon) run under competitive conditions. Blood samples were collected before the start of the run and immediately after the athletes crossed the finishing line. The complete blood cell count (CBC) was performed using Advia 2120. No substantial changes were observed for hematocrit, hemoglobin and red blood cell count after the run in both groups (all p >0.05), whilst significant post-run increases were observed for white blood cell (WBC) count (non-diabetics: 13.7±3.6x109/L versus 6.0±1.2x109/L, p <0.001; diabetics: 9.3±2.2x109/L versus 6.1±1.0x109/L, p <0.001), platelet count (non-diabetics: 249±58x109/L versus 239±56x109/L, p=0.011: diabetics: 257±50x109/L versus 221±41x109/L, p <0.001) and red blood cell distribution width (RDW) (non-diabetics: 14.7±0.4% versus 12.4±0.4%, p <0.001; diabetics, 12.8±1.1% versus 12.4±0.8%, p=0.042) in both non-diabetic and diabetic subjects. The mean percent WBC increase was significantly higher in non-diabetic that in diabetic subjects (128% versus 52%; p <0.001), as was the RDW percent increase (19% versus 4%; p <0.001), whilst the percent increase of platelet count was over 4-fold higher in diabetic that in non-diabetic subjects (17% versus 4%; p=0.003). These results clearly attest that the acute hematologic response to medium distance endurance exercise may be different between diabetic and non diabetic subjects.

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PHYSICAL EXERCISE AND OXIDATIVE STRESS IN ELDERLY

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Aging is a complex phenomenon regulated by a few 'aging genes' including sirtuins (1) and depends on redox status (2), epigenetically controlled as well. Physical exercise, depending on intensity, type of training and duration, seems to modulate positively the redox state in aging and also in many oxidative stress-related pathologies such as type II diabetes, arthritis, neurodegenerative and cardiovascular diseases which represent comorbidities in ederly. It was demonstrated the depletion of intracellular GSH and tGSH levels in the muscles of aged animals with disuse evidencing their susceptibility to oxidative stress (3). Moreover physical activity, especially if moderate and regular, seems to activate mechanisms of adaptation, reducing body's vulnerability to oxidative stress and improving systemic metabolic activities. We examined the effects of regular exercise (3 times/week for 2 months) on the levels of radical species (dROMtest), thiol groups (SHptest), plasma antioxidant capacity (BAPtest), 8-OH-guanosine (COMET test) and some redox-related proteins (HSP27, HSP70, Sirt1 and 2) in 40 elderly subjects with type 2 diabetes and/or hypertension, aging from 65 to 74 years. In addition plasma enzymatic activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) were evaluated (4). The enrolled subjects showed oxidative stress before exercise with medium-high levels of radical species, high oxidation of purine bases and reduced antioxidant capacity. After training some beneficial effects on the glycemic control of some patients, a global improvement of oxidative stress level and a significant reduction of DNA oxidation were observed. Furthermore, as an adaptive response, HSP27, HSP70, Sirt1 and Sirt2 expression were upregulated. Our results suggest that in elderly with comorbidities, these biochemical parameters are involved into aging and redox responses induced by physical exercise. Furthermore, physical exercise stimulates adaptive responses by modulating redox status and some stress related-proteins. Therefore, these biomarkers may be used in preventive and predictive medicine to identify individuals at higher risk for developing oxidative stress related pathologies as well as in personalized therapy. References: 1. Sauve AA, et al. Annu Rev Biochem 2006;2. Harman D. J.Gerontol. 1956;3. Chen CN, et al. Biol Sci Med Sci 2008;4. Tomasello B et al. Oncol Lett 2017.

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SENSIBILIZZARSI ALLA DONAZIONE DI SANGUE MIGLIORANDO LO STILE DI VITA

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Introduzione: Negli ultimi anni, sovrappeso, dislipidemie, ipertensione arteriosa e obesità sono aumentati in modo preoccupante provocando un maggior rischio di malattie celebrovascolari. A tal proposito il Centro trasfusionale di Aversa ha avviato un programma di screening volto a indagare lo stile di vita e le abitudini alimentari dei donatori di sangue afferenti al nostro SIT.
Metodi: Lo studio è stato condotto da gennaio 2017 a gennaio 2018. I donatori di sangue sono stati 2.000 tra cui rispettivamente 1.000 donne (50%) e 1.000 uomini (50%) di età compresa 35-65 anni. I parametri valutati sono stati: colesterolo totale, colesterolo HDL,trigliceridi, misurazione della pressione arteriosa,glicemia,anamnesi nutrizionale e valutazione antropometrica (rilevazione peso, altezza, circonferenza, indice di massa corporea).
Risultati: I dati ottenuti mostrano che: le donatrici di età compresa 35-49 anni (30%) prestano maggiore attenzione all'apporto calorico e all'attività fisica. Gli esami clinici eseguiti sono nella norma e mostrano un BMI a basso rischio. Le donatrici di età compresa 50-65 (20%), sono poco attente ad una corretta alimentazione, seguono una terapia antipertensiva, svolgono una vita sedentaria mostrando un BMI di rischio moderato. Gli uomini invece di età compresa 35-49 (33%), seguono una terapia antipertensiva, con valori di colesterolo totale >200 mg/dl (230±10) e trigliceridi >180 mg/dl (200±15), scarsa attività fisica e un BMI di alto rischio. Diversamente i donatori di età compresa tra i 50-65 (17%), praticano assiduamente attività fisica, sono molti attenti a seguire una dieta mediterranea con esami clinici nella norma e un BMI a basso rischio.
Conclusioni: Dall'analisi dei dati possiamo affermare che adottare uno stile di vita sano significa principalmente smettere di fumare, alimentazione corretta e attività fisica adeguata. Quest'ultima è oggi considerata come un vero e proprio farmaco efficace sia nella prevenzione/gestione delle principali malattie croniche sia nel controllo del peso che è uno dei fattori di rischio principali per malattie cardiovascolari, oncologiche e metaboliche. Per questo una dieta appropriata e una vita sana sono le migliori garanzie di salute e qualità del sangue donato.

P241
IRISIN, OXIDATIVE STRESS AND INFLAMMATORY BIOMARKERS IN RESPONSE TO A HALF-MARATHON IN TRAINED ATHLETES

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Objective: Irisin, oxidative stress and inflammatory biomarker changes after half-marathon are scarce, and generally limited to immediately post-race evaluation. Aim of the study was to evaluate biomarker changes before a half-marathon and during a 48-h recovery period.
Methods: Blood samples, taken before and soon after the run, and after 24 and 48 h of recovery from male trained runners (n=15, 39-60 years), were assessed for an Oxidative-INDEX (score reflecting both oxidative/ antioxidant counterparts), leukocyte telomere length (LTL) procoagulant activity of circulating microparticles (MP-PCA), and inflammatory parameters obtained by the hemochrome (white blood count, WBC; neutrophils, N; monocytes; lymphocytes; platelets; mean platelet volume, MPV; neutrophil-to-lymphocyte ratio; platelet-to-lymphocyte ratio; lymphocyte-to-monocyte ratio) and irisin.
Results: The Oxidative-INDEX ($p < 0.01$), MP-PCA ($p < 0.05$) and WBC ($p = 0.07$) N ($p < 0.05$), MPV ($p < 0.01$) increase after exercise and during recovery, irisin progressively decrease ($p < 0.05$), whereas the other biomarkers did not significantly change.
Conclusion: Changes in oxidative stress, MP-PCA, some inflammatory biomarkers, and irisin after half-marathon could retain a cell-damaging role or may represent an adaptation mechanism to regular endurance training. In any case, an adequate supply of antioxidants could be considered in this exercise setting.

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CONFRONTO TRA METODI: TNIH VS TnI-ULTRA SU ADVIA CENTAUR XPT

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Abbiamo valutato, da Ottobre 2017 a Marzo 2018, le performance analitiche di un nuovo metodo immunochemiluminescente per troponina I (TNIH Siemens Advia Centaur XPT), recentemente immesso sul mercato (marchio CE marzo 2017) ed implementato sull'analizzatore ADVIA CENTAUR XPT, in confronto con il metodo TnI-ultra Siemens utilizzato di routine nel nostro laboratorio.TnI-ultra e TNIH sono immunodosaggi sandwich a tre siti. Il primo utilizza un anticorpo di rilevazione (epitopi 27-40) policlonale di capra marcato con estere di acridinio e due anticorpi di cattura (epitopi 41-49 e 87-91) monoclonali murini biotinilati. Il LOD è di 6 ng/L, con cut-off globale di 40 ng/L. Il secondo utilizza un anticorpo di rilevazione monoclonale ricombinante Fab anti-TnI umano marcato con estere di acridinio e due anticorpi di cattura monoclonali murini biotinilati, diretti verso epitopi diversi della molecola; il LOD è di 2.21 ng/L, con cut-off globale 47.34 ng/L.Sono stati considerati 355 campioni di siero di pazienti (M 210 e F 145; età 31-84 aa) afferenti al DEA e/o ricoverati nel nostro policlinico, sui quali era stata dosata la troponina con kit TnI-ultra, con valori distribuiti nel range di misura da <6 a 120 ng/L. Successivamente sugli stessi sieri è stata dosata la troponina con kit TNIH. 195 campioni inferiori a 6 ng/ L con il kit TnI-ultra, sono risultati invece tutti dosabili (2.5-10 ng/L) con il kit TNIH, a conferma della dichiarata maggiore sensibilità analitica del metodo. Per i restanti 160 campioni, i risultati ottenuti con le due metodiche hanno mostrato la presenza di un errore sistematico, evidenziato con l'analisi di regressione lineare Passing Bablok ($y = 3.481 + 0.6916\ X$), $r = 0.797$ e un bias significativo (9.77%) con il plot di Bland-Altman. Abbiamo inoltre valutato la concordanza tra i risultati di tutti i sieri e abbiamo ottenuto un valore del 90.34%. La precisione del metodo è stata valutata utilizzando un siero di controllo a bassa concentrazione, 13 ng/L (Liquid Cardiac Marker Plus lot. n° 23635 Bio-Rad) effettuando 3 replicati per 5 giorni ottenendo un CV del 10.1%. La presenza di un bias significativo tra i metodi molto probabilmente potrebbe dipendere dai differenti anticorpi utilizzati nei kit e dai diversi epitopi verso cui sono diretti.

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CONVALIDA DI UN SECONDO METODO ANALITICO SIEROLOGICO NELLA VALIDAZIONE BIOLOGICA DEL SANGUE E DEGLI EMOCOMPONENTI – A.O.R.N. A. CARDARELLI DI NAPOLI

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Obiettivo dello studio. Nello screening delle unità di sangue e degli emocomponenti la falsa positività sierologica, definita come reattività non confermata, è un evento relativamente frequente ed è legata al livello di aspecificità insito nei test utilizzati. In routine, il Centro di Qualificazione Biologica dell'A.O.R.N. A. Cardarelli di Napoli utilizza per lo screening sierologico delle unità di sangue donate la piattaforma Vitros®3600 Ortho Clinical Diagnostics con metodica chemiluminescenza potenziata. Scopo di questo lavoro vuole essere una valutazione preliminare dello strumento Elecsys e411 Roche Diagnostics quale secondo sistema analitico all'interno del percorso di validazione biologica per definire i test sierologici risultati allo screening ripetutamente reattivi (RR) ma non confermati con metodica Immunoblot. Metodi. Lo strumento è stato sottoposto al processo di convalida. Sono state pianificate le seguenti analisi: precisione intraserie e interserie, sensibilità, specificità e accuratezza per i parametri HBsAg, HCV Ab, HIV 1/2 Ag/Ab e Treponema Ab; sensibilità analitica per HbsAg e antigene p24. Tali prove sono state effettuate con preparazioni di riferimento inviate dal Centro Nazionale Sangue. Risultati. Le prove di convalida hanno dato esito positivo per tutti i parametri valutati con performance migliori rispetto a quelle dichiarate sulle istruzioni per l'uso dei vari test. In fase preliminare abbiamo analizzato 25 campioni da seroteca risultati falsi positivi al sistema Vitros di cui 20 HCV e 15 HIV e tutti sono risultati negativi. Conclusioni. Il sistema Elecsys e411 (metodica elettrochemiluminescenza), da poco introdotto nel laboratorio viene utilizzato quale controllo di risultati dubbi nonché quale metodo alternativo a Vitros per il controllo dei campioni RR non confermati così come previsto da normativa vigente. L'introduzione di un secondo sistema analitico presenta molteplici vantaggi: è un elemento indispensabile nel protocollo di riammissione del Donatore con reattività aspecifica al metodo di screening, consente di recuperare alla donazione un numero consistente di Donatori con effetti sulle scorte di sangue disponibili e consente di fornire al Donatore informazioni basate su evidenze scientifiche sul loro reale stato di salute.

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IL LEAN THINKING: CASE REPORT DEL SERVIZIO DI MEDICINA DI LABORATORIO P.O. BOLOGNINI DI SERIATE – ASST BERGAMO EST

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Introduzione: L'attività di laboratorio nei processi pre-analitici, analitici e post analitici, deve essere costantemente controllata e presidiata. L'applicazione del Lean Thinking, metodo di management aziendale recentemente applicato anche in Sanità, ha permesso nel Servizio di Medicina di Laboratorio del P.O. Bolognini di Seriate, l'analisi dei flussi e la revisione in ambito organizzativo di diverse attività. Un focus particolare volto al miglioramento delle performance, è stato attivato sulla fase pre-analitica individuando aree prioritarie meritevoli di interventi per abbattere gli sprechi, ridurre i tempi di produzione, standardizzare i processi e ottimizzare le risorse disponibili coinvolgendo tutta l'equipe. La mappatura dei flussi ha evidenziato come priorità la rilevazione di campioni ematici con almeno un'anomalia (emolisi, ittero, opalescenza, coaguli, fibrina e riempimento anomalo) spesso causa di aumento del TAT (Turn Around Time), spreco di risorse ed errore analitico. Si è proceduto ad un monitoraggio (ispezione visiva) di tutti i campioni ematici pervenuti nel Corelab, prima e dopo retraining dei prelevatori, ridefinendo e dettagliando le corrette modalità di prelievo ematico, conservazione e trasporto del materiale biologico.
Obiettivi: Ridurre il numero di campioni con anomalie legate al prelievo Ridurre il TAT, eliminando le attività superflue e ottimizzando le risorse disponibili Mantenere i requisiti di qualità e sicurezza dei risultati Organizzare un ambiente di lavoro stabile e standardizzato.
Risultati: Da ottobre 2017, prima valutazione e raccolta dati, a gennaio 2018, seconda valutazione dopo retraining dei prelevatori, la percentuale dei campioni ematici con almeno un'anomalia si è ridotta circa del 50%; permangono l'emolisi e il riempimento anomalo dei tubi, causando spesso l'impossibilità al completamento di tutti i test richiesti.
Conclusioni: L'applicazione del Lean nella nostra realtà ha innescato un processo di miglioramento continuo con primi esiti soddisfacenti riscontrati nell'ottimizzazione del tempo uomo/analisi, nel miglioramento in termini organizzativi dei processi e nel mantenimento degli standard quali-quantitativi.

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IMPLEMENTATION OF A HPLC METHOD FOR THE EVALUATION OF URINARY 4-HYDROXYPHENYLACETIC ACID IN PATIENTS WITH NEUROENDOCRINE TUMORS

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Neuroendocrine tumors (NETs) are a group of neoplasms that originate from neuroendocrine cells. They can occur in various anatomic locations, most commonly in gastro intestinal tract, lungs, and pancreas, and they may be associated with symptoms caused by peptides and hormones release. In neuroendocrine tumors associated to the carcinoid syndrome, tumor cells produce hormones, such as serotonin, as well as other proteins like chromogranin A that can be used as biomarkers for the diagnosis and progression of NETs. Determination of urinary levels of 5-hydroxyindoleacetic acid (5-HIAA), the main metabolite of serotonin, is routinely used as tumor secretory marker to investigate and monitor patients with carcinoid syndrome. Measurement of urinary 5-HIAA is usually performed using high performance liquid chromatography (HPLC). However, with this methodology (Bio-Rad VMA/HVA/5-HIAA), we observed also the presence of 4-hydroxyphenylacetic acid (PHPAA), physiologically present in urine of healthy people, that interferes with the quantification of 5-HIAA, when the concentrations of both PHPAA and 5-HIAA are particularly high. In this condition, the retention time difference between 5-HIAA and the PHPAA is about 0.65 minutes, which makes it difficult to correctly quantify 5-HIAA. To allow a better separation and quantification of 5-HIAA and PHPAA, we developed a HPLC method, using a different analytical column (Agilent Eclipse Plus C18 5 μm, 4.6 x 150 mm) and a different mobile phase (citrate buffer pH 4,5 and acetonitrile in elution gradient) instead of the original method. These changes improved the separation between 5-HIAA and PHPAA, obtaining a retention time difference of 1.45 minutes, allowing a more accurate and specific quantification of both compounds. We analyzed the urines of 6 patients with NETs using the modified method. All patients showed high level of PHPAA compared to the reference values, while only 3 patients showed high levels of 5-HIAA. Therefore our preliminary data suggest that, using the modified method, we could quantify PHPAA that could be a useful marker, together with 5-HIAA, for the management of patients with neuroendocrine tumors.

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MULTICENTER STUDY FOR EVALUATION OF THE REFERENCE RANGE FOR TSH IN ITALY (ELAS TSH ITALIAN STUDY)

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Background: The aims of this study were: 1) to calculate reliable TSH reference intervals using laboratory databases; 2) to evaluate the relationship between TSH, sex and age values in different large Italian populations.
Methods: The TSH values stored in the Laboratory Information System (LIS) of clinical laboratories of 4 Italian City Hospitals, including 146,801 TSH measurements (with the respective age and sex data of individuals) were taken in consideration. Assuming a lognormal distribution, to log-transformed TSH values were applied the Dixon's iterative principle in order to exclude the outliers. At the end of this iterative process 142,821 logtransformed TSH results remained. The four clinical laboratories measured serum TSH concentrations using the same TSH immunoassay method (Access TSH 3rd IS, using DxI platform).
Results: TSH reference interval calculated in the present study (0.362 – 5.280 mIU/L) is similar to that suggested by the manufacturer for the Access TSH 3 rd IS assay (0.45 – 5.33 mIU/L). TSH values in females were significantly higher than those found in males (Females: mean= 2.06 mIU/L; SD= 1.26 mIU/L; N= 101243; Males: mean= 1.92 mIU/L; SD= 1.19 mIU/L; N= 41578; $p < 0.0001$). Moreover, a negative linear relationship was observed between TSH throughout all range age values (from 0 to 105 years).
Conclusions: The results of the present multicenter study confirm that data mining techniques can be used to calculate clinically useful reference intervals for TSH. From a pathophysiological point of view, our results suggest that some Northern populations of Italy might still suffer some harmful effects on thyroid gland due to mild to moderate iodine intake deficiency. Specific clinical trials are needed to confirm these results.

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VALUTAZIONE DELL'EMOGLOBINA GLICATA (HbA1c) MISURATA MEDIANTE ELETTROFORESI CAPILLARE E CONFRONTO CON IL METODO CROMATOGRAFICO

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Sommario: L'HbA1c è un marcatore cardine per il monitoraggio dei pazienti diabetici. In questo lavoro, al fine di saggiare la veridicità del dato e la comparabilità tra le diverse metodiche analitiche, abbiamo confrontato i valori di HBA1c misurati mediante Elettroforesi capillare (EC) con quelli ottenuti con il metodo cromatografico (HPLC).
Metodi: In questo studio sono stati analizzati 130 campioni di sangue intero (K3EDTA) provenienti da pazienti che richiedevano la determinazione della HbA1c con metodica HPLC (G8 Tosoh, Biosciences). Gli stessi campioni sono stati analizzati nel medesimo giorno anche con il metodo EC (V8 Nexus, Helena Medical System, Italia). I campioni sono stati scelti in base al valore di HbA1c compreso tra 3-13% (gruppo 1, n =130). In questo gruppo sono inclusi anche 27 campioni di pazienti anemici con Hb <10 g/dL (gruppo 2, n= 27).
Risultati: I risultati ottenuti con la EC hanno mostrato valori percentuali più bassi di HbA1c rispetto a quelli misurati con il metodo HPLC (y = 0,912x+0,3276; r = 0,971; P <0,0001). Anche i valori di HbA1c dei pazienti anemici, ottenuti mediante EC, sono risultati sottostimati rispetto ai valori misurati con HPLC (y = 0,8858x + 0,4772; r = 0,925; P <0,0001). I controlli di qualità misurati mediante HPLC per il livello 1 sono risultati 5,05% +/- 0,16; CV 3% (valori attesi: 4,9% +/- 0,15; CV 3%); per il livello 2 sono risultati 9,67% +/- 0,08; CV 1% (valori attesi: 9,8% +/- 0,25; CV 2,5%). I CQI misurati mediante EC sono risultati 5,46% +/- 0,2; CV 3,8% (valori attesi: 5,4% +/- 0,2; CV 3,7%); per il livello 2 sono risultati 6,9% +/- 0,21; CV 3,1% (valori attesi: 7,1% +/- 0,2; CV 2,8%).
Conclusioni: Sulla base dei risultati ottenuti riteniamo che l'Elettroforesi capillare (EC) è una metodica che ben correla con il metodo di riferimento HPLC e potrebbe essere utilizzata per l'analisi della HbA1c.

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PERFORMANCE EVALUATION OF ATELLICA IM 1600 ANALYZER ASSAYS IN A CLINICAL CHEMISTRY LABORATORY

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Background: Introduction of new instruments and methodologies in the clinical laboratories necessitates validation studies to ensure that new methods will meet acceptable standard of performance. Studies were performed in our laboratory to assess the analytical performance of immunoassays (IM) for the Atellica® IM 1600 Analyzer with respect to verification of precision, linearity, and method comparison with current assays on Siemens ADVIA Centaur® XP System.
Methods: Eight immunoassays were chosen for the study. Precision verification was performed according to EP15-A3, method comparison to EP09-A3, and linearity to EP06-A. For verification of precision, three or four concentration levels were used; each level of QC materials and sample pools were tested as one run per day with five replicates per run, for five days (25 total replicates per sample for each assay). Method comparison studies were performed on 4 immunoassays using at least 40 serum samples that covered a wide assay range, from low to high serum concentrations; in particular four concentration ranges were chosen for each analyte. The number of levels of linearity material ranged up to eight depending on the assay; three replicates for each level of linearity material samples were performed for each assay. Siemens Healthineers supported the study by providing systems and reagents and contributed to data analysis.
Results: Within-run (repeatability) CVs for immunoassays ranged from 1.0% to 5.8% and Within-Lab (total) CVs from 1.1% to 6.7%. Within-Run and Within-Lab CVs were similar or lower compared to the ones declared in the IFUs by the manufacturer. Method comparison studies showed good correlation for the Atellica® IM 1600 results compared with ADVIA Centaur® XP for the 4 immunoassays tested. Linearity was checked for 8 assays and only Ferritin showed non-linearity when results above 430.13 ng/mL were included.
Conclusions: Evaluation of the Atellica® IM 1600 in our laboratory setting demonstrated a good precision on QC materials and sample pools for all the tested assays. In our hands precision results were better than those declared by the IFUs. Method comparison studies between Atellica® IM 1600 and ADVIA® Centaur® XP were satisfactory even in the presence of constant or proportional error. Moreover, linearity of the immunoassays was successfully verified across the claimed intervals.

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PERFORMANCE EVALUATION OF THE ATELLICA CH 930 ANALYZER ASSAYS IN A CLINICAL CHEMISTRY LABORATORY

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Background: Introduction of new instruments and methodologies in the clinical laboratories necessitates validation studies to ensure that new methods will meet acceptable standard of performance. Studies were performed at our institution to assess the analytical performance of clinical chemistry (CH) assays for the Atellica® CH 930 Analyzer with respect to verification of precision and linearity, and method comparison with Siemens current assays on the ADVIA® 1800 System.
Methods: Nineteen clinical chemistry assays were chosen for the study. Precision verification was performed according to EP15-A3, method comparison to EP09-A3, and linearity to EP06-A. For verification of precision, from two to four concentration levels were used; each level of QC materials and sample pools were tested as one run per day with five replicates per run, for five days (25 total replicates per sample for each assay). Method comparison studies were performed using at least 40 serum samples that covered a wide assay range, from low to high serum concentrations; in particular four concentration ranges were chosen for each analyte. The number of levels of linearity material (LGC Maine Standards) ranged up to six depending on the assay; three replicates for each level of linearity material samples were performed for each assay. Siemens Healthineers supported the study by providing systems and reagents and contributed to data analysis.
Results: Within-Run (repeatability) CVs for clinical chemistry assays ranged from 0.2% to 4.8% and Within-Lab (total) CVs from 0.4% to 8.2%. Within-Run and Within-Lab CVs were similar or lower compared to the ones declared in the IFUs by the manufacturer. Method comparison studies showed good correlation of the Atellica® CH 930 results compared to ADVIA® 1800 System. Linearity was checked for sixteen assays and only LDL did not fulfill the acceptance limits for slight non-linearity detected at low end.
Conclusions: In our laboratory setting the evaluation of the Atellica® CH 930 Analyzer demonstrated a good precision on QC materials for all the tested assays. In our hands precision results were even better than those declared by the IFUs. Method comparison studies between Atellica® CH 930 and ADVIA® 1800 were satisfactory even in the presence of constant or proportional systematic error. Moreover, linearity of the assays was successfully verified across the claimed intervals.

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MONITORING OF LEVETIRACETAM IN SERUM. A SIMPLE METHOD OF DETERMINATION: LLE COUPLED TO GC-MS

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Introduction: LEV (LEV) is a second-generation anti-epileptic drug approved by the FDA in 1999. The exact mechanism of action of the drug is not known. It is approved for for the treatment of epilepsy as either monotherapy in the case of partial seizures or an adjuntive therapy for partial, myoclonic, and tonic-clonic seizures. The drug is rapidly and completely absorbed from the gastrointestinal tract after oral administration. It does not bind to plasma proteins. It is mainly excreted unchanged in the urine (67%). Conditions that may impair renal function may cause a lengthening of the drug half-life. Therefore, drug monitoring is important in many conditions such as the treatment of children, pregnant women, geriatric patients and patients with renal impairment. In the last year we dosed 1700 LEV.
Material and Methods: The samples were tested with Agilent 6890N GC and 5975 MS. The method developed consists in a liquid-liquid extraction (LLE) of LEV from the aqueous phase, based on its solubility in chlorinated solvents, which is followed by a quantitative determination in GC-MS. Extraction and recovery tests were carried out with two different extraction solvents:CH_2Cl_2 and $CHCl_3$.

In flat vials are placed 200 μl of drug free human serum previously added with standard solution, 50 ul of internal standard and 600ul of solvent. The vials are then shaken with vortex and then centrifuged. A net phase separation is obtained between the solvent and the aqueous, this allows the sampling system in GC-MS to draw directly extract with the sampler needle. The GC-MS system performs the chromatographic run in 4'45'', the retention time of the LEV is 2'30'' and the ISTD 3'50''. The comparative analysis obtained with the two solvents, show a higher $CHCl_3$ extraction capacity (15% higher recovery). The method developed with $CHCl_3$ was also compared with a method in HPLC-UV. Were sent 37 samples, in the range of concentrations from 1.67 to 54.45 ug/ml and the correlation between the measurements was 0.947.
Results: The developed method shows good linearity in the range 2.5-45 ug/ml, covering the therapeutic range. The LOD is 0.5ug/ml and the LOQ 2 ug/ml. The simple linear regression between the HPLC-UV method and the LLE-GC-MS method performed on 37 samples is $y = 0.97 x + 0.52$ with $r = 0.971$ and a standard error 2.85.
Conclusions: The coupled LLE-GC-MS method described for the determination of LEV is simple specific and sensitive.

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PRE- AND POST-ANALYTICAL PHASES IN CSF AD BIOMARKERS ANALYSIS: COMPARISON OF FOUR CENTERS

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The cerebrospinal fluid (CSF) biomarkers, Aβ and tau proteins, have been progressively integrated in clinical practice for the diagnosis of Alzheimer's disease (AD). However, the interpretation of the results needs expertise and caution. Clinical discrepancies of the results and laboratory issues reduced the specificity and sensitivity of these markers. In particular, high variability in experimental performance, methodological platforms, sample collection handling and banking, use of reliable cut-off values, represent main limitation for biomarkers use. Recently, fully automated chemiluminescent (CLIA) methods have been developed, that are expected to reduce variability when compared to manual measurement.

We evaluated the differences in pre and post analytic variables between four main Laboratories (Padua, Perugia, Rome and Turin) using CSF biomarkers, which have introduced CLIA system for analysis.

Pre-analytical procedures were similar in all the laboratories (use of polypropylene tubes, speed of centrifugation, number of aliquots) except for the temperature for collection and processing (4°/in ice= 1 lab; room temperature=3 labs) and storage (-20°= 1 lab; -80°=3 labs). Slight differences were present in the interpretation of the results. Only one center provided interpretative comments on laboratory report. Value of normality for $A\beta_{42}$ differs for less than 10% between the laboratories using CLIA ($A\beta_{42}$ >700 pg/ml= 2 labs; 770= 1 lab; 781=1 lab), and thus were very similar for tau (400 pg/ml=3 labs; 420=1 lab) and p-tau (<61=3 Labs; 64,5=1 pg/ml). For $A\beta_{42,}$ the normal values with CLIA in laboratories were closer respect to those used for ELISA, which varied from >450 to >790.

Consensus for pre-analytical procedures for CSF processing is needed. Harmonization of normal ranges for CSF biomarkers has been quite improved with the advance of automated technologies. National and international initiative for standardization and harmonization of AD CSF biomarker analysis and interpretation are essential to define reproducible and consistent biomarker measurements, and finally to reduce debate in the AD field regarding biomarker values and clinical interpretation of the biomarker results.

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RATIONALIZATION AND IMPROVEMENT IN PCT ASSAY AVAILABILITY IN A MULTICENTRIC REALITY IN TUSCANY

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Tuscany Region recommendation about sepsis panel in Emergency Department include procalcitonin (PCT) assay 7 days a week, 24 hours a day. Our reality (USL Toscana Centro) consists of eight hospitals. Up to October 2017 one laboratory had no PCT availability, three had three different methods h12, one had a fourth method 6 days a week only in the morning, and three were sending samples to this one. Than 6 labs introduced Diazyme PCT assay on Beckman Coulter AU systems (480, 680 and 5800) h24, one moved to DiaSorin Liaison connected to Beckman Coulter Power Processor h24, the last one maintained h12 on Biomerieux VIDAS due to an existing tender assignment. This reorganization guarantees autonomy to each lab, improving TAT and reducing costs; moreover, there is much more methodology homogeneity. Diazyme assay is latex particle enhanced immunoturbidimetric with CE marked application on AUs; calibration curve has 6 points at 0.01, 0.95, 2.75, 11.75, 21.86 and 58.78 ng/mL; LoB at 0.06, LoD at 0.16, LoQ at 0.2 and linearity 52 ng/mL. The new test shows a good reproducibility and repeatability. Interestingly, 10% CV at 0.5 and 20%CV at 0.2 ng/mL. We shortened verification time, checking QCs performances especially at low end and linearity upon dilution (data presented to SIMPEL Congress 2017): 5.17% CV at 0.653 and 8.24% at 0.35 ng/mL; good recovery diluting x5 and x10. Considering four labs 6 months' results we run more than 10.000 tests. After one false positive due to lipemia we introduced serum indexes for PCT also; one false positive due to preanalytical factors managed the same day; nine samples were investigated for possible analytical HAMA interferences with Scantibodies HBT (Heterofilic Binding Tubes) and two of them were confirmed interfered. No false negative results complained.
Conclusion: the new PCT Test is useful for the diagnosis and therapeutic control of septic patients and improves laboratory efficiency when applied on a serum analysis platform.
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P253
EVALUATION OF THE CHORUS PSA KIT FOR THE DETERMINATION OF THE PROSTATIC ANTIGEN SPECIFIC TOLATE (tPSA)

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Introduction: PSA (prostate specific antigen) is a single chain glycoprotein produced by the prostate and periurethral glands and is secreted in the prostate ducts, where as a serine protease it cuts the proteins to make the seminal fluid more fluid. Any change in the anatomical structure of the prostate gland increases the passage of PSA into the bloodstream, therefore, high levels of PSA are indicative of benign or malignant pathological conditions. The total PSA (tPSA) is measured and it represents the complexed form with α1-ACT or α2-M plus the free form. The aim of this study is to evaluate the commercial kit, Chorus PSA, through the analysis of functional sensitivity and comparison methods with routine test (Cobas Roche). Materials and methods: A total of 250 anonymised serum samples (112 tPSA<1 ng/ml, 138 tPSA≥1 ng/ml) from the routine analysis of the General Laboratory in Careggi Hospital and characterized for tPSA by Cobas Roche System,were analyzed with the Chorus PSA Lot 400 Enzyme Immunoassay kit by Chorus instrument. Statistical elaborations of the comparison between methods were performed by Passing-Bablok and Bland-Altman analyzes using SPSS version 11.0, while for the evaluation of the functional sensitivity were used Variance Function Program 3.0. Results: The results showed that the comparison of 46 samples (tPSA<0.1 ng/ml) is not comparable because values are below the Chorus LOQ. For 66 samples (tPSA 0.1-1 ng/ml), the correlation was r=0.9870; Intercept (I)=-0.1449 and the average of the differences (AD) was 0.15 (+1.96 SD=0.383; -1.96 SD =-0.084). In total 204 samples (tPSA≥0.1 ng/ml), the Passing-Bablok analysis showed a correlation r=1.0584; I=-0.1673 while AD in Bland-Altman analysis was 0.049 (+1.96 SD=3.782; -1.96 SD=-3.685). The 138 samples (tPSA≥1 ng/ml) showed a correlation r=1.0597; I=-0.1680 and AD of 0.001 (+1.96 SD =4.54; -1.96 SD=-4.538). The evaluation of the functional sensitivity indicates CV=19% to measure tPSA 0.30 ng/ml. Conclusion. The functional sensitivity showed acceptable analytical performances for values tPSA≥0.30 ng/ml. As observed by comparison methods, analytical performances are optimal for tPSA≥1ng/ml samples. For tPSA<1 ng/ml samples, the Chorus assay showed acceptable results, with margins of improvement for tPSA <0.30 ng/ml samples.

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ANALYTICAL AND CLINICAL ACCURACY PERFORMANCE OF A POCT DEVICE USED FOR GLUCOSE SCREENING TEST IN PREGNANCY

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Introduction: The glucose tolerant test is commonly used to screen for gestational diabetes and Point-of-care Testing (POCT) devices are being used in this context in a certain number of laboratories.
Objectives: Aim of this study was the retrospective evaluation of the analytical and clinical performance of a POCT device in pregnant women referred to the Niguarda Laboratory phlebotomy center for glucose screening test.
Materials and methods: The study population consisted of 200 pregnant women. Capillary samples were analysed with the Accu-Chek® Inform II POCT device (Roche Diagnostics, Switzerland); serum obtained from tubes containing anti-glycolytic agents were tested on a Cobas 8000 analyzer (Roche). Passing and Bablok regression and Bland and Alltman difference analyses, by means of the MetComp ver. 1.0 (SIBIOC) software were carried out to determine analytical accuracy. Clinical accuracy was investigated by means of the Surveillance Error Grid (SEG v. 1.2.04) analysis. Data were compared to ISO 15197:2013 requirements.
Results: Minimum, Q1, Median, Q3 and Maximum glocose values (mM) were, for Cobas 8000 and the POCT device, respectively: 3.63/4.24/4.46/4.84/7.98 and 3.63/4.40/4.65/5.06/7.37. Passing and Bablok regression (Y= 0.165+1X; 95%CI: slope= 0.949;1.150; intercept= -0.509;0.403) showed no deviation from linearity (cusum test P>0.05) and the absence of any systematic or proportional errors. Bland and Altman analysis showed the presence of a statistically but not clinically significant bias (-0.16 mM; 95%CI= -0.21;-0.12; -3.58%; 95%CI= -4.57;-2.60). As regards clinical accuracy, 96.8% (6/188) of the differences (ISO requirement: 95%) between the POCT device and Cobas 8000 were within 0.83 mM for glucose leves <5.6 mM; furthermore, all differences for glucose levels ≥5.6 mM were within ±15% according to ISO 15197:2013. SEG analysis provided 99.5% results within zones A and B of the consensus grid for type 1 diabetes.
Conclusions: The Accu-Chek® Inform II POCT device showed good analytical and clinical performance meeting the ISO 15197:2013 requirements in a population of pregnancy women. This study is going to be completed by precision performance evaluation.

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EVALUATION OF DIAGNOSTIC TESTS FOR SYPHILIS: SIMULTANEOUS DETERMINATION OF TREPONEMAL AND NON-TREPONEMAL ANTIBODIES IN COMPLETE AUTOMATION

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Introduction: Routine laboratory diagnostic tests for syphilis consist of a screening system (syphilis screen) for fully automated treponemal antibodies and of manual TPHA and RPR (VDRL) tests in the case of positive results. The RPR test (VDRL) provides a visual interpretation of the agglutination method (carbon antigen card test), influenced by interferences and subjectivity in interpretation. The new BioPlex Multiplex Luminex system allows the simultaneous evaluation of treponemal and non-treponemal (RPR) antibodies with titration.
Methods: For the evaluation of the Bio-Rad Multiplex Luminex system (BioPlex 2200 Syphilis Total & RPR), we selected 41 positive Syphilis screen samples performed by the Siemens ADVIA CENTAUR chemiluminescence method for qualitative treponemal antibodies, analyzed with the BioPlex2200 syphilis Total & RPR and we compared the results both for the research of treponemal antibodies (Syphilis total) and for the research and titration of non-treponemal antibodies performed with the manual method in use: RPR (VDRL) DASIT company.
Results: We have found practically a total agreement for the research of treponemal antibodies with the two methods, while for the research of non-treponemal antibodies, we have detected 29.3% of samples analyzed in agreement and some discrepancies: 24.4% and 19.5% of positive samples for RPR (VDRL), respectively titer 1/2 and titer 1/4 negative for BioPlex RPR, 14.6% of positive samples RPR (VDRL) titer 1/8 BioPlex RPR negative, 9.8% of concordant results but with significantly different titers between the two methods.
Conclusions: The BioPlex 2200 Syphilis Total & RPR method has been able to detect total treponemal antibodies in agreement with the method in use, with the advantage of simultaneously showing non-treponemal antibodies (RPR) and titration, with an improvement in the specificity, reproducibility of the analytical data and elimination of possible interference (for RPR / VDRL titer of 1/2 or 1/4). However, the most significant discrepancies found need further investigation to clarify the RPR/VDRL outcome useful from a clinical and diagnostic point of view, above all for therapeutic monitoring.

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HEMOGLOBIN A2 EVALUATION DURING HbA1c QUANTIFICATION WITH CAPILLARY ELECTROPHORESIS

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Introduction: Various methods have been used for measurement of HbA1c. The high-performance liquid chromatography (HPLC) methods have been preferred, but recently capillary electrophoresis has been developed and adapted to the analysis of HbA1c. Capillarys Tera3 instrument (Sebia) uses an alkaline pH buffer and detects normal and abnormal levels of hemoglobins and the most common variants in the following order, from cathode to anode: A2/C,E,S,D,F,A0 and then the HbA1c. In particular HbA2 quantification plays a key role in screening programs for thalassemia: its decrease might suggest α-thalassemia and its increase β-thalassemia.
Methods: The quantification of HbA2 using both the Capillarys (Sebia) HbA1c program and the HPLC VARIANT IITM (Biorad) βthal short program for screening of variants were compared in order to assess the ability of HbA1c program to report a reliable HbA2 value for detection α and β-thalassemia. 161 patients were enrolled in this study, from each of them we calculated HbA1c and HbA2 with both methods.
Results: 161 samples were tested with Capillarys and HPLC to quantify HbA1c and HbA2. Median HbA2 (interquartile range; min-max range) were 3.2% (2.1-5.2; 1.0-7.1) for HPLC and 2.5% (1.8-4.2; 0.9-5.6) for Capillarys. Differences between methods (X:HPLC; Y:Capillarys) were evaluated by Passing-Bablok regression and Bland-Altman analysis. Passing-Bablok regression showed both significant systematic proportional (slope: 0.80; 95%CI 0.79-0.81) and constant error (intercept: 0.08, 95%CI 0.05-0.12). Mean bias was 0.62% (95%CI 0.57-0.68) with 95% of the differences ranging from -0.05 to 1.30%).
Using a cut-off of 3.2 % for HbA2, and the adjustment of the values for direct comparison, the calculation of inter rater agreement revealed an agreement of 89.4 % with a Cohen K index of 0.79.
Conclusion: These data suggest that Capillarys HbA1c program, compared with HPLC hemoglobin program, is suitable for a sort of “unplanned” first-level screening of α and β-thalassemia among diabetic patients with reliable measurement of HbA2. Furthermore, it shows shorter time and higher throughput for the analysis.

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METHOD SETUP AND VALIDATION FOR THE DETERMINATION OF URINE AMMONIUM BY ION CHROMATOGRAPHY

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Several approaches have been proposed to determine urine ammonium such as microdiffusion, phenol hypochlorite based method, specific electrodes, and urine anion gap. Such approaches, however, are considered long and time consuming, and often anion gap cannot be calculated. An enzymatic method based on glutamic dehydrogenase activity was proposed which represents the reference method for plasma ammonium determination so far. However, proteinuria, hematuria, bacteria, and drugs limit the utilization for urine analysis. Given these findings, and considering that urine ammonium quantitation could aid to investigate urinary stone disease and to monitor patients admitted in intensive care units (ICU), the aim of the present work is to setup and validate an ion chromatography (IC) assay for measuring urine ammonium.
Urine samples selected from Desio Hospital clinical chemistry laboratory and ICU, were 200-fold diluted in methansulfonic acid (MSA) then analyzed. IC was performed using a Perkin Elmer 200 instrument coupled to a CD20 Dionex suppressor, and equipped with an Ion Pac CS12A column working at isocratic flow (MSA, 1 mL/ min). Linearity, sensitivity, and precision were assessed as indicated by CLSI documents. The limit of quantitation was determined by considering the imprecision profiles at different concentrations ranging from 0.5 to 40 ug/mL. Drug interference was evaluated by comparing the slope of the calibration curves of ICU patient urines with MSA curve.

Calibration curves showed good linearity (R^2 >0.99) between 0-10 ug/mL and 10-50 ug/mL. Repeatability, assessed by evaluating the CV on two pools at low (L, 2 ug/mL) and high (H, 12 ug/mL) concentration, resulted in a CV of 4.1% and 0.3%, respectively. Within laboratory precision resulted in CVs of 7.4% (L) and 3.0% (H). The limit of blank was 0 ug/mL whereas the limit of detection was 0.04 ug/mL. The imprecision profiles indicated that the lowest concentration showing CV <5% is 0.5 ug/mL. Finally, the urine calibration curves from ICU patients subjected to antibiotic, sedative, and diuretic treatments, showed the same slope of MSA calibration curve, indicating the absence of interferences.
Acknowledgements: Regione Lombardia Bando Linea R&S per Aggregazioni cof. POR FESR 2014-2020 CUPE47H16001530009.

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NUOVA TROPONINA HIGH SENSITIVITY (DIMENSION VISTA; SIEMENS): VALUTAZIONE ANALITICA PRELIMINARE

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Scopo dello studio è la valutazione analitica di un nuovo metodo di dosaggio della troponina ad alta sensibilità: TNIH Dimension Vista® (Siemens Healthcare Diagnostics) con tecnologia LOCI. Materiali e metodi. Studio di precisione: tre livelli di controlli di qualità interni QCI (L. C. M. Plus Control LT Biorad) e un pool di sieri, sono stati testati per cinque giorni consecutivi. Concentrazione al 99°percentile della popolazione di riferimento: la troponina è stata misurata in 100 campioni di siero di donatori apparentemente sani, (50 maschi e 50 femmine). Determinazione del LoD e del Lob: 20 determinazioni in replicato del calibratore a concentrazione zero fornito dalla ditta produttrice. Studio di comparazione: la TNIH è stata confrontata con la troponina attualmente in uso (CTNI Vista). Entrambe le troponine sono state misurate su 100 campioni sierici di pazienti, afferenti alla U.O. di Medicina di Accettazione e Urgenza. Per l'analisi statistica è stato utilizzato il software Med Calc Risultati. Studio di precisione. QC livello 1: media (DS)=49.5 (3) ng/L; CV tra-serie=6.1%. QC livello 2: media (DS)=4246 (159) ng/L, CV tra serie= 3.8%. QC livello 3: media (DS)= 21705 (538) ng/L, CV tra-serie= 2.5%. Pool di sieri: media (DS)=40.15 (1.1) ng/L, CV tra-serie=2.7%. 99° percentile (95% IC) della popolazione di riferimento: Maschi = 15 (95% IC=14 -17) ng/L, Femmine = 12 (95% CI= 11-13) ng/L. La TNIH era misurabile nel 100% dei soggetti. LoB = 0,7 ng/L. LoD =1 ng/L. Studio di comparazione: CTNI: media (DS)=1272 (6167) ng/ L; range = 15-58000 ng/L. TNIH media (DS)=891(3014) ng/L; range = 2- 20466 ng/L. Equazione della retta di regressione di Passing and BabloK: y = -0.866 + 1.058 X; intercetta (95% IC) = -0.866 (-7.02 - 4.47); slope (95% IC) =1.058 (1.01 - 1.12); coefficiente di correlazione: 0.96 p<0.0001.Conclusione. I nostri dati preliminari mostrano che la TNIH ha una buona performance analitica. Studi sono in corso al fine valutare l'imprecisione analitica (CV=10%) della concentrazione al 99° percentile in una più ampia popolazione di riferimento.

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STABILITY STUDY OF COMMON CLINICAL CHEMISTRY TESTS IN HEPARINIZED PLASMA SAMPLES STORED IN THE REFRIGERATED MODULE OF FLEXLAB TOTAL LABORATORY AUTOMATION SYSTEM

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Background-Aim: A good storage system for archiving samples in required in Clinical Chemistry Laboratory.The Central Clinical Chemistry Laboratory of Spedali Civili in Brescia has got a Total Laboratory Automation (TLA) system (FlexLab, Inpeco), where the analyzed samples are archived at 4°C in a refrigerated storage module (RSM) after being sealed with an aluminum foil. The aim of the present study was to evaluate the stability of plasma heparinized samples maintained in the RSM up to 48 hours. Methods A total of 20 samples were collected in lithium-heparin containing tubes from Sarstedt. For each of these samples 21 clinical chemistry parameters (glucose, uric acid, urea, creatinine, total cholesterol, triglycerides, Na, K, Cl, calcium, albumin, phosphate, magnesium, total bilirubin, CK, LDH, AST, ALT, ALP, gGT and pCHE) were randomly analyzed at day 0 by one of the four Dimension Vista 1500 systems (Siemens Healtheers) connected to TLA. All samples were reanalyzed after 24 and 48 hours after recalling them from RSM. Internal Quality Control was assessed as in internal procedures. The variation in the results was calculated by comparing the value at at each time (Xt) with the initial value (X0), and was expressed as relative bias: (Xt/X0*100)-100. The mean relative bias was calculated for all samples. Stability was evaluated according to the allowable Total Error % (TEa), calculated as: ±1.65*CVa (%) + bias (%). CVa was calculated for each test as annual mean between day imprecision of the four instruments. Desirable bias according biological variations criteria was used for all analytes except to urate, triglycerides, bilirubin, CK, LDH, ALT, ALP and gGT were optimal bias was used and to K were minimum analytical goal for bias was used.RESULTS A total of 18 out 21 analytes were stable up to 48h. Glucose and phosphate were stable up to 24h, because a bias of -10,4% and of +8.80% was found at 48h, respectively. Potassium showed an increase of + 9.5% and + 26.2% after 24 and 48h, exceeding TEa limits. Conclusions: Automated refrigerated system connected to FlexLab is useful to store previously analyzed samples, avoiding the evaporation process due to the sealing of samples. The system ensures the ability to reanalyzed stored samples for the majority of clinical chemistry parameters evaluated.

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VALIDAZIONE DELLA STABILITÀ NEL TEMPO DEL DOSAGGIO AccuTnI+3 BECKMAN COULTER

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La variazione della cTnI/T nel tempo è un parametro importante sia nella diagnosi dell'infarto acuto del miocardio che nel suo monitoraggio. Dopo intervento di angioplastica coronarica sono di solito effettuati 4 dosaggi ogni 6 ore. Nell'informativa del dosaggio TnI Accu+3, Beckman Coulter raccomanda di "conservare i campioni sigillati a temperatura ambiente per un massimo di 2 ore e di centrifugare, separare fisicamente dal contatto con le cellule e refrigerare i campioni quanto prima". Questa indicazione rende difficile gestire i prelievi di monitoraggio fatti in reparto alle 6 di mattina, che, non essendo inviati al laboratorio in urgenza, sono centrifugati dopo le 2 ore raccomandate. Scopo: valutare la stabilità della AccuTnI+3 nel tempo. Materiali e metodi: Ciascun campione prelevato in LiEp è stato diviso subito dopo il prelievo in 13 aliquote, di cui una dosata immediatamente (T0) sia su DXI800 (Access AccuTnI+3 ref A98264) che su Access2 Beckman Coulter (Access AccuTnI+3, ref A98143); 6 aliquote sono quindi centrifugate al T0 conservando il plasma a 4°C (campioni CC); 6 sono conservate a 4°C senza nessun trattamento (campioni SC) e saranno centrifugate prima del dosaggio, dopo 2,4,6,8,10 e 24 ore. Risultati: La variazione % media rispetto al T0 su DXI800 è stata -2.77%, (y= -2.79 + 0.001x) per i campioni SC e -1.50% per i campioni CC (y= -1.18 - 0.035x); per Access2 è stata -1.50% per i campioni SC (y= -0.19 - 0.145x) e -1.59% per i campioni CC (y= -0.928 - 0.068x). Non c'è differenza nel diverso trattamento dei campioni sugli analizzatori considerati separatamente (p=0.269 DXI, p=0.414 Access2, test t a dati appaiati) né insieme (p=0.504). Conclusione: per i campioni SC (senza centrifugazione, situazione che rispecchia la realtà dei campioni nei reparti) si rileva per le misure su DXI un calo iniziale e poi il valore si mantiene costante nel tempo fino a 24h dal prelievo. Lo studio non ha mostrato variazioni significative nel tempo dei risultati del dosaggio della TnI. Non si sono rilevate significative differenze in caso di diverso trattamento dei campioni; sembra quindi possibile conservare il prelievo in Litio Eparina a 4°C senza centrifugazione e separazione anche per un tempo maggiore rispetto a quello indicato da Beckman.

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VALUTAZIONE DELL'ALLINEAMENTO DELLA MISURA DELL'EMOGLOBINA TRA SISTEMA POCT E LABORATORIO DI RIFERIMENTO

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Introduzione: La diffusione dei POCT ha permesso di soddisfare l'esigenza del clinico di avere esami in tempi rapidi e di qualità, sia in regime di urgenza, per una diagnosi tempestiva ed un'efficace terapia, che in elezione, per monitorare in tempo reale l'efficacia delle cure, anche con lo scopo di ridurre i tempi di degenza ed i relativi costi.
Obiettivo: Si è voluto verificare l'allineamento dei risultati forniti dagli emogasanalizzatori, siti nei reparti dell'azienda ASST Nord Milano, per emoglobina (Hb) con quelli ottenuti con l'analizzatore di ematologia in Laboratorio.
Materiali e metodi: Sono stati raccolti dalla normale routine in duplicato 1048 campioni provenienti da diversi reparti del P.O. Bassini. Tutti i campioni di sangue intero sono stati prelevati contemporaneamente con provetta K2-EDTA per l'analisi con sistema XE-2100 (DASIT Sysmex) in laboratorio e con siringhe eparinate per emogasanalisi per la determinazione su sistema RAIDPoint 500 (SIEMENS) direttamente in reparto. L'analizzatore RAIDPoint 500 fornisce la misura dell'Hb totale attraverso CO-Ossimetro integrato (metodo spettrofotometrico), mentre il sistema XE-2100 utilizza un metodo spettrofotometrico a 555 nm. La valutazione dell'agreement è stata effettuata su base grafica e su base statistica (Passing-Bablok).
Risultati: L'Hb media misurata (g/dL) è stata 11.6 (±2.3) su XE-2100, 12.3 (±2.2) su Sysmex. I dati ottenuti evidenziano una buona concordanza fra i due strumenti (r=0.97) e l'analisi di Passing-Bablok evidenzia una slope pari a 0.995 (intervallo di confidenza al 95%: 0.980/1.010) e un'intercetta di 0.743 (intervallo di confidenza al 95%: 0.577/0.910).
Conclusioni: L'analisi evidenzia che i risultati forniti dall'emogasanalizzatore sono correlabili con quelli del laboratorio di riferimento, seppure in presenza di un errore sistematico costante dovuto probabilmente a cause pre-analitiche (modalità di prelievo, conservazione del campione). Per ridurre tale errore è stato introdotto un fattore di correzione. I primi risultati hanno mostrato un alto grado di concordanza tra i due analizzatori con una riduzione significativa dell'errore sistematico costante (intercetta=0.269; intervallo di confidenza al 95%: -0.634/1.172). Ulteriori analisi saranno necessarie per verificarne l'utilità.

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VALUTAZIONE DELL'IMPATTO OPERATIVO DI UN METODO TURBIDIMETRICO PER IL DOSAGGIO DELLA CALPROTECTINA IN UN LABORATORIO HUB

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La calprotectina è una proteina contenuta all'interno del citoplasma dei granulociti neutrofili ed è un marcatore sensibile di flogosi. La sua misura è attualmente utilizzata nella diagnosi delle malattie infiammatorie croniche e nel monitoraggio di recidiva o progressione nei pazienti in terapia medica o sottoposti a chirurgia. Il dosaggio della calprotectina Buhlmann fCAL turbo è un metodo immunologico turbidimetrico arricchito con particelle (PETIA) che può essere utilizzato sugli analizzatori di chimica clinica correntemente presenti in Laboratorio. Presso Synlab Italia questo metodo è stato implementato sull' AU5800 (Beckman,Italia) in sostituzione di un metodo immunoenzimatico (Calprest, Eurospital, Italia), col vantaggio di effettuare l'esecuzione di corse analitiche quotidiane. Dal nostro data base sono stati estratti i carichi di lavoro per la calprotectina nei periodi maggio-dicembre 2016 (Caprest, n=12945) e maggio-dicembre 2017 (Buhlmann fCAL, n=16538). Sebbene diversi, è stato comunque possibile valutare la diversità dei relativi "TAT" (dal check-in al risultato e dal check-in alla firma del referto). L'impiego della turbidimetria ha consentito il completamento del 100% delle misure a 156 ore dal check-in contro il 99,9% a 240 ore utilizzando il metodo Calprest, e la firma del 97.6% dei referti a 144 ore contro il 97.8% a 180 ore impiegando il metodo Elisa. Le mediane dei due "TAT" osservate sono state rispettivamente 36,4 per la turbidimetria e 86,3 ore per il metodo Elisa. I "TAT" comprendono i tempi di trasporto al Laboratorio HUB di Castenedolo (BS) che variano da poche ore a 12 ore, per i soggetti abitanti in Italia meridionale. L'implementazione in routine del metodo turbidimetrico ha consentito quindi la riduzione significativa del "TAT" senza incidere negativamente sulla Qualità Analitica, valutata nel periodo preliminare all'introduzione in routine del nuovo metodo (dati non forniti).

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CAN ANOTHER METHOD BE USEFUL IN TYPING CRYOGLOBULINS?

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Background: Cryoglobulins are immunoglobulins wich precipitated below 37°C from patient's serum and redissolve when rewarmed in vitro. Based on the composition of the immunoglobulin content three distinct types of cryoglobulins can be distinguished. Type I cryoglobulins consist of IgM, IgG or IgA monoclonal immunoglobulin; Type II are characterized by the combined presence of a monoclonal immunoglobulin with rheumatoid factor activity and polyclonal immunoglobulins and Type III are composed of polyclonal IgM and IgG immunoglobulin. Despite lack of standardized criteria, there is a good consensus on the protocol used for detection and a lesser extent for typing of serum cryoglobulins. Aim of the study: to assess the analytical performance of immunofixation (IFE) and the capillary electrophoresis technique (CZE) for typing of cryoglobulins. Methods: Ten serum samples from patients with different disorders related to cryoglobulinemia were studied. Blood samples were collectioned in 10 ml pre-warmed tube, transported to laboratory in device at 37°C and separated of serum fraction by centrifugation at 37°C. Three fraction were storaged at 4°C for 3-7 days, displayed for presence of precipitate, incubated one fraction at 37°C for checking resolution and washed three times at 4°C to remove serum protein and dissolved in PBS at 37°C for immunofixation. To perform CZE urine technique one rate of purified cryocrit was dissolved in a solution containing 225 ml of dialysis buffer and 75 ml of Fluidil® solution (Sebia). Results: Seven samples confirmed typing obtained by IFE, three samples instead showed Type II IgM k monoclonal crioglobulins with significant differences between the two methods. Conclusions: The present study proposes to use CZE urine in a complementary way with the traditional test for typing of cryoglobulins especially in cases where monoclonal IgM immunoglobulins can give rise to a wrong interpretation with the IFE. CZE associated with immunotyping displays a higher sensitivity compared to the IFE because eliminated visible artifacts on agarose gel due to the intrinsic characteristics of monoclonal IgM.
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CONSOLIDAMENTI E TEMPI DI PRE-ANALISI: SIERO O PLASMA PER LA DETRERMINAZIONE DELLA GLICEMIA?

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Nell'ambito della riorganizzazione Regionale della rete dei laboratori in AULSS8 Berica, è stata uniformata la matrice per gli esami di chimica, da siero a plasma. Il plasma offre numerosi vantaggi, ma presenta una maggiore glicolisi in vitro, potenziale errore per la determinazione della glicemia. Pertanto come azione preventiva sono state introdotte centrifughe presso i punti prelievo, al fine di separare attraverso il gel, il plasma dagli eritrociti. Scopo di questo studio è verificare l'effetto di questa azione sulla determinazione della glicemia. Materiali e metodi. Sono state confrontate le concentrazioni di glucosio in 10.000 campioni di pazienti esterni consecutivi, raccolti in siero (VacutestKIMA cod.610174) e centrifugate all'arrivo in laboratorio (tempo medio di consegna 90 minuti) con le concentrazioni di glucosio di 10.000 pazienti esterni, raccolti in litio eparina con gel separatore (VacutestKIMA cod. 12550) ottenuti successivamente e centrifugati presso le sedi di raccolta entro 30 minuti dal prelievo. La determinazione del glucosio è stata effettuata sul sistema Cobas C702 ROCHE/HITACHI mediante riferimento enzimatico con esochinasi e rilevazione fotometrica. Risultati: La mediana delle concentrazioni di glucosio del gruppo "siero" è di 95 mg/dL interquartile (IQ) 85-113, valori min/max 32-476. La mediana delle glicemie determinate in plasma e separate entro 30 min. è di 104 mg/dL, IQ 94-122, min/max 26-510. La differenza mediana è risultata di 9 mg/dL, con un range 8-11 mg/dL, proporzionale alla concentrazione del glucosio nel sangue. La percentuale di soggetti con glicemie inferiori a 70 mg/dL è passata dal 2,32% a 0,47%, mentre le glicemie >125 mg/dL dal 17.60% al 22,65%.Conclusioni: nonostante il fenomeno della glicolisi in vitro sia maggiore in campioni raccolti in litio eparina rispetto al campione coagulato, una corretta gestione della fase preanalitica e il pretrattamento con centrifugazione separativa migliora la qualità dei campioni preservando le concentrazioni del glucosio. La misurazione sulla matrice plasma e la nuova procedura di centrifugazione ha permesso di ridurre del 2% il numero di ipoglicemie "false", mentre ha aumentato del 5% le iperglicemie. L'introduzione del plasma ha di fatto migliorato la misura delle glicemie.

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ANALISI SU XN-9000 DEL CONTENUTO DI EMOGLOBINA RETICOLOCITARIA E DELLA PERCENTUALE DI GLOBULI ROSSI IPOCROMICI: INTERVALLI DI RIFERIMENTO E COMPARAZIONE CON STRUMENTAZIONE ADVIA 120.

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Il contenuto di emoglobina reticolocitaria (CHr) e la % di globuli rossi ipocromici (HYPO%) sono utili per la valutazione precoce della carenza di ferro nei pazienti in dialisi. In loro sostituzione, l'equivalente di emoglobina reticolocitaria (Ret-He) e la % di eritrociti ipocromici (%Hypo-He), analizzabili su piattaforma Sysmex XN 9000, rappresentano una alternativa a quanto.

Scopo dello studio è stabilire gli intervalli di riferimento (IR) per Ret-He e %Hypo-He in una popolazione di soggetti idonei come donatori di sangue, comparare i dati ottenuti in un gruppo di 100 campioni di pazienti dializzati con Sysmex e Siemens, eseguendo l'analisi di CHr e HYPO% (ADVIA 120) e Ret-He e %Hypo-He (XN-9000) entro le 3 ore dal prelievo, valutare la riproducibilità dei dati dopo 12 h di conservazione del campione a 4°C.

I dati preliminari ottenuti su 100 soggetti donatori evidenziano differenze di genere statisticamente significative solo per emoglobina e Hypo-He: per Ret-He non esistono differenze significative di genere e sono sovrapponibili a quanto descritto in letteratura. Nel confronto dei valori dei parametri Sysmex vs Advia (pazienti dializzati), la correlazione di Pearson ($r=0.911$; $p<0.0001$) e il Bias plot di Bland Altman evidenzia un buon accordo tra i due parametri (Bias medio=0.28 pg) nonostante si osservino, in accordo con altri studi, valori più bassi di Ret-He a Chi < 27pg e valori tendenzialmente più alti di RET-He a CHr >35 pg. Assumendo come riferimento CHr <29 pg (cut-off linee guida), la curva ROC evidenzia una eccellente concordanza diagnostica di Ret-He (valore AUC=0.987), con sensibilità diagnostica pari a 100% e specificità diagnostica a 94.4%.

Per quanto riguarda Hypo-He, la correlazione vs Hypo % Advia non è lineare, ma dà un buon coefficiente di correlazione Pearson ($r=0.748$; $p <0.0001$). I valori di cut-off suggeriti dalle curve ROC per Hypo-He non sono identici a Hypo % Advia: per positività Hypo% >6 (linee guida), il cut-off ottimale di Hypo-He risulta essere >0.70 (Sensibilità 71.9% - Specificità 86.1%). Per positività Hypo % >10, il cut-off ottimale di Hypo-He risulta essere >0.90 (Sensibilità 79.5% - Specificità 82.1%).

I cut-off ottenuti saranno validati in un successivo studio clinico.

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CLINICAL VALIDATION OF THE NEW AUTOMATED ALDOSTERONE LIAISON XL ASSAY FOR ADRENAL VEIN SAMPLING

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Background: Measurement of aldosterone is the cornerstone in Primary aldosteronism (PA) workup, for both screening and final diagnosis (as confirmatory test). Albeit several automated aldosterone chemiluminescent assays have become recently available as reliable alternatives to the well-established radioimmunometric methods (RIA), little information is available on the diagnostic performance of the former methods using samples collected by adrenal vein sampling (AVS). AVS for aldosterone measurement is still considered the 'gold standard' procedure to differentiate aldosterone-producing adenoma (APA) and bilateral idiopathic hyperaldosteronism (IHA).This study was aimed to assess the clinical utility of the new Aldosterone Liaison XL assay for AVS sampling compared with a traditional RIA method. Data We were also compare with a reference liquid chromatography-mass spectrometry (LCMS) assay. Materials and Methods: The study population consisted of 11 patients (10 males and 1 females) undergoing AVS, Aldosterone was measured with RIA (DSL800, Beckman Coulter), Liaison XL (Diasorin) and BSN LC-MS (BSN, Italy) on AbSciex 4500. Samples were taken step by step from both adrenal veins, and the inferior vena cava (below and upper renal vein). Results: Results of serum samples (n=42 , two vein cava sample were missing) were analysed. The median values (2.5-97.5 percentiles) for RIA, Liaison XL and LC-MS were 289 ng/mL (58-48550 pg/mL), 203 ng/mL (64-39689 pg/mL) and 131 ng/mL (9-42627 pg/mL), respectively. Nonparametric regression of Passing & Bablok and the Spearman's correlation showed excellent performance for Liaison XL Aldosterone and LC-MS assay (Liaison XL: 0,46 x RIA + 41; $r= 0.93$, $p <0.001$; LC-MS:0,41 x RIA -21; $r= 0.94$, $p <0.001$). In all patients the lateralization ratio were confirmed with both Liaison XL and LC-MS (6 patients had IHA; APA were 1 right and 5 left, respectively). Conclusions: The rate of lateralization at AVS was identical using RIA, LC-MS or LIAISON XL for measuring aldosterone. We hence conclude that Liaison XL assay is suitable for rapid quantification of aldosterone after AVS.

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COMPARISON OF ANDROSTENEDIONE RESULTS BY A RADIOIMMUNOASSAY, A CHEMILUMINESCENT IMMUNOASSAY AND LIQUID-CHROMATOGRAPHY COUPLED WITH TANDEM MASS-SPECTROMETRY

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Background: The radioimmunoassay (RIA) has been the method of choice for steroids analysis until the advent of use-frendly and high throughput liquid-chromatographs coupled with mass-spectrometry detectors (LC-MS). Immunoassays (IAs) for Androstenedione (A) based on different tracers than radiolabels seem to suffer particularly of the pitfalls of the technology, but conversely accurate assays are necessary as widely used in clinical practice. Recently a new chemiluminescent immunoassay (CLIA) by DiaSorin S.p.A. promises to be more accurate than RIA and IAs in general. Objective: To assess the relations between a RIA a CLIA and a LC-MS method for A quantification. Methods: A was measured in 47 serum samples with Immunotech & DSL RIA, Beckman Coulter Inc, USA; CHS MSMS Steroid Kit Wallac Oy Perkin Elmer, Turku Finland; Liajson Androstenedione DiaSorin S.p.A. Saluggia, Italy. The relation between the assays was assessed by the Bland and Altman analysis (BA), the Passing and Blablok regression (PB) and the concordance coefficient (ρc). LC-MS was assumed as reference method and statistical significance was set at $p < 0.05$. Results. LC-MS and RIA had almost the same sensitivity and it was 8 times higher than CLIA (0.12, 0.10, 0.84 nmol/L respectively). RIA and CLIA imprecision (higher total CV%) was the same (12%) and it doubled that of LC-MS (5.2%). The BA showed that RIA overestimates both other methods (mean of differences%: LC-MS vs RIA= -44%, RIA vs CLIA= 43.3%) and that the best agreement was found between LC-MS and CLIA (mean of difference% LC-MS vs CLIA= -2.9%) nevertheless, the wide range between the limits of agreement (LC-MS vs RIA -123%/+34.4%, LC-MS vs CLIA -64.4%/+58.7%, RIA vs CLIA -6.5%/+93.1%) demonstrated they are not equivalent assays. The poor concordance correlation coefficients were consistent with this finding: LC-MS vs RIA: ρc= 0.29 (0.12 - 0.43), vs CLIA: ρc= 0.72 (0.54 - 0.83), RIA vs CLIA: ρc= 0.41 (0.28 - 0.53). Conclusions: This study demontrated CLIA Androstenedione DiaSorin provides results more aligned with LC-MS than with RIA Immunotech & DSL, though the two methodologies are not equivalent. A further, larger sample-based, study, increasing the power of the statistical estimations, shoud be made to confirm these preliminary findings.

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EVALUATION OF LONG-TERM STABILITY OF GLUCOSE IN SODIUM FLUORIDE, EDTA AND CITRATE BUFFER CONTAINING TUBES

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Background-Aim: Measuring the glucose concentration in whole blood samples is critical due to glycolysis. NACB, ADA and the Working Group on Diabetes of SIBioC recommend for the correct determination of glycemia the use of tubes containing the ternary mixture of NaF, EDTA and citrate.The aim of the present study was to evaluate long-term stability of glucose in samples containing the acidified mixture. Methods: 72 fasting and non fasting glucose samples were collected in GlucoEXACT tubes containing a liquid form of the acidified mixture of NaF, EDTA and citrate. In a first group of 38 samples glucose stability was evaluated for 5 days long after blood drawing and in a second one of 34 samples glucose stability was evaluated afterword 1 and 2 weeks. In the day of blood drawing, samples were centrifuged at 20°C for 15 min at 2500g, and glucose determined by an hexochinase method on a Dimension Vista 1500 system, then samples were stored at 2-6°C in the primary tubes until next determinations.Glucose determinations were made at day 1,2,3,4 and 5 (group 1) and at 1 and 2 weeks without any previous centrifugation.Sample stability was evaluated according to desirable bias based on biological variation studies (<±1.95%). Results: In group 1 mean (range) glucose concentrations were: G0: 94,34 (61.06-139,29), G1:94,77 (60,68-141,24), G2: 94,77 (60,68-141,24), G3: 95,13 (62,10-144,20), G4: 92,71 (60,20-138,00), G5: 93,87 (59,45-137,93) mg/dL with mean bias of +1,10% at G1 and +0,48%, +0,82%, -1,69% and -0,32% at G2, G3, G4 and G5, respectively. In group 2 mean (range) glucose concentrations were: G0: 96,81 (63,51-153,03), W1:96,01 (63,34-153,02), W2: 96.76 (61.86-149.27) mg/dL with a mean bias of -0,94% at W1 and of -0.08% at W2.Mean relative bias was within desirable one at all time points. Conclusions: Tubes containing a mixture of NaF, EDTA and citrate buffer in a liquid form are an efficient tool allowing a long-term stability of glucose when store refrigerated at 4°C. Other studies are needed to evaluate stability of glucose at different temperatures such as ambient temperature (20-25°C) and at 37°C to better clarify the performances of this new suggested tubes.

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UPGRADE OF AN LC-MS/MS METHOD FOR DETERMINATION OF LACTULOSE AND MANNITOL

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Introduction: The lactulose/mannitol (L/M) test has been the most widespread dual-sugar test used to assess intestinal barrier function.
Among available techniques used to determine lactulose and mannitol in urine samples, a valid solution for carbohydrate analysis is HPLC separation with electrospray ionization tandem mass spectrometry that guarantees high level of selectivity and reproducibility.
We recently published the validation of an ESI-HPLC-MS/MS method for lactulose and mannitol quantification using a NH_2 stationary phase and according to other published method we used raffinose as internal standard both for lactulose and mannitol. As suggested by international guidelines, the use of labeled internal standard guarantees the highest reliability in terms of reproducibility and accuracy. For this reasons we update our published method introducing an isotopic based internal standard calibration and improving chromatographic method with a BEH amide column.
Method: To 10 µL of urine samples, controls and standards were added 240 µL of IS solution and, after mixed, a 200 µL aliquot was transferred into a glass vial for the injection to UPLC-MS/MS. The chromatographic separation was performed using a BEH amide column operating at a flow rate of 200 µL/min and eluted with a linear gradient from 90 to 40 % acetonitrile in water. Total run time is 5 minutes. The mass spectrometry operates in electrospray negative mode.
Results and discussion: Limit of quantification was 10 mg/L for mannitol and 2.5 mg/L for lactulose. The assay was linear up to 1000 mg/L for both mannitol and lactulose. Eight calibration curves, analyzed over a period of three weeks, display an R^2 always higher than 0.99 for both analytes. The within-run precision and accuracy ranged from 0.7 to 2.9% and 97.2 to 101.2%, respectively. The between-run precision and accuracy ranged from 1.9 to 4.7% and 94.8 to 97.5%, respectively. Recovery was always higher than 90.2% for both lactulose and mannitol and matrix effect for both lactulose and mannitol was lower than 15%.
With this method we have a real improvements in terms of accuracy, precision and reproducibility making this method great for routine purpose.

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VERIFICA DEL METODO PER LA DETERMINAZIONE DELL'ERITROPOIETINA SU ADVIA CENTAUR SECONDO IL PROTOCOLLO CLSI EP15-A3

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Introduzione: Recentemente è stato implementato dalla ditta Siemens il dosaggio della eritropoietina, ADVIA Centaur EPO. Per per valutare se il nuovo metodo avesse le stesse prestazioni analitiche di quello in uso (Access EPO Beckman Coulter) o se fosse necessario stabilire nuovi intervalli di riferimento e/o limiti decisionali è stato seguito il protocollo CLSI EP15-A3. Materiali e metodi: Per valutare la precisione del metodo abbiamo predisposto 3 pool a contenuto basso (7,64 U/L), medio (81,06 U/L) e alto (174,8 U/L) di eritropoietina che sono stati testati seguendo lo schema 5 x 5 (5 replicati per 5 giorni). Per verificare invece la presenza di bias, 83 campioni (compresi tra 1,2 e 1472 U/L) sono stati dosati con il metodo in uso e con il metodo in validazione. L'accettabilità delle prestazioni è stata fatta stimando l'errore totale consentito sulla base dell'utilità clinica. Per l'analisi statistica è stato usato utilizzando il software MetComp. ver 1.0 (Vidali e GdS Sibioc statistica per il laboratorio). Risultati: La ripetibilità entro il laboratorio è risultata essere: CV3.5% (pool high), CV 4,16% (pool medium) e CV 5,52% (pool low), è stata confrontata con quanto dichiarato dal produttore ricercando il parametro T e il valore critico C, ed è stata giudicata accettabile per tutti i livelli considerati. Comparazione dei metodi: la regressione di Passing-Bablock (y=1,3007 + 1,0017x) ha mostrato una deviazione non significativa dalla linearità e la presenza di un errore sistematico costante privo di impatto clinico. L'analisi di Bland Altman infatti mostra un bias non significativo. Accettabilità delle prestazioni: l'intervallo di riferimento dell'eritropoietina è compreso tra 5 e 28 U/L. Ipotizzando un errore totale massimo intorno al 16% per concentrazioni vicino alle 20 U/L, la prestazione del metodo risulta accettabile (diagramma MEDx Chart). Per concentrazioni invece molto basse intorno a 5 U/L l'errore totale massimo è intorno al 35%, accettabile dal punto di vista clinico. Conclusioni: Il metodo Siemens per la determinazione dell'eritropietina su Advia Centaur ha dimostrato ripetibilità conformi a quanto dichiarato dal produttore ed è comparabile con il metodo usato precedentemente in laboratorio. È stato quindi implementato senza ricalcolo degli intervalli di riferimento.

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DETECTION OF INTRATHECAL IMMUNOGLOBULIN SYNTHESIS USING KAPPA FREE LIGHT CHAINS INDEX

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Introduction: Intrathecal immunoglobulin synthesis occurs in several disorders of the central nervous system and it is part of current generally accepted diagnostic criteria for Multiple Sclerosis (MS). The detection of oligoclonal IgG bands (OBs) in cerebrospinal fluid (CFS) by isoelettrofocusing (IEF) followed by immunoblotting is the current "Gold Standard" approach for evaluating intrathecal immunoglobulin synthesis. This reference method of oligoclonal IgG determination has shown high sensitivity in different studies but it is time-consuming and subjective. The measurement of the K-free light chains index (KFLCi) has been recently proposed as a faster, cheaper and easier alternative test for the assessment of intrathecal immunoglobulin synthesis.
Methods: KFLC index and IgG index quantification and the presence of OBs were assessed in 183 consecutive patients: 47 with MS, 86 with other neurological inflammatory diseases (NID) and 50 with neurological non inflammatory diseases (NNID). Our aim was to evaluate the diagnostic accuracy of a nephelometric assay for K-FLC determination in CFS and serum.
Results: Both IEF and KFLCi showed a similar accuracy as diagnostic tests for MS. KFLCi was significantly higher in patients with MS (79.61 ± 93.23 vs. NID 28.91 ± 57.61 vs. NNID 7.55 ± 23.83; p <0.0001). KFLCi yielded a diagnostic accuracy comparable to CSF OBs (Negative Pattern 1 (NP1) 4.00 ± 5.60; Positive Pattern 2 (PP2) 85.37 ± 89.13; Negative Pattern 4 (NP4) 27.14 ± 44.10; Negative Pattern 5 (NP5) 2.40 ± 0.67; Positive Pattern 3 (PP3)undetectable; p <0,0001). KFLCi value of 5.2 was found through ROC curve analysis to identify clinically negative patients (AUC 0.938; 95% CI 0.881-0.996; sensitivity 93.6%; specificity 82%).
Conclusion: Our results suggest that KFLCi in CFS is a promising biomarker of intrathecal synthesis and can accurately discriminate MS patients. It is an operator indipendent test, it can be performed on a routine basis and it could be useful for the disease monitoring with a sensitivity and specificity comparable to the identification of OBs in CFS trough Gold Standard tests.

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BREATH TEST AL LATTOSIO: RELAZIONE TRA SINTOMI E RISULTATI

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Background: L'intolleranza al lattosio è la forma più comune di malassorbimento alimentare e colpisce persone di ogni età. Lo strumento più appropriato per la diagnosi è il Breath test. Scopo del presente lavoro è determinare la comparsa di eventuali sintomi durante l'esecuzione del test e quali siano i più comunemente associati.
METODI: Sono stati valutati i risultati dei test eseguiti su 300 pazienti: 147 maschi e 153 femmine di ogni di età, afferenti al Centro Prelievi dell'Ospedale Bambino Gesù, nel periodo Maggio 2017- Maggio 2018. Sono stati registrati i sintomi riferiti dai pazienti dopo l'assunzione del lattosio ai tempi 60, 90, 120, 150 e 180 minuti. Il dosaggio dell'idrogeno espirato è stato eseguito mediante gas-cromatografo Quintron.
Risultati: Il 53% dei pazienti è risultato positivo al test in percentuali simili tra maschi e femmine (53,5% vs 46,5%). Dolori addominali, diarrea e meteorismo sono i sintomi maggiormente segnalati, nausea in minima parte. I dolori addominali si manifestano: 56,0% dei test positivi, con picchi a 90' (39,6%) e 120' (39,0%), e 36,2% dei test negativi con un picco a 60' (20,8%). Diarrea: 24,0% dei test positivi, con picchi a 90' (12,6%) e 120' (10,7%), e 10,6% dei test negativi, con picco a 60' (4,3%). Meteorismo: 16,4% dei test positivi con valori quasi costanti e 4,9% dei negativi con valori similari. Nausea: 19,5% dei test positivi e 9,2% di quelli negativi e si osserva in prevalenza nei pazienti di sesso femminile, soprattutto nei primi tempi di somministrazione del lattosio (a 60' il 10,5% delle femmine vs 4,8% dei maschi).
Conclusioni: I sintomi rilevati sono presenti con frequenza maggiore, ma non significativa nei pazienti con test positivo ed i più frequenti sono risultati: dolori addominali, diarrea e meteorismo. I sintomi si distribuiscono diversamente nelle varie fasi del test: nei soggetti negativi tendono a scomparire nelle fasi finali dell'esame mentre nei positivi sono ancora riscontrabili, andamento maggiormente evidente per meteorismo e diarrea. Non sembrano esserci correlazioni legate all'età o al sesso, ad eccezione del sintomo della nausea, la cui frequenza è maggiore nel sesso femminile.

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RISCHIO DI TRASMISSIONE DI EPATITE B TRASFUSIONALE DA DONATORI PORTATORI DI OBI

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Premessa: La Normativa attuale limita gli esami obbligatori per accertare l'assenza di infezione da HBV sui donatori, alla ricerca dell'HBsAg e al test NAT-HBV. Esistono, tuttavia, condizioni caratterizzate da livelli di HBsAg indosabili con i comuni kit commerciali (come per mutazioni del gene che codifica per l'HBsAg) o da livelli di HBVDNA estremamente bassi (<100 IU/ml), rilevabili solo con metodiche altamente sensibili. I portatori di OBI sono caratterizzati da negatività per HBsAg e livelli di viremia bassi, spesso fluttuanti. Rimane, pertanto, la remota possibilità di introdurre nell'uso clinico unità di donatori con OBI. METODI: È stata valutata la prevalenza di OBI tra i donatori del SIMT di Agrigento nel periodo 2012-2017, attraverso l'uso di test NAT eseguiti con kit Ultrio Elite Panther (Grifols®). Risultati: Al SIMT di Agrigento afferiscono circa 3000 donatori l'anno. Nel periodo esaminato sono stati esclusi definitivamente 11 donatori NAT-positivi per HBV ed HBsAgnegativi. Sono tutti donatori periodici, senza segni bio-umorali di danno epatico, con valori di ALT nella norma e donazioni precedenti sempre valide. Su tutti è stato eseguito un pannello completo per marcatori-HBV: il titolo dell'anti-HBs è risultato <10mIU/ml in 7 e compreso tra 10 e 70 mIU/ml in 4 donatori; in tutti i casi è stata evidenziata positività per HBcAb-tot con negatività per HBcAb-IgM. Due donatori, dopo 6 mesi, sono risultati negativi al test NAT. Conclusioni: Tra il 2008 e il 2011 sono stati accertati in Italia 2 casi di infezione trasfusionale da HBV, documentando l'identità di sequenza donatore/ricevente. La particolare conformazione del genoma virale, nel quale il gene pol (che codifica per la polimerasi) e il gene s (che codifica per l'antigene di superficie) si trovano sovrapposti, si presta a mutazioni che, interessando contestualmente entrambi i geni, comportano alterazione dell'HBsAg, riduzione della replicazione virale e sviluppo di farmaco-resistenza. Una certa quota di OBI è sostenuta da varianti HBV "e minus" a bassa replicazione virale o da alterata espressione dell'HBsAg: è quindi possibile che unità prelevate da donatori con OBI vengano validate per uso clinico, esponendo i riceventi al rischio di contrarre infezione da HBV. Da quanto esposto e considerato che il DM 2-11-2015 prevede l'esclusione permanente dalla donazione dei soggetti affetti o con pregressa infezione da HBV, riteniamo auspicabile implementare gli esami di legge con il dosaggio dell'antiHBctot, per escludere donatori anti-HBc positivi e ridurre, così, ulteriormente il margine di pericolo derivante dal fallimento degli attuali test di screening.

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SPESA SANITARIA, RIORGANIZZAZIONI, PIANO DI RIENTRO, APPROPRIATEZZA E CONSUMISMO SANITARIO: LA CRISI ECONOMICA E LA MEDICINA DI LABORATORIO

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Dal 2007 l'evoluzione tecnologica e la crisi economica hanno indotto cambiamenti professionali ed organizzativi nella sanità europea, dall'89 in Inghilterra (riforma Tatcher) al 2001 in Italia con la riforma costituzionale. Un decennio con radicali e trionfali politiche sanitarie (oltre la riforma Thatcher, quella Amato-De Lorenzo in Italia, Clinton in USA e Dekker in Olanda) rovinosamente cadute. Il SSN oggi affronta il duplice problema dell'aumento della vita media e dei costi della salute con politiche di risparmio e di razionalizzazione. Il cittadino è in mezzo,tra due fuochi, se malato si confronta con ticket, liste d'attesa e tetti di spesa. La ns spesa sanitaria pubblica nel 2017 supera i 110 miliardi di euro (8,3% del Pil, circa 1.800 euro medi annui/abitante). Inferiore in Europa a quella di Regno Unito, Francia e Germania. A livello di spesa più basso la Polonia (876 dollari procapite). Le famiglie partecipano per una quota del 22,8% (1,9% del Pil). Il confronto europeo mostra che la ns quota di spesa sanitaria privata è vicina a quelle di Germania, Austria, Irlanda e Francia. La spesa sanitaria italiana è per 6,5 punti percentuali pubblica e per 1,8 punti a carico delle risorse delle famiglie.Il peso è più alto nel Mezzogiorno (2,0%) rispetto al Centro-Nord (1,8%) perchè si tratta di un bene primario incomprimibile. Le regioni in cui la quota è più elevata (superiore al 2% del Pil) sono Calabria, Molise, Friuli-VG, Campania, Puglia e Piemonte. Nella distribuzione della spesa il contributo delle famiglie è relativamente più basso nel Mezzogiorno (17,7%) che nel Centro-Nord (24,0%, max 25,3% nel Nord-ovest). La quota maggiore è in Friuli-VG (27,2), ai livelli più bassi Basilicata, Sicilia e Sardegna. Calcolata per famiglia la spesa sanitaria privata è pari a 955 euro per il Mezzogiorno e 1.265 euro per il Centro-Nord: confermando l'aspetto legato ai differenziali di reddito tra le ripartizioni. I campani utilizzano un massimo di 6,17 esami di laboratorio pro capite rispetto ai 9,78 della Toscana, ai 9,72 dell'Emilia ed ai 8,66 del Veneto. La relazione della Corte dei Conti al parlamento sulla spesa sanitaria 2016 ha evidenziato che il SSN migliora i conti e riduce il deficit ma si ricorre di più al privato e l'aumento dell'età apre la porta a nuovi rischi tutti da valutare.

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CHANGES IN TRANSCRIPTIONAL LEVELS OF RANKL AND INFLAMMATORY BIOMARKERS IN PBMCs ARE DEPENDENT ON VITAMIN D STATUS

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Vitamin D deficiency is common among general population, and interesting observations demonstrated that vitamin D status is involved in the risk of many chronic diseases including cardiovascular diseases, cancer, autoimmune and infectious diseases (1). However consequences of vitamin D deficiency should be associated to molecular pathways in order to characterize the involvement of vitamin D in different cell dysfunctions and to find new potential therapeutic targets. Some pleiotropic effects of vitamin D associated with immune response such as cytokine production and mechanisms of monocyte activation have been evaluated (1). Among several molecular pathways involved in immune response, the RANK/RANKL/OPG system is also implicated in the orchestration of immune functions affecting lymphocyte differentiation, dendritic cell survival and T-cell activation (2). Therefore, this study aimed to investigate the expression of RANKL, OPG and some inflammatory markers in peripheral blood mononuclear cells (PBMC) from healthy subjects with different vitamin D status.

We enrolled 32 healthy subjects (mean age 59.2 ± 8). 25-OH-D3 plasma concentrations were assessed by HPLC. The gene expression levels of RANKL, OPG, TNF-α, IL-13 and ICAM in PBMC were evaluated by qRT-PCR.

The expression of RANKL increased in subjects with vitamin D levels lower than 52 nmol/L in comparison to subjects with normal vitamin D levels (2.5 times, p=0.03), whereas OPG expression was not detected independently from vitamin D status. The increased expression of RANKL was associated with increases of ICAM (3.3 times, p=0.002) and TNF-α (3 times, p=0.008). Additionally, a reduction of about 75% in the anti-inflammatory cytokine IL-13 has been observed (p=0.04).

Our preliminary data give evidence of the modulatory effects of circulating vitamin D levels on gene expression of biomarkers of immune activation in PBMC, and provide a first step in applying analysis of RANKL expression in peripheral blood cells to characterize molecular mechanisms of immune/inflammatory response that may be associated with hypovitaminosis D conditions.

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DAISY-LIKE CRYSTAL NELLE URINE DI TRE PAZIENTI PORTATORI DI TRAPIANTO RENALE

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Nel nostro laboratorio analizziamo 65000 sedimenti urinari/anno per screening o richiesta dei nefrologi con l'analizzatore automatico sediMAX (Menarini Firenze), inoltre i campioni di provenienza nefrologica (1500/anno) sono anche visti in microscopia manuale.

Abbiamo osservato in 3 pazienti trapiantati renali cristalli come quelli descritti da Fogazzi [1] quali "daisy-like crystals" (DLcr), ma non ancora del tutto caratterizzati chimicamente. Essi hanno struttura tondeggiante che ricorda una margherita, formata da triangoli di diversa ampiezza convergenti in un punto centrale.

Paziente 1, agosto 2005: maschio, 63 anni. P-creatinina, 1,58 mg/dL (intervallo di riferimento 0,67-1,17); U-chimico-fisico: densità, 1,015; pH, 7,0, gli altri parametri negativi; sedimento: nulla da segnalare tranne DLcr.

Paziente 2, luglio 2008: maschio,58 anni. P-creatinina, 1,52 mg/dL; U-chimico-fisico: densità, 1,015; pH, 7,0; esterasi leucocitaria 3+, gli altri parametri negativi; sedimento: 60 leucociti/µL e DLcr.

Paziente 3, gennaio 2018: maschio, 62 anni. P-creatinina, 1,25 mg/dL; U-chimico-fisico: densità, 1,006; pH, 7,0; gli altri parametri negativi; sedimento: nulla da segnalare tranne DLcr.

Nei 3 pazienti i DLcr erano 10-20/µL e erano visibili anche in microscopia in campo scuro ma non in luce polarizzata.

I campioni avevano pH alcalino, nessun paziente aveva una dieta ricca in vegetali né assumeva farmaci noti per indurre cristalluria.

Segnaliamo tali cristalli rari per contribuire ad identificarne ulteriori aspetti e potenziali associazioni cliniche.

I DLcr potrebbero essere più frequenti di quanto segnalato perché: 1-il campione potrebbe non essere visto in microscopia manuale, qualora segnalato dal sediMAX come non patologico; 2-non tutti i laboratori hanno il contrasto di fase e in questo caso il riscontro di DLcr è difficoltoso; 3-è possibile ritenerli erroneamente dei contaminanti; 4-motivazione e curiosità dell'osservatore sono fondamentali per tener conto di tutti i riscontri; 5-non sempre il tempo disponibile è sufficiente per tali approfondimenti, visto l'alto numero di sedimenti esaminati quotidianamente nella maggior parte dei laboratori.

Fogazzi GB, et al. An unusual type of crystalluria (appearing only once every 130 years?). Nephrol Dial Transplant. 2004;19:1907-9.

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EUROPEAN BIOLOGICAL VARIATION STUDY (EuBIVAS) OF THE EFLM- WITHIN AND BETWEEN-SUBJECT BIOLOGICAL VARIATION DATA OBTAINED FROM 91 HEALTHY SUBJECTS FOR IMMUNOGLOBULIN G, IMMUNOGLOBULIN A, AND IMMUNOGLOBULIN M

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Aim: European Biological Variation Study (EuBIVAS) sera samples were used to obtain new biological variation (BV) estimates for Immunoglobulins G (IgG), A (IgA) and M (IgM).

Method: A cohort of 91 healthy subjects (38 males and 53 females, 21-69 years old) were bled for 10 consecutive weeks in six European centers. An equivalent and stringent pre-analytical protocol was followed at each center. Separated sera, were delivered in dry ice to the San Raffaele Hospital, Milan, and stored at -80°C prior to analysis in duplicate within a single run on a Roche Cobas c702. Roche control materials at two different levels were analyzed in duplicate in each analytical run. The data were subject to outlier analysis prior to CV-ANOVA, to determine the BV estimates with confidence intervals at 95% (CI). Homogeneity of analytical CV (CVA) and of intra-subject BV (CVI) were examined. Trend analysis was performed to verify the steady-state status of the subjects. The normality assumption of the distributions were verified. Analytical performance specifications (APS) were obtained using CVA and BV estimates, choosing the lowest value of CV when statistical differences between sexes were found.

Results: Statistical difference between sexes in CVI estimates was found for IgA: 2.83% (2.6-3.1) and 3.51% (3.3-3.8) for males and females respectively. Both CVI values were significantly lower than the CVI reported on line (5.4%). CVI values obtained for IgG (2.83% (2.7-3.0)) and for IgM (3.86% (3.6-4.1)) were also lower than those reported on line (4.5 and 5.9% respectively). CVA calculated by ANOVA on sample's replicates, was below desirable APS for imprecision based on current BV data only for IgG (1.28 vs 1.41%). On the contrary, CVA for IgA (1.49%) and IgM (2.2%) were slightly higher than APS (1.41 and 1.93% respectively). Differences between sexes in mean values concentration were found for IgM, so that between-subjects BV estimates (CVG) were considered separately for males and females. CVG estimates obtained for all Immunoglobulins were similar to those reported on line.

Conclusion: All CVI obtained were clearly lower than those currently used reported in the Westgard website. The new BV data deliver lower analytical goals for imprecision for all Immunoglobulins.

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LC-MS/MS BASED 25(OH)D STATUS IN A LARGE CENTRAL EUROPEAN OUTPATIENT COHORT – GENDER AND AGE SPECIFIC DIFFERENCES

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Background: Developed countries have a high prevalence of vitamin D deficiency. In previous studies 25(OH)D was predominantly measured by immunoassays. The present study assessed serum 25(OH)D in a very large European outpatient cohort by liquid-chromatography-tandem-mass-spectrometry (LC-MS/MS).
Material and Methods: 74,235 serum 25(OH)D results generated under routine conditions between 2015 and 2016 were extracted from the laboratory information system of the Department of Clinical Pathology at Bolzano Hospital (Italy). In 3,801 cases parathyroid hormone (PTH) was requested in parallel. Serum 25(OH)D was measured by a NIST-972 aligned commercial LC-MS/MS method. The distribution of serum 25(OH)D concentrations in males and females of different age groups, the prevalence of $25(OH)D_2$ and seasonal variability were studied.
Results: The average 25(OH)D concentration in the entire cohort was 68.6 nmol/L (7.5 - 1880 nmol/L). Females had a 7 nmol/L higher average 25(OH)D concentration than males, which increased significantly with age. 37.9% and 28.3% of males and females, respectively, had a deficient 25(OH)D concentration of <50 nmol/L. 620 samples (0.84%) had measureable amounts of $25(OH)D_2$. In samples with a normal PTH 25(OH)D was 9 nmol/L higher than in the entire cohort. Seasonal variation ranged between 20-30% and was most pronounced in young individuals. $25(OH)_2$ remained constant throughout the year.
Conclusion: Vitamin D status in South Tyrol is rather good. Higher 25(OH)D concentrations in individuals with normal PTH suggest a functional deficit in a substantial number of subjects. Seasonal variation of serum 25(OH)D is a relevant aspect in young and middle-aged adults, but becomes less relevant in elderly subjects. $25(OH)D_2$ is of minor practical importance in South Tyrol.

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MID-REGIONAL PRO-ADRENOMEDULLIN PREDICTS POOR OUTCOME IN NON-SELECTED PATIENTS ADMITTED TO INTENSIVE CARE UNIT

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Background: currently, mortality risk and outcome in critically ill patients can be predicted by scoring systems, such as APACHE and SAPS. Nevertheless, they can't provide prognostic information immediately and require a significant data collection burden. The introduction of prognostic biomarkers that could be easily measured upon admission in Intensive Care Unit (ICU) could help to overcome these limitations. The aim of our study was to assess the prognostic value of plasma MR-proADM at ICU admission in non-selected patients in comparison to APACHEII and SAPSII scores. Methods: this is an observational, single-centre study. Eligible patients were all consecutive adult patients admitted to the ICU of the University Hospital of Palermo from June to December 2017. APACHEII and SAPSII scores were calculated and intra-hospital Length of Stay (LOS) was recorded. Plasma MR-proADM levels were measured by TRACE-Kryptor on admission (T0) and after 24h (T24) at the Laboratory Medicine Unit of the hospital. The primary endpoint was intra-hospital mortality. Results: 126 consecutive patients were enrolled. Forty patients died during hospital stay, the median LOS was T0 and T24 plasma MRproADM levels were higher in non-survivors in comparison to survivors (1.93 [1.10–4.93] nmol/L vs 1.07 [0.8–1.6] nmol/L, P=0.0002 for T0; 2.37 [1.29–4.19] nmol/L vs 1.18 [0.87–1.77] nmol/L, P=0.0001 for T24) and they were correlated with LOS (r=0.28; P=0.0014 at T0; r= 0.26; P=0.005 at T24). Notably, a weaker correlation was found between SAPSII and LOS (r=0.18; P=0.036) while no correlation was detected between APACHEII and LOS (r=0.15; P=0.08). Multivariate analysis showed that T0 MRproADM levels were significant predictors of mortality (OR: 1.27 95%CI: 1.03-1.55; P=0.022; adjusted for gender, age and cause of admission). ROC curves analysis revealed that plasma MR-proADM on ICU admission identified non-survivors with high accuracy, (AUC: 0.71; 95%CI: 0.62-0.78; P=0.0002) and it was not inferior to the one of APACHEII and SAPSII scores, as documented by the pairwise comparison of ROC curves (P=0.95 for APACHEII; P=0.08 for SAPSII).Conclusion: our findings pointed out a role of MR-proADM as a reliable prognostic tool in critically ill, non-selected patients admitted to ICU.

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RISULTATI INDAGINE CONOSCITIVA SUI FABBISOGNI FORMATIVI ESPRESSI DAI SOCI SIBioC NEL 2018

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Introduzione: Il Comitato Scientifico SIBioC Provider ha promosso, nel 2018, un'indagine conoscitiva sui fabbisogni formativi dei soci SIBioC al fine di soddisfarli al meglio.
Materiali e metodi. Il questionario era composto da 16 quesiti: uno sulle motivazioni, due sugli ambiti di interesse, due sulla tipologia e sei sulle modalità di fruizione dell'offerta formativa, tre su quella soddisfatta degli enti di appartenenza, due sulla Scuola di Formazione Permanente in Medicina di Laboratorio (SPML). Il questionario è stato inviato a tutti i soci via mail con SurveyMonkey e pubblicato sul sito SIBioC per 4 mesi.
Risultati: Hanno partecipato all'indagine 495 soci (4455 risposte, media 9 risposte/socio). L'analisi dei dati mostra che per il 14% dei soci l'acquisizione dei crediti ECM non è la motivazione principale dell'aggiornamento. L'ambito di maggior interesse è l'ematologia di laboratorio (47%), seguita dai marcatori tumorali (32%), l'analisi dei liquidi biologici (31%) e la coagulazione (30%), con un marcato interesse sull'approfondimento dei metodi (72). Il 47% dei soci indica come ideale una fruizione dei corsi mista: FAD e residenziali. Chi ha indicato i FAD (34%) giustifica la preferenza con la possibilità di scelta della tempistica più conveniente per seguire le lezioni (80%). Per i corsi residenziali le modalità di fruizione indicate sono: giornate infrasettimanali (57%) non consecutive (51%) con la possibilità di momenti formativi sul campo (69%). L'86% e l'83% dei soci rispettivamente ritiene utile avere: le presentazioni dei docenti prevalentemente di formato elettronico e un contatto diretto con i docenti. L'83% dei soci dichiara che l'ente di appartenenza organizza eventi formativi e, nel 48% dei casi, per partecipare a corsi extra-aziendali usa risorse proprie. Nel 47% dei casi le esigenze formative sono soddisfatte dalle Società Scientifiche. Il 93% dei soci ritiene utile la SPML con la costituzione di un albo di specialisti consultabile online (89%).
Conclusioni: I risultati mostrano l'importanza della formazione erogata da SIBioC sia con corsi FAD che residenziali, la necessità di potenziarla in alcune are di interesse e di ricercare nuove forme di interazione fra docente e discente.

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SERUM URIC ACID IN ERECTILE DYSFUNCTION

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Erectile dysfunction is a common disease characterized by endothelial dysfunction. The aetiology of ED is often multifactorial but evidence is being accumulated in favor of the proper function of the vascular endothelium that is essential to achieving and maintaining penile erection. Uric acid itself causes endothelial dysfunction via decreased nitric oxide production. This study aims to evaluate the serum uric acid (SUA) levels in 180 ED patients, diagnosed with the International Index of Erectile Function-5 (IIEF-5) and 30 non-ED control. Serum uric acid was analyzed with a commercially available kit using ModularEVO (Roche, Monza, Italy). Within-assay and between-assay variations were 3.0% and 6.0%, respectively. Out of the ED patients, 85 were classified as arteriogenic (A-ED) and 95 as non-arteriogenic (NA-ED) with penile-echo-color-Doppler. Uric acid levels (median and range in mg/dL) in A-ED patients (5.8, 4.3-7.5) were significantly higher ($p < .001$) than in NA-ED patients (4.4, 2.6-5.9) and in control group (4.6, 3.1-7.2). There was a significant difference ($p < .001$) between uric acid levels in patients with mild A-ED (IIEF-5 16-20) and severe/ complete A-ED (IIEF-5 $\leq$ 10) that were 5.4 (range 4.3-6.5) mg/dL and 6.8 (range 6.4-7.2) mg/dL, respectively. There was no difference between the levels of uric acid in patients with different degree of NA-ED. Our findings reveal that SUA is a marker of ED but only of ED of arteriogenic aetiology.

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TO PROVIDE CORRECT ESTIMATES OF BIOLOGICAL VARIATION - NOT AN EASY TASK. THE EXAMPLE OF S100-β PROTEIN AND NEURON-SPECIFIC ENOLASE

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Introduction: The biological variation data critical appraisal checklist (BIVAC) (1), to evaluate studies on biological variation (BV), if correctly used, point out the reliability of the BV data published. To emphasize the utility of BIVAC, intra-subject BV (CVI) data for S100-β (S100B) and neuron-specific enolase (NSE) proteins, from the European Biological Variation Study (EuBIVAS) samples, are compared to a recently published study that does not comply with all BIVAC quality items (QI) (2). Both proteins are tumor markers particularly used for melanoma (S100B) and neuroendocrine tumors (NSE).

Methods: EuBIVAS involved 6 European labs that collected weekly serum samples for 10 weeks from 91 healthy volunteers. The samples, stored at -80 °C until analysis, were measured in duplicate using a Roche Cobas e801, reagents, calibrators and control materials Roche. The outlier detection was performed for replicates and samples. Homogeneity of analytical CV and of CVI were examined. The subjects' steady-state for the whole study period was examined. CVI were estimated using CV-ANOVA.

Results: The EuBIVAS final number of results used to obtain CVI, respectively for NSE and S100, were 1609 and 1728, while Johnson et al. used only 40 results (10 subjects, 4 samples measured in singleton) (2). The sizes of the confidence intervals (CI) around the CVI data calculated by Johnson et al. are larger about 20 times than EuBIVAS CI. Therefore, also if EuBIVAS CVI [NSE, 10.9% (10.3-11.5); S100, 10.2% (9.6-10.7)] are definitely lower than those are already published [NSE, 22.1% (9.9-34.3); S100, 18.9% (8.5-29.4)], they seem not significantly different because of their overlapping.

Discussion: When assessing the Johnson study (2) by the BIVAC (1), several QI are not fulfilled. The BIVAC awards overall grades A, B, C and D indicating decreasing compliance with the QI (1). Deviations from the QI may lead to reporting of too high CVI estimates (1). The study published by Johnson at el (2) would receive a grade C and the EuBIVAS a grade A. It is likely that publications that miss, or fail to address, essential detail regarding the QI deliver less reliable, and in most cases, probably too high estimates of CVI.

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MICROBIOME AND REPRODUCTIVE HEALTH : A SYSTEMATIC REVIEW

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Introduction: the study of human microbioma in the reproductive system with 16S ribosomial RNA profiling show an incresing interest on fertility health and reproductive outcome.Material and methods:A web search conducted on Medline,Plos,Google scholar on the terms “Microbioma” “fertility” “Microbiota” “reproductive tract” showed 22 publications according PRISMA statement. Results: Two groups depending in Lactobacillus dominance > 90 % LD or 90 %< NLD,and a lower implantation (LD 60,7% vs. NLD 23.1%), a PH values was 6.6-8.51 and independent of bacterial composition in endometrial microbiota for Moreno. For Himan Lactobacillus dominates vaginal microbioma and L.Crispatus at 65 %, according with 42% in the study of Forney. The 16SrRNA gene amplicon sequencing was used to detect abnormal vaginal microbioma in 36 /130 (28%) with Gardnerella or Atopobium vaginae (Haahr 2016), otherwise 9% of woman with bacterial vaginosis obtained a pregnancy. A study on fallopian Tube revealed a high presence of Staphilococcus when Pseudomonas is low and difference was found between microbial population in left and right tubes, the ampolla and isthmus (Pelzer 2018). The same Haahr confirm the prevalence (16%) of bacterial vaginosis in patients with tubal infertility. (Haahr 2018). In 33 pazients undergoing in vitro -fertilization embryo trasfer Lactobacillus 86 % in vaginal swabs with 95 % di PCR amplified and cloned rDNAs sequences read for L.Crispatus, an ottimal ratio was designed lactobacillus: Flavobacterium 2.5. This study used QIIME software for read Shannon diversity index (SDI) and Chao 1 for richness species (Hyman 2011). Franasiak nel 2016 performed a samples from 33 pazient undergoing in vitro fertilization, a time of embryo trasfer, with a 5 mm of the trasfer chateter with a DNA free tube and showed a 278 genus calls, where lactobacillus species were the higher read. Conclusion: The reproductive health of the woman found in the microbioma a target of interests for studies to understand the mutualistic bacterial community. The microbioma of fallopian tubes, endometrium, vagina with ribosomial DNA non culture dipendent sequencing today is obiect of further investigation on pregnancy outcomes in infertility treatment.

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EFFECT OF CRYSTALLOIDS WITH DIFFERENT STRONG ION DIFFERENCE ON HEMODILUTION AND PH IN PATIENTS UNDERGOING MAJOR SURGERY

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Background: The choice of dose and type of crystalloid administered during surgery is still controversial. Crystalloids influence acid-base status depending on the amount of fluid infused and its electrolyte composition. Strong Ion Difference (SID = [Na+] + [K+] – [Cl-]) is the major determinant of pH variations produced by crystalloids. The kidney reacts to crystalloids preserving acid-base homeostasis primarily by changing reabsorption and excretion of chloride, thus modifying urinary strong ion difference (SIDu).
The aim of our preliminary analysis is to assess the ability of incremental fluid infusion to achieve consistent hemodilution in vivo.
Secondary purpose is to analyze the renal response to acid base disturbances caused by different crystalloids and different volumes.
Methods: Healthy adult patients undergoing spinal surgery were recruited to this prospective, observational study and assigned to receive either 0.9% saline (S), lactated Ringer’s (LR) or rehydrating solution (RS) throughout surgery. We excluded patients receiving diuretics and those undergoing a surgery with unexpected bleeding.
For each solution, we evaluated the effects of two fluid boli of different volumes (10 mL/kg and 20 mL/kg) on hemodynamic status, fluid balance, acid-base equilibrium and urinary electrolytes.
Results: We included 13 patients (male 38%, mean age 45 year-old). Hemodilution was effectively achieved with a significant increase between the first and the second bolus (7% [05-10] vs 10% [8-18], p=.039). There was a clear trend towards increase in Cl concentration in patients receiving S (p=.08), while Na did not show significant difference among groups over times (p=.7). Accordingly, a trend in SID reduction was observed in patients receiving S(p=.10). Cl variation was more pronounced in S group, as well (2 [1.4-2.5] and 1.8 [1.5-3.6] after first and second bolus respectively, p=.059]. Interestingly, despite a trend in increase of plasmatic Cl, no difference in urinary Cl were detected at any time point.
Conclusion: Our preliminary analysis showed that an effective increase of hemodilution was achieved with infusion of different volumes of crystalloids, but also that an increase of Cl concentration consequent to saline infusion did not imply a parallel increase of Cl elimination.

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TOWARDS COLLABORATIVE TECHNOLOGY TRANSFER MODELS FOR LIFE SCIENCE: THE CASE OF "InnovaSIBioC"

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The ecosystem of innovation at the international level is characterized by a research system that each year produces a substantial portfolio of scientific results. These findings often remain far from an industrial application and are poorly exploited commercially, not generating the expected revenues. Companies, for their part, may find it difficult to evaluate the actual applicability of these findings and to understand their potential because of their embryonic stage of development or either their performance or their repeatability has not been tested sufficiently. In the last years, also customer needs are becoming more complex and the industrial system must develop useful products and processes based on frontier technologies. Thus, the traditional technology-push and demand-pull models appear obsolete in a highly dynamic and complex society. In the life science sector, the collaborative models are the benchmark for the future; the traditional model of fully integrated companies has evolved towards a collaborative discovery business model that requires the collaboration among customers/patients, companies and researchers. Thus, new technology transfer models are emerging; they are co-development models where the research and the industrial systems work together to validate (proof) the scientific findings (concept) from the very early stage of the innovation process by taking into account multiple and heterogeneous actors. To date, few empirical analyses in scientific studies investigate the real applications along with the motivation underlying the co-development activity of the new technology transfer models. This element represents a gap in technology transfer. In this context, the present work attempts to enrich the literature on co-development technology transfer models in the life science sector. The main research question is: Which are the main drivers of co-development in life science sector? We use a "theory building research", through a case study: the "Innovasibioc", proposed by a prestigious organization in the life science field that is SIBIOC (Società Italiana Di Biochimica Clinica E Biologia Molecolare Clinica). It is the first Italian experience of co-development technology transfer process in life science sector.

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NON-BLOOD SOURCES OF CELL-FREE DNA FOR NON-INVASIVE CANCER MOLECULAR PROFILING IN CLINICAL PATHOLOGY AND ONCOLOGY

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Liquid biopsy consists of the quantification and qualification of cell-free DNA fragments (cfDNA) and of tumour-derived (ctDNA) that are shed in the bloodstream (plasma and serum) and in other biological human liquids. CfDNA quantification and mutation analysis can be applied to the diagnosis, follow-up and therapeutic management as novel oncologic biomarkers. In addition, many studies have shown that cfDNA and ctDNA quantification are able to assess target therapy resistance and improve patient surveillance. Differently to several solid cancers (i.e. lung, gastrointestinal, pancreatic, ovarian, bladder, breast), some tumor-types release a low amount of DNA into the bloodstream hampering their diagnosis through standard liquid biopsy procedures. These tumors, as such as brain, kidney, prostate, and thyroid cancer, which are characterized by low concentration levels of cell-free tumoral DNA (ctDNA), are in direct contact with other body fluids. Therefore, non-blood body fluids may be alternative sources for cfDNA and ctDNA isolation and quantification. The most striking example is given by seminal plasma cfDNA, which can be analyzed through cost-effective procedures, as such as fluorimetric and elettrophoretic techniques, and yet may provide powerful information capable to revolutionize the diagnosis and management of prostate cancer (PCa) patients and other genitourinary tumors. Other non-blood sources of cfDNA/ctDNA useful as novel oncologic biomarkers are: cerebrospinal fluids for brain tumor, urine genitourinary cancer, sputum for lung cancer, saliva for head and neck squamous carcinoma, pleural effusion for malignant mesothelioma and lung cancer and stool for pancreatic and colorectal cancer. In the near future the analysis of cfDNA from non-blood biological liquids will become routine clinical practice for diagnosis and management of cancer patients.

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UN APPROCCIO DI GENE TARGETING PER LA FIBROSI CISTICA (FC)

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L'SFHR (Small Fragment Homologous Replacement) è un approccio di gene editing in grado di modificare stabilmente una sequenza genica utilizzando piccoli frammenti di DNA (SDF) come templato. La risposta cellulare all'SFHR coinvolge la riparazione del DNA, il ciclo cellulare e geni specifici (Trex1). L'SFHR può essere usato a scopo terapeutico per correggere il gene CFTR (Cystic Fibrosis Transmembrane conductance Regulator) mutato ripristinando la sequenza wild-type. È possibile espandere cellule epiteliali derivate da diversi tessuti umani attraverso la metodologia CRC (Culture Reprogramming Condition). Ottenere grandi quantità di queste cellule con alta efficienza e con proprietà stem-like da pazienti, apre nuove prospettive per il gene targeting della FC.L'efficienza di SFHR è stata ottimizzata mediante un sistema reporter di MEF (Mouse Embryonic Fibroblast) mutati per la proteina eGFP. La correzione è rilevata mediante FACS in seguito al ripristino della fluorescenza. La nucleofezione è eseguita con il sistema Amaxa (Lonza). La tecnica CRC consiste nella coltura di cellule epiteliali primarie su un feeder layer di fibroblasti murini irradiati in presenza dell'inibitore Y-27632. È stata eseguita la caratterizzazione delle FC-CRC ottenute da brushing nasale di pazienti FC. Sono in corso gli studi per la valutazione della correzione del CFTR, in seguito a SFHR, mediante digital PCR e analisi di frammenti.MEF sincronizzati in fase G2/M, trattati con agenti ipometilanti e con inibizione del gene Trex1 mostrano la migliore efficienza di correzione (0,071%). Le FC-CRC esprimono i marcatori tipici di staminalità e mantengono la capacità di ri-differenziare in epitelio respiratorio. L'mRNA del CFTR è espresso a livelli fisiologici. L'analisi di sequenza del gene e mRNA del CFTR ha escluso l'insorgenza di mutazioni de novo nelle CRC confermando il genotipo delle cellule epiteliali da cui sono derivate. Le FC-CRC rappresentano un modello cellulare utile per ottimizzare terapie personalizzate per ciascuna mutazione. L'unione dei risultati ottenuti dal modello MEF e il modello FC-CRC, permetterà la migrazione dell'SFHR dalla fase di ottimizzazione a quella di correzione di mutazioni FC, eventualmente applicabile come terapia cellulare personalizzata ex vivo.

50° Congresso Nazionale della Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica (SIBioC - Medicina di Laboratorio)

Napoli, 16-18 ottobre 2018

Indice degli Autori