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

SS01-01
FONDAMENTI E PRINCIPI DELL'HTA

C. Favaretti

Roma

La valutazione delle tecnologie sanitarie (*Health Technology Assessment* - HTA) è un elemento cruciale in tutti i sistemi sanitari.
Il concetto di tecnologia sanitaria è più ampio di quello al quale normalmente si fa riferimento. Le tecnologie sanitarie comprendono, infatti, non solo le attrezzature sanitarie e i dispositivi medici, ma anche i farmaci, i sistemi diagnostici, le procedure mediche e chirurgiche, i percorsi assistenziali e gli assetti strutturali, organizzativi e manageriali nei quali viene erogata l'assistenza sanitaria. Le tecnologie sanitarie sono quindi rappresentate da tutte le applicazioni pratiche della conoscenza che sono utilizzate per promuovere la salute e prevenire, diagnosticare e curare le malattie.
Il rapido aumento delle conoscenze scientifiche e delle loro possibili applicazioni, l'aumento dei costi e l'incertezza nella scelta allocativa delle risorse, l'intreccio tra questioni scientifiche, sociali ed etiche, la frequente confusione tra il mezzo (le tecnologie) e il fine (la salute dei singoli e della comunità), l'incertezza dei legami tra efficacia clinica e tecnologie rendono sempre più necessaria una esplicita attività di valutazione.
La valutazione delle tecnologie sanitarie è proprio la complessiva e sistematica valutazione multidisciplinare delle conseguenze assistenziali, economiche, sociali ed etiche provocate in modo diretto e indiretto, nel breve e nel lungo periodo, dalle tecnologie sanitarie esistenti e da quelle di nuova introduzione.
Tradizionalmente, la valutazione delle tecnologie sanitarie rappresenta il ponte tra il mondo tecnico-scientifico e quello dei decisori. Ma la valutazione delle tecnologie sanitarie è anche un'occasione strutturata d'incontro tra le diverse esigenze e aspettative di tutte le parti interessate all'assistenza sanitaria, che ne consente il successivo bilanciamento su criteri espliciti e condivisi tra le parti stesse. È il contesto nel quale i decisori politici, chi ha responsabilità organizzative, i professionisti, i pazienti e i fornitori contribuiscono al processo decisionale (cosa fare, come fare, quando fare, se fare,…..) e rispondono reciprocamente di tali decisioni (accountability).
La valutazione delle tecnologie sanitarie è quindi uno degli strumenti di governance integrata che le strutture sanitarie, ai diversi livelli, possono utilizzare per gestire l'assistenza sanitaria. Può essere applicato al livello generale delle scelte legislative e delle decisioni degli organismi di regolazione nazionale e regionale, al livello intermedio delle scelte gestionali nelle singole aziende sanitarie e al livello delle scelte professionale compiute dai singoli nella pratica assistenziale quotidiana.
Quest'ultimo aspetto è essenziale per la sostenibilità dei sistemi sanitari del futuro.

SS01-CO01
FROM HARMONIZATION TO THE BEST PRACTICE: EVIDENCE ABOUT POINT-OF-CARE TESTING

V. Pecoraro[1], M. Mele[2], T. Trenti[1]

[1]*Laboratorio Tossicologia, e* [2]*Rete Aziendale PoCT, Dipartimento interaziendale ad attività integrata Medicina di Laboratorio e Anatomia Patologica, Nuovo Ospedale Civile Sant'Agostino Estense, Modena*

Background: Point of Care testing (PoCT) has been developed to provide rapid test results and improve patient management. PoCT for immunoassay are commonly utilized in emergency situations. Most published studies focus on analytical performance of PoCT, neglecting the importance of patients' outcomes (1). We reviewed the analytical performance and diagnostic accuracy of PoCT specifically planned for immunoassay, comparing results obtained in PoCT and in standard laboratory settings, and we evaluated the possible impact of rapid results on patient management.
Methods: We searched Medline and Embase for studies reporting immunoassay results obtained in both PoCT and central laboratory scenarios. We extracted data on study details and assessed the methodological quality of the studies. We evaluated the analytical characteristics and diagnostic accuracy of six PoCT: Tn, PCT, intraoperative PTH, BNP, CRP and N-Gal.
Results: We analyzed 114 scientific papers. The majority of the studies (89,78%) were judged at low risk of bias. Thirty five studies (31%) reported analytical data, showing satisfactory correlations between results obtained through PoCT instruments and those in a central laboratory scenario. Fifty-four studies (47%) evaluated the diagnostic accuracy of PoCT as acceptable for Tn (Sn 74%,Sp 94%), BNP (Sn 82%,Sp 88%) and CRP (Sn 85%,Sp 85%) only. Clinical impact was evaluated in 30 studies. Of these, 2 studies measuring PTH and BNP reported a limited impact on diagnostic decisions. Seven studies measuring CRP showed a significant reduction of antibiotic prescription. Several authors of Tn studies reported improved care through early diagnosis of ACS following PoCT.
Conclusions: PoCT immunoassay results seem to be reliable and accurate in terms of diagnosis for Tn, BNP and CRP. The satisfactory analytical performance of PoCT, together with its excellent practicability, suggests that it could be a reliable tool in clinical practice, but, at present, no data shows a significant improvement in outcomes for patients treated in according to a PoCT protocol. The availability of quick test results seems to offers clinical advantages, but the impact of PoCT results is poorly documented in clinical studies.

1. Pecoraro V, Germagnoli L, Banfi G. Point-of-care testing: where is the evidence? A systematic survey. Clin Chem Lab Med 2014;52:313-24.

SS01-CO02

IQCP ED ELEVATA AUTOMAZIONE PER LA GESTIONE PREANALITICA DELL'ACCETTAZIONE CAMPIONI IN LABORATORIO

G. Visconti, L. Dumitrescu, C. Barichello, M. Valongo, M. Pradella, S. Schiavon, L. Zardo

Laboratorio Analisi, Ospedale "San Giacomo" - ULSS 8, Castelfranco Veneto (TV)

La fase di accettazione dei campioni in laboratorio rappresenta il punto d'incontro di due importanti fasi del processo analitico: preanalitica e analitica, si convalida l'adeguatezza del campione raccolto a garanzia del processo analitico che deve iniziare. Scopo del lavoro è descrivere i vantaggi derivanti dallo sviluppo di un'area di accettazione ad alta tecnologia che controlla le fasi di raccolta, verifica, check-in, smistamento e trattamento dei campioni in arrivo presso il Laboratorio, avvalendosi di due strumenti Automate 1250 (Beckman) interfacciati al middleware Halia (Noemalife). E' stato sviluppato un Individualized Quality Control Plan (IQCP) con una prima fase di raccolta di informazioni relative alla strumentazione, picchi di lavoro, esigenze analitiche, quindi una mappatura del processo per identificare e valutare le principali criticità. Si è proceduto poi alla riorganizzazione della raccolta, alla configurazione della strumentazione e all'implementazione di regole di autoverifica e controllo. Il flusso di lavoro creato prevede: -raccolta dei campioni nei centri prelievo e nei reparti su appositi rack destinati al carico su Automate, riducendo sia i tempi di raccolta che di carico strumentale; -separazione, grazie a regole presenti sul middleware Halia, dei campioni da centrifugare in area smistamento da quelli da consegnare non centrifugati ai settori analitici, e successivo smistamento automatizzato delle provette, riducendo gli errori di distribuzione; -controllo automatizzato delle provette mediante riconoscimento del colore tappo e tipo di provetta, permettendo di intercettare errori di raccolta; -preparazione automatizzata dei rack in uso nelle stazioni analitiche, riducendo i tempi di caricamento strumentale; -controllo automatizzato del volume di siero/plasma, intercettando campioni con volume inadeguato; -aliquotazione automatizzata, riducendo sia i tempi in fase analitica che i rischi legati al maneggiamento di campioni aperti. Lo sviluppo di un'area accettazione gestita da strumentazioni ad alta prestazione e da regole appropriate del middleware ha consentito di ridurre l'impatto degli errori di raccolta dei campioni nella processazione degli stessi, intercettando e gestendo la criticità prima che il campione arrivi alla stazione analitica.

SS02-05

THROMBOPOIESIS AND PLATELETS MORPHOLOGY

G. Specchia

Bari

The history of identification of the platelets dates back to about 1800, when they were firstly defined as "dust of the blood", then in 1841 Addison called them "extremely minute …granules and finally, in 1882, they were denominated "Platelets" by Bizzozzero. The term "Megakaryocytes" was coined by Howell in 1890. Since then, a series of experimental studies in vitro and in vivo has led to a knowledge of the complex molecular mechanisms that regulate both physiological and pathological Megakaryocytpoiesis/Thrombopoiesis. Megakaryocytopoiesis is the complex multistep process that takes place prevalently in the bone marrow, originating from the progenitor hemopoietic stem cells (HSCs) and ending with the production of polyploid Megakaryocytes that can release up to 10^{11} platelets (Thrombopoiesis) per day into the circulation to repair vascular damage and prevent hemorrhagic events. The proliferation/maturation process of Megakaryocytic Progenitors occurs through the biological mechanisms of endomitosis, producing cells with a polyploid nucleus and ample cytoplasm that express specific membrane antigens like CD41, CD42, CD61. The Thrombopoiesis process adds the crucial point in the phase when fragmentation of the pseudopodal projections of the mature megakaryocytes membrane gives rise to the proplatelets. This process involves a massive reorganization of the megakaryocytes membrane and cytoskeleton components, including actin and tubulin. The entire process of proliferation and differentiation of the Megakaryocytes is rigorously controlled and regulated by exogenous and endogenous factors as well as the transcriptional factors and epigenetic mechanisms. The prevalent extrinsic control mechanism of Megakaryocytopoiesis is carried out by Thrombopoietin (TPO) and its receptor MPL, expressed by the Megakaryocytes and their precursors. In turn, for signaling, they require the activity of the tyrosine kinase Janus 2 (JAK2). Thus, the TPO-MPL axis regulates both megakaryocytic maturation and platelets production. TPO acts in synergy with other cytokines like SCF, IL11 and EPO to promote the proliferation of HSCs. In addition, it has been demonstrated that after the activation of c-Mpl by binding with TPO, a series of intracellular molecular pathways is triggered, that involve JAK2 and then STAT3,STAT5, leading to the production of Bcl-x_L SOCS, Cyclin D, p27 etc. Among the transcription factors that control megakaryocytopoiesis and thrombopoiesis, GATA1 and FLI1 play an important role. In addition, FLI1 acts in concert with other factors like GATA1-FOG1,ETS1 to activate the expression of many specific receptors of the megakaryocytes and platelets such as MPL, GP2B (CD41), GP9 (CD42). The discovery of the various molecular mechanisms that

regulate the physiology of normal thrombopoiesis has contributed to a better knowledge of the pathogenesis underlying some hereditary/congenital platelets conditions as well as chronic myeloproliferative neoplasms. Today, light has been shed on the genomic defects involved in hereditary conditions that have long been known, and characterized by functional defects, such as the Bernard Soulier syndrome, “grey platelets” syndrome, Chediak-Higashi syndrome, Glanzman Thromboasthenia, etc. Hereditary thrombocytosis is rare, prevalently of dominant autosomal type, like those due to mutations of TPO, others to the MPL and JAK2 genes, but these are different from the acquired mutation V617F of JAK2 on exon 14 identified in 2005 in chronic myeloproliferative neoplasms (CMPN-ce). All diseases are characterized by specific morphological features of platelets.

SS02-06

PLATELETS: TECHNOLOGICAL ADVANCEMENTS TO MEET THE NEEDS OF CLINICAL MEDICINE

G. Da Rin

Laboratory Medicine, ASL 3 Bassano del Grappa

The assessment of the platelet count is essential in clinical hematology.
Historically, manual platelets count, using phase contrast microscopy, was common practice. The manual method is time-consuming and subjective.
The introduction of automated full blood counters resulted in a dramatic improvement in the accuracy and precision, particularly in severe thrombocytopenic patients.
The automated analytical procedures for platelet counting are: impedance analysis, optical light scatter/fluorescence analysis using various analysers and immunoplatelet counting by flow cytometry. (1) The impedance method detects cells by increase in electrical impedance when a cell passes through an aperture in the flow cytometer. Platelets are hereby detected by cell size because the increase in impedance is proportional to cell volume. For optical light scatter method, cells are also analysed based on volume when cells pass through a laser beam in the flow cytometer. If a two-angled method is used, light is two-dimensional permitting additional analysis of cell granularity. More accurate methods have been developed but are available on a few analysers. They include an immunological method with application of a platelet-specific antibody or fluorescent labeling of platelets prior to counting in the flow cytometer. Although currently in development, image analysis could become the standard practice in the future.
Other indices related to platelet counts are provided by hematologic analyzers.
They include mean platelet volume (MPV), platelet distribution width (PDW) and the fraction of large platelets. Derived platelet parameters are highly dependent upon the individual technology and are influenced by the anticoagulant and delay time from sampling to analysis.
The mean platelet component (MPC) based on the refractive index using an optical method corresponds to platelet density, and a decrease in density suggests platelet activation or defective biogenesis. Several clinical studies have documented an association between hemostasis and platelet size as they found MPV to be an independent risk factor for thrombosis, and large platelets are selectively consumed during massive bleeding. (2) No definite conclusion can be made about the value of any of the new platelet parameters to differentiate reactive thrombocytosis from clonal proliferation. (3) Reticulated platelets are newly released platelets; they contain RNA and are identified by flow cytometry and the use of nucleic acid specific dyes. Reticulated platelets are a laboratory tool to differentiate hypoproduction from accelerated platelet destruction and to predict platelet recovery after chemotherapy and stem cell transplantation (2,3).

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SS02-07

DIFFERENTIAL DIAGNOSIS OF THROMBOCYTOPENIAS

P. Noris, F. Melazzini

Department of Internal Medicine, IRCCS Policlinico San Matteo Foundation and University of Pavia, Pavia

The introduction into the routine laboratory practice of automated blood cell counters has much increased the prevalence of thrombocytopenia in the general population over the last decades. The main difficulty the clinicians must deal with is the correct recognition of inherited thrombocytopenias (ITs) from immune thrombocytopenia (ITP). This distinction can be difficult especially when a low platelet count is incidentally found in adulthood, and therefore categorized into acquired forms. The most important tools to prevent a misdiagnosis, and consequently inappropriate treatments, are careful medical history, physical examination, and microscopy examination of peripheral blood films (1). The presence of other family members with low platelet count, long lasting bleeding tendency even more pronounced than expected based on the degree of thrombocytopenia, and congenital or acquired additional defects, strongly supports the hypothesis of an IT. Nevertheless, recessive

forms and de novo mutations must always be taken into account. Physical examination is important not only to detect the clinical signs of thrombocytopenias, as cutaneous or mucosal bleeding, but also to recognize the low platelet count as part of a syndromic IT: defects may affect all organs and apparatus, some of them are evident, while others must be sought carefully, eventually by imaging studies (1). Morphologic examination of peripheral blood smears is mandatory since it is the most informative tool for the differential diagnosis of thrombocytopenias: ITs are often characterized not only by morphological abnormalities of platelets, but also of leukocytes and/or red blood cells (2). Most of ITs are usually characterised by platelets of increased dimension, although the degree of this enlargement varies among the different inherited disorders. A classification of ITs based on platelet diameter has been suggested and it takes into account the percentage of platelets larger than 3.9 μm, which corresponds approximately to half the diameter of normal red blood cells. According to this classification, ITs are classified into four categories: with giant platelets, with large platelets, with normal or slightly increased platelet size, with normal or slightly decreased platelet size (3).
On the other hand, ITP is similarly characterized by increased platelet dimensions, largely overlapping those of ITs: a lower percentage of large platelets in ITP turned out to be a useful parameter for a correct differential diagnosis with IT with giant platelets (3, 4). Abnormality of platelet granules may also suggest the congenital origin of an isolated thrombocytopenia: in some ITs granules may be reduced or absent, more frequently associated with platelet macrocytosis. Polymorphonuclear leukocytes containing peculiar inclusion bodies (Döhle-like bodies) are specific for MYH9-related disease when associated with the presence of very large platelets, while some abnormalities of red cells morphology may suggest the diagnosis of rarer ITs. A few complex laboratory tests will substantiate the suspected diagnosis which is strongly recommended to be confirmed at molecular level given the predisposition to hematological cancer or to the appearance of additional defects worsening the quality of life, typical of some ITs (2).

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SS02-08

THROMBOCYTOSIS AND RISK OF THROMBOSIS IN MYELOPROLIFERATIVE NEOPLASMS

A. Falanga, V. Milesi, C. Giaccherini

Immunoematologia e Medicina Trasfusionale e Centro Emostasi e Trombosi, Osp. Papa Giovanni XXIII, Bergamo

Thrombotic events are very frequent and represent the main cause of morbidity and mortality in patients with Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs), mainly Polycythemia Vera (PV) and Essential Thrombocythemia (ET) [1]. The pathogenesis of blood clotting activation in these diseases is multifactorial, and involves various abnormalities of platelets, erythrocytes, and leukocytes, as well as dysfunctions of endothelial cells. Typically, elevations in the number of circulating blood cells as well as modifications in the cellular hemostatic properties occur.
Patients with MPN can have a different thrombotic risk. Understanding this risk is very important as the treatment decision in these patients is highly dependent on their thrombotic risk category. According to age (>60), history of thrombosis, and, more recently, the presence or not of JAK2V617F mutation and/or cardiovascular risk factors: patients are stratified in “high-risk”, “intermediate” or “low-risk” categories [2].
Thrombocytosis is typical of these diseases, particularly of ET, but its role in the pathogenesis of thrombotic events is still controversial. No studies to date have demonstrated a statistically significant correlation between the platelet count and thrombosis in either PV or ET. In the ECLAP study, neither the currently proposed therapeutic target of $400x10^9$/L nor any other platelet count thresholds were able to predict a higher risk of thrombosis. In young ET patients (aged <60 years) with extreme thrombocytosis (platelet count ≥$1,000x10^9$/L), and without a previous history of thrombo-hemorrhagic complications, the incidence of major thrombosis and hemorrhage during the follow-up was similar between those who were treated with prophylactic cytoreductive therapy and those who did not receive such therapy. These findings suggest that current treatment should not primarily aim at lowering the platelet count [3]. Paradoxically, extreme thrombocytosis (i.e., platelets >$1,500x10^9$/L) is rather associated with hemorrhagic manifestations in patients with ET. Recently, biological studies of circulating thrombotic markers, to characterize the presence of a hypercoagulable state in patients with MPN, show that platelet qualitative abnormalities, more than platelet count, are associated with hypercoagulability in these patients. Specifically, several studies have demonstrated that platelets circulate in an activated status and possess a high prothrombotic potential. These findings suggest that in these diseases the control of platelet activation over the platelet count is an important goal of treatment strategies.

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SS02-CO03

CORRECTION WITHOUT PRE-WARMING OF COLD AGGLUTINATION IN THE RET OPTICAL CHANNEL OF SYSMEX XE-2100/XN 9000 AND MINDRAY BC 6800 HEMATOLOGICAL ANALYZERS. PRELIMINARY DATA

A. La Gioia[1], F. Fiorini[1], M. Lorubbio[3], M. Bombara[2], F. Balboni[8], A. Marini[4], P. Pezzati[3], M. Quercioli[5], M. Fiorini[2], L. Francioni[1], E. Marchese[6], F. Maglione[7]

[1]*Patologia Clinica Osp. Lotti, Pontedera*
[2]*U.O.C. Medicina di Laboratorio Ospedale di Livorno*
[3]*Laboratorio Generale, Azienda Ospedaliero Universitaria Careggi, Firenze*
[4]*Patologia Clinica, Ospedale Versilia*
[5]*Centro Regionale Controllo di Qualità AOU-Careggi, Firenze*
[6]*Laboratorio Analisi I, AUO Policlinico-V. Emanuele, Catania*
[7]*U.O.C. Patologia Clinica Venosa-Melfi, ASP - Potenza*
[8]*Laboratorio Analisi Istituito Fiorentino di Cura e Assistenza, Firenze*

Introduction: Cold agglutinin disease (CAD) is an autoimmune hemolytic anemia (AIHA) caused by an immune reaction against red blood cell self-antigens. The presence of agglutination on the EDTA tubes causes a decrease of RBC count and an increase of MCV. The MCHC increases in parallel with the artefactual decrease in the number of RBC. The warming at 37 degrees causes the reversibility of the agglutination and the normalization of the RBC counting as well as of the MCHC. It has been reported that the RBC value and the MCHC were not influenced by cold agglutination in the optical counting of RBC (RBC-O) in the RET channel of the Sysmex XE-2100 analyzers. In these cases, the RBC-O values are similar to the RBC counting performed after warming at 37 degrees in the impedance channel (RBC-I).We studied this phenomenon in a wider series of increased MCHC in order to evaluate the possibility to use the RBC-O counting instead of the RBC-I after warming.
Methods: 35 samples with increased MCHC (range 375–2411 g/L; median 540) were tested on the Sysmex XN 9000 and XE-2100 as well as on Mindray BC-6800 for Hemoglobin, RBC-O and for RBC-I after 2 hours of 37 degrees warming. MCHC in the optical channel was calculated by using a research parameter “Most Frequent Value” (MFV). Statistical analysis was performed using Analyse-it software version 3.90.1 (Analyse-it software Ltd; Leeds, UK).
Results: The RBC-O counting resulted in a resolution of the agglutination in 32 out of 35 samples. The comparison from two counting in 24 out of 27 samples analyzed with Sysmex technology showed a Passing Bablok regression y =,132 + 1,01 x (95% CI of slope 0,87 -1,12 and intercept -0,304 to 0,635). The calculated MCHC-O compared with the measured MCHC after 37 degrees warming showed a Passing Bablok regression y = -48,33 + 1,134 x (95% CI of slope 0,52–2,08 and intercept -365,1 to 153,0). Both RBC-O and MCHC -O resulted acceptable even by adding 8 Mindray samples to Sysmex samples.
Conclusions: In 91,4% of the cases the RBC-O counting without pre warming resulted in a resolution of the agglutination. The MFV is proved a reliable surrogate of MCV to calculate the MCHC. For these reasons the Manufacturers should be encouraged to develop a reflex test for automatic execution of both RBC-O count and calculated indices in cases of the presence of cold agglutinins.

SS02-CO04

BIOLOGICAL VARIATION OF PLATELET PARAMETERS DETERMINED BY THE SYSMEX XN-1000 HEMATOLOGY ANALYZER

M. Seghezzi, B. Manenti, P. Dominoni, A. Crippa, L. Cerutti, S. Buoro

SMeL Generale di Base - Analisi Chimico Cliniche Azienda Socio Sanitaria Territoriale Papa Giovanni XXIII

Aim of the study: Biological variation data (BVD) allow the derivation of important parameters for the interpretation and use of laboratory test, including indices of individuality (II), assessment of significance of change in serial results (reference change values, RCV). As BVD can be impacted by analytical technology, we evaluated BVD of hematology parameters provided by the Sysmex XN1000. In this study we present the results for PLT parameters (PLT count; PDW, Platelet Distribution Width; MPV, Mean Plaletet Volume; P-LCR, Platelet Large Cell Ratio; PCT, platecrit).
Material and methods: Blood samples were collected by the same phlebotomist at weekly intervals over 5 weeks from 21 ostensibly healthy volunteers (12 women, 9 men with ages ranging from 26 to 64 years old) and analyzed in duplicate within 2 hours using a single Sysmex XN analyzer. The analytical (CVa), within-subject (CVi) and between-subject (CVg) component of variations were calculated by nested ANOVA from replicated analyses after Cochran’s test and Reed’s criterion for outlier identification. Shapiro-Wilk test was applied separately to the results of each individual to check data distribution.
Results: CVa ranged from 1.0% (PLT) to 3.1% (PDW); CVi and CVg were 6.4 and 15.4 for PLT, ranging from 2.3

(MPV) to 7.0 (P-LCR) and from 7.3 (MPV) to 21.2 (P-LCR) respectively; Index of Individuality (II) was lower than 0.5 for all platelet parameters. RCV was 18.1 for PLT, 7.3 for MPV, 14.5 for PDW, 21.1 for P-LCR, 17.8 for PCT.
Conclusions: As shown by the results of CVa, the analytic variation of Sysmex XN for PLT parameters are very low, particularly for PLT count (CVa=1.0%). CVi and CVg were compatible or slightly lower in comparison with published data on desiderable quality specifications. The II was <0.5 for all PLT parameters, indicating high individuality and limited diagnostic utility of reference intervals in the detection of unusual results in an individual. Therefore, comparison of serial results to detect significant changes should be evaluated by means of RCV. The improvement of precision of new analytical technologies implies greater confidence in the use and documentation of BVD, that is an essential prerequisite mainly in development of clinical application of new parameters.

SS03-09
CSF ANALYSIS IN ACUTE MENINGOENCEPHALITIS: BIOCHEMICAL AND CYTOLOGICAL APPROACH

G. Bernardi, E. Corsini, M. Lazzaroni

Clinical Pathology and Medical Genetics Laboratory, Fondazione IRCCS INN C. Besta, Milano

Biochemical and Cytological analysis offer initial etiological diagnosis of acute meningoencephalitis, while microbiological and viral results confirm diagnose, identify causative agent and optimize treatment. Due to high morbidity and mortality, rapid diagnosis for prompt and appropriate treatment is necessary. Each hospital laboratory has to ensure urgently CSF glucose, Total Protein (TP) and cytometry. In bacterial meningitis glucose consumption differentiate them from viral ones, CSF/serum glucose ratio gives better sensitivity and specificity, but serum is not always available in emergency. TP rise much in bacterial meningitis and in a lower degree in viral meningitis, international and national guidelines (1, 2) suggest to prefer to TP CSF/serum Albumin quotient, but in emergency total protein is largely preferred. Cell count and their differentiation is essential for diagnosis, reference method is microscopy and counting chamber, but cell type differentiation needs expert operators. Modern Blood cells analyzers have now specific CSF counting programs, each one has different performances that are well described in literature (2). As meningoencephalitis involves high number of cells, sensitivity is not a priority. Most attention has to be made in cell differention as granulocytes are typical in bacterial meningitis, lymphocytes in viral one and monocytes in resolution phases. A lot of new biomarker have been recently proposed, possibility to test them in emergency is an important selection criteria. There are a lot of publications on CSF C Reactive Protein, IL6 and Procalcitonin. Antigens from various agents are tested in CSF to anticipate cultural results, Galactomannan antigen in CSF has been recently inserted in diagnostic criteria (3). Problems arise from interpretation results as intrathecal fraction is usually not detected and absolute values are influenced by barrier function and serum values that may be very high in case of invasive pathogen disease. Viral meningoencephalitis have usually a benign clinical course, but sometimes can worsen, so lumbar puncture is made in post-acute phase when viral PCR test are negative, unfortunately CSF serology is rarely performed according to CSF standards (1, 2), mainly because serology and biochemistry are made in different laboratories. So while there is perfect harmonization in routine CSF analysis, other tests need greater collaboration among the various laboratory specialties.

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SS03-10
BACTERIAL MENINGITIS

S. Andreoni

Novara

Meningitis is an infection of the protective membranes covering the brain and spinal cord. Acute bacterial meningitis is the most common form of meningitis. Approximately 80 percent of all cases are acute bacterial meningitis. Bacterial meningitis can be life threatening.
The bacteria most often responsible for bacterial meningitis are common in the environment and can also be found in your nose and respiratory system without causing any harm. Sometimes meningitis occurs for no known reason. Other times it occurs after a head injury or after you have had an infection and your immune system is weakened. Outbreaks of bacterial meningitis also occur in living situations where you are in close contact with others, such as college dormitories or military barracks.
Community-acquired bacterial meningitis is still a significant cause of morbidity and mortality. The organisms that cause bacterial meningitis differ somewhat by geographic region and by age. In premature babies and newborns up to three months old, common causes are group B streptococci and bacteria that normally inhabit the digestive tract such as

Escherichia coli. Older children are more commonly affected by Neisseria meningitidis (meningococcus) and Streptococcus pneumoniae and those under five by Haemophilus influenzae type B. In adults, Neisseria meningitidis and Streptococcus pneumoniae together cause 80% of bacterial meningitis cases. Risk of infection with Listeria monocytogenes is increased in persons over 50 years old. The introduction of pneumococcal vaccine has lowered rates of pneumococcal meningitis in both children and adults

The epidemiologic features of bacterial meningitis have changed dramatically over the past decades with the advent of the Haemophilus influenzae vaccine.

The most common symptoms are fever, headache and neck stiffness. Other symptoms include confusion, vomiting, and an inability to tolerate light or loud noises. Young children often exhibit only nonspecific symptoms, such as irritability, drowsiness, or poor feeding.

To diagnosis bacterial meningitis, CSF examination is mandatory. CSF culture is the “gold standard” for diagnosis, and it is obligatory to obtain the in vitro susceptibility of the causative microorganism and to rationalize treatment. CSF Gram staining, latex agglutination testing are additional diagnostic tools that might aid in etiological diagnoses, especially for patients with negative CSF cultures. If lumbar puncture cannot be performed, serum inflammatory marker, blood culture, skin biopsy, and urine antigen testing may provide supportive evidence to diagnose bacterial meningitis.

More specific technologies are under development to enable more confident diagnosis of the major etiologic agents of meningitis. One example of a technology that could potentially be applied broadly to meningitis is that of a PCR multiplex panel.

Bacterial meningitis is treated with antibiotics. A general intravenous antibiotic with a corticosteroid to bring down the inflammation may be prescribed even before all the test results are in. In addition to antibiotics, it will be important to replenish fluids lost from loss of appetite, sweating, vomiting.

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SS03-11

MENINGITIS WITH A CLEAR CEREBROSPINAL FLUID

C. Giraldi

Cosenza

Introduction: The infections of the central nervous system are very heterogeneous due to the broad spectrum of microorganisms (bacteria, viruses, fungi, helminths) that cause them, and on account of the clinical aspect. Based on the clinical syndromes they can be classified into: acute meningitis, subacute and chronic, acute and chronic encephalitis, space-occupying lesion syndrome, myelitis, neuritis, radiculitis, (the latter in particular are often mediated by bacterial toxins).

Meningitis/encephalitis: They are inflammation of the meninges, usually of infectious origin, which may evolve, if not properly treated, in meningo-encephalitis or brain-myelitis.

Acute meningitis in adults is characterized by high fever, headache gravativa, exacerbated by contacts, movements, lights, sounds, cerebral vomiting, and stiff neck. Viral meningitis is characterized by the onset of acute meningeal symptoms and fever and can affect all ages, but it is most common in young children. The main ethiologic agents are enteroviruses, responsible for 46% of viral meningitis, followed by herpes simplex -2 (HSV-2) (31%), varicella zoster (VZV) (11%) and HSV-1 (4%). Following the introduction of the vaccine, the incidence of viral meningitis due to mumps and measles has been reduced, while meningitis caused by enteroviruses, has slightly increased especially those caused by Coxsachie B and Echovirus. Enterovirus meningitis may be accompanied by mucocutaneous manifestations including vesicles localized on the hands, feet and mouth, herpangina and generalized maculopapular rash. HSV also causes viral meningitis as a complication of primary genital infection (especially HSV-2) and is not always associated with clinical symptoms. Meningitis due to VZV is most commonly observed in association with the reactivation of the virus and can occur in 50% of cases in the absence of skin lesions. Even the primary HIV infection is an important cause of meningitis whose neurological symptoms can occur in 17% of cases of seroconversion. Viral meningitis agents involved in forms of the traveler and that affect humans almost exclusively in the warm seasons, a period in which arthropod vector activities are present and they are: West Nile virus (WNV), St. Louis encephalitis virus, Tick- viruses borne encephalitis (TBE) Virus and Tuscany (TOSV).

Encephalitis is an inflammation of the brain tissue supported by an infectious cause, viral, and less frequently, bacterial or parasitic. The diagnosis etiology is only possible in half of the cases.

The symptoms are: agitation, altered state of consciousness and possible seizures. In nursing infants usually it prevails the seizure symptoms.

The encephalitis can be primitive or represent the secondary complication of a viral infection or a vaccination. The most frequent forms of primary encephalitis are due to HSV-1, HSV-2, VZV, enterovirus and influenza virus A. The HSV encephalitis is the most frequent, with a 'incidence of about 1/million per year. It can be the consequence of a primary infection or a reactivation and is caused in 90% of cases by HSV-1

and 10% in HSV-2. The HIV patients, with immunosuppression, may develop encephalitis mainly by: HSV, VZV and cytomegalovirus. The arbovirus encephalitis, transmitted by mosquitoes and ticks (fever of St. Louis, Eastern and Western equine fever, fever of California, neuro-invasive forms of WNV, tick-borne encephalitis "tick-borne encephalitis"), affect humans almost exclusively in the warm seasons, a period in which the activity of the arthropod vector is present.
Laboratory diagnosis of meningitis or encephalitis: The cerebrospinal fluid (CSF) undergoes microbiological, physical, chemical and biochemical examination. Blood cultures are also performed as support together with other microbiological tests of various biological samples not belonging to the neurological district. Serum can also be tested.
Collection and transport of CSF sample and other samples: LCR: is taken by lumbar puncture (LP) or lumbar puncture in the space between the 4th and the 5th lumbar vertebra, collected in sterile tubes with screw cap and conical bottom. The sequential division into three aliquots help gradually reduce contamination with blood from the tissue perforated during the execution of the lumbar puncture. The sample should be collected, preferably before beginning antimicrobial therapy and sent to the laboratory within 15-30 minutes of collection and no later than a maximum of two hours along with blood samples for a possible serological diagnosis, swabs (nasal, pharyngeal and vesicular), feces for the eventual search of enteroviruses and blood cultures.
Examination of the CSF: The examination of the CSF includes: macroscopic and microscopic evaluation, chemical-physical investigations, direct research of bacterial and fungal antigens, culture and susceptibility and specific gene amplification test.
In viral meningoencephalitis the CSF has an increased protein content, cell count with a predominance of peripheral blood mononuclear cells which are between >5<500 GB/mL (lymphocytosis may be absent at the beginning of the infection). The glucose levels are usually normal or low in the course of mumps virus infection, HSV, enteroviruses, HIV, WNV, and LCMV. Microscopic examination can reveal red blood cells which may be present especially in encephalitis caused by HSV. In viral meningitis (a clear CSF) specific gene amplification tests for each virus are diagnostic.

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SS03-12

THE CRUCIAL ROLE OF CLINICAL LABORATORY: TOSCANA VIRUS AND LISTERIA EXPERIENCES

P. Pauri

UOC Patologia Clinica, Jesi - AV2- ASUR Marche; Study Group SIBioC "Infectious disease diagnostics"

The diagnosis of acute meningo-encephalitis in the emerging setting is very important for many reasons: to begin the specific therapy as soon as possible, for prognostic evaluation, to identify and limit epidemic disease, to plan public sanitary approaches for control and prevention and to improve the local epidemiologic knowledge.
The synergic actions between Clinical Laboratories and Public Health Services are more relevant in cases of meningitis and meningo-encephalitis caused by Toscana Virus and Listeria which are zoonotic infections.
The project founded by the Italian National Center for Disease Prevention and Control (CCM) called "Surveillance of the zoonoses and the vectorial diseases: rationalization of the diagnostics methods and of information flows for the planning of interventions in the medical and veterinarian fields" started in 2014 in Marche, Emilia-Romagna, Lombardy and Sicily. The project is based on the evidence that the laboratory plays a strategic role in the clinical diagnostic process as well in the notification of zoonoses to the competent health authorities.
In 2016, in the Italian Society of Clinical Biochemistry and Laboratory Medicine (SIBioC), the "Infectious Disease Diagnostics Study Group" was born. Its initial task is a survey to evaluate the "state of art" in the Italian laboratories, of diagnostic serology comprehensive of zoonoses.
The *Toscana Virus* (TOSV) belongs to the Bunyaviridae family and it is the most frequent pathogen for the

aseptic meningitis with a good prognosis. This pathogen is spread by *Phlebotomus perniciosus* and *Phlebotomus perfliewi*, also known as vectors for human and canine leishmaniosis. For this reason, the TOSV meningitis is widespread in summer time both in the center of Italy (30-52% of the cases) and in the Mediterranean basin (1,2,3). The laboratory diagnosis must be considered in all those cases of meningitis with clear liquor and lymphocytosis that occur from June to October.

Gene amplification as well as IgG and IgM antibodies research with recombinant antigens are first choice tests. This approach allows us to avoid useless tests and to reduce the hospitalization. As a consequence, surveillance programs and disinfestation campaigns can be started.

Listeria monocytogenes (LM) rarely causes sepsis and hard meningitis. It is a food pathogen which causes an high number of hospitalization with a mortality rate from 20% to 30%. In the ministerial protocol (2015), Listeriosis is classified as an invasive bacterial disease (4) that is mandatory to notify. Since 2015, there is in Marche Region an epidemic outbreak which has involved 23 people with 3 deaths. The infection, clinically characterized by invasive and systemic forms, is caused by the same genotype (cluster) that was identified by using the pulsed field gel electrophoresis (PFGE) and next generation sequencing (NGS).

According to CCM projects, Clinical Laboratories, Clinicians, Prevention Departments and Veterinarians have operated in a coordinated and integrated way. Also Public Health Services as well as the Zoo-prophylactic Institutes of Marche and Umbria, Abruzzo and Molise Regions were involved.

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SP04-15

THERAPEUTIC DRUG MONITORING OF ANTI-TNF BIOLOGIC AGENTS: WHAT BENEFIT FOR PATIENTS?

V. Pecoraro, E. De Santis, T. Trenti

Laboratorio Tossicologia, Dipartimento interaziendale ad attività integrata "Medicina di Laboratorio e Anatomia Patologica", Nuovo Ospedale Civile Sant'Agostino Estense, Modena

Background: Infliximab and adalimumab are biologic blockers anti-inflammatory drugs directed against TNFα, that reduce the inflammatory response of autoimmune diseases. Biologics are immunogenic, and consequent anti-drug antibodies (ADA) are usually determined for therapeutic drug monitoring to optimize dosing of anti-TNFα (1). We performed a systematic review to evaluate the impact of anti-drug antibodies (ADA) on therapeutic response.

Methods: We considered studies enrolling adult patients affected by Rheumatoid Arthritis (RA), Ulcerative Colitis (UC) and Psoriatic Arthritis (PsA) in therapy with infliximab or adalimumab. We searched electronic databases MEDLINE and Embase. We collect data about study and population characteristics, treatment dosage, determination of ADA, adverse events and reaction to site of infusion. We combined data in meta-analysis, calculating risk ratios (RR) for each study. We evaluated heterogeneity calculating I^2. P-values <0.05 were considered as statistically significant. Analyses were performed with the RevMan 5.3 and Stata 11 softwares.

Results: We included 16 studies enrolling 2289 patients. Of these, 2166 patients (95%), evaluated in 13 studies, were affected by RA, 85 by UC (1 study) and 38 by PsA (2 studies). DAS28 score at baseline ranged from 5.2 to 6.5. The effect of ADA on treatment response was evaluated in 8 studies, showing a significant reduction of response (RR 0.75, 95%CI 0.6 – 0.94) in patients with ADA+ respect to patients ADA-. The heterogeneity between studies was low (I^2=6.3%). The therapy with infliximab or adalimumab produced, reaction of infusion site in 73 patients (3%), infection in 168 (7%) patients and serious adverse events in less of 1% of patients (20 out of 2289).

Conclusion: Our analysis showed that detectable ADA reduced anti-TNFα response. ADA could interfere with drugs and compromise their effects. Moreover, they cause injection site reaction and infections. The development of ADA and consequent worse therapeutic response is well documented (2, 3), but the usefulness of determination of ADA remains unclear (4). At moment, there are any indications about use of immunogenicity test to guide therapy. We need of more information to define the usefulness of this test for patients management.

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SP04-16

CONTROL AND VALUE OF LABORATORY IN THE DIAGNOSTIC APPROPRIATENESS

C. Bonaguri[1], A. Melegari[2]

[1]*Diagnostic Laboratory Department, Parma Hospital, Parma*
[2]*Diagnostic Laboratory Department, NOCSAE Hospital, Modena*

Background: The limitation of inappropriate test requests and the identification of a balance between available economic resources and increasing health needs is crucial for modern Healthcare Services. In this perspective, harmonization of testing algorithms is a goal. To improve the appropriateness of the test requests in autoantibody testing, reliable and universally accepted diagnostic algorithms need to be defined and implemented; these algorithms should have been developed using the available guidelines found in the current scientific literature and should be shared by all physicians working in clinical immunology. The most appropriate strategy for requesting autoimmune rheumatic disease laboratory testing should encompass selective criteria, should begin from a clinical suspicion, followed by a logical succession of analyses performed with sensitive tests at an early stage and specific tests for confirmation. We have specifically focused on antinuclear antibodies (ANA), Extractable Nuclear Antigens (ENA) and double-stand (dsDNA).
The Emilia-Romagna Region, on March 1th, 2013 has released the first coding for ANA Reflex testing, a common guideline for autoantibody testing in Autoimmune Rheumatic Disease, which places ANA test at the first analytical level whereas the following steps are directly guided by the laboratory. With this algorithm, we can also specify the presence/absence of anti-mitochondria antibodies (AMA) . The natural history of PBC may be significantly improved when the disease is diagnosed at an early stage. Anti-mitochondria antibodies (AMA) are the serological mainstay for the diagnosis of PBC at present, but they cannot be detected in a significant percentage (i.e., 10-15%) of PBC patients, thus resulting in a delayed diagnosis. Some antinuclear antibodies (ANA),such as anti-Multiple nuclear dots and anti-Membranous/Rime-like which target the sp100 and gp210 antigens respectively, are regarded as PBC-specific surrogate biomarkers,although controversial data exist about their diagnostic and prognostic value.
Methods: We have compared both the number of ANA, ENA and dsDNA tests and the percentage of positive results at the second level tests observed in the first three years after the implementation of ANA Reflex (May-Dicember 2013, 2014, 2015) in Autoimmune Laboratories of Parma and Modena.
Results: During the years after ANA Reflex implementation we have observed that the requests of ANA Reflex showed a significant increasing trend of total ANA requests in both Parma (45%, 54%, 70%) and Modena (54%, 61%, 80%). We have also found that ENA and dsDNA requests during this period showed a reduction in Parma and in Modena, accompanied by a substantial increase of positivity, with an improvement in selected positive cases.
Conclusions: Close collaboration and audit between clinicians and laboratory personnel will enable standardization and widespread implementation of diagnostic algorithms for a more efficient use of immunological tests in the diagnostic evaluation, prognostic assessment, and monitoring of patients with systemic autoimmune diseases.

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SP04-CO05

THE NEW TEST FOR MONITORING ANTI-TNFα THERAPY: FROM LABORATORY TO THE CLINICAL PRACTICE

E. De Santis[1], A. Melegari[1], C. Bonaguri[2], T. Trenti[1]

[1]*Diagnostic Laboratory Department, Nocase of Modena*
[2]*Diagnostic Laboratory Department, Parma Hospital*

Background: Biologicals directed against TNFα have improved the treatment of autoimmune disease. However, there are patients who do not respond or show a loss of efficacy becouse of the formation of anti-drug antibodies (ADA).
Aim: Evaluating the test for individual monitoring of therapy, the relevance of ADA detection and the relationship with clinical response and some laboratory parameters.
Materials and methods: We enrolled 73 patients treated with Infliximab (IFX): 12 with Ulcerative Colitis and 25 with Crohn's disease from Gastroenterology Unit of Parma, 6

with Rhetumatoid Arthritis, 6 with Psoriatic Arthritis and 24 with Ankylosing Spondylitis from Rheumatolgy Unit of Modena. Blood samples were collected before the next IFX somministration and we tested: IFX drug level, ADA and some aoutoantibodies characteristic of each disease. Results: From Gastroenterology: 18,92% of patients show IFX in therapeutic range without ADA, 24,33 IFX in subtherapeutic level without ADA, 13,51% IFX in therapeutic range with ADA, 43,24% with IFX in subtherapeutic level with ADA. From Rheumatolgy: 21,4% of patients show IFX in therapeutic range without ADA, 16,7% IFX in subtherapeutic level without ADA, 45,2% IFX in subtherapeutic level with ADA and 16,7% FX in therapeutic range with ADA. The correlation with clinical response suggest that the presence of ADA could interfere with efficacy of therpay but there is no significant association beetwen ADA and other serological parameters.
Conclusion: These tests for monitoring therapy are easy and quick but with a high cost. The detection of ADA and IFX levels is important tools in order to prevent adverse events and therapy failure but we have to optimize this approach trought collaboration between clinical and laboratory.

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SP04-CO06

DIAGNOSI DI MALATTIA CELIACA NEI BAMBINI CON ETA' INFERIORE AI DUE ANNI: QUALE SIGNIFICATO ATTRIBUIRE ALLA DISCORDANZA TRA MARCATORI SIEROLOGICI ANTI-GLIADINA DEAMIDATA (DGP) IgA E IgG?

P. Mella, M. Tani, L. Vedovelli, S. Cresceri, G. Bonelli, B. Milanesi

Servizio di Patologia Clinica, ASST del Garda

Le più recenti linee guida nazionali ed internazionali per diagnosi di malattia celiaca nei bambini con età inferiore ai due anni raccomandano l'uso degli anticorpi di ultima generazione anti gliadina deamidata (DGP) IgA e IgG, combinati al gold standard anti transglutaminasi (tTG) IgA e all'anti-endomisio (EMA) di conferma. Gli algoritmi proposti attualmente prevedono, ove le IgA risultino quantitativamente nella norma, di confermare la negatività del dosaggio delle anti tTG con la ricerca dei soli anticorpi anti DGP IgG, reputati secondo numerosi studi i più sensibili per questa fascia di età, oppure con entrambi i DGP di classe IgA e IgG. Non è chiaro tuttavia come interpretare il dato nel caso in cui uno dei due anticorpi risulti negativo e l'altro positivo, nè alcuna delle linee guida ritiene necessario eseguire l'EMA per ridimere la discordanza. Il nostro studio si è proposto di valutare la prevalenza di discordanza tra DGP IgA e DGP IgG su tutti i bambini di età inferiore ai due anni, testati in routine nell'arco di 16 mesi per sospetto diagnostico di malattia celiaca. Inoltre abbiamo ritenuto utile eseguire in tutti i casi discordanti il test EMA, avendo la più elevata specificità tra tutti i marcatori attualmente in uso per celiachia. Su 159 bambini di età compresa tra i 7 e i 23 mesi analizzati con metodo quantitativo F.E.I.A., risultati con valori di IgA nella norma e negativi per tTG IgA, 20 bambini pari al 12% del totale hanno mostrato positività per almeno uno dei due marcatori DGP. L'esecuzione del test EMA con metodo I.F. è risultato negativo per tutti i 20 bambini analizzati. Un solo bambino è risultato positivo per DGP IgA e dubbio per DGP IgG; dei restanti 19 bambini tutti hanno mostrato valore negativo per DGP IgA, di cui 4 con valore dubbio e 15 con valore positivo per DGP IgG. E' interessante notare che la positività per DGP IgG è risultata in tutti i casi moderata. Questo risultato lascia aperta la discussione su come interpretare il dato. Si può ipotizzare una associazione tra positività alle sole DGP IgG e sensibilità al glutine non celiaca, patologia emergente per la quale è noto che gli unici marcatori presenti nel 50 % dei casi sono gli anti gliadina (AGA) IgG di prima generazione, mentre non è ancora chiaro il ruolo dei DGP.

SS06-20

NEXT GENERATION SEQUENCING (NGS) APPLICATIONS IN THE STUDY OF METABOLIC LIPID DISEASES

A. Di Costanzo

Department of Internal Medicine and Medical Specialties, "La Sapienza" University, Policlinico Umberto I Hospital, Rome

In an age when the majority of monogenic human disease genes have been identified, one of the challenges for the incoming generation of human geneticists will be to unravel the mechanism of polygenic and multifactorial diseases such as primary or secondary disorders of lipid metabolism, metabolic syndrome (MetS), Type 2 diabetes (DT2), hepatic steatosis (NAFLD) which lead to cardiovascular disease (CVD).
These complex phenotypes are caused by a multitude of genetic and environmental factors acting in concert, which involves the inheritance and expression of a phenotype being determined by many genes at different loci, with each gene exerting a small additive effect. This implies that the different effects are cumulative, i.e. no one gene is dominant or recessive among another.
Genome-wide association studies (GWAs) have revealed several *loci* and several common single nucleotide polymorphisms –SNP- (Minor Allele Frequencies, MAF ≥5%) associated with multifactorial metabolic diseases and plasma lipid levels. However, the proportion of risk of lipid phenotypes explained by GWAS-identified loci remain modest [1].
It has been suggested that rare (MAF ≤1%) or low-

frequency variants (MAF=1%-5%) with moderate/strong effects that are not captured by GWAS, could explain a part of the 'missing heritability' [2-3]. An effective way to identify these rare/low-frequency variants is to re-sequence the candidate genes or the whole-genome in subjects with extreme phenotypes or perform a case-control studies. This strategy has already been successfully employed for various candidate genes involved in lipid metabolism, where multiple rare variants in combination with common variants were found to contribute to inter-individual variation in plasma lipid levels [4-5].

Mutational analysis of many genes, however, requires massive parallel sequencing technologies (NGS) to be accomplished reliably and within a reasonable time. In the last few years, the advent of NGS technologies has revolutionized the approach to genetic studies, making whole-genome sequencing a possible way of obtaining global genomic information. NGS has been shown to be successful in identifying novel causative mutations of rare or common Mendelian disorders. The identification of rare and frequent genetic variants can be very important in clinical practice to detect pathogenic mutations or to establish a profile of risk for the development of pathology. The purpose of this presentation is to discuss the recent applications of NGS in the study of several multifactorial metabolic lipid diseases, also discussing some relevant studies conducted to evaluate the genetic variance of plasma lipid-lipoprotein traits. We will also illustrate some preliminary data from our experience in NGS technologies used to identify genetic markers of NAFLD disease.

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SS06-21

LABORATORY DIAGNOSTICS OF DYSLIPIDEMIAS: HARMONIZATION OF RESULTS AND REPORTING

F. Ceriotti

Clinical Laboratory Service, San Raffaele Hospital, Milano

Dyslipidemias represent in western countries a relevant modifiable cardiovascular risk factor. Lipid measurements are the tool to identify the presence of dyslipidemias and to monitor their treatment. The existence of agreed upon decision limits (even if different in Europe and USA) requires a high degree of trueness and precision of the measurements of all the lipid components. For this reason it is extremely important to control all the sources of pre-analytical and analytical variability. Pre-analytical. The patient has to be in a stable metabolic state, without any inflammatory disease (that reduces TC) and the blood drawing has to be performed after a sitting position of at least 5 min and with a short tourniquet time to avoid possible hemoconcentration. Fasting is preferable even if a recent consensus statement indicates that it is not required in most cases. Analytical. Due to the fact that the decision limits for all lipids are not on the tails of the distribution of the population values, but in the middle, even a small bias introduces a large misclassification of subjects, so the use of methods able to provide traceable results is mandatory. For total cholesterol (TC), LDL-Cholesterol (LDL-C), HDL-Cholesterol (HDL-C) and Triglycerides (TG) a reference measurement system exists and it is maintained by the Centers for Disease Control and Prevention (CDC) (Atlanta, GA) that coordinate a network of reference laboratories. For TC and TG the reference measurement system is based on a pure primary standard (cholesterol and triolein) and ID-GC-MS reference methods (for TC also the Abell-Kendall method), for HDL-C and LDL-C pure cholesterol remains the primary standard, but the results depend on the CDC method used for the separation of the class of lipoproteins.

Producing accurate lipid results is important, but without a proper reporting the outcome for the patient may be null or even negative. And reporting the lipids results is particularly complex. A recent survey in Italy has shown an incredible heterogeneity. In fact the use of population based reference intervals is not recommended because of the unhealthy lipid concentrations typical of the western populations. On the contrary it is suggested to use decision limits based on desirable lipid concentrations, necessary to reduce the cardiovascular risk. Unfortunately the desirable lipid concentrations depend on the patient's characteristics, some of these characteristics (smoke, hypertension, diabetes, previous cardiovascular events, etc.) are not known by the laboratory. To avoid the making of complex reports with several possible references, it has been decided to propose a uniform simplified reporting indicating the desirable values for a patient at intermediate – low level of cardiovascular risk (targets: TC ≤5.00 mmol/L, LDL-C ≤3.00 mmol/L, HDL-C ≥1.00 mmol/L (males), ≥1.20 mmol/L females, TG ≤1.70 mmol/L). These references have to be accompanied by a comment indicating that, in case of subjects with high cardiovascular risk, lower targets should be considered. Only combining correct analytical results and proper reporting, the service for the patient will be really effective.

SS06-CO07

GENETIC SCREENING OF FAMILIAL HYPERCHOLESTEROLEMIA: KEY ROLE IN CARDIOVASCULAR PREVENTION

C. Giacobbe[1], M.D. Di Taranto[2], A. Morrone[2], G. Marotta[3], M. Gentile[3], P. Rubba[3], G. Fortunato[2]

[1]*CEINGE S.C.ar.l. Advanced Biotechnology, Naples*
[2]*Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples*
[3]*Department of Clinical Medicine and Surgery, University of Naples Federico II, Naples*

Introduction. Familial Hypercholesterolemia (FH) is a common autosomal dominant disorder characterized by high levels of total and LDL cholesterol, associated with increased cardiovascular risk (1). Early identification of FH patients can be useful to establish an adequate therapy and prevent cardiovascular accidents. We aim to screen the main genes involved in FH. Patients and Methods. We enrolled 466 subjects with clinically diagnosed FH, of whom 342 were unrelated.The promoter and exon 18 of the LDLR gene, exons 26 and 29 of the APOB gene as well as all exons of the PCSK9 and LDLRAP1 genes were amplified by PCR and directly sequenced. MLPA was performed to identify large rearrangements in the LDLR gene. Results. The screening of candidate genes revealed mutations in 265/342 unrelated FH patients, of whom 239 have mutation in LDLR gene, 4 in PCSK9 gene, 2 in APOB gene and 1 in LDLRAP1 gene; 12 patients are homozygotes or compound heterozygotes. We also observed that the values of LDL cholesterol gradually incresead among the homozygous or compound heterozygous patients, heterozygous patients with a null mutation (splicing, nonsense, duplication and deletion), heterozygous patients with missense mutations and patients without mutations, with statistically significant difference (p=0.001). Conclusions. The screening of the LDLR, APOB, PCSK9 and LDLRAP1 genes revealed a mutation rate equal to 77.5%. Carriers of radical mutations showed a severe lipid phenotype suggesting a strict follow up and an early initiation of therapy. In conclusion, molecular diagnosis is an effective instrument to early detect affected patients with a view to improving the prevention of fatal cardiovascular events.

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SS06-CO08

CLINICAL APPLICATION FOR THE STUDY OF THE CHOLESTEROL METABOLISM BY MASS SPECTROMETRY

V. Leoni[1], A. Nauti[1], C. Caccia[2]

[1]*Laboratorio Analisi Chimico-cliniche, Ospedale di Circolo di Varese, ASST-Settelaghi, Varese*
[2]*Laboratorio di Patologia Clinica e Genetica Medica, IRCCS Carlo Besta, Milano*

Cholesterol is a structural elements of the plasma membrane. About half of the circulating cholesterol is absorbed and half is synthesized from acetyl-CoA. High serum cholesterol is related to higher cardiovascular risk. Evidences of a metabolic impairment were described in several neurodegenerative diseases (NDs), as Alzheimer, Huntington and Multiple Sclerosis. Oxysterols (-OHC) are mono-oxygenated cholesterol metabolites formed by autoxidation (7-ketocholesterol, 7KC) or by the activity of specific enzymes expressed in tissues as liver, brain and lung. Methods. Sterol and oxysterol profiling in serum (or plasma) is measured by isotope dilution mass spectrometry after alkaline hydrolysis and liquid to liquid extraction. Results. Plasma lathosterol, lanosterol and desmosterol (precursor sterols) are markers of the cholesterol biosynthesis. They increase in hypercholesterolemia and decrease in the statin therapy. They are markedly increased in some rare autosomal recessive metabolic diseases and reduced in NDs. In presence of inhibition of the citric acid cycle and OXPHOS, the lower formation of ATP and NADHH significantly reduces the cell and tissue levels of sterol precursors. Plasmatic 24OHC (formed in brain by neuronal CYP46A1) depends on the number of metabolically active neurons in the cerebral cortex and decreases in proportion to the degree of brain atrophy in several NDs. Plasma 27OHC (formed by CYP27A1) increased significantly in hypercholesterolemia and more than 100% in SPG5, an autosomal recessive disorder characterized by peripheral neurodegeneration. In the presence of atherosclerosis with carotid thickening, the concentration of 7KC (and 27OHC) is increased. 7KC is involved in apoptotic mechanisms observed in ischemia-reperfusion and demyelination process. Conclusions: The sterol and oxysterol profile is a diagnostic test for rare inherited metabolic diseases and provides information about the whole cholesterol metabolism. The evaluation of the clinical and diagnostic significance of this metabolomic information in aging and degenerative diseases is in progress: new pathogenetic mechanisms, new biomarkers and new therapeutic targets have been identified so far.

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SS07-24

CELLULE CIRCOLANTI: QUALI CERCARE NELLA BIOPSIA LIQUIDA NELL'OTTICA DELLA MEDICINA DI PRECISIONE

E. Cortesi

Roma

Gli studi di sequenziamento genico hanno dimostrato un significativo livello di eterogeneità non solo tra tumori diversi ma anche nel contesto di uno stesso tumore (eterogeneità intra-tumorale), come risultato della complessa evoluzione molecolare del cancro. Simili premesse spiegano la considerevole difficoltà nel definire accuratamente, per ogni tumore, le alterazioni molecolari dominanti e complicano lo scenario nell'era delle terapie anti-tumorali a bersaglio molecolare. Benchè altamente informativa, l'analisi del tessuto tumorale presenta notevoli limitazioni legate al concetto di eterogeneità tumorale. Infatti, essa rappresenta l'immagine statica e parziale di una popolazione di cellule in continua trasformazione e non fornisce, pertanto, informazioni rappresentative dell'intera massa tumorale né dell'evoluzione nel tempo della malattia. Dal punto di vista teorico, una caratterizzazione affidabile ed esaustiva del genotipo tumorale dovrebbe consentire di analizzare il profilo genetico delle cellule tumorali, seguirne l'evoluzione durante il corso della malattia, nelle sue diverse fasi e in seguito ai trattamenti e capire come il profilo genetico del tumore possa influenzare la risposta del singolo paziente ai trattamenti farmacologici per guidare di conseguenza la scelta delle terapie. Quanto detto rappresenta un obiettivo piuttosto lontano se lo strumento di cui ci si avvale è l'analisi del tessuto tumorale ottenuto da biopsia che, come procedura invasiva e non scevra da rischi per il paziente, risulta difficilmente ripetibile con la frequenza che un monitoraggio "in tempo reale" del genotipo tumorale richiederebbe. La biopsia liquida, intesa come l'analisi di cellule tumorali circolanti (CTCs), proteine o frammenti di acidi nucleici derivati dal tumore e presenti nel sangue periferico, in un prossimo futuro può rappresentare un approccio innovativo rispetto al più tradizionale studio del tessuto tumorale.

SS07-CO09

FREQUENCY OF BRCA1 AND BRCA2 LARGE GENOMIC REARRANGEMENTS (LGRs) IN MUTATION-NEGATIVE OVARIAN CANCER WOMEN CANDIDATES TO OLAPARIB TREATMENT

R. Rizza, A. Costella, G.L. Scaglione, D. Guarino, C. Santonocito, A. Minucci, P. Concolino, E. Capoluongo

Laboratory of Molecular Biology, Institute of Biochemistry and Clinical Biochemistry, Catholic University of Sacred Heart, Policlinico "A. Gemelli", Roma

Background: Ovarian cancer is the leading cause of death from gynecological malignancies. Olaparib is an oral PARP inhibitor, used as post-chemotherapy maintenance in high-grade serous ovarian cancer (HGSC) patients with germline or somatic mutations in BRCA1 or BRCA2. However, emerging data may be able to help expand its role into tumors with other homologous recombination deficits (while also determining if all BRCA1/BRCA2 mutations respond equally). Large genomic rearrangements (LGRs) have recently been identified in hereditary breast and/or ovarian cancer (HBOC) families and account for a small but still significant proportion of cases in several populations. 81 BRCA1 and 17 BRCA2 variants large genomic rearrangements have actually been described and are responsible for up to one-third of the identifiable BRCA mutations.
Patients and Methods: Fifty-seven patients with HGSC, were fully screened for BRCA1/2 mutations by massive parallel sequencing. All samples negative were tested for BRCA1 and BRCA2 rearrangements using the Multiplex Amplicon Quantification (MAQ), following the manufacturer's protocol. Amplification products were analyzed with 3500 - Genetic Analyzer (Applied Biosystems Warrington, UK) and results were analyzed by the software MAQ-S v2.0 (Multiplicom) for quantification of copy number variation (CNV).
Results: The MAQ method has detected 11 LGRs in BRCA1 gene, divided as follows: 4 deletions in exons 1-2, 2 macrodeletions from exon1 to exon 13, 1 deletion in exon 3, 1 deletion in exon 14 and 1 in exon 17. Between the rearrangements detected, we identified two novel rearrangements: exon 3 duplication and exon 1-6 deletion in BRCA1 gene.
Conclusion: The treatment of recurrent ovarian cancer remains a big challenge to clinicians. Clinical and molecular characterization of LGRs identified in HGSC patients is necessary for therapeutic management. For this reason, we recommend the use of specific approach, as MAQ technique, after massive parallel sequencing in order to ensure an improvement of quality of NGS pipeline.

SS07-CO10

IDENTIFICAZIONE DI MUTAZIONI SOMATICHE IN PAZIENTI ONCOLOGICI: CONFRONTO TRA I RISULTATI OTTENUTI SU TESSUTO, SINGOLE CELLULE TUMORALI CIRCOLANTI E DNA LIBERO CIRCOLANTE

F. Salvianti[1], F. Galardi[2], G. Rotunno[3], F. De Luca[2], M. Pestrin[2], A.M. Vannucchi[3], A. Di Leo[2], M. Pazzagli[1], P. Pinzani[1]

[1]*Dip. Scienze Biomediche, Sperimentali e Cliniche, Università di Firenze, Firenze*
[2]*Dip. Oncologia Medica Sandro Pitigliani, Ospedale di Prato, Istituto Toscano Tumori, Prato*
[3]*Dip. Medicina Sperimentale e Clinica, Univ. di Firenze*

Introduzione: Le cellule tumorali circolanti (CTC) e il

DNA libero circolante (cfDNA) rilasciati nel torrente circolatorio dal tumore primitivo e dalle sedi metastatiche sono considerati una "biopsia liquida" del tumore in grado di riflettere la complessità e l'evoluzione della malattia in ogni suo stadio. In un precedente studio abbiamo dimostrato la fattibilità di un protocollo per l'analisi mutazionale a livello di singole cellule tumorali circolanti in pazienti con carcinoma mammario metastatico (MBC) (1). Scopo del presente studio è l'ottimizzazione di un protocollo per la ricerca di mutazioni somatiche nel cfDNA mediante Next Generation Sequencing e il confronto con i dati ottenuti dall'analisi di singole CTC isolate dallo stesso prelievo di sangue. Sebbene i due componenti principali della biopsia liquida, CTC e cfDNA, siano stati già molto studiati separatamente, pochi sono gli studi che confrontano le informazioni ottenute da questi due diversi comparti per un approccio terapeutico personalizzato. Materiali e Metodi: Il cfDNA è stato estratto da 2 ml di plasma utilizzando il kit QIAamp Circulating Nucleic Acid Kit (Qiagen). I campioni di DNA sono stati sequenziati con il sistema Ion S5 Sequencer utilizzando Ion AmpliSeq™Cancer Hotspot Panel (Thermo Scientific) che consente di studiare regioni "hot spot" di 50 oncogeni o geni oncosoppressori frequentemente mutati in pazienti oncologici. I risultati ottenuti utilizzando il cfDNA di 4 pazienti MBC sono stati confrontati con quelli di 3-5 CTC isolate dallo stesso prelievo e del tumore primitivo. Risultati: Dal confronto abbiamo evidenziato un'eterogeneità intra-paziente nello stato mutazionale in tutti i casi analizzati. I geni con il maggior numero di varianti di sequenza erano TP53, con 9 diverse mutazioni e PIK3CA, con 3 differenti mutazioni. In 2 su 4 pazienti cfDNA ha mostrato una mutazione, non presente nelle CTC e nel tessuto. Conclusioni: Questo studio preliminare suggerisce che cfDNA e CTC rappresentano due aspetti complementari della biopsia liquida. I risultati ottenuti sono a supporto della fattibilità dell'utilizzo della biopsia liquida come strumento utile nell'ambito della medicina personalizzata per la gestione dei pazienti con MBC.

SS08-26

THE CLINICAL VALUE OF PHARMACOTOXICOLOGY LABORATORY REPORTS IN DETERMINING THE "USE/NON-USE" OF DRUGS

C. Leonardi

Direttore Dipartimento Dipendenze e Medicina Penitenziaria, ASL Roma 2

According to the Italian occupational health legislation, every worker has to be checked by an occupational specialist in function of his occupational risk.
Distress occurs when repeated or excessive stimuli exceed the adaptive capacity of the individual, resulting in manifestation of a pathological condition that can involve various systems like the cardiovascular, nervous, endocrine, gastrointestinal and the immune systems. Any heavy or stressful work conditions are fertile ground for the establishment of the psychopathological situation and/or may contribute to cause a chronic stress-related illness.
Bus drivers of public transport are the workers with higher levels of stress. The traffic of the city, relationships with passengers, the responsibility for people transporting, stressful shifts often in the conditions of isolation, the inability to decide when have a work break, shift work and repetitive tasks are critical stress factors for these workers.
Cocaine is the best stimulant drug and it is one of the most addictive drugs in the world. Hundreds of thousands of individuals are victims of the nervous effects caused by cocaine. These effects may be different in every person. Since it is one of the top stimulants, it is able to cause some very powerful effects and for these reasons it is still one of the most abused drugs in the world.
While the use of cocaine in the workplace is quite common, heroin is less used among workers.
Cocaine may successfully mask fatigue; however, high dosages may to impair judgment and to interfere with the ability of the driver to concentrate. Coordination and vision are impaired. There is an increase in impulsive behaviours with tendencies to take more risks and create confusion within the user. A person using cocaine maintains the illusion of being alert and stimulated, although physical reactions are impaired.
Also our statistics show that more transportation workers are using illegal drugs, with a large spike in cocaine use.
Cocaine harms both employees and employers, and despite the downward trend, it is still a serious issue. It is a very addictive drug, and when used at work, it can have disastrous effects.
Worker's distress may be a cause for this prevalent use and abuse of cocaine.

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SS08-29
NEW PSYCHOACTIVE SUBSTANCES AND NEW BIOLOGICAL MATRICES

S. Pichini, E. Marchei, M.C. Rotolo, M. Pellegrini, S. Graziano, R. Pacifici

Istituto Superiore di Sanità, Roma

New psychoactive substances (NPS) are increasingly emerging on illegal drug market. In 2015 Europol and European Monitoring Centre for Drugs and Drug Addiction reported the identfication of more than 150 new substances mainly sold by internet web sizes and whose effects are naively reported by usres in web forums. Many cases of co-consumption of NPS and other substances have also been reported. Hence, the development of analytical methods aiming at the detection of a broad-spectrum of compounds (NPS and "traditional" drugs) are needed in case of seizures of unknown products or intoxiaction by unknown substances.
We will provide a critical overview of the analytical methods for detection and quantification of NPS and traditional substances in non biological (seizures) and biological matrices.
Systematic toxicological analysis in case of completely unknown samples will be also illustrated.
Focus will lie on advances in sample preparation, analytical techniques and new biological matrices for clinical and forensic purposes. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) makes it possible to determine low concentrations not only in serum, plasma or whole blood or urine, but also in alternative matrices like oral fluid, dried blood spots, hair, nails and non biological matrices. Ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS), recently introduced in our analytical laboratory makes it possible to quantify a high number of compounds within a shorter run time with a minimum use of solvents and simple matrices extraction.
Analytical methods using these techniques can identify new synthetic cannabinoids, new synthetic cathinones, phenetilamines-derived substances, triptamines, etc..
Systematic toxicological analysis is currently applied to conventional clinical and forensic matrices but also to new biological matrices used to disclose NPS use, such blood dried spots which represent a future challenge to non invasively investigate on NPS related intoxications.

SS08-CO11
NEW PREGABALIN ASSAY: POSSIBLE ABUSE

A. Calcinari, M. Brugia, A.M. Margarucci Gambini, M. Piaggesi, S. Marinelli, M. Galeazzi

Laboratorio Analisi, Azienda Ospedaliero Universitaria Ospedali Riuniti, Ancona

Pregabalin (PRG) is a gamma-aminobutyric acid (GABA) analogue indicated for the treatment of neuropathic pain, add-on therapy for partial seizures in epilepsy and generalized anxiety disorder, benzodiazepine addiction. We aimed to evaluate the possible abuse liability of PRG among patients from drug addiction service (Ser.T.) and ER with suspect of benzodiazepines abuse. We retrospectively analyzed 110 urine patient samples (average age 36,3±14,7, age range 15-81 years old) coming from ER (39), from Ser.T. (61) and blood transfusion department (10) of Ospedali Riuniti di Ancona, Italy. Blood trasfusion department samples were employed as negative control. Samples were assayed on CDx90 analyzer using immunoturbidimetric ARK Pregabalin Urine Assay (Tema Ricerca Srl, Italy). Application was elaborated in collaboration with Tema Ricerca. Positive samples were confirmed with chromatographic method (HPLC).78 patients (71%) were regular user of benzodiazepines, 15 of them (19%) have been found positive for PRG, also confirmed by HPLC. 11 PRG positive patients (73,3%) were found with very high level of PRG (>2000 ng/ml). These patients showed positivity even for other drugs of abuse such as cannabinoids, opiates, cocaine, amphetamines or alcohol. Our preliminary study confirms that PRG abuse is taking place and the potential hazard of this drug should not be underrated. Misuse of this therapeutic drug represents a serious emerging problem that must be monitored carefully through a rapid and reliable assay, now possible by automatic analyzers.

SS08-CO12
EMERGING DRUGS ADDICTION: NIR SPECTROSCOPY AND CHEMOMETRICS FOR THE EARLY DETECTION OF NEW PSYCHOACTIVE SUBSTANCES

R. Risoluti[1], A. Gregori[2], L. Ripani[2], S. Materazzi[1]

[1]*Dipartimento di Chimica, Università "Sapienza", Roma*
[2]*Reparto Investigazioni Scientifiche (RIS), Arma dei Carabinieri, Roma*

Near infrared spectroscopy (NIRs) proved to be a fast and non destructive tool for the detection of different compounds in forensic matrices [1]. This study investigated the feasibility of using NIR coupled to chemometrics calibration to detect new psychoactive substances in street samples. The capabilities of this approach in forensic chemistry were assessed in the determination of new molecules appeared in the illicit market and often claimed to contain "non-illegal" compounds, although exhibiting important psychoactive effects. The study focused on synthetic molecules belonging to the classes of synthetic cannabinoids, phenethylamines and cathinones. For each sample, NIR spectra in reflectance mode were acquired and chemometric tools based on Principal Component Analysis (PCA) were used to built a multivariate model of

prediction for the screening of new emerging substances. The approach was validated comparing results with officials methods and successfully applied for “in site” determination of illicit drugs in confiscated samples, in cooperation with the Italian Scientific Investigation Department (Carabinieri RIS) of Rome. The achieved results allow to consider NIR spectroscopy analysis followed by chemometrics as a fast, cost-effective and useful tool for the preliminary determination of new psychoactive substances in forensic science.

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SS09-30
L’ORGANIZZAZIONE DELLA RETE PIEMONTESE

G. Bracco

Torino

La riorganizzazione dei Laboratori analisi della Regione Piemonte è stata definita con Delibera di Giunta regionale per la prima volta nel 2007: si trattò di una dichiarazione intenti, cui non seguì reale attuazione. Negli anni successivi numerosi provvedimenti regionali hanno preso in considerazione le analisi urgenti, l’appropriatezza prescrittiva (si veda il TSH riflesso e molto altro), la Genetica.
Dal 2010 la Regione è in piano di rientro. Uno degli aspetti di riorganizzazione presi in considerazione negli accordi con i Ministeri è stato il piano relativo ai Laboratori analisi. Nel 2015 la D.G.R. 50 ha descritto la nuova rete dei Laboratori, prevedendo cinque strutture ospedaliere dove concentrare la diagnostica specialistica, e sette strutture ospedaliere (hub) in cui si eseguano analisi di grande automazione. La D.G.R. prevede che nei laboratori spoke vengano eseguite le analisi urgenti, con un numero massimo di analisi analoghe a quelle relative ai pazienti ricoverati.
Gli obiettivi 2016 dei Direttori generali delle Aziende Sanitarie comprendono l’attuazione del progetto, che si prevede completata entro l’anno per l’accentramento della specialistica, mentre per la grande automazione è richiesto che venga bandita almeno una gara entro settembre 2016.
Sono stati presi in considerazione tutti gli aspetti necessari alla riorganizzazione dei laboratori: costi, flussi informativi, personale, forniture, trasporti, informatica, spazi.
Nel corso del 2016 è stata aggiudicata la prima gara di grande automazione in un’Area sovrazonale, con un minore costo per strumentazione e diagnostici previsto in circa 21 milioni di euro in sette anni, pari al 42% della spesa storica, per una popolazione di 800.000 assistiti, su un totale di 4.400.000 dell’intera Regione.
Tra gli aspetti centrali nella riorganizzazione si cita l’attenzione per la qualità assistenziale, l’appropriatezza prescrittiva, l’equità di accesso, l’attenzione alla riduzione del numero di professionisti negli anni prossimi.
Il Direttore generale di ogni Azienda sede di laboratorio hub è responsabile dell’attuazione del progetto, in solido con gli altri Direttori generali, mentre il Direttore sanitario della stessa Azienda ne è il referente. E’ stato inoltre individuato un referente regionale per l’attuazione dell’intero programma, con il compito di sollecitare e indirizzare le Direzioni aziendali, e riferire mensilmente all’Assessorato sullo stato di avanzamento nelle diverse Aree sovrazonali (sei in Piemonte, con una popolazione che varia da 450.000 a 900.000 assistiti per ciascuna Area).
Passi successivi, volti a facilitare la riorganizzazione, sono l’assegnazione alla Società di Committenza Regionale di incarico di bandire gara per unificare i sistemi informativi dei Laboratori analisi, e il progetto di affrontare l’organizzazione dei trasporti a livello in regionale, in sinergia con Politecnico e Ires, e prendendo in analisi anche i Servizi trasfusionali, i Laboratori di microbiologia, le Anatomie patologiche.

SP10-36
SINAPTOPATIA: LINK TRA INFIAMMAZIONE E NEURODEGENERAZIONE

D. Centonze

Università Tor Vergata, Roma & IRCCS Neuromed, Pozzilli (IS)

Allo scopo di comprendere il coinvolgimento della trasmissione sinaptica nel danno neurodegenerativo della sclerosi multipla (SM), abbiamo studiato in anni recenti gli effetti della infiammazione sulla trasmissione sinaptica eccitatoria glutammato-mediata nel cervelletto del topo con encefalomielite autoimmune sperimentale (EAE).
I nostri dati hanno messo in evidenza un profondo sovvertimento delle correnti sinaptiche eccitatorie registrate elettrofisiologicamente in tale modello, con conseguente danno eccitotossico dei neuroni cerebellari. Abbiamo identificato la interleuchina 1beta (IL-1b) come la principale citochina infiammatoria responsabile di tale alterazioni e il trattamento in vivo dei topi con EAE con un inibitore della IL-1b somministrato per via intratecale non solo correggeva le alterazioni sinaptiche descritte ma riduceva anche la gravità clinica della malattia.
Abbiamo inoltre dimostrato il coinvolgimento delle cellule astrogliali nelle alterazioni sinaptiche indotte dalla EAE e dalla IL-1b, poiché tali cellule erano attivate dalla infiammazione e andavano incontro a una drammatica riduzione della espressione sulla membrana e della attività del trasportatore per il glutammato. Abbiamo infine dimostrato la presenza di linfociti T attivati nel

cervelletto dei topi con EAE e il loro ruolo nelle alterazioni sinaptiche associate a tale modello di sclerosi multipla. L'incubazione di fettine cerebellari di topi di controllo con linfociti T attivati estratti dalle milze dei topi EAE era infatti in grado di riprodurre le alterazioni sinaptiche tipiche dei topi EAE attraverso il rilascio della IL-1b.

SP10-CO13

THE DIFFUSION, STANDARDIZATION AND HARMONIZATION OF CEREBROSPINAL FLUID BIOMARKERS ANALYSIS FOR THE DIAGNOSIS OF ALZHEIMER'S DISEASE: THE ITALIAN SELFIE

G. Sancesario[1], S. Toniolo[2], S. Di Santo[2], D. Chiasserini[3], M. Pieri[6], L. Parnetti[3], M. Musicco[5], G. Bernardi[4], S. Bernardini[6], Study Group of Clinical Biochemistry of Biological Fluids other than Blood - SIBioC, SINdem-ITALPLANED

[1]*Dept. of Clinical and Behavioural Neurology, Santa Lucia Foundation IRCCS, Rome*
[2]*Dept. of System Medicine, Tor Vergata University, Rome*
[3]*Dept of Medicine, Center for Memory Disturbances, University of Perugia, Perugia*
[4]*IRCCS C. Besta Neurologic Foundation, Milan*
[5]*Epidemiology and Biostatistics Unit, National Research Council, Milan*
[6]*Dept. of Experimental Medicine, Tor Vergata University, Rome*

Although the use of cerebrospinal fluid (CSF) biomarkers, amyloid β1-42, tau and phosphorylated tau, has diagnostic and prognostic values 1, its diffusion is still limited in clinical practice, and only a restricted number of patients receive an integrated clinico-biological diagnosis. By a survey, we aimed at doing a "selfie" of the use of CSF biomarkers in the clinical practice in Italy, to understand the diffusion of CSF analysis and the distribution of CSF laboratories. Moreover, we considered pre analytical procedures, the methods used, the cut offs and the participation to Quality Control programs. We conducted a nationwide survey in March-May 2016, using an online questionnaire, sent to the members of SIBioC, SINdem-ITALPLANED and to main Neurological Clinics all over Italy (n=1815). Anonymous data were collected and analyzed. Based on our "selfie" study, in Italy AD biomarker' CSF analysis is currently performed in 24 laboratories, which can be distinguished as "internal laboratories" performing analysis just for their own hospital (9/24), and "centralized laboratories" (15/24), which provide biomarkers analysis for their own and for a network of neighboring hospitals. Indeed, 15 hospitals lack the service of an internal laboratory and send CSF samples to centralized laboratories. The distribution of laboratory centers varies along the territory. Surprisingly, nine regions lack CSF laboratories. The number of analyses is generally less than ten or less than twenty per month (39.13 and 47.83% of the responses); only in a few laboratories it is more than 20 per month (13.04%). In 70.59% of the laboratories there is a standardization of pre analytical procedures; only some laboratories (54.17%) participate in International Quality Control Programs. Moreover, there is no harmonization of cut-offs for the three biomarkers among centers. In Italy, the use of CSF biomarkers in clinical practice is patchy. The aim would be to offer to a larger number of patients an early and more accurate diagnosis and therapy. A standardization of pre analytical procedures and harmonization of cut offs are needed.

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SP10-CO14

ANTI-AQUAPORIN-4 ANTIBODIES LONGITUDINAL TITRATION IN NMO PATIENTS TREATED WITH RITUXIMAB: INVOLVEMENT IN THE PATHOGENESIS OF THE DISEASE, RITUXIMAB IMPACT AND CLINICAL PRACTICE

F. Marnetto, A. Sala, P. Valentino, L. Granieri, A. Bertolotto

Neurologia 2 - Centro Riferimento regionale Sclerosi Multipla (CReSM) & Neuroscience Institute Cavalieri Ottolenghi, Osp. S. Luigi, Orbassano (TO)

Neuromyelitis optica (NMO) is a severe autoimmune disorder of the central nervous system with inflammatory lesions restricted to the optic nerves and spinal cord. NMO is associated with autoantibodies to the water channel aquaporin-4 (AQP4). Chimeric monoclonal antibody Rituximab (RTX) have been shown to be effective in NMO; RTX reinfusion timing is based on monitoring the percentage of memory CD19+ B cells. Numerous studies analysed AQP4-Antibody (AQP4-Ab) titres, its correlation with the clinical course of disease, or during different treatments, to support a pathogenic role of these antibodies, with heterogeneous results. Our aims were to investigate the biological value of AQP4-Ab titer in the mechanism of disease and during RTX therapy, and to evaluate the usefulness of AQP4-Ab titration in the clinical practice. We determined AQP4-Ab titre in 322 serum samples from 7 NMO patients treated with RTX for a long follow-up (median 65 months), using an immunofluorescence cell based assay commercialized by Euroimmun. We found that 1) AQP4-Ab titres correlate with clinical disease activity with higher titres during and preceding clinical relapses compared with remission periods. 2) AQP4-Ab levels are reduced under RTX treatment; moreover, RTX induce a marked reduction in AQP4-Ab titres 3 months after infusion, as also during a 6 years follow-up. 3) AQP4-Ab levels were further analysed based on clinical outcomes (Annualized Relapse Rate and Expanded Disability

Status Scale) evaluated for each patient to define treatment responsiveness: in the long term follow up, AQP4-Ab levels decrease in the majority of responder patients, while an increase in Ab titer occurs in the non responder one. This different trend is observed after the first two-years of therapy. 4) Correlation between every increase of CD 19+ B cells, AQP4-Ab titer and clinical relapses was also evaluated showing a low association. Results obtained demonstrate a good relationship between AQP4-Ab levels, clinical state and response to RTX therapy, supporting their involvement in the pathogenesis of NMO; in particular RTX reduce Abs levels in responder patients, providing information for the treatment efficacy after the first two years of therapy.

- Granieri L, Marnetto F, Valentino P, et al. Evaluation of multiparametric immunofluorescence assay for standardization of neuromyelitis optica serology. PLoS One 2012;7:e38896.

SS12-42

THE USE OF GLYCATED ALBUMIN IN THE MANAGEMENT OF DIABETES MELLITUS

E. Dozio

Milano

Glycated albumin (GA) is considered useful for assessing the degree of protein glycation directly dependent on glucose exposure. The conventional biomarkers employed for screening and monitoring diabetes mellitus (DM) include fasting glucose, postprandial glucose and glycated hemoglobin (HbA1c). Although they provide useful information, in some clinical conditions they are inadequate. GA is an interesting biomarker for diabetes mellitus: 1) as it is an intermediate-term marker of the glycemic status, it gives more information than the short-term (glycemia) and long-term (HbA1c) biomarkers currently employed in clinical practice; 2) in specific clinical conditions (altered erythrocyte lifespan, pregnancy and end-stage renal disease) it should be preferred to HbA1c; 3) it is probably also useful for diabetes mellitus screening and risk stratification of diabetes-related complications. HbA1c may be affected by any condition affecting erythrocyte lifespan (hemolytic anemia, hemorrhage, folate and vitamin B12 deficiency anemia, aplastic anemia, nephropathy) and hemoglobin metabolism (variant hemoglobin, thalassemia). Unlike HbA1c, GA is not influenced by their lifespan and is also independent of iron deficiency. In pregnancy HbA1c suffers some limits as an indicator of glycemic control since it raises from the second to the third trimester probably due to iron deficiency. Unlike HbA1c, GA is not affected by iron deficiency and, as an intermediate-term glycemic marker (albumin turnover is shorter than erythrocyte lifespan - 20 vs. 120 days), it enables pregnant women with DM to maintain a stricter glycemic control, important to lower the risk of fetal and maternal complications. GA is also a useful biomarker for monitoring diabetes mellitus in newborns due to the high levels of fetal hemoglobin. Patients with diabetes and end-stage renal diseases under dialysis also cannot be efficiently managed with HbA1c, because of the reduced persistence of red blood cells due to mechanical disruption, lower hemoglobin and erythropoietin concentration. GA may be a better indicator of their glycemic status. GA may be a useful diagnostic tool for diabetes screening in the general population and in individuals with a pre-diabetic condition. GA also rises sooner than HbA1c when glycemic status worsens, probably due to albumin biochemistry and its half-life . This means that GA is more useful as an indicator of glycemic status in all those conditions requiring short-term control of changes in glycemia, such as after the start or modifications of diabetes treatments. GA may be also directly implicated in the development of different complications related to diabetes, playing a role as a pathogenic molecule. However, in some specific disorders GA levels are either lower or higher than the mean plasma glucose concentration, mainly because of changes in the albumin metabolism.
In conclusion, the introduction of this biomarker in clinical practice could help clinicians in the diagnosis and monitoring of diabetes mellitus and in planning measures to prevent long-term diabetes complications.

SS16-51

UTILITÀ DIAGNOSTICA DEL DOSAGGIO DELLA OLOTRANSCOBALAMINA NEL DEFICIT SUBDOLO DI VITAMINA B12 IN DIVERSI SETTING CLINICI

V. Rocco, M. Fumi, Y. Pancione, L. Rocco, S. Sale

UOC Patologia Clinica, AORN G. Rummo, Benevento

I medici, tendono a pensare agli stati di deficit con i loro segni classici e generalmente floridi e, nel caso del deficit di cobalamina, questi segni comprendono principalmente l'anemia megaloblastica e le disfunzioni neurologiche. La teoria che l'anemia megaloblastica non sia invariabilmente presente nella presentazione clinica del deficit di cobalamina è stata proposta 90 anni fa e fu vigorosamente respinta a quel tempo. E' stato successivamente ben documentato che l'anemia e la macrocitosi sono spesso assenti nel deficit di cobalamina.
Il deficit subdolo, moderato o marginale, è definito come evidenza metabolica della carenza, senza manifestazioni iniziali di anemia o malattia neurologica e, sia che si tratti di uno stato iniziale, che di un fenomeno transitorio o di uno status prolungato nel tempo, questa condizione è molto comune. Certi gruppi di popolazione, come gli anziani, gli adolescenti, le donne in gravidanza, i vegetariani e i vegani sono particolarmente proni al deficit franco o subdolo di vitamina B12. Ad esempio, negli anziani sopra i 60 anni,

esso è presente in circa il 20% della popolazione. Numerosi sono, inoltre, gli studi che hanno verificato la correlazione tra i livelli della vitamina B12 e malattie neurologiche come: declino cognitivo, demenza, malattia di Parkinson, Alzheimer e cefalea così come malattie gastro-intestinali come il morbo di Crohn. In questi lavori i test utilizzati ai fini della valutazione dello status sono stati soprattutto la vitamina B12 totale, l'omocisteina e l'acido metilmalonico. Per la limitata sensibilità e specificità del test di screening standard, il livello della vitamina B12 sierica totale, per la limitata specificità dell'omocisteina (elevata anche in situazioni di carenza di folati) e a causa della difficoltà di esecuzione routinaria del test per l'acido metilmalonico, è stato proposto come indicatore alternativo potenzialmente utile dello stato della vitamina B12 la olotranscobalamina (holoTC) cioè la B12 plasmatica legata alla transcobalamina che è la frazione della B12 totale che è disponibile per l'uptake tissutale.
Sulla base di queste premesse e sulla scorta di numerosi case reports relativi ad anemie megaloblastiche e a disordini neurologici con Vitamina B12 totale nella norma e olotranscoblamina ridotta, abbiamo valutato le 200 richieste di vitamina B12 ricevute in 60 giorni consecutivi senza limitazioni relative a presunta inappropriatezza nella richiesta. Per la valutazione dello stato della B12 sono stati utilizzati sia test biochimici, come la vitamina B12 totale, i folati sierici, l'omocisteina e la olotranscobalamina, che test ematologici come l'MCV, il rapporto MCV reticolocitario/MCV dei globuli rossi e la presenza di neutrofili plurisegmentati. Lo scopo è stato quello di provare l'utilità della olotranscobalamina come test per la valutazione routinaria dello stato della vitamina B12.

SS17-54
PARAPROTEINEMIAS AND THE KIDNEY

G. Quattrocchio, D. Roccatello

Struttura Complessa a Direzione Universitaria di Nefrologia e Dialisi & CMID, Ospedale G. Bosco e Università di Torino

The finding of a monoclonal gammopathy (or paraproteinemia) in the serum and/or urine of a patient suggests the existence of a clonal proliferation of B-lymphocytes or plasma cells, and could represent either an overt malignant disease such as multiple myeloma (MM) and Waldenström macroglobulinemia or a premalignant condition called monoclonal gammopathy of undetermined significance (MGUS). Monoclonal proteins are usually detected by protein electrophoresis (PEP) combined with immunofixation electrophoresis (IFE) of the serum and/or of a sample of urine. However, sometimes it can be very challenging to detect serum or urinary monoclonal immunoglobulins, particularly in cases of isolated immunoglobulin light chain disorders. Recently, serum immunoglobulin free light chain (FLC) assays with accurate quantification of both light chain isotypes and automatic calculation of the k/lambda FLC ratio have been introduced in the laboratory armamentarium leading to major improvements in early diagnosis and management of patients with paraproteinemias.
The kidney is frequently involved in monoclonal gammopathies and a wide variety of histopathological lesions with different clinical presentations can be encountered, depending on the amount and type of the circulating paraprotein (intact immunoglobulin or fragments like heavy and light chains). The term monoclonal gammopathy of renal significance (MGRS) has recently been introduced, and encompasses renal disorders caused by a monoclonal immunoglobulin secreted by a nonmalignant B-cell clone generally consistent with MGUS. Kidney biopsy is warranted to identify the exact nature of the lesion and to evaluate the severity of renal disease in order to define an adequate treatment.
We present the clinical picture, the complex diagnostic workup and the individual therapeutic choices of three typical patients with three different types of paraprotein-related kidney diseases: 1) Cast nephropathy, 2) Light chain-associated (AL) amyloidosis, and 3) Monoclonal Ig deposition disease.
Serum and urinary PEP, IFE and FLC assays are indispensable to detect monoclonal paraproteins. Bone marrow aspirate and biopsy show the percentage of bone marrow plasma cells allowing the differentiation between MGUS, smoldering MM and overt MM. Kidney biopsy is crucial to identify the type and extension or renal lesions. The treatment is based on different chemotherapeutic regimens, which in some situations can be combined with removal of circulating paraproteins by means of extracorporeal therapies, such as plasma exchange, high cut-off hemodialysis and supra-hemodiafiltration with endogenous reinfusion. Interdisciplinary collaboration between hematologists and nephrologists is necessary, particularly in sharing a common therapeutic approach in some patients with MGRS.

SS19-59
STANDARDIZATION OF FECAL CALPROTECTIN ANALYSIS

A. Padoan

Department of Laboratory Medicine, University-Hospital of Padova

Fecal calprotectin (fCal) supports diagnosis and monitoring of inflammatory bowel diseases (IBDs). Different cut-offs are recommended for different clinical applications: 50 μg/g to rule-out IBDs, 150 to 300 μg/g to rule-in for IBDs relapse. A pre-analytical phase

(sample weighting and dilution) precedes the analytical fCal measurement, which might be based on ELISA, chemiluminescent, RIA or turbidimetric assays, the overall variability of a single stool sample analysis depending on both the pre- and analytical phases. The intra-spot CV was estimated to be 25%, while the overall biological variability 58%, being this value even higher when among diseased patients (1,2). Both analytical and biological variability might have a relevant impact on results interpretation, especially when values are close to the established cut-offs, thus limiting the efficacy of decision making strategies based on single test results. One of the main efforts of Clinical Laboratories should be oriented in reducing the analytical component of this variability by standardization and improvement in quality control by internal and EQA schemes. Currently, non-stool internal quality materials and EQA schemes with stool samples are used, but no standard material suitable for fecal sample analyses is available. In the absence of a standard reference material, a marker of accuracy is measurement uncertainty (MU). MU could be calculated by imprecision, trueness and bias uncertainty. By analysing 18 EQA results, we estimated fCal MU to be 23 μg/g and 175 μg/g for mean values below or above 200 μg/g respectively, confirming a concentration related MU. An added calprotectin analyte is now under evaluation for EQA schemes. We evaluated whether a recombinant standard (PRCA, DiaSorin Inc., USA) is suitable for fCal imprecision assessment by PhiCal Calprotectin ELISA (Immundiagnostik, Germany). MALDI-TOF/MS of PRCA confirmed its purity, showing only S100A8 and S100A9 peaks, which form the hetero-dimer Calprotectin. PRCA dissolved at the final concentration of 840, 420 and 210 ng/μL were diluted 1:25 in stool extraction buffer before ELISA, to reproduce the entire pre-analytical and analytical process. The expected results (336, 168 and 84 ng/mL) chosen were close to 300, 150 and 50 ug/g diagnostic cut-offs. Biases were: -1.2%, -4% and +6.6% from two replicated measures for the 336, 168 and 84 ng/mL targets respectively. Inter-assay imprecisions (11 independent assays) were: mean=331.84 ng/mL, CV=6%; mean=175.7 ng/mL, CV=9%; mean=100.3, ng/mL, CV=21%. In conclusion, a recombinant standard calprotectin might be useful as internal quality control for fCal assay.

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2. Calafat M, Cabré E, Mañosa M, et al. High within-day variability of fecal calprotectin levels in patients with active ulcerative colitis: what is the best timing for stool sampling? Inflamm Bowel Dis 2015;21:1072-6.

SS23-66

A CASE OF HEMATOLOGICAL INVOLVEMENT IN CELIAC DISEASE

F. de Liso[1], M. Ronchi[1], C. Matinato[1], N.N. Fantini[2], R. Maiavacca[1]

[1]*Lab. Analisi Chimico Cliniche e Microbiologia, e* [2]*Lab. Ematologia, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano*

We report a case of a pregnant 39 year-old woman (22th week) with a condition of leucopenia/neutropenia (WBC 2.400/mmc; N 400/mmc), iron-deficiency anemia (RBC 3.620.000/mmc, Hb 9.4 g/dL, MCV 82.3 fL, serum ferritin 7 ng/mL) and a slight elevation of transaminase (ALT 34 U/L, AST 38 U/L) whose causes were unclear, without family history of congenital anemia and leucopenia. The patient has been characterized by this hematological setting for ten years and medical management has been consisted by the treatment of symptoms (iron therapy). She arrived at our outpatient pregnancy clinic and the gynecologist required an hematological counseling. The hematologist decided to investigate the immunological status, assuming a possible autoimmune condition, which could justify the patient's long-term alterations. At first, anti-nuclear (ANA) and anti-double stranded DNA (anti-dsDNA) antibodies were required and were negative, but a fibrillar filamentous fluorescence of cytoplasm on HEp-2 cells (the gold standard substrate for detecting ANA) was observed. In order to better understand this fluorescence, the laboratory decided to evaluate anti-smooth muscle antibodies (ASMA) on rat Liver-Kidney-Stomach (LKS) section. Unexpectedly, a fluorescence reticulin-like was revealed and this result suggested to investigate the presence of celiac disease (CD). CD is an immune-mediated small intestinal enteropathy. Traditionally patients with CD have intestinal symptoms but over time the newly diagnosed patients can present with a wide range of symptoms and signs, including anemia and adverse pregnancy outcomes. According to guidelines, diagnosis of CD is by serology and duodenal biopsy (postponed until postpartum), and in this case we could perform only the serum panel for a new diagnosis of CD consisting in the assessment of total IgA (tIgA, to rule out a deficit of tIgA), IgA to tissue transglutaminase (TTG) and, if positive, IgA to endomisium (EMA). As the tests were strongly positive (TTG>128 U/mL, EMA positive), clinicians decided to start the gluten free diet with an improvement of WBC count (6.100/mmc), Hb levels (11 g/dL), iron status (ferritin 21 ng/mL) and transaminase values (ALT 18 U/L, AST 21 U/L) already after one month.

- Ludvigsson JF, Bai JC, Biagi F et al. Diagnosis and management of adult coeliac disease: guidelines from the British Society of Gastroenterology. Gut 2014;63:1210-28.

SS23-67
UN CASO DI ANEMIA MULTIFATTORIALE

F. Tosato, E. Piva, A. Schiavinato, M. Plebani

Dipartimento Medicina di Laboratorio, Azienda Ospedaliera Università di Padova

Introduzione: Il deficit di vitamina B12 deve essere prontamente identificato al fine di evitare l'instaurarsi di danni neurologici irreversibili. Tuttavia la condizione può essere mascherata, anche in caso di anemia, dalla presenza di altre concause.
Metodi: Una donna di 52 anni è stata ricoverata per vertigini, offuscamento della vista, sudorazione profusa e vomito comparsi da 3 giorni. La paziente lamentava astenia, dispnea e dolore retrosternale che si risolvevano rapidamente con il riposo. L'emocromo ha rivelato una grave anemia (Hb 60 g/L) normocitica (MCV 93,4 fL) normocromica (MCH 30,8 pg), con globuli bianchi, formula leucocitaria e piastrine nella norma. Gli esami ematochimici hanno evidenziato un significativo aumento di LAD (5426 U/L) e una diminuzione di aptoglobina (<0,08 g/L), inizialmente interpretati come segni di emolisi. All'osservazione microscopica dello striscio di sangue periferico era presente una marcata anisopoichilocitosi delle emazie con frammenti eritrocitari ma, ad una più attenta osservazione, si sono riscontrati ovalociti, dacriociti ed una ipersegmentazione dei granulociti neutrofili. Tali caratteristiche morfologiche e le informazioni strumentali hanno fatto sorgere il sospetto di un deficit di vitamina B12, confermato biochimicamente (B12 50 ng/L) e causato da anticorpi anti parete gastrica ed anti fattore intrinseco. Nonostante il deficit di B12, dato il valore di MCV, sono stati valutati il bilancio marziale e la presenza eventuale di disordini emoglobinici. La determinazione dell'assetto emoglobinico ha rivelato la presenza di un picco attribuibile ad HbS (33,1%), mentre risultava aumentato il recettore solubile della transferrina.
Risultati: E' stata quindi formulata una diagnosi di emoglobinopatia S con deficit di vitamina B12 e carenza marziale. Dopo supplementazione di ferro e B12, la paziente ha ottenuto la risoluzione della sintomatologia e la normalizzazione del valore di emoglobina. Tuttavia MCV ed MCH sono diminuiti, pertanto è stata rivalutata con diagnosi finale di disordine HbS/alfa talassemia.
Conclusioni: Il riscontro morfologico delle caratteristiche tipiche del deficit di vitamina B12 non deve essere trascurato in presenza di normale MCV, in quanto, in presenza di altre concause, tale deficit può essere mascherato.

SS23-68
RUOLO DEL LABORATORIO NELLA VALUTAZIONE DI UN DONATORE DI ORGANI CON SOSPETTA EMOFILIA

E. Milletti[1], C. Bellini[1], D. Fineschi[2], E. Franceschini[2], A. Silvietti[2], L. Terzuoli[1], D. Vannoni[1], C. Scapellato[2], R. Guerranti[1], R. Leoncini[1], P. Calzoni[2]

[1]*Dip. Biotecnologie Mediche, Università di Siena/UOC Lab. di Patologia Clinica, AOU Siena*
[2]*UOC Lab. di Patologia Clinica, AOU Siena*

Il Centro Regionale Allocazione Organi e Tessuti della Toscana ha richiesto la consulenza della UOS Coagulazione del Laboratorio di Patologia Clinica per escludere una sospetta patologia emorragica in un soggetto di 82 anni, potenziale donatore di fegato. Tale organo, insieme alla cornea, può essere utilizzato anche se il donatore ha un'età maggiore di 80 anni a differenza di altri organi che possono essere espiantati fino a 65 anni. Il paziente, colto da emorragia cerebrale, giungeva al ricovero classificato come paziente emofilico, ma il fegato risultava compatibile per un soggetto in urgente attesa di trapianto. Un paziente emofilico non può donare il proprio fegato, ma è stato riferito che il soggetto in realtà non aveva mai avuto fenomeni di sanguinamento, neppure lievi. Vista l'importanza di poter utilizzare questo organo, venivano richiesti ulteriori accertamenti, anche se i test coagulativi di primo livello risultavano tutti nella norma (PT sec=11,5, INR=1,05, aPTT sec=28, aPTT ratio=0,99). È noto che si possono ottenere quadri di emofilia A acquisita transitoria per patologie autoimmuni, neoplastiche, etc, che potrebbero avere indotto in precedenza a diagnosticare il suddetto soggetto come emofilico senza ulteriori accertamenti. L'obiettivo era la valutazione dell'idoneità all'espianto di fegato destinato al trapianto e l'urgenza della risposta induceva ad eseguire i dosaggi del FVII, FVIII e VWF (ACL TOP CTS 500; HemosIL-FVII deficient plasma; HemosIL-FVIII deficient plasma; HemosIL-VWF: RCo; HemosIL-von Willebrand Factor Antigen) che sono risultati nella norma (FVII=74%, FVIII=55%, VWF:Ri Cof=180% e VWF: Ag=213%) e, data l'importanza del caso, sebbene il deficit di FVII e FVIII siano il presupposto necessario per la ricerca degli inibitori circolanti, è stato comunque eseguito il parallelismo dei FVII e FVIII, che non ha evidenziato presenza di inibitori. L'assenza di alterazioni coagulative ha autorizzato a procedere con l'espianto del fegato. Per questo motivo è stata possibile la donazione del fegato su un ricevente maschio di 59 anni.

- Coppola A, Favaloro EJ, Tufano A, et al. Acquired inhibitors of coagulation factors: part I-acquired hemophilia A. Semin Thromb Hemost 2012;38:433-46.

SS23-69

DYNAMIC CHANGE OF TNI IN PRESENCE OF ST SEGMENT ELEVATION BUT NO OBSTRUCTIVE CORONARY DISEASE

C. Bellia[1], B. Lo Sasso[1], G. Bivona[1], G. Novo[2], M. Ciaccio[1]

[1]*Sezione Biochimica Clinica e Medicina Molecolare, Dipartimento di Biopatologia e Biotecnologie Mediche, Università degli Studi di Palermo*
[2]*Cattedra di Cardiologia, AOUP Policlinico P. Giaccone, Palermo*

Introduction: the diagnosis of STEMI is based on the detection of new ST segment or T wave changes at ECG in presence of chest pain. Typically, a high increase of TnI is observed. Case description: The patient, a 66 years old woman, was admitted at the Emergency Department of Policlinico P. Giaccone, Palermo, for non radiating chest pain occurred in the last hours after an emotional stress. Her medical history comprised a positive familiar history for cardiovascular disease, arterial hypertension, gastroesophageal reflux disease, anxiety-depressive syndrome, dysthyroidism. On admission, blood pressure was 110/65 mmHg; ECG showed diffuse ST-T abnormalities with an elevation of ST segment; cardiac markers were TnI: 3.79 ng/ml and CPK: 374 U/L. After three hours TnI was 3.88 ng/ml and CPK was 377 U/L; after six hours cardiac markers concentrations declined (TnI: 2.88 ng/ml; CPK: 339 U/L) and became negative after 48 hours. Echocardiography showed apical akinesia and hyperkinesia of the basal segments of left ventricle (LV) with moderately impaired LV function (EF=43%). No acute atherosclerotic lesions and no evidence of significant coronary artery stenosis were detected on angiography. Routine laboratory tests results were: AST: 33 U/L; ALT: 29 U/L; total cholesterol: 218 mg/dl; HDL cholesterol: 53 mg/dl; triglycerides: 88 mg/dl; glucose: 106 mg/dl; creatinine: 0.98 mg/dl; WBC: 9.76 10^3/microL; RBC: 4.49 10^6/microL; Hb: 12.1 g/dl; PLT: 310 10^3/microL. Given the moderate increase of TnI, the elevation of ST segment and the lack of stenosis or acute atherosclerotic lesions, the diagnosis of Takotsubo cardiomyopathy was suspected. Moreover, the Tn/EF ratio was 9, significantly lower than the cut-off of 60 proposed for differential diagnosis with AMI. Cardiac MRI ruled out both myocardial infarction and myocarditis and confirmed the diagnosis of Takotsubo cardiomyopathy. Supportive therapy with ACE-inhibitors, spironolactone and acetylsalicilic acid was initiated. After 4 days, echocardiography showed the complete recovery of LV function. Conclusion: a slight increase of TnI in presence of typical ST segment ECG abnormalities should be interpreted with caution, especially when it does not correlate with a significant but transient left ventricular dysfunction.

SS23-70

A CASE OF VENTRICULAR WALL RUPTURE PREDICTED BY AN EXTREMELY INCREASED RDW VALUE

G. Lippi[1], R. Aloe[2], G. Cervellin[3]

[1]*Section of Clinical Biochemistry, University of Verona, Verona*
[2]*Laboratory of Clinical Chemistry and Hematology, Academic Hospital of Parma, Parma*
[3]*Emergency Department, Academic Hospital of Parma, Parma*

We describe the case of a 86-year old women, admitted to the emergency department (ED) of the University Hospital of Parma for acute dyspnea. The patient had a history of percutaneous transluminal coronary angioplasty (PTCA), chronic obstructive pulmonary disease (COPD) and osteoporosis, and was currently taking aspirin, clopidogrel, atorvastatin, phenytoin, lansoprazol, as well as calcium and vitamin D supplementation. At presentation she appeared dyspneic at rest, subcyanotic and with visible jugular vein distension. Immediately upon admission the patient underwent electrographic assessment, which was suggestive for a ST elevation myocardial infarction (STEMI) of the inferior wall and right ventricle, along with urgent laboratory testing. The main results of lab testing were a troponin I value of 54480 ng/L (reference range [RR] <60 ng/L), hemoglobin 77 g/L (RR 120-140 g/L), BNP 1885 pg/mL (RR <150 pg/mL) and estimated glomerular filtration rate (eGFR) 17 ml/min (RR >60 ml/min). Notably, the value of red blood cell distribution width (RDW) was 19.8%, thereby extremely increased compared to its RR (<14.8%). Due to the clinical status, ECG findings, increased troponin concentration and very high RDW value, the patient was immediately transferred to catheterization lab, where she eventually died 45 min afterward for ventricular wall rupture and cardiogenic shock. Albeit many laboratory data were indeed over the respective RRs, none of these (except RDW) was really predictive of such a dramatic complication of STEMI. This conclusion is in accord with current evidence attesting that RDW is an independent predictor of adverse outcome after myocardial infarction, exhibiting a predictive accuracy that is often greater than that of other and more expensive biomarkers. Despite the unfavourable outcome, this case report should be seen as a paradigmatic example of how the association between clinical perception and simple laboratory tests may provide a valuable aid for the clinical decision making in the ED. We hence conclude that major focus should be directed toward underestimated laboratory parameters such as the RDW by the emergency physicians, wherein the presence of a high degree of anisocytosis may always be considered an important negative prognostic factor.

SS23-71
UN CASO DI LES SUBCLINICO

A. Gubbiotti, L. Rosengart, A. Malgrande, G. Piccone

U.O.C. di Patologia Clinica, Az. Osp. San Camillo-Forlanini, Roma

Una donna di anni 51, di costituzione fisica longilinea e in stato di buona salute, nel 2010 inizia a manifestare linfonodi latero-cervicali, rush cutaneo e/o eritema,che regrediscono con terapia topica cortisonica. Gli esami ematochimici di routine sono nella norma; nelle urine compare lieve proteinuria (30 mg/dl). Successivamente continuano a presentarsi linfonodi e manifestazioni cutanee di lieve entità; la paziente gode di buona salute. Alla fine del 2015 uno dei noduli dietro al nuca si ingrandisce e viene asportato, il quale risulta istologicamente reattivo-infiammatorio. Si ripetono indagini di base che risultano nella norma, nell'esame chimico-fisico delle urine compare di nuovo proteinuria (50 mg/dl), emazie, alcuni cilindri ialini. Non vengono prescritti esami di approfondimento. Nel corso dei mesi successivi compaiono un esteso eritema dietro le spalle, ulteriori linfonodi laterocervicali, dimagrimento ed astenia. Si ripetono esami di base e si riscontra ancora proteinuria (70 mg/dl). La paziente,che lavora in ospedale,chiede consiglio al laboratorio. Si decide di approfondire: compare una proteinuria elevata (1965 mg/24 ore), albuminuria all'elettroforesi urinaria, consumo elevato di C3 (31 mg/ml) e C4 (<7 mg/ml). Si passa quindi ad indagini di autoimmunità che rilevano presenza di anticorpi anti dsDna, anticorpi antinucleo con pattern finemente granulare ad alto titolo; inoltre in immunoblotting si evidenzia la presenza di anticorpi diretti verso l'antigene SM. Ci sono quindi tutti i criteri per la diagnosi di les che viene formulata dal reumatologo. La paziente, sottoposta subito a terapia cortisonica, viene inviata dal nefrologo per la biopsia renale al fine di valutare il grado di severità del danno d'organo. In conclusione se si fossero effettuati esami di approfondimento nella direzione dell'autoimmunità sin dai primi segni clinici (comparsa di linfonodi, manifestazioni cutanee e proteinuria), si sarebbe potuta formulare una diagnosi precoce di LES subclinico, il danno renale sarebbe stato contenuto e la patologia sarebbe stata sotto controllo. Ancora una volta risulta indispensabile il dialogo tra clinica e laboratorio.

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SS23-72
CSF KAPPA AND LAMBDA FREE LIGHT CHAINS FOR THE DIAGNOSIS OF MULTIPLE SCLEROSIS

D. Lopergolo, M.T. Dell'Abate, D. Scribano, C. Zuppi, T. De Michele

Laboratorio Analisi 1, Fondazione Policlinico Universitario A. Gemelli, Rome

As today, oligoclonal band (OCB) analysis by mean of isoelectric focusing is considered the Gold Standard technique for Multiple Sclerosis (MS) diagnosis. Nevertheless, the technique is cumbersome and in the evaluation of samples yielding dubious OCB responses (i.e. a single band), OCB analysis reveals interpretative limitations. Notably, in our laboratory experience about 15% of total OCB analysis during the last year revealed dubious responses. Recently, free light chains (FLCs) has been suggested as potential diagnostic value in the context of MS. Thus, the kappa and lambda FLCs assay for intrathecal FLC quantification has emerged as a promising tool for supporting MS diagnosis. Here we describe 2 patients who underwent FLCs quantification in cerebrospinal fluid (CSF) and serum using Freelite™ (The Binding Site Ltd, UK), a nephelometric assay. Case 1: A 43 years old man presented with visual impairment and left homonym hemianopia. Microbiological examinations on CSF and serum were negative. The CSF diagnostic revealed a negative IgG index, positive CSF/serum albumin ratio (6.1) and OCB analysis indicated a borderline aspect (a single supernumerary OCB). CSF and serum κ and λ FLCs were within the reference range while κFLC index was positive (13,6). Visual evoked potentials and MRI were indicative of retrobulbar optic neuritis suggesting a diagnosis of clinically isolated syndrome (CIS). Case 2: A 55 years old woman arrived to the hospital emergency with visual impairment in left eye lasting one week and mild retro-orbital pain. The CSF diagnostic revealed a negative CSF/serum albumin, negative IgG index and OCB analysis indicated a borderline aspect (a single supernumerary OCB). Interestingly, CSF κ and λ FLCs were respectively 1.90 and 0.45 mg/L and κFLC index was positive (56,3) while serum FLCs were within the reference range. Evoked potentials and MRI were suggestive for optic neuritis and also indicated brain parenchymal signal abnormalities consistent with MS diagnosis. Conclusions: The FLCs CSF test may be a valid alternative to support MS diagnostic protocols especially in the event of dubious or unclear OCB results, in order to confirm or reject all suspicions. FLCs assay may be performed as initial screening upon sample request submission.

SS23-73

FREE LIGHT MEASUREMENT IDENTIFIES RELAPSE AND PROMPTS TO RECONSIDER AMYLOID TYPING IN A PATIENT WITH AL AMYLOIDOSIS

P. Milani[1], M. Basset[1], F. Russo[1], M. Nuvolone[1], F. Lavatelli[1], T. Bosoni[2], L. Pirolini[2], A. Foli[1], R. Albertini[2], G. Palladini[1], G. Merlini[3]

[1]*Centro per lo Studio e la Cura delle Amiloidosi Sistemiche, Fondazione IRCCS Policlinico San Matteo, Dip. di Medicina Molecolare, Università di Pavia, Pavia*
[2]*Lab. Analisi Chimico Cliniche, Fondazione IRCCS Policlinico San Matteo, Pavia*
[3]*Direzione Scientifica, Fondazione IRCCS Policlinico San Matteo, Pavia*

The detection and quantification of amyloidogenic monoclonal light chains (LC) is necessary for the diagnosis and evaluation of response to therapy in AL amyloidosis (Palladini et al. JCO 2012). A 69 years old woman with nephrotic syndrome (proteinuria 5 g/24h), macroglossia and perorbital purpura was evaluated in another center in December 2001. A monoclonal IgGλ was detected in serum and the urine immunofixation was negative. Creatinine was 0.98 mg/dL (u.r.l. 1.17 mg/dL) and echocardiography was normal. The abdominal fat aspiration was positive. The diagnosis of AL (λ) amyloidosis with renal and soft tissue involvement was made. In April 2002, the patient was started on melphalan and dexamethasone, and, after 4 cycles, was evaluated at our center. We documented a negative serum and urine immunofixation, with reduced proteinuria (2 g/24h). Treatment was continued until cycle 6, and complete remission was confirmed in June 2002. Free light chain (FLC) measurement became available at our center in June 2003. FLCs were measured by the Binding Site assay on a BNII Dade-Behring nephelometer. FLC ratio on July 2003 was normal until June 2006, when proteinuria increased (8 g/24h), serum creatinine was 1.9 mg/dL, serum and urine immunofixation was still negative, but elevated κ-FLC concentration was documented (102 mg/L,κ/λ ratio 5.69). Since progression occurred in the absence of λ monoclonal protein, but κ-FLC increased, we reconsidered the initial diagnosis. Thus, we repeated the abdominal fat aspirate for amyloid typing by immune-electron microscopy, that revealed κ-LC deposits. On frozen serum stored at the first evaluation at our center κ-FLC was 19 mg/L (κ/λ ratio 1.16). The final diagnosis was AL-κ amyloidosis. The disease had responded to first line therapy, as confirmed by FLC measurements on frozen sera. A relapse was documented with κ-FLC increase. Thus, we started second line therapy with bortezomib and dexamethasone and, after 8 cycles, a second complete remission was obtained. In this case the FLC allowed the identification of amyloidogenic LC, that were not visible at immunofixation, enabling the timely detection of relapse. The identification of the LC forming the deposits and follow-up including FLC measurement are vital in AL amyloidosis.

SS23-74

MONITORING RESPONSE ASSESSMENT DURING TREATMENT WITH ANTI-CD38 IN A PATIENT WITH LIGHT CHAIN MULTIPLE MYELOMA

G. Topini[1], A. Fiorini[2], V. Panichi[1], R. Buzi[3], M.T. Muratore[3], M. Montanaro[2]

[1]*Lab. di Citofluorimetria, Osp. S. Anna, Ronciglione*
[2]*UOC Ematoogia, Osp. S. Anna, Ronciglione*
[3]*Lab. Analisi Chimico-Cliniche, Osp. Belcolle, Viterbo*

Daratumumab (DARA) is a human CD38-directed monoclonal antibody indicated for the treatment of patients (pts) with Multiple Myeloma (MM) who have received at least 3 prior lines of therapy including a proteosome inhibitor (PI) and an immunomodulatory agent (IA) or who are double refractory to a PI and an IA. We treated a 61 yrs old woman affected with light chain (LC) K MM with DARA from 31st of March till now. The patient was diagnosed with LC K MM in sept 2008. At diagnosis bone marrow (BM) plasmacells (PC) showed this cytometry pattern: CD138+, CD38+,CD56- . BJ was >2 g/dl, she had anemia, not renal failure. She underwent autologous BM transplantation after PAD induction (Doxorubicine, Bortezomib, Dexametasone) and then in the following relapses she was treated with bortezomib, thalidomide, lenalidomide, pomalidomide, cyclophosphamide,melphalan (M). In Jan 2016 she had a futher disease progression with worsening of renal failure.BM aspiration showed 75% PC. At this stage of progressive disease we mainly monitored disease by free light chain (FLC) assay (Binding Site) because BJ could be underestimated in presence of renal failure. KFLC assay was 1225 mg/L (3.30-19.40), λ FLC 4.96 mg/L (5.70-26.30), ratio 246.98 (0.26-1.65). She started renal adjusted M without improvement. K FLC assay increased to 9929 mg/L, λ 1.71 mg/L and ratio increased to 5806, BJ K 625 mg/L, creatinine 7 mg/dl. So we decided to start DARA 16 mg/kg body with weekly iv infusion for the first 8 weeks (wks), then every 2 wks till 24 weeks and then every 4 wks until disease progression including our patient in a compassionate-use programme. K-FLC assay was performed during therapy and showed an improvement: after 2 courses K FLC was 881 mg/L, ratio 1276, after 5 K-FLC was 103.16 mg/L, ratio 87.42, after 6 K FLC 147.9 mg/L, ratio 30.89, after 8 K FLC 119.79 mg/L, ratio 19.41, after 10 K FLC was 90.92, ratio 8.89. BM aspiration after 9 courses revealed 4% PC: CD138+, CD38-, CD56- with down regulation of CD38 by DARA. Actually patient is clinically stable with improvement in blood count; she started however dialysis in May 2016. Therefore assessment of FLC assay is very important in this subset of MM pts with LC disease and with renal failure

for monitoring response during therapy. Furthermore, DARA monotherapy, showed encouraging efficay in heavily pretreated and refractory pts with MM like this one.

SS23-75

BIOCHEMICAL AND GENETIC CHARACTERIZATION OF A CHOLESTEROL BIOSYNTHESIS DEFECT: A NEW CASE OF STEROL-C4-METHYL OXIDASE DEFECT IN A YOUNG ITALIAN MALE

M.P. Lenza[1], C. Sica[1], M. Gelzo[1], E. Procopio[4], A. Dello Russo[1], G. Frisso[3], G. Corso[2], F. Salvatore[3]

[1]*Dip. Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli Federico II*
[2]*Dip. Medicina Clinica e Sperimentale, Università degli Studi di Foggia*
[3]*CEINGE Biotecnologie Avanzate s.c. a r.l.*
[4]*Sez. Malattie Metaboliche e Muscolari ereditaria A.O.U. Meyer*

Cholesterol plays a pivotal role in cell membrane physiology and in the biosynthesis of steroids, bile acids, and vitamin D. Inborn defects of cholesterol biosynthesis are a group of metabolic disorders presenting with multiple congenital anomalies, growth delay, and psychomotor disabilities. Recently, a new defect of cholesterol biosynthesis, involving the sterol-C-4-methyl oxidase (SC4MOL) enzyme, has been described in four patients as an autosomal recessive disease due to the defect of demethylation of C4-methylsterols (1). Herein, we describe a new case of SC4MOL deficiency. His clinical history reported bilateral congenital cataracts at the age of 8 months; at 4 years old, he showed psychomotor development delay and learning disabilities; at the age of 15 years, he showed small stature, microcephaly, cerebellum hypoplasia, obesity, and behavioural disorder. Despite numerous clinical, biochemical, and genetic examinations, such as array-CGH, X-fragile test, mitochondrial DNA sequencing, amino acids and organic acids in plasma and urine, the diagnosis was missed until the age of 19 years. Based on these evidences, a cholesterol biosynthesis defect was suspected. Sterol analysis by GC-FID and GC-MS showed higher levels of C4-monomethyl- and C4-dimethylsterols in plasma and red blood cell membranes, suggesting a defect of SC4MOL enzyme. Sequencing of the SC4MOL gene showed that the patient is compound heterozygote for two mutations: c.731A>G (p.Y244C), a known mutation, which substitutes an amino acid within highly conserved metal-binding domain; c.605G>A (p.G202E), not previously described, occurring in a trans-membrane site of fatty acid hydroxylase region. It is absent in EXAC database and in 250 healthy Caucasian individuals. Bioinformatics analysis suggests that this substitution may have a pathogenic role. Both his parents are found heterozygous. Therefore, genetic result supports the diagnosis of SC4MOL deficiency. In conclusion, integration between plasma and red blood cell membranes sterol analysis and genetic analysis allows to reach the definitive diagnosis. In addition, genetic result allows to differentiate among overlapping phenotypes, and to establish the exact reproductive risk.

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SS24-77

PROJECT ON QUALITY INDICATORS OF IFCC WG-LEPS

M. Plebani, L. Sciacovelli

Department of Laboratory Medicine, University-Hospital of Padova

According to the International Standard for medical laboratories accreditation, ISO 15189:2012, “The laboratory shall establish quality indicators to monitor and evaluate performance throughout critical aspects of pre-examination, examination and post examination processes” and “The process of monitoring quality indicators shall be planned, which includes establishing the objectives, methodology, interpretation, limits, action plan and duration of measurement”. Therefore, the establishment of quality indicators (QIs) covering the entire testing process should be considered “a must” for complying with the requirements. The laboratory should, therefore, establish and periodically review QIs to monitor and evaluate performance throughout critical aspects of pre-, intra- and post-analytical processes. In fact, there is a large consensus on the vulnerability of the extra-analytical phases and on the need to improve extra-analytical procedures and processes, the current debate is about the strategies to be adopted to establish performance specifications (PSs) and tools for reducing extra-analytical errors. Errors in extra-analytical phases decrease the value of laboratory information, increase unjustified laboratory costs and affect patient safety.
The Working Group of the International Federations of Laboratory Medicine “Laboratory Errors and Patient Safety” has launched a project on a model of quality indicators (MQI), available at the website www.ifcc-mqi.com. The project aims at the harmonization of QIs use in Laboratory Medicine through the management of External Quality Assurance Program (EQAP), the organization of meetings and scientific sessions, and the publications of papers in scientific journals. The harmonization of QIs, in addition to support compliance with the ISO 15189 requirements, allows the identification of a reliable state-of-the-art, which is the first step in defining the improvement goals, and contributes to the reduction of the errors and the improvement of patient safety. All laboratories, at

international levels, have been called to contribute to the success of the project and to participate in the EQAP through the use of a common MQI, collection of data, and reporting of statistical data. The data collected from several laboratories worldwide have provided valuable insight on the state-of-the-art, especially as they were obtained using a harmonized list of QIs and with a homogeneous reporting system. A criterion to establish PSs has been proposed for each indicator in order to make easy the interpretation of QIs results and identification of action priorities. The definition of three different performance goals allows laboratories to evaluate how they are placed in comparison with other laboratories and if improvement actions are possible: the lower percentiles (25th) represent the better performances; the higher percentiles (75th) the worst performance.
The use of PSs is very important, first and foremost, to guide improvement programs and, in addition, represents a benchmark allowing inter-laboratory comparison of performances.

SS24-78
RECOMMENDATIONS FOR IDENTIFICATION AND MANAGEMENT OF CRITICAL VALUES IN CLINICAL LABORATORIES

G. Lippi

Section of Clinical Biochemistry, University of Verona, Verona

The critical values, which are also conventionally referred to as “panic” or “alarm” values, are meant to emphasize a test result associated with an immediate and tangible risk for patient health, thus requiring timely notification to the healthcare personnel in charge of the patients, who should promptly establish the most appropriate therapeutic interventions (1).
Although the implementation of critical values reporting is now universally recognized as a vital standard for the good laboratory practice and a critical element for the accreditation process according to the ISO 15189 standard, the process of identification, routine implementation, notification and clinical management of critical values remains challenging around the globe. Despite reiterated claims by the Joint Commission (National Patient Safety Goals 01.01.01), supranational accreditation bodies and the IFCC working Group on Patient Safety, several lines of evidence attest that the importance of critical values reporting is still underrecognized and underestimated worldwide, nor validated practices for management in the laboratory are widely implemented (2, 3). However, the application of standardized and universally agreed procedures for management of critical values appears now unavoidable to achieve a worldwide harmonization. In this perspective, valuable information can be taken from the guidelines issued by the Italian Society of Clinical Biochemistry and Laboratory Medicine (SIBioC), the Italian Society of Laboratory Medicine (SIMeL), with the Italian Committee for Standardization of Laboratory and Haematological Methods (CISMEL), through the Intersocietary Study Group (GdS) on "Standardization of extra-analytical variability of laboratory results" (4). In fact, the document is aimed to provide consensus recommendations for selection, application and management of critical values in clinical laboratories. Indeed, the development and introduction into hospital information systems of innovative information technology will facilitate to develop effective policies of critical values management, aimed to ultimately increase patient safety.

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SS24-CO15
CHECK-LIST IN LABORATORY MEDICINE: AN IMPORTANT TOOL TO IMPROVE THE PATIENT SAFETY

A. Aita[1], R. Marin[1], E. Piva[2], L. Sciacovelli[2], M. Plebani[1]

[1]*Department of Medicine, University of Padova*
[2]*Department of Laboratory Medicine, University-Hospital, Padova*

Background: Poorly standardized activities, human factors, patient crowding could affect the blood collection process leading to the pre-analytical errors that impact on patient safety. The introduction of Checklist (CL) in laboratory medicine, prerogative of the surgical setting, could be considered an important tool to avoid the recurrence of procedural errors, ensuring the patient safety. Aim. This work aims to describe results concerning the implementation of a Check list (CL) on blood collection procedures in Laboratory Medicine.
Methods: The project, focused on 3 outpatients phlebotomy sites (SMa, SMb and PN), involved 25 physicians and 15 nurses for a month. It concerns: analysis of literature and operating procedures carried out; selection of check-points (CPs); evaluation of CL draft; staff training; a brief period of experimentation; problems of analysis; release of a final CL.
Results: 5661 CL were filled on 9469 venepunctures (59.8%). The percentage of CPs filled in SMa, SMb and PN were respectively: 100- patient identification and label-sample-identification matching; 77.8, 71.0 and 58.5-vein selection; 97.8, 96.7 and 98.4-needle selection;

83.0, 85.8 and 90.6-tourniquet application time; 99.0, 97.9 and 98.7-tubes filling; 98.4, 97.4 and 98.7-tubes mixing; 28.0, 33.02 and 11.2-temperature transport; 20.8, 29.3 and 1.3-time transport.
Conclusions: The CL was very helpful for staff in training, but difficult to use during the patients crowding. The insufficient number of prepared CL copies and difficulties over communication concerning purpose and methodology affected the project results. In order to prevent errors, a CL has to: include only critical CPs, be shared by all staff, be an integral part of a quality management system. Today, the CL focused on blood collection, is used in daily practice as a support tool to prevent and reduce procedural errors, in order to ensure the patient safety.

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SS24-CO16

MISIDENTIFICATION IN LABORATORY MEDICINE: RESULTS OF THE TUSCANY SURVEY - RISK MANAGEMENT STUDY GROUP SIBIOC AND SIE

C. Scapellato[1], F. Balboni[2], M. Quercioli[3], P. Pezzati[4], G. Toccafondi[5], P. Casprini[6], A. Aita[7], R. Guerranti[1], M. Fiorni[8], G. Fuzzi[9], R. Tartaglia[5]

[1]*UOC Patologia Clinica, Azienda Ospedaliera Universitaria Senese*
[2]*Laboratorio Analisi, Istituto Fiorentino Cura e Assistenza, Firenze*
[3]*Centro Regionale di Riferimento per il Controllo di Qualità, Regione Toscana*
[4]*Dip. dei Servizi Lab. Analisi, AOU Careggi, Firenze*
[5]*Centro Gestione Rischio Clinico e Sicurezza del Paziente, Regione Toscana*
[6]*Dipartimento Medicina di Laboratorio, USL Toscana Centro*
[7]*Dipartimento di Medicina (DIMED), Università degli Studi di Padova*
[8]*UOC Medicina di Laboratorio, Livorno, Azienda USL Toscana Nord Ovest*
[9]*Ricerche Cliniche "Prof. Manfredo Fanfani", Firenze*

The Risk Management and Patient Safety study group SIBioC with the Italian Society of Ergonomics developed a survey directed to Tuscany clinical laboratories aimed to investigate the level of awareness on the misidentification issues. The underlining concept is that clinical laboratories can play a central role in disseminating safety culture. The survey was designed by a scientific board and was validated by three validators. The survey consists of 30 questions many related to quality procedures from the non-conformity management to proactive or reactive analysis following adverse events: the focus is on human factors. 21 Tuscany laboratories were and 18 answered (86%). All the Tuscany region is represented. 72% of structures declared to have accomplished ISO 9001:2008-2015 certification and 28% other accreditation typology. A structured preanalytical phase is present in the 67% of laboratories and it is working 24h24, 7 days7 in 50% of structures. Less than half (46%) declares to treat non-conformities with proactive actions (FMECA: 56% and 11% in another way) and to register corrective actions systematically. A misidentified sample is analysed after error correction in 73%, not analysed in the 40% and every action is traced 80%. The non-conformity is telephonically communicated 100%. There is the wide spread use of delta-check 80% with the purpose to identify misidentification errors although in the vast majority (70%) there is not a structured procedure. In the 70% of cases a warning note is added. When a misidentification occurs, the clinical risk management is informed (60%) and in 87% of cases a clinical audit or M&M take places, followed by implementation plan. The IFCC project proposed by WG-LEPS is known only by the 33% of Tuscany laboratories. The results of the survey show that in Tuscany the safety culture is well disseminated and that laboratory personnel are well aware of the importance of preanalytical issues; however, there is room for improvement especially in the application of proactive techniques.

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SS25-81

START UP: RESEARCH AND DEVELOPMENT IN LIFE SCIENCE

F. Cariati[2,3], V. D'Argenio[1,2,3], N. Di Paola[4], R. Vona[4], R. Tomaiuolo[1,2,3]

[1]*Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli Federico II*
[2]*CEINGE-Biotecnologie Avanzate scarl*
[3]*KronosDNA srl*
[4]*Dipartimento di Economia, Management, Istituzioni, Università degli Studi di Napoli Federico II*

In healthcare, in order for something to be classed as an innovation, it has to produce a positive impact, and improve processes. The innovation delivery is able to intensify the innovation effects in terms of improved patient experience, improved quality and decreased costs. Although the importance of academic research to long-term economic growth is generally recognized, however the Italian context of medical research has not yet expressed its full economic potential.
The life sciences place specific barriers to the ability to identify business opportunities and transforming research results into marketable products.

In order to identify solutions that are able to create the ideal conditions to trigger and develop the academic entrepreneurship in the Life Sciences (LS), the accelerator project focuses on the academic entrepreneurship activation mechanisms in healthcare, and the acceleration tools for healthcare academic spin-off companies.
In particular, the entrepreneurial path of One4Two will be described; it refers to a diagnostic kit that allows to perform the screening for genetic and chromosomal disorders related to infertility of couples in a single analytical run, by using a cutting-edge genomics method.
One4Two arises from the idea of a women's team. The original team worked for many years in scientific translational research and is now aspiring for entrepreneurship. Subsequently, the team has been enriched by the management component.
In effect, the typical and value-added feature of a start-up is the multidisciplinary composition of its founder team. It includes the medical and entrepreneurially-vocated component and the managerial one.
Through the launch of multidisciplinary research and educational paths, the know-how gained from the startup will be transferred to other academic spin-offs in healthcare, thus promoting the conditions that improve the academic research outcomes thanks to the consolidation of the University driving role in the innovation ecosystem, the enhancement of patenting activities and the contribution to the local socioeconomic development due to the spin-off companies (the so called “third mission” of the Universities).

SS25-82
FROM TRADITIONAL BIOMARKERS TO CIRCULATING MICRORNAS IN CANCER

M. Montagnana

Sezione di Biochimica Clinica, Dipartimento di Neuroscienze, Biomedicina e Movimento, AOUI Verona

The National Academy of Clinical Biochemistry (NACB) defines tumor markers as “..surrogate indicators that increase or decrease the clinician’s suspicion that future clinically important events, such as cancer onset, recurrence, or progression or patient death, will or will not happen, and/or that a specific treatment will decrease the risk of such events” (1). These molecules may be produced and released either by tumor cells or by host cells, and their presence may be detected in the serum or other biological fluids, behaving as an indicator of the tumor presence. Typically, an ideal tumor marker should be easy to measure and reproducible, a positive result should only occur in patients with malignancy and quantitative levels would correlate with stage and response to treatment. Unfortunately, no one of the currently available tumor markers meets this ideal.
Epigenetics, conventionally defined as heritable change in gene expression that is not attributable to alteration of the DNA sequence, represents a new avenue in cancer research. Reliable evidence is accumulating that epigenetic mechanisms may play a key role in cancer progression and as well as in the onset of chemotherapy resistance (2). Moreover, since blood can easily be collected through a minimally invasive procedure, and also provides the ideal substrate for miRNAs analysis, the assessment of non-coding RNAs (ncRNAs) has been proposed as a valuable perspective for early diagnosis of different cancers (3). However, the clinical transferability of such tests is uncertain due to pre-analytical, analytical and post-analytical variables (4).
Ovarian and endometrial cancer represent the most frequent gynecological cancer in developed countries. In patients affected by this neoplasia, 5-years survival rate is elevated when the diagnosis is made at an early stage, but it dramatically decreases when the cancer is diagnosed at stage IV. Given the high mortality rate of patients diagnosed with advanced cancers, the goal of gynecologists is to make a timely diagnosis and establish an early surgical and/or chemotherapic treatment.
In the last years, our research group performed some studies aimed to evaluate the diagnostic performance of both traditional, as CA125 and Human Epididymis Protein 4 (HE4), and epigenetic biomarkers in ovarian and endometrial cancer.

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SS25-83
NEURODEGENERATIVE DISEASES AND OLIGOMERS: THE ROLE OF CLINICAL BIOCHEMISTRY

G.M. Sancesario

Dept. Clinical and Behavioural Neurology, Santa Lucia Foundation, Roma

Neurodegenerative diseases that belong to the group of proteinopathies share a common pathogenic mechanism, that is the formation of extracellular and/or intracellular deposits of abnormal misfolded proteins. This is the case of Alzheimer's, Parkinson's, Lewy Bodies, Huntington's and prion diseases, among others, in which distinct protein aggregates composed by amyloid β-protein (Aβ), tau, prion protein (PrP), α-synuclein (α-syn) and

huntingtin can be found in the brain of patients and seems to cause these disorders. Although such aggregation-prone proteins have entirely different sequences, they have the common propensity of fold into β-sheet-rich structures that contain one or more shared conformational epitopes and trigger different mechanisms of neurotoxicity and cognitive or functional impairment.

Recent evidences suggest that the formation of insoluble aggregates is preceded by soluble aggregates or oligomers that are not only correlated to fibril formation, but are also neurotoxic compounds. These emerging concepts are exemplified by Alzheimer's disease (AD), in which amyloid - β oligomers (o-Aβ) adversely affect synaptic structure and plasticity [1], and Parkinson's disease (PD), for which in vivo and in vitro studies suggest a toxicity of soluble α-syn oligomers (o-αSyn) for nigral dopaminergic neurons. The proposed role of o-Aβ and o-αSyn in the brain pathology of AD [2] and PD [3] led to an extensive search of their presence in the CSF because, unlike insoluble fibrils deposited in brain tissue, soluble oligomers can diffuse in the CSF and, if detected in sensitive assays, they could constitute a diagnostic tool to be included in Clinical Laboratory Medicine of neurodegenerative diseases.

Several methods have been recently developed in the attempt to analyze diffusible oligomers in the CSF, but results on the normal levels and range are controversial. In AD, some studies reported increased levels of o-Aβ, whereas others showed largely overlapping or decreased level respect to control or different diseases. Similarly, CSF level of α-syn was lower in patients with PD and dementia with Lewy bodies than in those with AD, while other researchers have found no differences. Moreover, a significant decrease of t-α-syn and an increase of o-α-syn levels were found in PD patients [4] by using a specific double-ELISA, whereas other methods with excellent specificity for recombinant in vitro oligomerized α-syn and oligomers in brainstem lysates of human α-syn transgenic mice failed to detect oligomeric α-syn in CSF [5]. Probably, the large variability in the results using different techniques could be due to the heterogeneity of oligomers, the presumably very low concentrations and the high background of monomeric forms.

The research in the field of CSF oligomers in AD and PD seems to be a winding road. A large variability in the results and the lack of a reliable method for assay still hamper the use of CSF oligomers in Clinical Biochemistry as new biomarkers for neurodegenerative disease. A great effort is required to validate a reliable method of analysis and harmonization of results.

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SS25-CO17

POSTPRANDIAL CHOLESTEROL METABOLISM: A CLUE TO THE IMPACT OF TM6SF2 RS58542926 VARIANT ON LIVER AND CARDIOVASCULAR DISEASE IN NAFLD?

R. Gambino[1], M. Cassader[1], F. Saba[1], S. Pinach[1], N. Alemanno[1], G. Musso[2]

[1]*Department of Internal Medicine, University of Turin, Italy*
[2]*Gradenigo Hospital, Turin*

Background: Nonalcoholic fatty liver disease (NAFLD) encompasses a histological spectrum, ranging from simple steatosis to steatosis plus necroinflammation (NASH), which can be differentiated only by liver biopsy. Genetic factors contribute to the pathogenesis of NAFLD. Genetic variation in the transmembrane 6 superfamily member 2 protein (TM6SF2) at rs58542926 was shown to confer susceptibility to NAFLD1. Objective: We examined the impact of this polymorphism on postprandial lipoprotein subfractions and on postprandial changes in cytokeratin-18 fragments in normolipidemic biopsy-proven NAFLD patients and matched healthy controls. Methods: fifty-five nonobese, nondiabetic, normolipidemic biopsy-proven NASH patients and 55 age, sex, BMI-matched healthy controls underwent an oral fat load test, with measurement of plasma triglyceride-rich lipoprotein subfractions, total cholesterol, oxidized LDL, non-esterified fatty acids, E-selectin, ICAM, and cytokeratin-18 fragments. Results: TM6SF2 T-allele carriers showed a lower postprandial triglyceridaemia and nefaemia, and a striking redistribution of cholesterol from smaller, more atherogenic VLDL2, LDL and oxLDL particles to larger VLDL1 subfractions than C-allele carriers. On multiple regression analysis, IAUC VLDL1-Cholesterol during the oral fat load independently predicted NAFLD activity score (β=0.394, p=0.022), IAUC CK-18 (β=0.412, p=0.018) and fibrosis score (β=0.402, p=0.019), while IAUC oxLDL predicted circulating E-selectin (β=0.418, p=0.011) and ICAM-1 (β=0.451, p=0.012). Conclusions: These findings may partially explain the impact of TM6SF2 C>T variant on liver injury and CVD risk in NAFLD. TM6SF2 C>T variant modulates dietary cholesterol fluxes, with T-allele diverting toxic cholesterol away from the vessel walls into the liver, thereby promoting liver injury. Consistently, circulating VLDL-Cholesterol closely correlated with hepatic cholesterol content, inflammation, fibrosis, and cell injury in NASH. If confirmed by larger follow-up studies, these findings may

provide the rationale for evaluating approaches using cholesterol-lowering medications to alleviate hepatic cholesterol overload and liver injury in TM6SF2 T-allele carriers, irrespective of fasting blood cholesterol levels.

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SS25-CO18

FULLY AUTOMATED MASSIVELY PARALLEL SEQUENCING (MPS) LIBRARY PREPARATION FOR GERMLINE BRCA1/2 MUTATION TESTING BY OMNIA LH100 AND LH75 (MASMEC) SYSTEMS

M. De Bonis[1], A. Costella[1], V. Costantino[2], A. Minucci[1], E.D. Capoluongo[1]

[1]*Laboratory of Clinical Molecular and Personalized Diagnostics, Institute of Biochemistry and Clinical Biochemistry, Catholic University, Rome*
[2]*R&D Department Masmec Biomed-Masmec SPA Division, Modugno, Bari*

Introduction: Library preparation for MPS is one of the most critical, on.bench and time-consuming steps in the MPS workflow. Automation of library preparation phase is the best option to avoid the risk of human-introduced error. Our objective was to report a rapid, automated solution to prepare Multiplicom-NGS based protocol for Illumina MiSeq® sequencer for BRCA1/2 genes and to analyze the performance of MPS in terms of number of sequences/run, coverage uniformity and number of variants detected. A total of 120 samples were used to evaluate the automated preparative process. Methods. Complete protocol for BRCA1/2 genes amplification by the BRCA MASTR Dx kit (Multiplicom, Niel, Belgium) was performed using OMNIA LH 100 and OMNIA LH 75 automated workstations designed and produced by MASMEC Biomed (Modugno, Bari). The LH 100 was equipped with a robot (X-Y-Z), 8 independent pipette channels and a layout with two racks for reagents and DNA samples, 9 deck positions for 96 well plates and different size tips and two heating-cooling units for controlled temperature steps. The LH 75 was prepared with a single pipette and a magnetic tool for analyzing 12 samples at the same time and 6 deck positions for 96 well plate and different size tips. Both workstations were controlled by MASMEC Framework software and were provided with UV lamp for decontamination to reduce the risk of cross-contamination. Results. OMNIA platforms were accurately customized to set up libraries preparation and purification process of 12 patients simultaneously per run in only 8-9 working hours (respect to 1-2 working days for manual execution). Because the workstations require very little hands-on time, 12 consecutive samples were prepared for the next automated amplification cycle while the first 12 samples were purified. Thanks to perfect setting of ratio between magnetic beads and DNA amount, a high-quality of BRCA1/2 libraries and MPS performance were obtained. Discussion. This study describes a new automated solution for fast and reproducible BRCA1/2 library preparation for MPS using a robotic workstation. Automated library preparation and MPS performance were comparable to a standard manual library preparation. The throughput of our pipeline was high positively improved by introducing these machines in our routine workflow.

SP26-CO19

IDENTIFICATION AND QUANTIFICATION OF URINARY MONOCLONAL PROTEINS BY CAPILLARY ELECTROPHORESIS IN AL AMYLOIDOSIS

P. Milani[1], G. Palladini[1], F. Russo[1], V. Valentini[1], M. Basset[1], T. Bosoni[2], G. Ferraro[1], L. Pirolini[2], A. Foli[1], F. Lavatelli[1], F. Belvisi[2], M. Nuvolone[1], R. Albertini[2], G. Merlini[3]

[1]*Centro per lo Studio e la Cura delle Amiloidosi Sistemiche, Fondazione IRCCS Policlinico San Matteo, Dip. di Medicina Molecolare, Università di Pavia, Pavia*
[2]*Lab. Analisi Chimico Cliniche, Fondazione IRCCS Policlinico San Matteo, Pavia*
[3]*Direzione Scientifica, Fondazione IRCCS Policlinico San Matteo, Pavia*

Identification and quantification of urinary monoclonal proteins (uMPs) is fundamental in the diagnosis and monitoring of monoclonal gammopathies (Kyle et al. Leukemia 2010). We prospectively assessed the performance of the Sebia Capillarys urine protein capillary electrophoresis (UPCE) and immunotyping in 75 patients with AL amyloidosis. Samples were tested with: homemade high-resolution agarose gel immunofixation electrophoresis (hr-IFE) of serum and concentrated (10 times) urine; commercial semi-automated agarose gel immunofixation of urine (Sebia Hydragel BJ on Hydrasys 2, SHBJ); UPCE and immunotyping (Sebia Capillarys 2 Flex Piercing Urine); quantification of circulating free light chains (FLC) by Freelite and N latex FLC. Urinary MPs were quantified using Sebia Phoresis software tools. Sixty-eight patients in whom uMPs were detected by hr-IFE were included in the study. A uMP was detected by UPCE in 62 cases (91%), and was quantifiable in 55 (81%). The median uMP excretion was 130 mg/24h (range 10-1610). Nine of the 12 patients with dFLC <50 mg/L (Freelite) had a quantifiable uMP (median 90 mg/24h). The uMP was also quantifiable on SHBJ in 51 patients (75%). There was a good correlation between measurements of uMP excretion on UPCE and SHBJ (Pearson's r = 0.87, 95%CI 0.78-0.92). So far, 16 patients with quantifiable uMP and dFLC (Freelite) >50 mg/L were treated and had response data at 3 months. Five subjects responded

with a median 69% dFLC decrease (range 51-90%). In all of them uMP excretion decreased (median 100%, range 30-100%). Among non-responders, only one had a relevant reduction in uMP (from 740 to 250 mg/24h, dFLC from 746 to 619 mg/L) with stable renal function. Post-treatment UPCE was also available in 5 cases with baseline dFLC (Freelite) <50 mg/L. In 2 of them the uMP was still visible but was no longer quantifiable, in 2 it remained stable and in one uMP increased from 20 to 40 mg/24h. UPCE can identify uMPs in patients with AL amyloidosis with a good sensitivity, and can quantify uMP excretion as low as 10 mg/24h. Changes in uMP excretion can be monitored during therapy, including patients with low FLC disease. Further studies are warranted to evaluate the response assessment.

SP26-CO20

ASSESSMENT OF IMMUNONEPHELOMETRY AS SCREENING METHOD FOR LABORATORY DIAGNOSIS OF BENCE JONES PROTEIN

P. Natali, M. Varani, G. Patelli, S. Tagliavini, D. Carra, T. Trenti

S.C. Medicina di Laboratorio NOCSAE, Modena

Introduction: Free light chains κ and λ (FLC) monoclonal immunoglobulin in serum and urine (BJP) are markers of B-cell proliferative disorders. Immunofixation electrophoresis agarose gel in urine (uIFE) is the reference method for the determination of BJP, while the Immunonephelometric assay (INA) to detect FLC in urine is accepted as an initial screening method to exclude negative samples. The aim of the present study was to compare the performance of INA and uIFE methods in urine samples and to estimate the capability of INA to select BJP negative samples.

Methods: The urine samples were received by Corelab NOCSAE Modena. One thousand of unconcentrated ones were analyzed by INA method, with FLC antiserum (New Scientific Company -NSC) by Nephelometer Beckman Coulter: we considered negative the urine samples with both the concentration of FLC κ or λ ≤10 mg /L, as suggested by NSC, and FLC κ or λ ≤5 mg /L, as indicated in the literature. Within 48 hours the same 1000 urine samples were tested by uIFE, with antiserum anti-heavy chains, anti-FLC κ and λ (Sebia): those that showed a homogeneous band in the FLC κ or λ track were considered as positive. The findings of both INA FLC >10 mg/L and FLC >5 mg/L (INA+) with uIFE results were compared.

Results: Seventy-one urine were found positive for BJP (7.1%). Data obtained with cut off FLC κ or λ >10 mg/L: urine 39 uIFE+ and INA+; 815 uIFE- and INA-; 114 uIFE – and INA+; 32 uIFE+ and INA-. Sensitivity 55%; Specificity 88%; LR + 4.5; LR- 0.5; Accuracy 85%; Choen's kappa 0.3. Data obtained cut off FLC κ or λ >5 mg/L: urine 53 uIFE+ and INA+; 693 uIFE- and INA-; 236 uIFE – and INA+; 18 uIFE+ and INA-. Sensitivity 75%; Specificity 75%; LR+ 2.9; LR- 0.3; Accuracy 75%; Choen's kappa 0.2.

Conclusions: The results obtained using INA to select the negative urine for BJP are unsatisfactory considering both proposed cut off of FLC ≤10 mg/L and FLC ≤5 mg/L. The correlation between the results of INA and uIFE methods is poor, as a high number of false positives were found and an even higher number of false negatives. Therefore INA is an unsuitable screening method even only to detect negative samples.

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Torino, 18-20 ottobre 2016

Riassunti Poster

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P092-P094	Diabete e sindrome metabolica
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Nota dell'Editore: i riassunti sono stati riprodotti senza alcuna revisione dal materiale direttamente fornito dagli autori.

P001

HIGH SENSITIVITY OF THE BASOPHIL ACTIVATION MARKER CD203C IN A DIAGNOSTIC PROTOCOL FOR PARIETARIA SENSITIZATION: A CASE REPORT

M. Caruso[1], R. Emma[1], L. Fabbella[1], G. Di Fazio[1], S. Bellinvia[2], R. Polosa[1]

[1]*Dipartimento di Medicina Clinica e Sperimentale, Laboratorio di Immunologia Sperimentale, Università degli Studi di Catania, Catania*

[2]*Dipartimento di Medicina Interna e d'Urgenza, A.O.U. Policlinico-OVE, Catania*

Aims: We report the case of a 26-years-old man who showed seasonal allergy symptoms, including sneezing, nasal itching and rhinorrhea, from two years. Allergic status was investigated by skin prick test (SPT) and measurement of specific IgE to aeroallergens (ImmunoCAP–Thermo Scientific). SPT resulted negative for all the environmental allergens tested. Instead, we observed a slight increase of IgE to Parietaria (0.14 kUA/L) as a "possible sensitization". Moreover the patient resolved all symptoms by antihistamines. Our purpose is to verify if the slight increase of IgE to parietaria is confirmed by the Basophil Activation Tests (BATs) by using CD203c and CD63 markers(1).

Methods: On peripheral blood sample of the patient we performed the analysis of expression of the two markers CD63 and CD203c on basophils. Analysis was carried out in two series of tubes, one for each marker. Both negative (PBS) and positive (anti-IgE) controls were prepared for each test. Aliquots of blood were incubated with two different dilutions (1:1, 1:10) of allergen (mix parietaria – Bial) in a Phosphate Buffered Solution. Stained samples were analysed by Flow Cytometry. Positivity was established by the Stimulation Index (SI) defined as the ratio between the percentage of positive cells in allergen-stimulated samples and in unstimulated samples.SI≥3 was consider as positive.

Results: The flow cytometric analysis of CD63 was negative for both the dilutions of Parietaria allergen, 1:1 (SI =0,87) and 1:10 (SI=0,65). Instead, the patient showed a positive response to parietaria stimulation for CD203c with both of the allergen dilutions, 1:1 (SI=4,67) and 1:10 SI=3,22).

Conclusion: The results showed that BAT performed with the activation marker CD203c is in accordance with the result of specific IgE. Similarly, BAT performed with the degranulation marker CD63 reflects the negative result of skin prick test. Thus, CD203c seems to be a useful marker able to confirm a slight sensitization to Parietaria, doubtfully detected by ImmunoCAP,whereas CD63 and skin prick test fails to detect it.

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P002

SMALL RNA ANALYSIS TO IDENTIFY NOVEL DIAGNOSTIC AND THERAPEUTIC MARKERS FOR COW'S MILK ALLERGY

V. D'Argenio[1], V. del Monaco[2], L. Paparo[3], F.E.D. De Palma[1], R. Nocerino[3], F. Salvatore[1], R. Berni Canani[3]

[1]*CEINGE-Biotecnologie Avanzate and Department of Molecular Medicine and Medical Biotechnologies, University of Naples Federico II, Italy*

[2]*CEINGE-Biotecnologie Avanzate, Naples, Italy*

[3]*Department of Translational Medical Science, University of Naples Federico II, Italy*

Epigenetic mechanisms have been involved in several allergic diseases, including cow's milk allergy (CMA). According to this hypothesis, we have recently assessed that alterations in the DNA methylation of the Th1 and Th2 cytokine genes affect CMA (1). Since also small RNAs have been implicated in the pathogenetic hypothesis of allergies, to address this issue, we used a next-generation sequencing (NGS)-based approach to analyze the miRNA profiles in CMA children respect to healthy children.

Totally, 18 subjects were enrolled, 10 CMA patients and 11 healthy subjects. Peripheral blood samples were collected from each study subjects to obtain RNA. After RNA quality assessment, small RNA libraries were prepared and sequenced according to manufacturer's indications (Illumina).

Differential expression analysis highlighted 5 up-regulated and 20 down-regulated miRNAs in CMA children versus healthy controls. Notably, we were able to identify one miRNA significatively differentially expressed between CMA patients at diagnosis and healthy controls that has been predicted to regulate the expression of genes involved in interleukin's production.

If confirmed by further evaluations, our finding could provide a novel CMA biomarker for disease diagnosis and/or monitoring.

1. Berni Canani R, Paparo L, Nocerino R, et al. Differences in DNA methylation profile of Th1 and Th2 cytokine genes are associated with tolerance acquisition in children with IgE-mediated cow's milk allergy. Clin Epigenetics 2015;7:38.

P003
VERIFICATION OF THE ACCURACY OF THREE GLUCOSE POINT-OF-CARE TESTING (POCT) DEVICES FOR THEIR USE IN A HOSPITAL SETTING

E. Aloisio, E. Frusciante, A. Dolci, M. Panteghini

Centro di Ricerca per la Riferibilità Metrologica in Medicina di Laboratorio (CIRME), Università degli Studi, Milano

Background: Inaccuracy in glucose POCT can lead to inappropriate therapeutic decisions. Here we evaluated the performance of three POCT glucometers by comparing results to those by an automated system traceable to higher-order references.
Methods: 31 heparinized venous blood samples were collected and assayed in duplicate for whole blood glucose concentrations with Roche Accu-Check Compact Plus, Nova Biomedical NovaPro and OKBiotech OKmeterDirect glucometers. All systems were calibrated to report plasma-equivalent results. Samples were then centrifuged and plasma glucose was immediately determined by the hexokinase assay on Abbott Architect c16000 platform. The traceability of Abbott assay was checked by comparison with the hexokinase reference procedure performed on three samples. POCT performance was evaluated according to CLSI POCT12-A3 criteria [max 5% of results >±12 mg/dL (for reference results <100 mg/dL) or >±12.5% (for reference results ≥100 mg/dL)] and consensus error grid (CEG) analysis.
Results: The Abbott assay was perfectly standardized (mean bias, 0.13%). Sample glucose concentrations were from 62 to 326 mg/dL, with haematocrit spanning from 0.27 to 0.58 L/L. Average CV on duplicates was 2.2% Roche, 3.3% Nova and 5.9% OKBiotech. All meters gave more than 5% of results (Roche 19.4%, Nova 16.1% and OKBiotech 22.6%) outside the CLSI criteria. However, all results, except two borderline values for OKBiotech, were within the low-risk zone according to CEG.
Conclusions: By using CLSI acceptability criteria, the evaluated glucometers were not accurate enough for clinical use. CEG analysis suggests, however, that this inaccuracy would not have any significant impact on patient outcome.
Sacks DB, Bruns DE, Horton J et al. Point-of-care blood glucose testing in acute and chronic care facilities; approved guideline – Third edition. The Clinical and Laboratory Standards Institute 2013, document POCT12-A3.

P004
CREATININA IN REGIME DI POCT: DUE ANALIZZATORI A CONFRONTO

R. Daniele[1], A. Marcantoni[1], E. Radin[2], M. Di Benedetto[1]

[1]S.C. Analisi Cliniche, Osp. U. Parini, Aosta
[2]S.C. Nefrologia e Dialisi, Osp. U. Parini, Aosta

La disponibilità di emogasanalizzatori che misurano anche la creatinina rappresenta un utile supporto nella gestione dei pazienti con patologie renali sia in situazioni di urgenza che in strutture assistenziali ubicate lontano dagli ospedali. In questo lavoro sono state confrontate le prestazioni dell'analizzatore portatile Epoc Reader (ER, Alere) e dell'ABL827flex (De Mori) comparandole con quelle del Cobas8000 (Roche). Tutti i metodi si basano sulla determinazione enzimatica. La valutazione è stata effettuata su campioni di sangue venoso prelevati da 40 pazienti (20 maschi e 20 femmine, età media 57 anni) afferenti al nostro Centro Prelievi nell'arco di 2 settimane. I valori di creatinina erano compresi tra 0,36 e 3,74 mg/dL per lo strumento ER, tra 0,53 e 3,59 per l'ABL, tra 0,53 e 3,60 per il Cobas. La differenza media tra le determinazioni ottenute dall'analizzatore ER e quelle del Cobas è stata pari a -0,11 mg/dL (LOA = Limits Of Agreement: -0,28 e +0,07 mg/dL), mentre tra ABL e Cobas si è riscontrata una differenza media di -0,06 (LOA: -0,15 e +0,02). Esaminando il grafico secondo Bland & Altman si evidenzia che, per entrambi gli analizzatori, l'entità della differenza con il metodo di riferimento è indipendente dal valore di creatinina. Applicando la formula CKD-EPI è stato poi calcolato il filtrato glomerulare (GFR). Le differenze tra i due analizzatori e il Cobas sono state riportate in una griglia secondo Shermock basata sui valori delle 6 classi di stadiazione della malattia renale cronica: la collocazione nella classe di filtrato risulta corretta per entrambi gli strumenti, ad eccezione della fascia compresa tra 80 e 86 mL/min/1,73 m^2, in cui si osserva una sovrastima (minore per ABL che per ER) rispetto al metodo di riferimento. La sovrastima osservata è comunque di scarso impatto clinico, tranne in due casi in cui la differenza di GFR era superiore a 20 mL/min/1,73 m^2. Si ritiene opportuno estendere lo studio a pazienti con malattia renale cronica più avanzata per evidenziare eventuali classificazioni scorrette che potrebbero incidere in maniera clinicamente significativa sulla gestione del paziente.
Zaninotto M, Miolo G, Guiotto A, et al. Quality performance of laboratory testing in pharmacies: a collaborative evaluation. Clin Chem Lab Med 2016. doi: 10.1515/ cclm-2016-0104. Epub ahead of print.

P005
L'EMOGASANALIZZATORE PORTATILE: L'EVOLUZIONE DEL RUOLO DEL LABORATORIO NEL MONITORAGGIO DEL PAZIENTE CRONICO

N. Jordaney, M.F. Condello, G. Bonfant, M. Di Benedetto

S. C. Analisi Cliniche, Osp. U. Parini, Aosta

L'aumentata disponibilità di emogasanalizzatori (EGA) da banco e portatili in regime di Point-of-care testing (POCT) per l'uso extraospedaliero (assistenza sanitaria di base, domiciliare, ambulatoriale, zone geografiche di difficile accesso, residenze sanitarie) ha permesso di ridurre il TAT. Il monitoraggio clinico di pazienti con patologie croniche viene agevolato dalla disponibilità di un risultato immediato di pH, gas ematici, elettroliti, ematocrito, emoglobina e metaboliti, consentendo appropriate decisioni relative a ossigeno terapia, equilibrio acido-base, idratazione, con la garanzia di un livello di qualità analitica analogo al Laboratorio o almeno adeguato alla finalità terapeutica.
Materiali e metodi: il sangue di 40 soggetti esterni è stato raccolto in siringhe con eparina bilanciata, immediatamente analizzate sul l'EGA portatile ER (Epoc Reader, Alere) e su quello da banco ABL825flex (De Mori) e in provette con K_2 EDTA processate sul DxH800 (Beckman Coulter).
Risultati e discussione. Confronto tra i due EGA: le medie dei valori di pH ed elettroliti (Na^+, K^+, Ca^{++}, Cl^-) sono sovrapponibili; la lieve differenza media della pCO_2 (3,5 mmHg) tra ER e ABL induce un aumento del valore di bicarbonati di ER di circa 2 mmol/L (7%) clinicamente poco rilevante; sui valori di pO_2, si è riscontrata una lieve sottostima (2,5 mmHg). Confronto EGA vs DxH (riferimento): sullo strumento ER, il parametro Hb calcolato in base all'Hct, risulta sovrastimato del 10% rispetto al DxH, che ne esegue una determinazione diretta; per l'ABL, che usa un metodo di dosaggio analogo al DxH, la differenza media è di 0,2 g/dL; l'Hct ER, misurato con metodo conduttimetrico, mostra una differenza media con DxH (2,9 g/dL) maggiore di quella ottenuta con ABL (1,1 g/dL) che calcola l'Hct a partire dell'Hb; ne consegue una riduzione dell'utilità di Hb e Hct ER ai fini diagnostici. Vista la facilità d'uso, l'immediatezza dei risultati e la possibile connettività, si ritiene che i risultati ottenuti siano accettabili ai fini del monitoraggio di pazienti cronici in contesti ambientali extraospedalieri.
Leino A, Kurvinen K. Interchangeability of blood gas, electrolyte and metabolite results measured with point-of-care, blood gas and core laboratory analyzers. Clin Chem Lab Med 2011;49:1187-91.

P006
POINT OF CARE TESTING (POCT): VALUTAZIONE DELL'IMPRECISIONE ANALITICA

F. Marciano[2], A. Miscioscia[1], M. Savoia[1]

[1]D.A.I. MedLab – A.O.U. Federico II, Napoli
[2]Sc. Spec. Biochim. Clin., Univ. Studi di Napoli Federico II

Scopo del lavoro: Il Laboratorio deve governare l'intero processo pre-analitico, analitico, e post-analitico dei POCT, garantendo la conformità alle regole adottate in Laboratorio e l'omogeneità delle procedure nei diversi siti. L'impiego del Controllo di Qualità Automatico (CQA) permette l'esecuzione automatica, programmata dal Laboratorio, del Controllo di Qualità Interno (CQI) sulle singole strumentazioni. In questo studio abbiamo valutato l'imprecisione analitica dei 16 emogasanalizzatori POCT dell'AOU Federico II di Napoli.
Materiali e metodi: Sistemi a cartuccia, RAPID Point 500 (Siemens) che misurano: pH, $PaCO_2$, PaO_2, Na^+, K^+, Cl^-, Ca^{2+}, HCT, Glucosio, Lattato, Bilirubina non coniugata, tHb, HHb, O_2Hb, COHb, MetHb. La cartuccia CQA contiene soluzione tampone bicarbonato, Na^+, K^+, Ca^{2+}, Cl^-, CO_2, O_2, Azoto, Glucosio, a 3 livelli di concentrazione. Sono stati valutati, impiegando lo stesso lotto di CQA, i CV per singolo parametro e singolo POCT, nell'arco di 3 mesi. L'accettabilità dei dati segue le regole di Westgard e se i valori non rientrano negli intervalli stabiliti, il parametro viene automaticamente disattivato. Il Software (RAPID Comm,Siemens) dedicato alla gestione dei sistemi emogasanalitici consente l'acquisizione dei dati dai singoli POCT collegati in rete al Laboratorio.
Risultati: CV medi osservati per i 3 livelli: pH, $PaCO_2$, PaO_2, Na^+, K^+, Cl^-, Ca^{2+}, Glucosio e tHb, tra 0,01(pH) e 2,4(pO_2); Bilirubina non coniugata: 2,3-3,9; Lattato: 2,8-4,6. Le frazioni dell'Hb, mostrano CV medi più elevati, 0,94-9,1, anche in considerazione dei bassi livelli misurati. Su 69964 controlli (x_m = 97 determinazioni, per parametro e singolo livello) il 99,7%, sono risultati compresi negli intervalli di x_m ±1 DS e solo 0,26% tra 1 e 2 DS e lo 0,04% tra 2 e 3 DS, spingendoci a restringere l'intervallo di accettabilità sempre entro x_m ± 2 DS.
Conclusioni: La qualità dei risultati in POCT deve essere assicurata e continuamente monitorata dal Laboratorio. Il contenimento dell'imprecisione analitica, insieme a programmi di VEQ (Valutazione Esterna di Qualità) rappresentano strumenti indispensabili nel garantire l'affidabilità dei sistemi analitici in uso.
Di Serio F, et al. Documenti SIBioC – Biochim Clin 2011;35:242-52.

P007
DROPLET DIGITAL PCR APPLICATION FOR HEPATITIS DELTA VIREMIA QUANTIFICATION

A. Olivero[1], M.L. Abate[1], G. Niro[2], G.P. Caviglia[1], C. Rosso[1], A. Ciancio[1], R. Fontana[2], A. Smedile[1], M. Rizzetto[1], G.M. Saracco[3]

[1]Dep. of Medical Sciences, University of Turin, Turin, Italy
[2]Gastroenterology, CSS Hospital, San Giovanni Rotondo, Italy
[3]Dep. of Oncology, University of Turin, Turin, Italy

Introduction and aim: Quantification of Hepatitis Delta Virus (HDV) viremia is critical in management of HDV patients. Droplet Digital PCR (ddPCR) is a recent technology that allows a direct measurement of viral nucleic acid with absolute quantification (1). Our aim was to design a new assay for HDV-RNA viremia quantification using ddPCR technology and compare it to a Real Time quantitative PCR (RT-qPCR) assay.
Methods: Primers and probe were designed from conserved regions of aligned sequences of different HDV genotypes. Quantitative linearity and range were determined analyzing dilution series of the WHO HDV standard and of an "in house" cDNA standard. For diagnostic evaluation quantitative data obtained analyzing 25 HDV-RNA positive patients samples were compared.
Results: Both methods showed excellent linearity with estimated slope coefficients ranging from 0,97 to 1,02 and R^2 values very closed to 1. ddPCR had a quantitative linear dynamic range between 1,00E+02 to 1,00E+07 copies/ml, the linear range for RT-qPCR was from 5,00E +02 to 1,00E+08 copies/ml. Using as reference the WHO HDV standard the conversion factor from copies/ ml to IU/ml was 0,944 for ddPCR and 0,888 for RT-qPCR. A significant degree of correlation (r_s = 0,9) was reported comparing viral load data obtained by the two different methods in the evaluation of 25 HDV-RNA clinical samples at different concentration. Both assays were able to quantify HDV-RNA viremia from samples of HDV genotypes 1, 2, 5, 6, 7 and 8.
Conclusions: Our preliminary data suggest that ddPCR is a highly accurate technique with a precision and reproducibility comparable to RT-qPCR. Further studies are required to evaluate the limit of detection and performance for low viral load. However ddPCR with its feature of absolute quantification independent from PCR reaction efficiency and a calibration standard curve could be a viable alternative to RT-qPCR.
1. Hudecova I. Digital PCR analysis of circulating nucleic acids. Clin Biochem 2015;48:948-56. doi: 10.1016/ j.clinbiochem.2015.03.015. Epub 2015 Mar 28. Review. PMID: 25828047.

P008
EVALUATION OF MIR-203 EXPRESSION LEVELS AND PROMOTER METHYLATION STATUS IN SERUM OF ENDOMETRIAL CANCER PATIENTS

M. Benati[1], M. Montagnana[1], E. Danese[1], E. Paviati[1], S. Giudici[2], M. Franchi[2], G. Lippi[1]

[1]Department Neurosciences, Biomedicine and Movement Sciences, Clinical Biochemistry Section, University of Verona
[2]Department Surgical Sciences, Dentistry, Gynecology and Pediatrics University of Verona

Aim: Endometrial cancer (EC) is the fourth most frequent female cancer in Europe. In attempt to facilitate early diagnosis, many studies identified putative biomarkers for gynecologic cancers including circulating microRNAs (miRs) and aberrant promoter methylation status. Some previous studies investigated the miR-203 expression profiles in EC tissues and normal endometrial tissues, concluding that miR-203 expression is regulated by methylation promoter. Aim of this work study was to compare the expression of miR-203 and the promoter methylation levels in serum of EC patients and healthy controls (HC).
Methods: 45 EC patients (63.9±12.0 years) and 30 HC (63.4±13.1 years) were enrolled prior to surgical management. RNA extraction was performed by using the mirVANA PARIS Kit (Applied Biosystems). MiR expression was determined by quantitative RT-PCR (Applied Biosystems). The expression levels of miR were normalized to miR-16 and calculated using the $2^{-\#Ct}$ method. A quantitative methylation-specific PCR (MSP) technique was used to analyze the miR-203 promoter methylation status. Differences between groups were evaluated assessed by Mann-Whitney test (for continuous variables) and chi-squared test (for categorical variables), whereas correlations were calculated by using Spearman's test. The diagnostic performance of miR and methylation status was estimated calculated by means of receiver operator characteristic (ROC) curves. The level of statistical significance was set at $p < 0.05$.
Results: Serum levels of miR-203 were higher in EC patients compared to HC (p=0.0015). Aberrant miR-203 methylation was detected in 11/45 (24.4 %) of EC patients and in 2/30 (6.6%) of HC (p=0.04). The expression levels of miR-203 were not significantly correlated with the promoter methylation status. The area under the curve of miR-203 expression and methylation promoter levels was 0.70 and 0.60, respectively.
Conclusion: Aberrant miR-203 methylation was found to be a molecular event in the cancerogenesis. However, we found that miR-203 serum expression levels in EC retains better diagnostic performance than methylation levels.
Huang YW, et al. Hypermethylation of miR-203 in endometrial carcinomas. Gynecol Oncol 2014;133:340-5.

P009

PROGNOSTIC CORRELATES OF THE IMMUNE GENE EXPRESSION PROFILE DETERMINED DIRECTLY ON LIVER TISSUE SAMPLES OF HEPATOCELLULAR CARCINOMA PATIENTS

C. Carone[1], A. Olivani[2], T. Trenti[1], G. Missale[2], E. Cariani[1]

[1]Lab. of Toxicology, Dept. of Laboratory Medicine and Pathology, Nuovo Ospedale S. Agostino-Estense, Modena, Italy
[2]U.O. Infectious Diseases and Hepatology, Azienda Ospedaliero-Universitaria di Parma, Parma, Italy

Background and aims: Several studies support the relevance of anti-tumor immune response for the clinical outcome of human hepatocellular carcinoma (HCC) [1]. In the present study we evaluated the possible prognostic impact of immune gene expression profile determined directly on liver tissue samples.
Methods: RNA was extracted from frozen liver tissue samples of HCCs (n. 12) and paired non-tumorous tissues (n. 10) using the Norgen's Total RNA Purification Kit.
The immune gene expression profile was performed by the nCounter ® GX Human Immunology v2 system (NanoString Technologies) which relies on direct hybridization of RNA with fluorescent barcoded probes complementary to 579 immune response-related genes. For inter-assay variability evaluation, a commercial normal liver RNA sample was tested in each experimental session. Statistical analysis of results was carried out by nSolver ® Analysis, TIGR MeV and Graph Pad Prism softwares.
Results: Inter-assay CV ranged from 18 to 2% for low and high-expression housekeeping genes, respectively; while mean signal (mean of low-level positive controls) to background (mean of negative controls) ratio was 4.5.
Unsupervised clustering of immune gene expression profiles identified two main clusters of expression both in tumorous and in non-tumorous liver samples. Each expression cluster was associated with different median time to HCC recurrence (tumorous tissue: 10.5 vs 42 months, p=0.03; non-tumorous tissue: 11 vs 42 months, p= 0.01). No significant relationship was observed between immune gene expression profile and overall survival.
Preliminary results showed that worse outcome was apparently related to lower expression of immune response-related genes in tumor tissue and to higher expression of immune activation-related genes in non-tumorous liver tissue.
Conclusions: The nCounter ® Human Immunology v2 system appears to be a reproducible, easy-to-use and reliable assay for the evaluation of immune gene expression that could be determined directly on liver tissue without the need of isolating infiltrating cells. Preliminary results show that immune gene expression profiles correlated to the clinical outcome of patients can be identified both in HCCs and in non-tumorous liver tissue samples.
1. Sachdeva M, et al. Immunology of hepatocellular carcinoma. World J Hepatol 2015;7:2080-90.

P010

CHANGES IN FRACTURE RISK-ASSOCIATED CIRCULATING MIRNA PROFILE CHANGES OVER AN 8-WEEK REPEATED SPRINT TRAINING PROTOCOL

G. Lombardi[1], V. Sansoni[1], S. Perego[1], G. Vernillo[2], M. Bonzanni[3], G. Merati[4], A. Barbuti[3], A. La Torre[2], G. Banfi[1]

[1]Lab. Biochimica Sperimentale e Biologia Molecolare, I.R.C.C.S. Istituto Ortopedico Galeazzi, Milano
[2]Dip. Scienze Biomediche per la Salute, Università degli Studi di Milano, Milano
[3]Dip. Bioscienze, Univ. degli Studi di Milano, Milano
[4]I.R.C.C.S. Fondazione Don Carlo Gnocchi, Milano

Introduction: Although the underlying mechanisms are largely unknown, bone metabolism is finely regulated by specific microRNAs. Changes in their expression are associated with metabolic bone disorders and increased fracture risk [1]. Exercise is a well-known determinant of bone metabolism but different training protocols differently affect bone [2, 3]. Currently, little is known about the effects of exercise on bone-specific miRNA expression. We studied the effect of a 8-week high-intensity training protocol on circulating levels of selected miRNA associated with fracture risk [4].
Methods: 18 male adults were assigned to either EXP group (n=9), who performed repeated sprint training (3 times/week, 18 maximal all-out 15m sprints with 17s of passive recovery), or CTRL group (n=9). Blood was taken before the start of the protocol (T0) and after 4 (T1) and 8 weeks (T2). miRNA-enriched total RNA was extracted from plasma and RT and Real-Time PCR were performed (Sp6 and Cel39: internal controls). miR-21-5p, miR-23a-3p, miR-24-3p, miR93-5p, miR-100-5p, miR-122-5p, miR-124-3p, miR-125b-5p, miR-148a-3p, miR-637 were tested. Relative expressions were calculated by the $2^{-\#\#CT}$ method using miR-425-5p as housekeeping. Hemolysis was checked by the miR-23a-to-miR-451 ΔCT ratio (positive if >7). Changes over time where tested by repeated measure one-way ANOVA; inter-group differences at each time-point were tested throughout t test.
Results: In our cohort, miR-637 and miR-124-3p were undetectable. In CTRL miRNA levels remained stable with a very high inter-individual variability. In EXP, miR-21-5p, miR-93-5p, and miR-125b-5p did not change. miR-23a-3p, miR-24-3p, miR-100-5p showed a net decrease between T0 and T2 ($p< 0.01$), even if miR-100-5p had a mid-time increase at T1. miR-122-5p and miR148a-3p decreased from T0 to T1 ($p <0.001$) and then returned to baseline at T2.
Conclusions: An 8-week long high-intensity training protocol downregulates the expression of circulating miRNA associated with fracture risk.
1. Gámez B, Rodriguez-Carballo E, Ventura F. J Mol Endocrinol 2014;52:R179-97.
2. Lombardi G, Sanchis-Gomar F, Perego S, et al. Endocrine Epub.
3. Xu J, Lombardi G, Jiao W, et al. Sports Med Epub.
4. Chen J, Qiu M, Dou C, et al. Drug Development Res 2015;76:235-45.

P011

K-RAS MUTATIONS DETECTION IN CIRCULATING EXOSOMES OF PATIENTS WITH PANCREATIC DUCTAL ADENOCARCINOMA: A STUDY ON ANALYTICAL FEASIBILITY

M. Pelloso[1], C.F. Zambon[1], A. Padoan[2], D. Bozzato[2], A. Aita[2], M. La Malfa[1], V. Aneloni[3], C. Pasquali[4], C. Sperti[4], D. Basso[1], M. Plebani[2]

[1]Dept. of Laboratory Medicine, University of Padova, Padova, Italy
[2]Dept. of Medicine-DIMED, University of Padova, Italy
[3]Unità Operativa Immunotrasfusionale, University Hospital of Padova, Italy
[4]Dept. of Surgical, Oncological and Gastroenterological Sciences DISCOG. University of Padova, Padova, Italy

Background: early diagnosis of pancreatic ductal adenocarcinoma (PDAC) is a key element to improve patients prognosis. k-ras gene mutations establish early in carcinogenesis and are present in almost 90% of PDACs. Exosomes shed in the extracellular compartment also by cancer cells are a non invasive and enriched source of circulating tumour DNA (exoDNA). Aim of this project is to verify whether the recognition of k-ras gene mutations in exoDNA is analytically feasible.
Methods: We verified pre-analytical processing (sample type, temperature, duration of storage, centrifugation protocol and exosome isolation procedures) on exoDNA. Blood from 5 donors was collected in serum and EDTA tubes and kept at room temperature (RT) or refrigerated (COLD). Samples were centrifuged one or two times after 30 minutes (30min) and 3 hours (3h). Aliquots (-80°C) were used for exosome isolation by means of two commercial kits: Total Exosome Isolation Kit (Life Technologies) (A) and ExoQuick precipitation solution Kit (System Biosciences) (B). DNA was extracted and quantified (fluorimetric assay).
exoDNA was obtained from sera of 11 PDAC patients (T2-3, N0, M0). Gly12Asp and Gly12Val k-ras mutations were analyzed by CAST PCR (Life Technologies). Results were compared with those from matched neoplastic and normal adjacent tissues.
Results: Mean efficiency of exoDNA isolation (ng DNA/mL of sample) was significantly higher in serum (15,94±11,51 ng/mL) than in plasma (5,13±2,27 ng/mL) ($p < 0,001$) being independent on the number of centrifugations. Among sera mean efficiency of exoDNA isolation was significantly higher in (RT, 3h) samples (kit A=27,75±9,03 ng/mL and kit B=30,61±7,72 ng/mL) than in (COLD, 30 min) samples (kit A=13,10±8,07 ng/mL and kit B=14,22±7,75 ng/mL) ($p < 0,05$). k-ras mutations were detected in 10/11 tumour samples (7/11 Gly12Val and 3/11 Gly12Asp). No mutation was detected in normal adjacent tissues. Mean concentrations of exoDNA extracted from PDAC patients' sera was (57,74±51,20 pg/μL) and no k-ras mutation was detected.
Conclusions: the overall low concentration of exoDNA extracted from peripheral blood hampers K-ras mutation detection due to a low probability of sampling tumour exoDNA in the analytical phase.
Kahlert C, et al. J Biol Chem 2014;289:3869-75.

P012

LA RICERCA E L'IDENTIFICAZIONE DELLA NEISSERIA GONORRHOEAE ESPERIENZA DEL LABORATORIO DELL'AZIENDA OSPEDALIERA SAN GIOVANNI ADDOLORATA DI ROMA

M. Cenci, P.M. Placanica, R. Thaller, M. Gaudio

U.O.C. Patologia Clinica, Azienda Ospedaliera San Giovanni Addolorata, Roma

La Neisseria gonorrhoeae (NG) è l'agente eziologico di una delle malattie sessualmente trasmesse più diffuse al mondo che negli uomini provoca secreto muco-purulento ma nelle donne è spesso asintomatica. Se non trattata può avere conseguenze gravi e permanenti come la sterilità.
La diagnosi si effettua mediante: esame microscopico (EM) del secreto uretrale; esame colturale (EC) che ne rappresenta il gold standard, a condizione che si rispettino le corrette modalità di conservazione e trasporto; biologia molecolare (BM).
Nel nostro studio 5572 pazienti, 5234 donne e 338 uomini, afferenti al nostro laboratorio (4540 tamponi cervicali, 639 vaginali e 393 urine) dal 1/12/12 al 30/9/15 hanno effettuato il test NG in BM.
E' stato usato il sistema Cobas 4800 (Roche) che identifica contemporaneamente la Chlamydia trachomatis (CT) e la NG in PCR Real time da campioni di urina per l'uomo, cervicali o vaginali (sensibilità diagnostica identica) o di urine per la donna.
Sono stati identificati 15 casi positivi per NG (0.27%): 12 uomini, età media 33 anni, (3,55%) e 3 donne, età media 44 anni (0,057%).
Gli uomini erano sintomatici: 4 provenivano dal centro prelievi e 8 dall'Ambulatorio di Immunologia Clinica (AIC) della nostra Azienda.
In tutti i 12 casi è stata richiesta la CT e l'EC, l'EM solo negli 8 casi dell'AIC.
Solo un uomo era positivo anche per CT. In tutti i 12 casi l'EC era negativo per NG. L'EM, nei casi richiesti, era positivo per diplococchi Gram negativi.
L'EC era positivo in 1 donna e negativo in 2 che non pensavano di essere affette da gonorrea: una delle 2 ripetè il test dopo 15 giorni, riferendo di non aver fatto terapia, con risultato negativo per NG (falso positivo della BM?)
Il nostro studio evidenzia come il test in BM abbia rappresentato la conferma al sospetto clinico di uomini sintomatici con EM positivo ed EC negativo. Nelle donne l'identificazione della NG può essere una scoperta accidentale e quindi non curata dalla paziente se non confermata da altri metodi come l'EC.
Vengono confermati i dati italiani sulla bassa prevalenza della gonorrea, maggiore comunque negli uomini specialmente sintomatici e rara nelle donne.
1. Papp J, et al. Recommendations for the Laboratory-Based detection of C.trachomatis and N.gonorrhoeae. MMWR Recomm Rep 2014;63:1-19.

P013

LA PREVALENZA DELLA CHLAMYDIA TRACHOMATIS NEL CENTRO ITALIA LA NOSTRA ESPERIENZA

M. Cenci, P.M. Placanica, R. La Mancusa, M. Gaudio

U.O.C.Patologia Clinica Azienda Ospedaliera San Giovanni Addolorata Roma

L'infezione da Chlamydia trachomatis (CT) è una delle malattie sessualmente trasmesse più comuni in Italia sia tra le donne (2,4%) che tra gli uomini (8,4%), con prevalenza meno elevata nei soggetti con età >19 anni (3,1%), nelle persone del Centro-Sud Italia (1,8%) e nei soggetti asintomatici rispetto a quelli sintomatici (2% vs 4,5%). E' prevalentemente asintomatica, specialmente nelle donne, ma deve essere tempestivamente identificata e trattata per evitare complicanze gravi come la malattia infiammatoria pelvica (MIP) con conseguente sterilità nella donna.
La diagnosi si effettua mediante PCR (Polymerase Chain Reaction), che oggi viene considerata il gold standard, mentre la ricerca anticorpale è utile nella MIP.
Nel nostro studio è stata identificata la CT mediante PCR in 5572 pazienti, 5234 donne (94%) e 338 uomini (6%), afferenti al laboratorio della nostra Azienda (4540 tamponi cervicali, 639 vaginali e 393 urine) dal 1/12/12 al 30/9/15. 4958 (89,0%) erano pazienti del centro prelievi afferente alla U.O.C. di Patologia Clinica, 519 (9,3%) dei reparti di Ostetricia e Ginecologia ed i restanti 95 casi (1,7%) degli altri reparti ed ambulatori aziendali.
La PCR Real time è stata effettuata sul Cobas 4800 (Roche). Nell'uomo la ricerca della CT con questo metodo è stata condotta sulle urine; nella donna su campioni vaginali o cervicali oppure sulle urine.
La CT è stata rilevata in 88 casi (1,58%): 77 donne, età media 34 anni, (1,47%), 59 su tampone cervicale e 18 su tampone vaginale; 11 uomini, età media 38 anni (3,2%), su urine.
Nel nostro studio, su pazienti del Centro Italia, si rileva una prevalenza dell'infezione da CT inferiore a quella italiana sia per le donne che per gli uomini ma molto vicina a quella del Centro-Sud Italia. Nelle donne valutate nel nostro studio, la prevalenza è simile ai soggetti asintomatici italiani; negli uomini, invece, ai pazienti sintomatici.
Quindi, identificare la CT precocemente con un metodo altamente sensibile e specifico come la PCR, è utile per poter trattare tempestivamente questa infezione ed impedirne la diffusione e le complicanze per i pazienti affetti.

1. Papp J, et al. Recommendations for the Laboratory-Based detection of C.trachomatis and N.gonorrhoeae. MMWR Recomm Rep 2014;63:1-19.

P014

NAFLD (NON-ALCOHOLIC FATTY LIVER DISEASE): UNA PATOLOGIA METABOLICA DI INTERESSE GENETICO MOLECOLARE

R. Gigli[1], G. Niro[1], M. Magri[1], A. Aiello[2], D. Prioriello[1]

[1]U.O.S. Biologia Molecolare, P.O."A.Cardarelli", Campobasso
[2]U.O. Diabetologia Endocrinologia, P.O."A.Cardarelli", Campobasso

La NAFLD, suddivisa in tre fenotipi clinici, steatosi, NASH (Non Alcoholic Steatohepatitis) e fibrosi, è la causa più frequente di epatopatia cronica non alcolica in occidente. Colpisce dal 3% al 10% della popolazione pediatrica mondiale, fino al 90% dei soggetti obesi. Più di un quarto dei soggetti con steatosi progredisce verso la NASH e verso una fibrosi significativa. Fino a qualche anno fa la mancanza di test diagnostici specifici e sensibili e l'invasività della biopsia epatica limitavano le conoscenze sulla reale prevalenza di questa patologia. In questo studio abbiamo analizzato i quattro polimorfismi correlati ai differenti aspetti istologici associati alla NAFLD. Sono stati esaminati 47 pazienti provenienti dal centro di Diabetologia ed Endocrinologia clinica, di età e sesso diversi che presentavano tutti valore di BMI elevato. I pazienti sono stati suddivisi in due gruppi: 20 con valori degli enzimi epatici, colesterolo e trigliceridi, al di sopra del limite superiore della norma e 27 con valori nel limite della norma. Mediante il test di discriminazione allelica si è proceduto alla genotipizzazione dei seguenti polimorfismi: rs3750861 in KLF6; rs13412852 in LPIN1; rs738409 in PNPLA3 e rs4880 in SOD2. Nella popolazione i genotipi presenti, correlati ad un rischio maggiore di NAFLD, erano espressi secondo queste percentuali: SOD2-TT (26%); KLF6-CC (91.5%); LPIN1-CC (27.66%); LPIN1-CT (70%); PNPLA3-GG (8.51%) PNPLA3-CG (42.55%). Nel primo gruppo, rappresentato da 20 pazienti con alterazione dei valori ematochimici, 7 mostravano un profilo genetico a più alto rischio di NAFLD; il restante esprimeva un profilo genetico a rischio intermedio. Nel secondo gruppo di pazienti, a indici biochimici normali, 15 mostravano un gradiente di elevato rischio di NAFLD; 12 un rischio minore per NASH e fibrosi. Tutti i pazienti con BMI elevato si ponevano nella fascia di rischio medio e alto di NAFLD. I polimorfismi studiati, pur non essendo conclusivi nell'identificazione di singoli geni candidati, permettono di individuare profili genetici associati a classi di rischio per la progressione della NAFLD e aiutano il clinico nel follow up del trattamento dei pazienti.

Bellentani S, Scaglioni F, Marino M, et al. Epidemiology of non-alcoholic fatty liver disease. Dig Dis 2010;28:155-61.

P015
INTERPRETATION OF NON-CODING VARIANTS IN INHERITED CARDIOMYOPATHIES ASSOCIATED TO SUDDEN DEATH

M.R. Pricolo[1], N. Detta[2], C. Mazzaccara[1], P. Coppola[1], F. Salvatore[2], G. Frisso[2]

[1]Dip. di Medicina Molecolare e Biotecnologie Mediche, Federico II Univ., Naples, Italy
[2]CEINGE-Biotecnologie Avanzate, Naples, Italy

Cardiomyopathies are inherited conditions characterized by structural or electrical alterations of myocardium, and represent the most common causes of sudden cardiac death (SCD) among young people. They are autosomal dominant diseases, with variable penetrance and expressivity. When a definitive mutation is found in the proband it is possible to search the mutation in relatives, identifying the asymptomatic subjects at risk of developing the disease. The most common variations found in cardiomyopathy-related genes are point mutations, which can be classified as pathogenic when cause the introduction of a stop codon, affect the canonic splicing site or if they are functionally characterized. The utilization of bioinformatics tools is useful to predict the pathogenicity of mutations, but they may be doubtful to analyze the effect of point mutations located in non-coding regions [Wallis Y ACGS /VGKL 2013].
We functionally tested 5 intronic variants (MYBPC3-c.506-2 A>C, MYBPC3-c.906-7C>T, MYBPC3-c.2308+3 G>C, SCN5A-c.393-5 C>A, ACTC1-c.617-7 T>C), found in 5 patients affected by inherited cardiomyopathies (hypertrophic cardiomyopathy or Brugada syndrome), related to SCD. The MYBPC3-c.506-2 A>C mutation was analyzed in mRNA extracted from peripheral blood of the patient. The analysis revealed the loss of the canonical splice site and the utilization of an alternative splicing site, causing the loss of first 7 nucleotides of the exon 5 (MYBPC3-G169AfsX14). When patients mRNA was not available, we generated minigene constructs, transfected in HEK-293 cells. The minigene assay showed the MYBPC3-c.2308+3 G>C and SCN5A-c.393-5 C>A produced an altered pre-mRNA processing, resulted in the skipping of the involved exons. On the contrary no alterations were found in ACTC1-c.617-7 T>C minigene. In addition, we are performing in vitro analysis of MYBPC3-c.906-7C>T variation.
In conclusion, the in vitro analysis of these mutations shows different splicing alterations consistent with the diagnosis, except for the ACTC1 variation. The identification of causative mutations is an integral part of the diagnostic process. Thus, the evaluation of pathogenic effects by in vitro analysis can be helpful for interpretation of non-coding variants and to make a correct molecular diagnosis.

P016
ASSOCIATION OF H558R POLYMORPHISM IN SCN5A GENE WITH FAMILIAL DILATED CARDIOMYOPATHY

C. Mazzaccara[1], A. Fusco[2], A. Redi[2], D. Bonaduce[3], G. Limongelli[4], F. Salvatore[5], G. Frisso[1]

[1]CEINGE-Biotecnologie Avanzate s.c.ar.l., Napoli, Italia; Dip. di Medicina Molecolare e Biotecnologie Mediche, Università Federico II, Napoli
[2]Dip. di Medicina Molecolare e Biotecnologie Mediche, Università Federico II, Napoli
[3]Dip. di Scienze Mediche Traslazionali, Università Federico II, Napoli
[4]U.O.C. di Cardiologia A.O. Monaldi, Seconda Università degli Studi di Napoli, Napoli
[5]CEINGE-Biotecnologie Avanzate s.c.ar.l., Napoli, Italia; IRCCS – Fondazione SDN, Napoli

Dilated cardiomyopathy (DCM) is a cardiomyopathy caused, frequently, by ischemic heart disease. Less common causes include structural heart disease, inflammation, infections and genetically-mediated forms (i.e. familial DCM; FDC) [Fazio G. Open Medicine 2014]. Approximately 40-50% of DCM cases are classified as Idiopathic Dilated Cardiomyopathy (IDC), after detectable causes have been excluded. Up to 35% of IDC has a genetic basis. To date several genes have been identified associated to DCM, among which SCN5A gene, encoding the cardiac sodium channel α subunit. The SNP rs1805124 (c.1673A>G; p.H558R) in SCN5A gene is associated with different cardiac disorders. However no studies have evaluated the prevalence of rs1805124 in DCM patients. We examined the association between the rs1805124 and the risk of DCM in 185 DCM cases and 251 age and sex matched controls. By family history screening and by evidence of coronary artery disease and/or myocardial infarction, we identified 56/185 FDC and 50/185 post-ischemic patients (pi-DC) respectively. Seventy-nine IDC patients, were also recognized according the known criteria. We valuated the allele and genotype frequencies of rs1805124, in FDC, IDC, pi-DC patients and controls and their association with DCM risk. In FDC (OR=7.39, 95% CI=2.88-18.96; p <0.0001) and DCM (OR=2.78, 95% CI=1.26-6.13; p=0.011) patients, the GG genotype was associated with increased risk of disease compared to the AA genotype. When we pooled the FDC and the IDC patients in the single ni-DC group, the association was still significant (OR=2.73, 95% CI=1.70-8.55; p=0.001), instead no difference was found comparing rs1805124 genotype frequencies in the pi-DC and IDC patients vs controls. Moreover logistic regression analysis, showed that GG carriers had a higher risk of DCM than AA + AG carriers, particularly in FDC subjects (OR=5.45, 95% CI=2.23-13.35; p<0.001) and ni-DC group (OR=3.14, 95% CI=1.43-6.92; p=0.006) but also in the whole DCM population (OR 2.21, CI=1.0-4.83; p=0.05. These results indicate that the GG genotype was significantly associated with DCM risk in non ischemic dilated cardiomyopathy, and particularly in familial cases. Further studies are needed to replicate our results in other ethnic groups with larger sample size.

P017

TECNICHE DI BIOLOGIA MOLECOLARE PER L'ANALISI DELLA MALATTIA MINIMA RESIDUA NEI LINFOMI FOLLICOLARI

G. Ercolano[1], A. Di Vita[1], E. Ciabatti[1], S. Grassi[2], F. Guerrini[1], C. Domenichini[1], G. Tarrini[1], S. Salehzadeh[1], S. Galimberti[1], M. Petrini[1], M.R. Metelli[1]

[1]Lab. di Biologia Molecolare, U.O. Ematologia, Dip. di Medicina Clinica e Sperimentale, Università di Pisa, Pisa
[2]Dip. di Biotecnologie Mediche, Università di Siena, Siena

Obiettivi. Il linfoma follicolare (LF) è un LnH indolente che origina dalle cellule B centrofollicolari dei centri germinativi. Il marker genetico-molecolare distintivo è la traslocazione dei cromosomi 14 e 18 [t(14;18)(q32;q21)]. Lo scopo dello studio è stato di caratterizzare a livello molecolare 45 pazienti (36-83aa) affetti da LF, trattati in 1° e 2° linea con Rituximab-Bendamustina, per valutarne i livelli di malattia minima residua (MMR) post-trattamento. Materiali e metodi. Tecniche di estrazione di DNA: colonna di silice e metodo del Salting Out. Nested PCR end-point e corsa elettroforetica su gel d'agarosio per valutazione qualitativa di BCL2@IGH-MBR e BCL2@IGH-mcr; PCR e corsa elettroforetica su capillare per analisi di frammenti per valutazione del riarrangiamento del gene IGH; Real-Time PCR (qPCR) per valutazione quantitativa del riarrangiamento BCL2@IGH-MBR.

È stata osservata una sensibilità dei metodi di $1x10^{-5}$ con diluizioni seriali di linee linfoidi clonali.

Risultati. BCL2@IGH è stato rilevato in 29 casi (64.4%) con PCR qualitativa e in 24 casi (53%) con qPCR (2 casi positivi per riarrangiamento mcr). 18 pazienti presentano infiltrazione midollare mediante analisi di biopsia osteomidollare; in 4 di questi (22%) non è stato riscontrato BCL2@IGH-MBR-mcr; i restanti correlano. In 8 pazienti senza infiltrazione midollare e con citofluorimetria negativa è stato riscontrato un marker molecolare (p=0.999). In qPCR il valore mediano pre-trattamento del tumor burden molecolare era di $1.6x10^{-2}$ copie.

Alla fine del trattamento, 26 di 29 casi PCR+ (90%) è risultato negativo a Nested PCR. In qPCR 18 di 20 casi valutabili (90%) sono diventati MMR negativi; i restanti 2 casi sono risultati "positivi non quantificabili" ($1x10^{-6}$ copie). Da notare una riduzione significativa della mediana del tumor burden molecolare di circa 4 Logs.

Conclusione. Questo studio retrospettivo sostiene l'efficacia di R-Benda sia in termini di risposta clinica che di eradicazione della MMR molecolare e soprattutto evidenzia che l'uso di metodologie con elevata sensibilità fornisce un valore aggiunto rispetto a tecniche diagnostiche convenzionali.

Dölken G. Detection of minimal residual disease. Adv Cancer Res 2001;82:133-85.

P018

DROPLET PCR: A NEW SENSITIVE METHOD FOR DETECTING BRAFV600E MUTATION IN HAIRY CELL LEUKEMIA

S. Salehzadeh[1], F. Guerrini[1], E. Ciabatti[1], S. Grassi[2], C. Domenichini[1], A. Di Vita[1], G. Tarrini[1], G. Ercolano[1], S. Galimberti[1], M. Petrini[1], M.R. Metelli[1]

[1]Lab. di Biologia Molecolare, U.O. Ematologia, Dip. di Medicina Clinica e Sperimentale, Università di Pisa, Pisa
[2]Dip. di Biotecnologie Mediche, Università di Siena, Siena

Hairy cell leukemia (HCL) is a rare B-cell chronic lymphoproliferative disease, characterized by the clonal expansion of mature B lymphocytes and by indolent course.

It mainly involves the bone marrow and spleen where a significant infiltration by neoplastic cells, the hairy cells, could be found, in addiction to a progressive bone marrow fibrosis that accounts for the peripheral multi-lineage cytopenia. The HCL owes its name to the presence of features cytoplasmic protrusions (hair) from the membrane of neoplastic lymphocytes. Recently a mutation of the gene BRAF has been associated to the pathogenesis of the disease. Mutations of this gene, coding for a serine threonine kinase, are mostly activating and somatic point mutations; this leads to excessive proliferation at the cellular level and to an increased resistance to apoptosis. About 90% of BRAF mutations include substitution of glutamic acid (E) with the valine (V) at position 600 of the protein chain (V600E), resulting in the constitutive activation of BRAF.

Our study enrolled 38 patients with indolent non-Hodgkin's lymphoma, including 19 with HCL in classic form, first in variant form and 18 with splenic marginal zone lymphoma diagnosis.

The BRAF V600E mutation analysis were performed on bone marrow samples by Real Time PCR (qPCR) and Droplet Digital PCR (dd-PCR).

The aim of our study was to compare these two different techniques and assess their sensitivity and specificity in order to identify which one would be the most valid method both for the differential diagnosis of HCL and for disease monitoring after drug treatment.

Our data demonstrated that:

1) the dd-PCR sensitivity is half a log higher than that offered by the qPCR ($5x10^{-5}$);

2) the dd-PCR specificity is comparable to that from qPCR (no cases affected by marginal lymphoma or variant HCL resulted BRAF-mutated);

3) its high sensitivity would allow employing dd-PCR as a suitable tool for minimal residual disease monitoring;

4) the feasibility and costs from dd-PCR are comparable to those coming from qPCR.

In conclusion, our study supports the introduction of dd-PCR as a routinary molecular technique in the scenario of indolent B lymphomas.

Tiacci E. BRAF mutations in hairy-cell leukemia. N Engl J Med 2011;364:2305-15.

P019

XENOBIOTIC METABOLISM-RELATED GENE VARIANTS ARE RISK MARKERS FOR IDIOPATHIC ENVIRONMENTAL INTOLERANCES

D. Caccamo[1], A. Gugliandolo[2], M. Currò[2], R. Ientile[1], L. Korkina[3], C. De Luca[4]

[1]*Operative Unit of Clinical Biochemistry, Polyclinic Hospital University, Messina*

[2]*Dept. Of Biomedical and Dental Sciences, and Morpho-functional Imaging, Polyclinic Hospital University, Messina*

[3]*Active Longevity Clinic "Institut Krasoty na Arbate", Moscow, Russia*

[4]*Centre of Innovative Biotechnological Investigations (Cibi-Nanolab), Moscow, Russia*

Idiopathic environmental intolerances (IEI) are a variety of pathological conditions, including multiple chemical sensitivity (MCS), fibromyalgia (FM), chronic fatigue syndrome (CFS), dental amalgam disease, electrosensitivity (EHS) and food intolerances, sharing the common feature of an aberrant response triggered by exposure to low doses of environmental xenobiotics (chemicals, drugs, heavy metals, radiations, microbial toxins, food compounds, synthetic implants, new biomaterials) in concentrations far below average reference levels admitted for environmental toxicants.
IEI symptoms are multi-organ, including psychosomatic, neurological, chronic muscular fatigue, chronic bronchitis and asthma, gastrointestinal, and autoimmune disorders, and appear mainly in adult life, with higher prevalence in women. Challenges for establishing differential diagnostic criteria for IEI lie in: lack of consensus on case definition; symptoms heterogeneity; individual sensitivity/genetic predisposition; not clear etiopathogenesis; variety of potential triggers; absence of dose-dependent responses; common comorbidity features with known autoimmune diseases like LES, rheumatoid arthritis, or vitiligo (1).
Given that the role of xenobiotic metabolism impairment in the individual hypersensitivity to xenobiotics was recently highlighted, we aimed to analyse the distribution of gene variants of xenobiotic-metabolizing enzymes in four cohorts of 170 MCS, 108 suspected MCS, 89 FM/FCS patients, all exhibiting alteration of redox and cytokine patterns at a variable extent, and 196 healthy controls. Using either allele discrimination method by Real-time PCR, with commercial TaqMan-based genotyping assays (Applied Biosystems, Monza), or allele-specific PCR with literature-derived allele-specific primers, we found significantly higher frequency of gene variants CYP2C9*2, CYP2C9*3, CYP2C19*2, CYP2D6*4, CYP2D6*41, and the haplotype GSTM1 null/GSTT1 null in patients compared with controls. Moreover, we found that the NOS2A -2.5 kb (CCTTT)n polymorphism may be useful for differential diagnosis of various IEI.
Our data demonstrate that these gene variants represent genetic risk markers for IEI and should be included in diagnostic panels.

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P020

RUOLO DEL LABORATORIO DI BIOLOGIA MOLECOLARE NELLE CONTAMINAZIONI IN AMBITO OSPEDALIERO DI PSEUDOMONAS AERUGINOSA

A. Scano[1], D. Pirroni[1], G. Serafi[1], P. Melis[1], F. Puggioni[1], S. Fais[1], A. Gigante[1], P. Ferraguti[1], M. Liciardi[2], V. Piras[3], F. Coghe [1], G. Orru'[1]

[1]*Laboratorio analisi Chimico-Cliniche e Microbiologia e Laboratorio Spoke di Biologia Molecolare, Dipartimento dei Servizi di Diagnosi e Cura, Azienda Ospedaliera Universitaria (AOU) Cagliari*

[2]*Istituto Zooprofilattico Sperimentale della Sardegna, Cagliari, Italy*

[3]*Sezione Odontoiatria, Dipartimento Scienze Chirurgiche, Università di Cagliari*

Obiettivi: Lo scopo del lavoro è stato valutare la presenza di P.aeruginosa (Pa) e le mutazioni responsabili della produzione di alginato in campioni provenienti da diversi riuniti odontoiatrici quali modello di possibili infezioni nosocomiali. In particolare il gene mucA codifica per una proteina coinvolta nella produzione di alginato in Pa, le mutazioni presenti nel promotore del gene o lungo la parte amino-terminale della proteina, modulano un'iper-espressione di alginato, conferendo al biofilm batterico una barriera pressoché impermeabile agli antimicrobici. Questo aspetto deve essere considerato durante l'utilizzo di microbicidi ossidanti quali H2O2, in grado di determinare mutazioni cromosomiche in Pa, inoltre i perossidi rappresentano i disinfettanti d'elezione nei riuniti, rendendo questi presidi ad alto rischio per contaminazioni di ceppi di Pa farmaco resistenti.
Materiali e Metodi: In 90 campioni prelevati su 20 riuniti odontoiatrici la presenza/titolo di Pa, e il profilo nucleotidico di mucA sono stati valutati attraverso PCR real time e Sequenziamento capillare (ABI 310) . Inoltre è stata valutata la massa del biofilm totale calcolando i genomi batterici con PCR quantitativa, amplificando una regione conservata per i batteri del gene rrs, la calibrazione è stata eseguita utilizzando ceppi di Pa a titolo noto e con profilo allelico conosciuto per il gene mucA.
Risultati: I risultati hanno evidenziato una contaminazione da Pa nell' 8% dei riuniti esaminati. Il 20% dei ceppi presentavano mutazioni missense nella regione codificante del gene (GGG/GCG in posizione 63).
Conclusioni: Il presente lavoro può rappresentare un metodo utile nello screening per la prevenzione di infezioni nosocomiali sostenute da Pseudomonas spp.

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P021

DIAGNOSI DI LABORATORIO PER INFEZIONE DA TREPONEMA PALLIDUM, VALUTAZIONE DELLE RESISTENZE AI MACROLIDI TRAMITE SEQUENZIAMENTO DEL GENE 23S rRNA

G. Orrù , A. Scano, G. Serafi, M. Pautasso, P. Melis, F. Puggioni, S. Fais, A. Gigante, G. Serreli, P. Ferraguti, F. Coghe

Laboratorio analisi Chimico-Cliniche e Microbiologia e Laboratorio Spoke di Biologia Molecolare, Dipartimento dei Servizi di Diagnosi e Cura, Azienda Ospedaliera Universitaria (AOU) Cagliari

Obiettivi: L'infezione sostenuta da Treponema pallidum (Tp) presenta, secondo i dati riportati dall'European Centre for Disease Control, ECDC e dell'OMS risultano allarmanti, sia per l'aumento di incidenza dei casi di sifilide, sia per la comparsa di ceppi resistenti o multiresistenti agli antibiotici. Lo scopo del lavoro è quello di utilizzare metodiche diagnostiche molecolari altamente specifiche che in tempi rapidi permettano di rilevare (anche a basse concentrazioni) T. pallidum e contemporaneamente rilevare le mutazioni genomiche a carico del gene 23S rRNA responsabili della resistenza ai macrolidi.
Materiali e Metodi: Da campioni provenienti da lesioni cutanee è stato estratto e poi amplificato il DNA tramite nested real time PCR, utilizzando primer specifici disegnati su una porzione del gene 23s rRNA. Il controllo positivo era rappresentato da un frammento di DNA a titolo noto contenente l'amplicone di PCR (606 bp). I campioni risultati positivi sono stati sequenziati con metodo capillare e comparati con la sequenza di riferimento GenBank NR_076531.
Risultati: La sensibilita' del metodo e' risultata pari a 100 genomi Tp/PCR, sono state rilevate 2 mutazioni principali nel target genico: (i) A2059G responsabile per resistenza alla Eritromicina e Azitromicina e (ii) A2058G per la Spiramicina.
Conclusioni: La sperimentazione eseguita dimostra l'affidabilità del metodo molecolare di cui il clinico può trarre vantaggio per un approccio terapeutico personalizzato e qualora il farmaco di elezione, la penicillina, non possa essere somministrato.
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P022

DIAGNOSI DI LABORATORIO PER INFEZIONE DA NOCARDIA SPP. TRAMITE PCR REAL TIME

F. Coghe , P. Melis , A. Scano, F. Puggioni, S. Fais, G. Serafi, P. Ferraguti, M. Pautasso, G. Serreli, A. Gigante, G. Orru'

Laboratorio analisi Chimico-Cliniche e Microbiologia e Laboratorio Spoke di Biologia Molecolare, Dipartimento dei Servizi di Diagnosi e Cura, Azienda Ospedaliera Universitaria (AOU) Cagliari.

Obiettivi: Diversi microrganismi appartenenti al genere Nocardia spp. possono causare infezioni eterogenee nell'uomo quali: malattie respiratorie, ascessi cerebrali, lesioni meningee, infezioni cutanee, articolari e oculari e le specie più frequentemente isolate sono N. asteroides e N. brasiliensis. La prognosi è favorevole, tranne nei casi di nocardiosi disseminata nei pazienti immunocompromessi. La diagnosi di laboratorio tradizionale a volte non è semplice e in genere si basa sull'osservazione microscopica del campione e sull'esame colturale. Lo scopo del lavoro è stato quello di utilizzare metodiche molecolari, quale la PCR real time, per arrivare a una diagnosi di laboratorio più veloce e specifica.
Materiali e metodi: Il DNA proveniente da diversi campioni, biopsie o tamponi cutanei prelevati da pazienti con sospetta Nocardiosi, e' stato sottoposto ad amplificazione tramite PCR real time (light-Cycler Roche). La PCR amplificava un segmento di 228 bp lungo il gene rrs specifico per Nocardia spp; nei casi dubbi o dove è richiesta l'identificazione di specie, e' stato possibile sequenziare l'amplicone con metodo capillare. La positività è stata valutata tramite l'analisi della curva di melting (Tm=91 °C) o tramite gel di agarosio. I risultati sono stati confrontati con il metodo colturale. Come controllo positivo è stato utilizzato e un ceppo di collezione di N. seriolae (ATCC 43993).
Risultati: La procedura utilizzata è risultata più veloce (4 ore) e sensibile rispetto al metodo colturale tradizionale, il limite di rilevamento della PCR è risultato essere pari a (500 copie DNA/PCR).
Conclusioni: La procedura descritta potrebbe dare dei benefici nella diagnosi di laboratorio per Nocardia spp. In particolare nei casi di campioni negativi alla coltura e positivi al microscopio per forme alcool resistenti.
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P023
EXPRESSION OF MICRO-RNA IN CENTENARIANS

F. Balzano[1], M. Deiana[2], S. Pasella[1], S. Pinna[1], A. Mannu[1], A. Zinellu[1], C. Carru[1], L. Deiana[1]

[1]Dip. Scienze Biomediche, Università, Sassari
[2]Ass. "Isola dei centenari", Sassari

Micro-RNA (miRNA) are a family of small non-coding ribonucleic acids that inhibits post-transcriptionally the expression of their target messenger RNA (1). We are interested in studying the involvement of miRNA in longevity and immune diseases. Human aging is an extremely complex process and miRNAs have proven to play a role in modulating the duration and the aging process. In this study we compared the different expression of seven microRNAs between ten human plasma healthy controls, 15 plasma samples of centenarians and 20 samples from patients with rheumatoid arthritis. All samples were recruited from the biobank longevity Akea project (project approved by the local bioethics).
We used the Life Technologies' protocol to isolate and quantify seven miRNAs from 45 plasma samples: 10 healthy human controls, 15 centenarians, 20 patients with autoimmune disorders. TaqMan MicroRNA assays were used to analyze the expression profiles of miR-125b, miR-425, miR-200b, miR-200c, miR-579, miR-212, miR-21 and miR-126. The relative expression of mature miRNAs was analyzed using software REST. The non-parametric bootstrapping test was used to assess differences in expression of miRNAs between cases and controls.
Our results show that miR-425, miR-21 and miR-212 significantly decreased in centenarians and in patients with immune system disorders compared with controls. Furthermore centenarians and immune disorder patients show the same miRNAs pattern. The similarity between centenarians and patients with autoimmune diseases is very interesting. The miR- 425, miR- 21, miR- 212 miRNAs were proven to be overexpressed in cancer progression. We can speculate that centenarians have survived diseases and cancer due to their lifestyle but also to a strong immune system. Recent studies linked altered miRNAs function to a number of diseases and age-related processes. The identification of miRNAs that modulate longevity will provide important insights into the molecular basis of aging. Considering the preliminary results obtained, the expression of miR-425, miR-21 and miR-212 seems to be of great interest in cancer, age-related processes and metabolic diseases.
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P024
CANCER STEM CELLS FROM HUMAN TUMOR CELL LINES: MOLECULAR AND PHENOTYPIC FEATURES

R. Campagna[1], D. Sartini[1], V. Pozzi[2], R. Seta[1], S. Fumarola[1], V. Pompei[1], M. Cecati[1], A. Santarelli[1], L. Lo Muzio[3], M. Emanuelli[1]

[1]Dip.Scienze Cliniche Specialistiche e Odontostomatologiche, Università Politecnica delle Marche, Ancona
[2]New York-Marche Structural Biology Centre (NY-MaSBiC), Università Politecnica delle Marche, Ancona
[3]Dip. Medicina Clinica e Sperimentale, Università degli Studi di Foggia, Foggia

The cancer stem cell theory states that a subset of tumor cells, termed cancer stem cells (CSCs), has the ability to self-renew and differentiate within the tumor bulk. According to this theory, CSCs are responsible for tumor initiation, growth, metastasis, resistance to therapy and recurrence.
In this work, a culture system was setup to enrich CSCs from Hep-2 (laryngeal cancer), T24 (bladder cancer), MG63 (osteosarcoma), CaCo-2 (colorectal cancer) and A549 (lung cancer) cancer cell lines, through sphere formation. Subsequently, magnetic-activated cell sorting (MACS) was used to further increase CSC enrichment. Molecular and phenotypic characterization of CSC-enriched cell populations was performed by exploring the expression levels of stem markers and in vivo evaluating the tumorigenic potential. The expression of the enzyme nicotinamide N-methyltransferase (NNMT) (1) was also investigated.
Real-Time PCR analysis and immunocytochemistry showed the upregulation of stem markers in CSC-enriched populations compared with control cells. After subcutaneous injection of Hep-2 cells into immunocompromised mice, CSC-enriched population yielded tumors of a much larger size compared with those generated by parental cells, suggesting a stronger ability of CSCs to form tumors in vivo. NNMT expression levels were markedly higher in CSC-enriched populations compared to parental counterpart.
The present study provides a useful methodology to enrich CSCs from tumor cell lines, allowing their molecular characterization. Moreover, NNMT overexpression in CSC-enriched populations seems to suggest a pivotal role of this enzyme in cancer growth and metastasis.
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P025

DNA LIBERO CIRCOLANTE NEL PLASMA COME MARCATORE MOLECOLARE E INDICATORE PROGNOSTICO NEI PAZIENTI CON TUMORE DEL POLMONE

C. Siracusa[1], P. Villa[1], S. Signorini[1], S. Besana [1], P. Brambilla[2]

[1]S.C. Analisi Chimico Cliniche, ASST di Monza, P.O. di Desio (MB)

[2]Dipartimento di Medicina e Chirurgia, Università degli Studi di Milano – Bicocca, Monza (MB)

Introduzione: L'efficacia delle terapie oncologiche target è legata alla selezione di pazienti potenzialmente responsivi attraverso l'identificazione di marcatori molecolari predittivi di risposta su campioni tissutali, a volte non sono adeguati, reperibili o sufficienti. La "biopsia liquida", alternativa per l'analisi molecolare, comprende cellule tumorali circolanti (CTC) e DNA tumorale libero circolante (cfDNA) nel plasma. L'analisi del cfDNA può costituire un utile strumento per la gestione dei pazienti con neoplasie maligne [1].

Scopo: Valutare in pazienti con tumore polmonare (LC) il ruolo del cfDNA nella diagnosi, follow-up ed evoluzione della malattia.

Materili e metodi: Da pazienti con LC, afferiti al nostro laboratorio nel periodo 2014-2016, sui quali è stata eseguita su tessuto l'analisi delle mutazioni degli esoni 18, 19, 20 e 21 del gene EGFR, si sta procedendo a raccogliere 1 campione di sangue venoso in una provetta da 8ml (K2-EDTA) per l'analisi di cfDNA. Il campione viene centrifugato entro 2 ore dal prelievo (2 centrifugazioni a 4°C per 10' a 2500g e 3500g), il plasma ottenuto viene stoccato a -80°C. Il cfDNA viene estratto mediante utilizzo del kit "Cobas cfDNA Sample Preparation"(Roche) e amplificato con RT-PCR su COBAS Z480 usando il kit "Cobas EGFR Mutation Test-v2"(Roche).

Risultati e conclusioni: è stata eseguita l'analisi molecolare del gene EGFR su 3 campioni di cfDNA plasmatico ottenuto da pazienti con LC in follow-up da 6 mesi. È stata rilevata una delezione sull'esone 19 e un'inserzione sull'esone 20 in campioni Wild Type (WT) su tessuto; un campione con delezione sull'esone 19 nel tessuto è invece risultato WT. L'esiguità dei campioni non permette ad oggi di trarre conclusioni significative ma l'analisi di cfDNA tumorale nel plasma di pazienti con LC è ormai una metodica non invasiva di necessaria implementazione nei laboratori di Biologia Molecolare per la diagnosi e gestione del follow-up e della terapia del paziente con LC. Il cfDNA diventerà uno strumento diagnostico per implementare la ricerca molecolare e nuove strategie per evidenziare cambiamenti genetici predittivi di chemioresistenza o chemiosensibilità [1].

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P026

DIFFERENTIAL miRNome OF HUMAN AMNIOTIC MESENCHYMAL STEM CELLS IN OBESITY GENDER RELATED

L. Iaffaldano[1], C. Nardelli[1], I. Granata[2], V. D'Argenio[1], V. Del Monaco[3], G.M. Maruotti[4], L. Del Vecchio[1], P. Martinelli[4], F. Salvatore[1], M.R. Guarracino[2], L. Sacchetti[3], L. Pastore[1]

[1]CEINGE-Biotecnologie Avanzate S.C.a R.L., Napoli; Dip. di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli Federico II, Napoli

[2]Laboratory of Genomics, Transcriptomics and Proteomics, Institute for High Performance Computing and Networking (ICAR), National Research Council (CNR), Napoli

[3]CEINGE-Biotecnologie Avanzate S.C.a R.L., Napoli

[4]Dip. di Neuroscienze e Scienze Riproduttive ed Odontostomatologiche, Università degli Studi di Napoli Federico II, Napoli

Experimental evidence indicates several differences between women and men, including the risk of developing to metabolic diseases. These sexual-related traits could depend on gene expression microRNA (miRNA) regulated, a class of endogenous non-coding RNAs (18–25 nucleotides long). They have also been implicated in fetal programming of obesity. We previously reported a different miRNA profile in the amnion of obese vs control women [1] and altered proteome in human amniotic mesenchymal stem cells (hA-MSCs) during obesity [2]. These results prompted us to investigate gender-related differences in gene expression miRNA regulated in Obese (Ob-) hA-MSCs, probably involved in metabolic changes. We isolated Ob- and Control (Co-) hA-MSCs from amnion at delivery. Here, we describe the miRNomes of 7 Co-hA-MSCs and 13 Ob-hA-MSCs from mothers of boys (3 M-Co, 6 M-Ob) or girls (4 F-Co, 7 F-Ob) by high resolution small RNA-sequencing. Interestingly, we have found that 19 miRNAs were down-expressed and one miRNA was up-expressed in F-Ob-hA-MSCs vs M-Ob-hA-MSCs ($p < 0.05$). The downstream target analysis of the gender-deregulated miRNAs predicted impairment of various mechanisms, such as: inflammatory, cellular stress and immune responses, cell defense, etc. In conclusion, we highlight that the obesogenic environment could influence the hA-MSCs epigenetics gender related.

1. Nardelli C, Iaffaldano L, Ferrigno M, et al. Characterization and predicted role of the microRNA expression profile in amnion from obese pregnant women. Int J Obes (Lond) 2014;38:466-9.

2. Capobianco V, Caterino M, Iaffaldano L, et al. Proteome analysis of human amniotic mesenchymal stem cells (hA-MSCs) reveals impaired antioxidant ability, cytoskeleton and metabolic functionality in maternal obesity. Sci Rep 2016;6:25270. doi: 10.1038/srep25270.

P027

RAPID GENOTYPING OF TWO WORLDWIDE G6PD VARIANTS BY HIGH-RESOLUTION MELTING ANALYSIS

M. De Bonis, G. Canu, E. De Paolis, A. Minucci, E.D. Capoluongo

Laboratory of Clinical Molecular and Personalized Diagnostics, Institute of Biochemistry and Clinical Biochemistry, Catholic University, Rome

Background: To date, about 190 G6PD variants are reported. These are mostly single nucleotide substitutions and present an evident geographic distribution. The Mediterranean and A- G6PD variants are particularly prevalent in Africa and Southern Europe. Our study was aimed to develop an assay for the rapid genotyping of these two variants by High Resolution Melting Analysis (HRMA).
Methods: For HRMA set up, we analyzed 60 patients 30 with G6PD Mediterranean and 30 with G6PD A- previously characterized by direct sequencing and 50 wild-type for both mutations. For test sensitivity and specificity additional 80 unknown samples originating from Sardinia, Apulia, Sicily and Campania regions, were tested. For PCR amplification three pairs of primers with the same annealing temperature were used: one for G6PD Mediterranean (c.563C>T, p.Ser188Phe) and two for G6PD A- (c.202G>A, p.Val68Met and c.376A>G, p.Asn126Asp). All primers used were designed to amplify a small fragment surrounding variants tested. G6PD A- mutations was carried out simultaneously by duplex PCR. Melting curve analysis was performed using the LightCycler 480 Gene Scanning Software Version 1.2 (Roche Diagnostic). To test definitively the HRMA specificity we test two different mutations affecting the same PCR/HRMA amplicons [G6PD Salerno (c.383T>G, p.Leu128Arg) and G6PD Murcia(c.209A>G, p.Tyr70Cys].
Results: From the normalized melting curves, differences plots we were able to clearly distinguish wild, hemi/ homozygous and heterozygous states for two mutations. For the G6PD Mediterranean, where the nucleotide change alters the number of hydrogen bonds, also the melting temperature was discriminates. In addition, the melting profiles of the G6PD Salerno and G6PD Murcia were different from both wild type allele that G6PD Mediterranean and G6PD A- alleles. Since the HRMA data of the known and unknown samples were 100% concordant with sequencing, we reached 100% sensitivity and specificity for both variants tested.
Conclusion: HRMA approach presented here is perfectly adapted for a simple, rapid, sensitive and cost-effective scanning of G6PD deficiency due to two worldwide G6PD variants on patients originating from Mediterranean area.

P028

DEVELOPMENT OF A HIGH RESOLUTION MELTING ANALYSIS FOR RAPID DETECTION OF A FOUNDER BRCA1 MUTATION IN THE ITALIAN POPULATION

M. De Bonis, E. De Paolis, L. Gentile, G. Canu, A. Minucci, E.D. Capoluongo

Laboratory of Clinical Molecular and Personalized Diagnostics, Institute of Biochemistry and Clinical Biochemistry, Catholic University, Rome

Introduction: Several different BRCA1 founder mutations have been described at high frequencies in families containing both ovarian and breast cancer from different countries. Some founder mutations are confined to geographically isolated regions or specific populations. BRCA1 5083del19 mutation is recurrent and specific to individuals of Italian descent with a founder effect on the Calabrian and Sicilian population. The correct identification of this type of mutation is one of pitfalls of next-generation sequencing (NGS) mainly due to insufficient coverage, read lenght or alignment quality. In view of this, we set up an assay for rapid genotyping of this “missing” founder mutation by High Resolution Melting Analysis (HRMA).
Methods: DNA samples were obtained from 30 subjects, 15 wild type (WT) and 15 mutated (M) for the BRCA1 5083del19, previously amplified by BRCA MASTR™ Dx (Multiplicom, Niel, Belgium), defined by NGS on the Illumina MiSeq® platform (Illumina Inc., San Diego, CA, USA) and confirmed by Sanger sequencing. A specific pair of primers in order to amplify the region containing the deletion was designed. PCR-HRMA were performed on the LightCycler® 480 Real-Time PCR System. Data, analyzed with LightCycler 480 Gene-Scanning Software version 1.2 (Roche Diagnostics), were normalized, temperature-shifted and converted to a derivative plot for analysis. Melting temperatures (Tms) were derived at the greatest dF/dT value of the derivative curve data.
Results: WT (95bp) and M amplicons (76bp) showed a clearly different melting profile; furthermore the WT and M samples presented evident differences in Tm (TmWT=79.3± 0.5; TmM =74.3±0.5). So, HRMA results were 100% concordant with direct sequencing.
Conclusion: Detection of the BRCA1 5083del19 mutation by BRCA MASTR™ Dx and NGS on the Illumina platform has some obvious limitations due to amplicon position of this mutation. HRMA is a suitable, alternative method for the 5083del19 detection. In fact, it is high throughput, cost-effective sensitive and specific method for identifying the BRCA1 5083del19 founder mutation, resulting in reduced cost of genetic testing and faster turnaround time on patients originating from Calabria and Sicily, where the HRMA could have important practical implications for BRCA1 5083del19 detection.

P029

LISTENING TO THE SOUND OF SILENCE: SYNONYMOUS CHANGES IN BRCA1/2 GENES THAT COULD AFFECT SPLICING

A. Costella, M. De Bonis, A. Minucci, E. Capoluongo

Lab. of Clinical Molecular and Personalized Diagnostics, Inst. of Biochemistry and Clinical Biochemistry, Catholic University, Rome

Introduction: The tumor-suppressor genes BRCA1 and BRCA2 predispose to hereditary breast and ovarian cancer syndrome (HBOC). Next-generation exome sequencing (NGS) has greatly improved detection of nucleotide changes, but the interpretation of most exonic variants remains challenging. Missense substitutions and silent variants create clinical uncertainty in the genetic counseling process. In silico assays are useful for the classification of exonic sequence variants in BRCA1/2. Here we report the case of three nucleotidic exonic changes that affect splicing.

Methods: We routinely perform BRCA1/2 genetic testing using the BRCA MASTR Dx assay (Multiplicom, Niel, Belgium) on the Illumina MiSeq® platform (Illumina Inc., San Diego, CA, USA). More than 1500 subjects, diagnosed as sporadic and/or familial HBOC patients, were analyzed in our laboratory in the last year. All patients obtained a genetic counseling before analysis. Genomic DNA was isolated from peripheral blood samples. All mutations were confirmed by Sanger sequencing. In addition, exonic nucleotidic changes within the first and the last ten nucleotides of each exon, were analyzed using Human Splicing Finder (HSF) [1], a useful tool that aims to help studying the pre-mRNA splicing.

Results: Here we report three variants that attracted our attention: the p.Pro3039Pro (c.9117G>A) in BRCA2, found in our laboratory for the first time in March 2016, but already described in literature as pathogenic mutation, and two variants never described before, the p.Asn103Asn (c.309C>T) in exon 7 of BRCA1 and the p.Val1454Val (c.4362A>G) in exon 14 of BRCA1. According to the analysis on HSF website, these variants, not causing aminoacid change, could indeed affect the normal splicing mechanism.

Conclusion: It is of crucial importance to make a correct interpretation of the NGS data and to be reckoned with silent variants that could hide a pathogenic role in HBOC. These data must be integrated with a good clinical evaluation and family history of the patients.

1. Desmet FO, Hamroun D, Lalande M, et al. Human Splicing Finder: an online bioinformatics tool to predict splicing signals. Nucleic Acids Res 2009;37:e67.

P030

EVALUATION OF A PIPELINE OF NEXT GENERATION SEQUENCING TO SEARCH FOR SOMATIC VARIANTS OF BRCA1 AND BRCA2 GENES IN OVARIAN CANCER FFPE SAMPLES

D. Guarino, C. Santonocito, P. Concolino, R. Rizza, A. Minucci , E. Capoluongo

Lab. of Molecular Biology, Institute of Biochemistry and Clinical Biochemistry, Catholic Universityof Sacred Heart, Largo A Gemelli, Rome

Ovarian cancer is the second most common gynaecological malignancy with an estimated incidence of 225 000 women per year worldwide and remains a significant cause of gynaecological cancer mortality. Ovarian cancer in patients with a BRCA1 or BRCA2 germline mutation is associated with several clinical characteristics including an increased likelihood of platinum sensitivity and improved survival compared with those with non-BRCA-related ovarian cancer. Recently it was demonstrated that BRCA mutated ovarian cancers respond better to approved PARP-inhibitors. The clinical relevance is that the significant activity of PARP inhibitors may not be limited to germline BRCA-mutated ovarian cancer, but indeed extends to a larger group of sporadic ovarian cancer patients. There is the need for efficient and timely methods to detect both somatic and germline mutations using formalin-fixed paraffin-embedded (FFPE) tissues and commercially available technology. We used a commercial kit that analyzed all exons and 50bp exon-intron junctions of BRCA1 and BRCA2 genes and next-generation sequencing (NGS) technology on DNA from 21 FFPE samples of high-grade serous ovarian cancers. We found 18/21 germline mutations and two somatic mutation. All mutations were confirmed by Sanger sequencing. Data analysis were performed by two software ANNOVAR and Amplicom Suite. Testing BRCA on formalin-fixed paraffin-embedded (FFPE) samples would permit the simultaneous assessment of both somatic and germline mutations using an easily accessible material that is routinely available in any pathology laboratory worldwide.

P031

NEXT GENERATION SEQUENCING-BASED ANALYSIS FOR THE COMPLETE CHARACTERIZATION OF THE AIRWAY MICROBIOME OF SARCOIDOSIS

V. D'Argenio[1], V. Precone[2], G. Casaburi[2], L.G. Moccia[3], I. Postiglione[2], M. Bocchino[3], A. Sanduzzi[3], F. Salvatore[1]

[1]CEINGE-Biotecnologie Avanzate and Department of Molecular Medicine and Medical Biotechnologies, University of Naples Federico II

[2]CEINGE-Biotecnologie Avanzate, Naples

[3]Department of Clinical Medicine and Surgery, Respiratory Medicine Section, University of Naples Federico II

Background: Sarcoidosis is a multisystem inflammatory disorder characterized by granulomatous inflammation that can affect any organ. Thoracic involvement is common and accounts for most of the mortality associated with the disease. The composition of the lung microbiome could contribute to both health and disease. Over 70% of the bacterial species on body surfaces cannot be cultured by currently available microbiological techniques. Next generation sequencing techniques that identify the 16S ribosomal RNA gene, have provided new insights into the depth and breadth of microbiota present both in the diseased and normal lung (1).
Aims: Here, we report the characterization of the entire lung microbiome of adult sarcoidosis patients to identify a signature specifically related to the disease.
Methods: Ten sarcoidosis patients and eight subjects with other chronic respiratory diseases were enrolled in the study. Genomic DNAs were extracted by bronchoalveolar lavage (BAL) samples and 16S rRNA next generation sequencing was carried out on the Genome Sequencer FLX instrument. Data analysis was performed using the QIIME community analysis pipeline.
Conclusions: More than 200 Mb equivalent to 771,032 sequences were totally obtained. High quality filtered sequences allowed the identification of 8,410 operational taxonomic units (OTUs). No significant differences were highlighted within the 2 groups, suggesting that microbial dysbiosis is not able to discriminate among different respiratory diseases.

1. Garzoni C, Brugger SD, Qi W, et al. Microbial communities in the respiratory tract of patients with interstitial lung disease. Thorax 2013;68:1150-6.

P032

THE CONTRIBUTION OF THE MASSIVELY PARALLEL SEQUENCING (MPS) TO THE BRCA1/2 MUTATION SPECTRUM AND FREQUENCY IN 1000 ITALIAN HIGH GRADE SEROUS OVARIAN CANCER (HGSOC) PATIENTS

M. De Bonis, A. Minucci, A. Costella, D. Guarino, R. Rizza, L. Gentile, C. Santonocito, P. Concolino, G.L. Scaglione, E.D. Capoluongo

Laboratory of Clinical Molecular and Personalized Diagnostics, Institute of Biochemistry and Clinical Biochemistry, Catholic University, Rome

Introduction: Germline mutations in BRCA1/2 genes have been detected in 8%–18% of patients with ovarian cancer. These genes have been studied extensively as a means to developing targeted therapy of these cancers. Ovarian cancer survival rates have improved only slightly in recent decades; however, treatment of this disease is expected to undergo rapid change as strategies incorporating molecular-targeted therapies enter clinical practice. The aim of this study was to assess the contribution of MPS to the BRCA1/2 mutation spectrum in Italian HGSOC patients.
Methods: All patients, obtained an interview and genetic counselling before molecular test and signed an informed consent, were analyzed for constitutional mutations throughout the entire BRCA coding regions. 1000 patients were PCR-enriched using the BRCA MASTR assay v2.0 (Multiplicom, Niel, Belgium) according to the manufacturer's instructions. Illumina MiSeq® platform was used for MPS.
Results: Of the 1000 patients, 263 (26%) carried a disease predisposing variant, including 180 (68 %) with a BRCA1 mutation and 83 (32 %) with a BRCA2 mutation. 109 frameshift (including 42 insertions and 67 deletions), 38 nonsense, 30 missense and 3 silent variants were identified in BRCA1; while in BRCA2 were identified 50 frameshift variants (including 13 insertions and 37 deletions), 16 nonsense, 16 missense and 1 silent mutation. We also detected 41 variants (20 in BRCA1 and 21 in BRCA2) (4%) classified as Variants of Uncertain Significance (VUS) in the reference BRCA database.
Among the mutations observed, 10 are the most recurrent: c.181T>G (2%), c.1360_1361delAG (3%), c.3756_3759delGTCT (5%), c.4117G>T (3%), c.4964_4982del19 (4%), c.5123C>A (3%), c.5263_5264insC (11%) in BRCA1; c.6468_6469delTC (2%) and c.9455_9456delAG (1%) in BRCA2. Screening of these variants allows a detection rate of 37%.
Conclusion: Our interesting results confirm that mutations in BRCA occur at a considerable frequency in Italian families with ovarian cancer. Although with inventing the new mutation screening methods like MPS knowing the most frequent BRCA mutations not be required, screening of some recurrent BRCA mutations make take some advantages for the diagnosis and treatment of Italian HGSOC patients, making the process of follow up of their family get faster and better.

P033

COMPETITIVE PCR-HIGH RESOLUTION MELTING ANALYSIS AS INNOVATIVE SCREENING METHOD FOR LARGE GENOMIC REARRANGEMENTS DETECTION IN BRCA1 GENE

E. De Paolis, G.L. Scaglione, M. De Bonis , P. Concolino, R. Rizza, A. Minucci , E. Capoluongo

Laboratory of Clinical Molecular and Personalized Diagnostics, Institute of Biochemistry and Clinical Biochemistry, Catholic University, Rome

Introduction: To date, 1.700 BRCA1 variants and 1.900 BRCA2 variants have been reported. However, only 81 BRCA1 variants and 17 BRCA2 variants are Large Genomic Rearrangements (LGRs). The aim of the study was to investigate a Competitive PCR-High Resolution Melting Assay (cPCR-HRMA) as an innovative screening method for BRCA1 LGRs analysis. The principle of the method is to amplify simultaneously sequences of unknown germline BRCA1 copy number variation (CNV) and unchanged Albumin CNV.
Method: We assessed the germline CNV status of exons 1, 2, 3 and 14 as training set of exons. cPCR-HRMA was performed in 30 wild type samples and in the following positive samples, previously analyzed by MLPA: 10 for exons 1 and 2 deletion, 5 for exon 3 deletion, 1 for exon 3 duplication and 4 for exon 14 deletion. HRMA was performed on target BRCA1 regions during exponential PCR phase in duplex reactions including Albumin as unchanged copy reference. Genotypes were assessed comparing the melting profiles and the fluorescence peaks height ratio (BRCA1/Albumin) in each reaction. Mean and SD of fluorescence peak height ratio of wild type samples was used to set an approximate scale of ratio for unchanged copy number (1x±SD), deleted (0.5x±SD) and duplicated (1.5x±SD) samples.
Results: Melting profiles showed a marked different behavior of both wild type and positive samples for all analyzed exons. Samples were correctly classified also by using fluorescence peaks height ratio scale. Furthermore, samples with point mutations and micro-rearrangements showed alternative typical melting profiles.
Discussion: MLPA and MAQ techniques are routinely used for CNV analysis in BRCA1/2 genes. These approaches are expensive and time consuming, therefore identification of screening methods is needed to optimize the diagnostic procedure. In this study, HRMA has proved an innovative, efficient and fast screening method for CNV BRCA1 evaluation. High sensitivity has made HRMA an attractive new tool, allowing an implementation of our diagnostic molecular workflow. For these reasons, we will try to apply this new molecular approach to CNV detection for all BRCA1 exons, considering it as a suitable strategy for better target MLPA test, using it only as confirmatory and definitive test.
Borun P, Kubaszewski L, Banasiewicz T, et al. Hum Genet 2014;133:535-45.

P034

THE CHALLENGES OF INTRODUCING ROUTINE G6PD RESIDUAL ENZYMATIC ACTIVITY PERCENTAGE INTO DIAGNOSTICS: GEMELLI HOSPITAL EXPERIENCE

E. De Paolis, G.L. Scaglione, M. De Bonis , A. Minucci, E. Capoluongo

Laboratory of Clinical Molecular and Personalized Diagnostics, Institute of Biochemistry and Clinical Biochemistry, Catholic University, Rome

Introduction: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common human enzymatic defect. Although the G6PD Residual Enzymatic Activity (AER) is commonly expressed in International Units per gram of Haemoglobin (U/gHb), it may be necessary to provide the AER percentage (AER%) value. G6PD AER% is required in clinical practice upon exposure to certain drugs and furthermore, normal G6PD AER% value represents a requirement for Italian Army enrolment. The aim of this study was to define G6PD AER% in our population in order to better stratify patients and to improve final diagnosis and treatments.
Methods: A total of 309 Italian male with normal haematological parameters underwent G6PD enzymatic test at Gemelli Hospital was included in this study. Red blood cells G6PD AER was determined by commercial kit (Sentinel Diagnostics, Milan, Italy). Adjusted median value of AER, considered as 100% of G6PD activity, was used to set cut-off levels of G6PD AER% (30%, 60%, 80% and 120%).
Results: Adjusted median value of AER was used to minimize the impact of severely deficient individuals. This value was calculated by exclusion of male with severe G6PD deficiency, equal to or less than 10% of the male median, and then by the determination of a new median. A total of 10 patients with AER% lower than 10% of the median value were excluded in the statistics analysis. In our population, the G6PD adjusted median value was estimated in 11.5 U/gHb (AER 100%). We established the following different deficiency classes as percentage of adjusted median value: total deficiency (AER<30%; AER<3.5 U/gHb), intermediate deficiency (AER 30-60%; AER 3.5-6.7U/gHb), partial deficiency (AER 60-80%; AER 6.8-9.1 U/gHb), normal activity (AER 80-120%; AER 9.2-13.8U/gHb) and increased activity (AER>120%; AER>13.8U/gHb).
Discussion: In our approach, evaluation of G6PD AER% represents an improvement in the correct interpretation of G6PD enzymatic activity test, adding information useful for patient management and characterization of deficient subjects. We further implement the definition of G6PD activity classes taking into account genetics features by combining data from both molecular and enzymatic assay.
Domingo GJ, Satyagraha AW, Anvikar A, et al. G6PD testing in support of treatment and elimination of malaria: recommendations for evaluation of G6PD tests. Malar J 2013;12:391.

P035
HB-LEPORE IN ASYMPTOMATIC FEMALE 71 YEARS OLD: CASE REPORT

G. Pedà[1], M. Amadei[1], S.T. Caravetta[1], M. Frasani[1], G. Grandi[1], M. Malpeli[1], F. Maradini[1], J. Uggeri[1], G. Lippi[2], L. Ippolito[1]

[1]U.O. di Patologia Clinica, Lab. Analisi, Ospedale di Vaio, Fidenza
[2]Lab. Chimica Clinica ed Ematologia, Azienda Ospedaliera Universitaria integrata di Verona, Verona

Introduction: Hemoglobin (Hb) Lepore is an abnormal hemoglobin characterized two normal α-chains and of two Lepore-chains. Two fusion-genes δβ and βδ are hence produced. Three different Lepore hemoglobins have been identified, differing from each other in the point at which the δβ fusion occurs. These three variants include Hb Lepore Hollandia (δ22/β50), Hb Lepore Baltimore (δ59/β86) and Hb Lepore Boston (δ87/β116). This hemoglobin variant is part of the hemoglobin genetic disorders implying defective hemoglobin synthesis and qualitative alterations of its composition due to loss/ replacement/addition of amino acids in hemoglobin chains. These disorders may occur both in homozygous or heterozygous state and are hereditary. Hb Lepore heterozygotes have similar clinical features as patients with minor β thalassemia.
Materials and methods: We describe here the case of a patient diagnosed with Hb Lepore at the Clinical Laboratory of Hospital Vaio - Fidenza (PR) by careful evaluation and comparison of different methods used in laboratory routine.
Results: The results of routine analysis revealed the presence of a Hb variant which co-eluted with HbA2. The red blood cell parameters and hemoglobin structure confirmed by electrophoresis showed the presence of a variant Hb Lepore at the heterozygous state (Hb Lepore + Hb A2 = 11.6%).
The additional results of hematologic testing were Hg: 11 g/L [12-15 g/L]; MCV: 70.6 fL [80-96 fL]; MCH: 21.7 pg [27-31 pg]; MCHC: 30.7 g/L [33-37]; ferritin: 57 ng/mL [10-120].
Conclusions: This case attests that the good laboratory practice allows a rapid diagnosis of a rare disease such as Hb Lepore, and also underpins the importance of using different methods and comparing them to have a consistent data that can guide clinical management. Additional genetic tests are performed, including also family members of the patient, to establish the precise origin of the mutation.

P036
ICTUS GIOVANILE DA CAUSA RARA: CADASIL

I. Cuomo[1], S. Abbadessa[1], S. Costanzo[1], F. Marciano[2], T. Cuomo[3]

[1]Dip. di Biochimica, Biofisica e Patologia Generale, Sez. di Immunopatologia. Seconda Università degli studi di Napoli
[2]Dip. di Biochimica e Biotecnologia Medica, Università degli studi di Napoli Federico II
[3]U.O.C. Neurologia, P. O. Umberto I Nocera Inferiore, ASL Salerno

L'Arteriopatia Cerebrale Autosomica Dominante con Infarti Sottocorticali e Leucoencefalopatia (CADASIL), è una patologia su base genetica a trasmissione autosomica dominante, causata da mutazioni del gene Notch3 localizzato sul cromosoma 19. Clinicamente è caratterizzata dalla comparsa, nell'età adulta intermedia, di ictus ischemici sottocorticali ricorrenti, declino cognitivo, emicranie con aura e manifestazioni psichiatriche. Il decorso è progressivo con esito fatale in alcuni anni. Descriviamo il caso di I.G.M. un uomo di 42 anni giunto presso l'U.O.C. di Neurologia per un disturbo transitorio dell'espressione verbale. All'anamnesi il paziente riferiva di una cardiopatia ischemica ipertensiva ed episodi transitori di deficit di forza a carico degli arti sinistri. La storia familiare evidenziò che il padre, morto all'età di 60 anni, aveva sviluppato un deterioramento cognitivo progressivo. Risultavano nella norma i valori dei parametri vitali(PA, FC, SO2), degli esami biochimici, emocoagulativi, degli autoanticorpi, dell'omocisteina, dei dosaggi di biologia molecolare dei fattori della coagulazione, l'ECD TSA, l'Eco TT, l'Eco TE. La RMN encefalo in T2W e FLAIR evidenziava lesioni sottocorticali bilaterali da riferire ad alterazioni gliotiche. Dalla valutazione neuropsicologica risutavano compromesse la memoria verbale, visuo-spaziale e le funzioni esecutive. Considerata la negatività dei fattori di rischio vascolari, l'età, l'anamnesi familiare positiva per deterioramento cognitivo ed la RMN, veniva richiesta consulenza genetica. L'analisi molecolare mediante sequenziamento diretto di tutti gli esoni del gene Notch3 ha permesso di identificare una mutazione nell'esone 4; lo studio, esteso alla famiglia, ha rivelato la presenza della stessa mutazione nel fratello.
Il paziente dimesso con diagnosi di CADASIL e terapia antiaggregante piastrinica, continua ad essere seguito per controlli periodici. In conclusione, in un giovane-adulto, in assenza di fattori di rischio vascolari ma con caratteristici segni clinici e lesioni alla RMN, bisogna considerare ipotesi diagnostiche rare ed avvalersi di consulenza genetica per porre diagnosi di patologie rare come la CADASIL.
Chabriat H, Joutel A, Dichgans M, et al. Cadasil. Lancet Neurol 2009;8:643-53.

P037
PREGNANCY IN A SPLENECTOMIZED BETA-THALASSEMIC PATIENT

F.B. Ronchi[1], P.P. Porcu[1], M. Canavero[2], I. Atzeni[2], S. Valveri[2], G. Serra[1]

[1]Servizio di Patologia Clinica, P.O. N. S. di Bonaria, San Gavino M.le, ASL Sanluri
[2]Struttura Trasfusionale, P.O. N. S. di Bonaria, San Gavino M.le, ASL Sanluri

The ß-thalassemia is a clinical condition of congenital chronic anemia requiring regular blood transfusions.
The resulting hypoxia and concomitant iron deposit in many organs, especially heart, liver and endocrine system may be responsible for gestational complications such as multiple organ damage of the mother with high fetal death likelihood.
In splenectomized patients the clinical condition may become worse for a high platelets number resulting in high thrombotic risk and for a higher chance of developing infections.
These conditions can be avoided through careful maternal and fetal surveillance, clinical and laboratory both, during the whole stage of pregnancy. In this regard, the literature does not report a case study worthy of note.
We describe the pregnancy of a 37 years ß-thalassemia patient splenectomized at 7 years old. She followed at the Transfusion Unit of San Gavino Hospital. The patient has always presented platelets values above the reference range (average $500x10^9$/L).
After several attempts to medically assisted fertilization has had a spontaneous pregnancy in August 2014.
Given the complexity of condition, the patient was followed up by a multidisciplinary team that has worked closely: gynecologists, medical transfusionists, clinical pathologists and hematologists.
The patient was enrolled in a diagnostic protocol including monitoring of following laboratory parameters: complete blood count (Siemens Advia 2120i), PT, PTT, fibrinogen (Instruments Laboratory Acl Top 500), C-Reactive Protein and infective surveillance.
After the first three-months of pregnancy it has been noticed an increase of leukocyte (WBC average 15.9, the maximum value $21x10^9$/L) and platelet (PLT average 550, maximum $686x10^9$/L).
It was decided to undertake a therapy with Seleparina 0.6 mg/day s.c. and perform weekly blood transfusion to keep hemoglobin values around 100 g/L.
Pregnancy followed a regular course without any complication. At the 37th week, as scheduled, it was carried cesarean delivery.
At birth the baby weighed 2610 g, with an Apgar score of 9/10.
Conclusion: thanks to the careful monitoring of the clinical and laboratory conditions of patient managed by multidisciplinary team has enabled the birth of the child. After 1 year, he is in excellent health.
Origa R, Piga A, Quarta G, et al. Haematologica 2010;95:376-81.

P038
WHEN MORE IS BETTER: AN UNUSUAL CASE OF NON-REACTIVITY OF IMMUNOGLOBULIN LIGHT CHAINS MIMICKING HEAVY CHAIN DISEASE

M.P. Campisi, I. Crespi, M. Marchiando, L. Miglio, E. Saliva, R. Serino, G. Bellomo

Lab.di Ricerche Chimico Cliniche, A.O.U. Maggiore della Carità di Novara, Novara

The heavy chain disease is a rare event, whose early recognition is critically relevant and is basically linked, following immunofixation, to the finding of immunoglobulin (heavy chain) band thickening without a concomitant detection of the same band in the light chain lanes. In march 2015 a serum sample of D.P.R (female, aged 51y) was submitted to the laboratory for the contemporary request of serum electrophoresis (SPE), immunofixation and quantification of both immunoglobulins and κ and λ free chains. The Ig concentrations were normal, the κ to λ ratio was reduced, the SPE was absolutely normal and the following immunofixation (IgG, IgA, IgM, κ and λ) showed a thickening of a band in the IgA lane. A following and deeper analysis (IgG, IgA, IgM, IgD, IgE, κ, κ free, λ and λ free) demonstrated a further faint IgG λ component. A year later the same diagnostic panel was requested for control. The Ig concentrations were normal, the κ to λ ratio was slightly reduced (for λ chain increase) and the SPE showed a shoulder in the γ region. The following immunofixation (IgG, IgA, IgM, κ and λ) showed again a thickening of the band in the IgA lane. A subsequent analysis (IgG, IgA, IgM, IgD, IgE, κ, κ free, λ and λ free) demonstrated the exclusive thickening of the IgA band without any IgG λ component. Capillary electrophoresis immunotyping with anti-IgG, IgA, IgM, κ and λ exhibited an expected decrease of the γ area (around 60% for IgG with a 2:1 ratio for κ and λ; no decrease for IgA and IgM). A further decrease in the ß area was detected exclusively for IgA with an inverse ratio for κ and λ. This suggested the presence of a small monoclonal IgA λ component. To further prove this hypothesis, serum underwent a first immunoprecipitatation with anti-λ and anti-κ antisera (separately) and the supernatants were analyzed by immunofixation (IgG, IgA, IgM, κ and λ). This procedure caused a complete disappearance of the IgA band thickening when the anti-λ antibody was employed for immunoprecipitation. The strategy described (following an apparent inappropriateness of the request) allowed to make a correct diagnosis of monoclonal component IgA/λ and to exclude a heavy chain disease.
Barkley K, Chari A. Diagnostic advanced in multiple myeloma. Curr Hematol Malig Rep 2016;11:111-7.

P039
L'IMPORTANZA DELLE CATENE LEGGERE LIBERE MONOCLONALI NEL PROCESSO DIAGNOSTICO DELL'AMILOIDOSI

F. Gulli[1], P. Piccioni [1], L. Cacciotti[2], U. Basile[3], M.L. Gozzo[1]

[1]Laboratorio centrale- Madre Giuseppina Vannini Hospital, Rome
[2]Institute of Cardiology - Madre Giuseppina Vannini Hospital, Rome
[3]Dipartimento Medicina di Laboratorio- Fondazione Policlinico Gemelli Rome

Viene illustrato un caso di un paziente maschio di 86 anni, giunto presso il pronto soccorso del nostro nosocomio riferendo dispnea e presenza di edemi periferici in data 10/02/2016.
All'anamnesi presentava fibrillazione atriale e ipertensione arteriosa e non riferiva altri precedenti cardiologici di tipo ischemico.
Gli esami emato-chimici presentavano un aumento costante e non progressivo della troponina T, associato ad una lieve piastrinopenia, ed un elettrocardiogramma con bassi voltaggi nelle derivazioni periferiche.Il paziente veniva ricoverato presso il reparto di cardiologia con la diagnosi di sospetta sindrome coronarica acuta e fibrillazione atriale.
Il giorno successivo, l'ecocardiogrammadoppler mostrava un' elevata impedenza acustica toracica, un ventricolo sinistro con spessori parietali aumentati ed un atrio sinistro ingrandito.
Veniva richiesto il dosaggio della troponina che rimaneva invariato, ed il dosaggio dell'NT-proBNP che risulta elevato.
In data 13/02/2016 veniva effettuata un'elettroforesi proteica che presentava un picco monoclonale (18.1%) in zona gamma globuline. Si eseguivano quindi immunofissazione sierica ed urinaria che evidenziavano una componente monoclonale di tipo IgG kappa e assenza della protenuria di Bence Jones. Il dosaggio delle catene leggere libere monoclonali (FLCs) mostrava una ratio Kappa/Lambda alterata pari a 11,45. La presenza di FLCs elevate in assenza di una Bence Jones positiva fa ipotizzare in associazione alla clinica una sospetta diagnosi di amiloidosi AL con interessamento cardiaco. I restanti valori emato-chimici risultavano nella norma.
Veniva eseguito l'agoaspirato su cresta iliaca posteriore superiore sinistra. L'esame microscopico mostrava l'8% di plasmacellule prevalentemente mature.
Per confermare il sospetto diagnostico il paziente è stato inviato presso un centro specialistico per eseguire l'agoaspirato del grasso periombelicale.
La concentrazione di NTproBNP e della troponina aumentate e della ratio delle FLCs alterata, sono espressione sia della compromissione emodinamica sia, verosimilmente, di quella quota di danno miocardico determinata dalla tossicità diretta delle catene leggere. Questi biomarkers sono utili sia per la stratificazione prognostica che per monitorare la risposta alla terapia.

P040
FALSA IPOGLICEMIA: UN CASO DI DIFFICILE SOLUZIONE

L. Bassi, S. Rizzardi, A. Sarzi Amadè, S. Sudati, V. Volpi, S. Testa

Laboratorio analisi chimico cliniche e microbiologia, ASST Cremona, Ospedale di Cremona

Introduzione: Il dosaggio della glicemia può presentare variabili preanalitiche.
Case report: Maschio, 69 anni, inviato dal curante al PS per astenia e stato soporoso con febbre. In trattamento con Bisoprololo 2, 5 mg, Rabreprazolo 20mg, Cardioasa 100mg, Ramipril 5mg dopo pregresso intervento per aneurisma aortico addominale (2014).
All'ingresso febbre 38°C, ECG lievemente alterato, ipoglicemia trattata immediatamente con 1 fiala di glucosio (GLU) al 5%, insufficienza renale acuto-su-cronica (IRAC). Per il persistere dello stato soporoso si ricoverava per sospetta encefalite virale, per la quale iniziava terapia specifica. Un nuovo controllo della glicemia, dopo infusione di soluzione glucosata, mostrava valori molto bassi (20 mg/dL), se pur in assenza di sintomi specifici e con esito POCT nella norma. Dopo 4 mesi il paziente veniva ricoverato in Nefrologia per approfondimenti dell'IRAC che evidenziavano componente monoclonale (CM) IgMK. La proteina Bence Jones risultava negativa con catene leggere libere k nel siero elevate (164,8 mg/dL), IgM=2204, 7 mg/dL, l'elettroforesi con immunofissazione evidenziava una CM IgMK=1,83 g/dL). La biopsia renale rilevava 4 glomeruli con catene k positive in depositi granulari globali e diffusi a livello delle anse e subepiteliali. Si riconfermava ipoglicemia severa in litio eparina (LIH), nella norma in Fluoruro ossalato e in POCT. Diagnosi alla dimissione: artrite reumatoide in macroglobulinemia di Waldenstrom.
Discussione: La discrepanza dei risultati rilevata in LIH e POCT in assenza di sintomatologia specifica,in concomitanza con la presenza di indici del siero lipemici positivi,ha indotto il successivo approfondimento diagnostico.
Infatti l'esecuzione del test con diversi anticoagulanti e nel siero ha permesso di evidenziare una interferenza analitica confermata dopo precipitazione con PEG. Tale analisi è risultata suggestiva per presenza di immunocomplessi circolanti associati a CM che ha portato alla diagnosi definitiva di Malattia di Waldenstrom.
Conclusioni: Il caso descritto mostra una "falsa ipoglicemia" causata da interferenza delle IgM con LIH ed evidenzia l'importanza dell'analisi e della valutazione degli indici del siero.
La positività dell'indice lipemico in presenza di plasma limpido è suggestiva per probabile presenza di immunocomplessi circolanti

P041
SERUM FLC TEST IN A LCMM PATIENT AFFECTED BY KIDNEY IMPAIRMENT

M. Nicosia, C. Ruscio, L. De Rosa, L. Pierelli

Lab. di Ematologia, U.O.C. di Ematologia e Trapianto Cellule Staminali, A.O. San Camillo-Forlanini, Roma

Background: Monoclonal gammopathies encompass a wide range of plasma cell disorders involving clonal B cells producing homogeneous monoclonal components (MCs). Laboratory testing plays a fundamental role in myeloma patient management, enabling the evaluation of both MC entity and type. Among tests, the serum free light chain test (FLC) has been acknowledged an important part among test panels both in national and international guidelines. More recently, the UK NICE Guidelines updated criteria for myeloma patient work-up has also included FLCs, whereas urine testing is no longer recommended. This is due to the fact that urine implies cumbersome manipulations and may be scarcely available. In such contexts, as in Light Chain Multiple Myeloma (LCMM), where only free light chains are secreted and may go undetected by less sensitive techniques, the FLC assay enables accurate detection of FLC.
Patient and Methods: We report the case of a 51 year old patient affected by LCMM referring to our hematology centre of the "San Camillo – Forlanini" hospital. The patient presented with FLC κLCMM, and severe kidney impairment. This implied that urine samples were unavailable. Throughout this time, the patient was uniquely monitored by means of serum FLC ("Freelite®" The Binding Site). The patient underwent cycles of therapy from 2008-2010 (before VEL-DEX and then LEN-DEX), when disease progression was observed. After 3 years, only serum FLC was able to detect relapse, anticipating clinical symptoms.
Results: Serum Freelite® enabled early detection of relapse, anticipating clinical symptoms. Indeed, detectable serum κFLCs were observed three weeks before appearance of clinical symptoms.
Conclusion: FLC quantification clearly enables to accurately and sensitively detect residual disease and FLC relapse before clinical signs were detectable. Thus, serum FLC monitoring throughout patient treatment is a fundamental step for MM patient work-up, as well as for guiding therapeutical choices, especially in cases such as LCMM, where MC are exquisitely detectable by such sensitive technique.

P042
UTILITA' DEL DOSAGGIO AMH NELL'IDENTIFICARE I TUMORI OVARICI

M. Brugia , A. Calcinari, A. Ceka, M. Galeazzi

Laboratorio Biochimica Clinica e Microbiologia, Azienda Ospedali Riuniti, Ancona

Donna di 32 anni si rivolge al proprio ginecologo per problemi di infertilità. Il medico le prescrive il dosaggio dell'ormone anti-Mulleriano (AMH) il quale viene eseguito in data 30/04/2015 nel nostro laboratorio che fa da centro di riferimento regionale per tale test, mediante metodo ECLIA (Cobas Roche). Risultato: 76 ng/ml. Per l'interpretazione di tale risultato la paziente viene indirizzata alla Clinica Ostetrica del nostro ospedale dove si reca il 18/06/2015 per una visita presso gli ambulatori e viene ripetuto il prelievo per il dosaggio dell'AMH. Risultato 96,7 ng/ml, riconfermato dopo 2 giorni. Il ginecologo, a seguito di questi valori e a colloqui telefonici intercorsi con il laboratorio, decide di procedere ad indagini ecografiche e evidenzia anomalie all'ovaio dx. La paziente viene sottoposta ad intervento chirurgico il 26/06/2015 e nel prelievo pre-operatorio vengono dosati anche CA19-9, CA125 e HE4 che risultano nella norma. L'esito istologico evidenzia un "tumore ovarico a cellule della granulosa". Il follow-up della paziente il 04/07/2015 evidenzia un valore di AMH di 2,39 ng/ml. La paziente inizia una gravidanza il 30/11/2015.

P043
SERUM FLC TEST IN A LCMM: AN ACCURATE AND SENSITIVE METHOD TO MONITORING THIS DISEASE

M. Nicosia, C. Ruscio, L. De Rosa, L. Pierelli

Lab. di Ematologia, U.O.C. di Ematologia e Trapianto Cellule Staminali, A.O. San Camillo-Forlanini, Roma

Background: Laboratory testing plays a fundamental role in myeloma patient management, enabling to evaluate monoclonal component entity and type. Among tests, the serum free light chain test (FLC) has been acknowledged an important part among test panels both in the International Myeloma Working Group guidelines and, more recently, in the UK NICE Guidelines updated criteria for myeloma patient work-up, especially in light of the fact that the assay enables to specifically and accurately detect and monitor monoclonal FLCs. This is of particular importance in special contexts, such as in Light Chain Multiple Myeloma (LCMM), where only free light chains are secreted and may go undetected by conventional techniques. Moreover, the FLC assay enables sensitive detection of FLC, thus anticipating relapse.
Patient and Methods: We report the case of a 71 year old woman affected by LCMM referring to our hematology centre of the "San Camillo – Forlanini" hospital. The patient presented with FLC λ MC, stage IIIA osteolytic lesions. Following therapy (three cycles of VTD) and total remission, the patient underwent autologous stem cell transplantation, obtaining a CR. Following the next 3 year, the patient relapsed and was resubmitted to therapy (VMP scheme).Throughout this time, the patient was monitored by means of serum FLC ("Freelite®" The Binding Site) and urine immunofixation (uIFE).
Results: Serum Freelite® enabled early detection of relapse, anticipating uIFE MC detectioin and clinical symptoms. Indeed, the increase of serum FLCλ was detectable about two month before uIFE and appearance of clinical symptoms.
Conclusion: FLC quantification clearly enables to accurately and sensitively detect FLC relapse, long before uIFE or even before clinical signs were detectable. Thus, serum FLC monitoring throughout patient treatment is a fundamental step for MM patient work-up, especially in these cases, such as LCMM, where MC are exquisitely detectable by such sensitive technique.

P044
UNUSUAL CONCOMITANT B-CELL CHRONIC LYMPHOCITIC LEUKEMIA AND LIGHT CHAIN AMYLOIDOSIS

G. Barilà, F. Lessi, F. Adami, L. Trentin, G. Semenzato, R. Zambello

Dept of Medicine, Hematology and Clinical Immunology section, Padua University School of Medicine, Padua

Light chain (AL) amyloidosis represents the most common type of systemic amyloidosis and is generally related to plasma cell dyscrasias but, rarely, can develop as a consequence of other B cell lymphoproliferative disorders. In this issue, we report a case of a 71 years old man affected from 2004 by B Cell-Chronic Lymphocytic Leukemia (B-CLL) and concomitant Monoclonal Gammopathy of Undetermined Significance (MGUS) IgM/κ. Due to the development of diffuse lymphoadenoapathy and anemia and thrombocytopenia, patient underwent in 2007 to bone marrow evalution with demonstration of 90% of CD5+/CD20+/CD23+/CD10-/sIgκ B Lymphocytes. As a consequence, patients started treatment with Rituximab, Fludarabine and Cyclophosphamide for 4 cycles obtaining a complete response and then he started periodic follow up. During the following 8 years M-protein progressively increased from 4,4 g/L to 10 g/L and Free Light Chains (FLCs) were first measured in July 2015 (sFLCκ=372mg/L, sFLCλ=9,8 mg/L, ratio=37,9). In March 2016 he underwent to progressive disease with development of anemia and marked increase of sFLCκ and ratio (910 mg/L, and 122 respectively); furthermore, an echocardiography of December 2015 demonstrated the presence of concentric cardiopathy with left ventricle wall thickening (14mm). Few weeks later, patient was admitted to the hospital for acute cardiac failure. Bone marrow evaluation was performed and a 100% of CD5+/CD20+/CD23+/CD10-/sIgκ infiltrating B-Lymphocytes carrying 17p deletion was discovered. To completion, peri-umbellical fat biopsy and was performed and κ chains deposition at immune-elettromicroscopy was discovered, setting diagnosis for concomitant AL κ chain-amyloidosis in relapsed B-CLL. Although AL amyloidosis is a generally associated to plasma cell discrasia, infrequently other B cell lymphoproliferative disorders may be responsible of the amyloidotic light chain production. In this patient, the B-cell κ restricted pathological clone was responsible for both CLL progression and amyloid production. Since sFLCκ and ratio were significantly increased almost one year before the development of symptomatic amyloidosis, sFLCs assay in B cell lymphoproliferative disorder should be performed in order to assess the presence of concomitant AL amyloidosis.

P045
LIGHT CHAIN MULTIPLE MYELOMA: A CASE REPORT

M. Simoncini[1], A. Vaccarino[1], M. Pagliaro[1], M. Cicilano[2], M. Bazzan[1]

[1]S.S.V.D. Ematologia, Osp. San Giovanni Bosco, Torino
[2]Lab. Analisi, Osp. San Giovanni Bosco, Torino

Background: Free Light Chain (FLC) assay is an emerging tool for evaluating patients with monoclonal gammopathy and plasma cell neoplasms, with relevant diagnostic, prognostic and therapeutic implications.
Case presentation: a 77-year-old patient with arterial hypertension, coronary artery disease, stage 0 B-chronic lymphocytic leukemia (CLL) and a long-lasting stable proteinuria, was admitted to the Nephrology Department for the recent onset of nephrotic syndrome and renal failure. The laboratory profile, including serum protein electrophoresis (SPE), was unremarkable, with the exception of proteinuria (5500 mg/24hrs), creatinine (2.5 mg/dL), lymphocytosis (Ly, 9.500/ mm3) and a severe hypogammaglobulinemia (IgA 18 mg/dL, IgG 233 mg/dL, IgM 20 mg/dL). Renal biopsy was performed, showing diffuse amyloidosis; however, immunohistochemistry for light chains was negative. Echocardiography and electroneuronography indicated infiltrative cardiomyopathy and axonal sensorimotor polyneuropathy, respectively. The neurological involvement and the absence of monoclonal spike on SPE led to the hypothesis of a genetic amyloidosis (e.g. due to transthyretin mutation). However, to rule out an AL-type amyloidosis, serum FLC was measured, showing significant impairment of the κ/λ ratio (<0.01). This finding suggested a monoclonal gammopathy involving light chains, therefore, bone-marrow biopsy was performed. It showed a massive infiltration of lambda clonal plasma cells, leading to the final diagnosis of light chain multiple myeloma (MM). According to the WHO criteria for MM treatment, a Bortezomib-based chemotherapy regimen was started. After three courses of therapy, despite clinical improvement, lambda free light chains showed a marked increase, leading to a new Melphalan-based therapeutic strategy.
Conclusion: measurement of FLC ratio was crucial for the diagnosis in this troublesome light chain MM case. It was the only non-invasive examination able to guide to the correct diagnosis of AL amyloidosis, despite the high suspicion of genetic amyloidosis. Moreover, it proved to be a reliable tool to monitor therapeutic response.

P046
INFERO-LATERAL ST-ELEVATION MYOCARDIAL INFARCTION (STEMI) IN A 29-YEARS PATIENT WITH CORONARY ECTASIA AND HIGH PLASMA LEVEL OF COAGULATION FACTOR VIII

M.A. Perrone[1], A. Intorcia[2], R. Morgagni[2], M. Marchei[2], F. Romeo[2], S. Bernardini[1]

[1]Cattedra di Biochimica Clinica e Biologia Molecolare Clinica, Università Tor Vergata, Roma
[2]Cattedra di Cardiologia, Università Tor Vergata, Roma

Background: Coronary-Artery-Ectasia (CAE) is a luminal expansion 1,5 greater than the diameter of the normal adjacent arterial segment. It can be either diffuse, affecting the entire length of a coronary artery, or localized. The pathophysiology of coronary ectasia remains widely unknown. High plasma levels of factor VIII of coagulation are linked to increased risk of thromboembolism. The increase of FVIII is an important risk factor for acute thrombotic events, even in the absence of a significant underlying atherosclerotic disease.
Case Report A 29 years old patient from Gambia with no risk factors or previous cardiac disease, was referred from E.R. to our cathlab for chest pain and ST segment elevation in inferolateral leads on ECG . Blood tests showed TnI 120 ng/ml. The angiogram documented the presence of an ectatic circumflex artery (CX) with an large endoluminal thrombus extended from the proximal segment to the distal segment, also extended to the first marginal branch. The other vessels, instead, were appearing free from a thromboembolic disease. A thrombo-aspiration was performed, extracting a large amount of tubular red thrombus (>1 cm), and a primary angioplasty was made with two drug eluted stent (2.5 x 30 x 3 mm and 15 mm on RMO) and a second stent 2.5 mm x 15mm on CX before PL. The procedure was optimized by a final Kissing Balloon around the M1- CX bifurcation. We evaluated the thrombophilia screening tests and we found an increase of coagulation factor VIII.
Conclusion: Myocardial infarction in young adults (<40 years) is a relatively rare phenomenon and its incidence varies between 2% and 10% according to various studies. The association between coronary artery ectasia and thrombophilia causes an increased risk of ischemic heart disease regardless of age and cardiovascular risk factors.
Hernández V, Muñoz N, Montero MA, et al. Acute myocardial infarction for thrombotic occlusion in patient with elevated coagulation factor VIII. Rev Esp Cardiol (Engl Ed) 2012;65:673-4.

P047
VALUTAZIONE FLC SU SIERO VS FLC SU SANGUE MIDOLLARE

R. Nuccorini[1], A. Girardi[2], S.P. Pascale[1], R. Penitente[2], E. Scavone[2], I. Attolico[1], A. Amendola[1], A. Matturro[1], G. Smaldore[2], M. Pizzuti[1]

[1]UO Ematologia, AOR San Carlo, Potenza
[2]UO Diagnostica di Laboratorio, AOR San Carlo, Potenza

In 28 pazienti (pz) affetti da Mieloma Multiplo (MM) abbiamo valutato contemporaneamente le FLC sul siero (sFLC) e sull'aspirato midollare (mFLC). In 3 pz il test è stato eseguito alla diagnosi e durante la terapia. In 25 (80%) abbiamo riscontrato concordanza tra i due dati (rapporto tra sK/L e mK/L compreso tra 0,8 e 1,2). In 5 (16%) abbiamo apprezzato una differenza fra i tipi di prelievo anche se il rapporto K/L era comunque alterato. In 1 (3%) il rapporto K/L era normale su siero e alterato su midollo. Si tratta di una donna di 63 anni con diagnosi di MM IgG k IIIA giunta c/o l'UO di Ematologia dell'AOR San Carlo di Potenza, il 13/06/2015, per astenia, inappetenza e dolori al rachide lombare. Alla diagnosi: Emoglobina 7,1 g/dL Globuli Bianchi 7100/ul Piastrine 236000/ul, creatinina 0,92 mg/dL, proteine tot. 14,30 g/dL, Calcio 9,4 mg/dL, LDH 213 U/L, B2 microglobulina 24,0 mg/dL, %γ 62,63, Componente Monoclonale (CM) 35%, Bence Jones assente, Plasmacellule (Pc) midollari>90%. In citofluorimetria: 77% di Pc con fenotipo patologico (38+138+56+19-K+). La risonanza magnetica mostra localizzazione di malattia. Eseguito il dosaggio del K/L sFLC 1,58 mentre mFLC 16,59. La pz inizia chemioterapia secondo protocollo Cy-BOR. sFLC è stato monitorato ad ogni ciclo di terapia: 2° 0,44 – 3° 0,40 – 4° 0,26. Rivalutazione di malattia ad ottobre 2015: Funzionalità epatica e renale nella norma, Pc1%, IF+, %γ 11,56, CM 4%, K/L sFLC 0,31. Status della malattia VGPR. Trapianto autologo il 07/12/2015. A 3 mesi: BOM 10% di Pc, IF +, %γ 8,51, K/L sFLC 2,37 (K:12,30–L:5,20), K/L mFLC: 1,34 (K:9,33–L:6,98). Status della malattia nCR, inizia il consolidamento con VTD. L'alterazione delle FLC è stata evidenziata alla diagnosi solo nel midollo. Seguendo le linee guida per la diagnosi del MM (monitoraggio su siero) l'alterazione delle FLC non sarebbe stata evidenziata. Il mK/L si è ridotto dopo terapia, invece sK/L non correlava con la malattia. Non sappiamo spiegare la discordanza dei test ed in letteratura non sono riportate valutazioni su midollo. La nostra esperienza deve essere approfondita su una casistica più ampia, indicherebbe però l'utilità di effettuare il test sia sul siero che sul midollo nei casi in cui l'esame su siero non mostra alterazione dei livelli di FLC.

P048
DIAGNOSI DI LIGHT CHAIN ESCAPE IN UN PAZIENTE AFFETTO DA MIELOMA MULTIPLO

K. Pocino[1], C. Napodano[1], T. Za[2], F. Gulli[1], V. De Stefano[2], U. Basile[1]

[1]Dip. di Medicina di Laboratorio, Fondazione Policlinico Universitario "A. Gemelli", Roma
[2]Istituto di Ematologia, Fondazione Policlinico Universitario "A. Gemelli", Roma

Uomo, 67 anni, si presenta a Giugno 2011 con diagnosi di Mieloma Multiplo asintomatico effettuata presso altro centro. Eseguiti quindi esami di ri-stadiazione: Hb 12.7 mg/dL; creatinina sierica 0.82 mg/dL, calcemia 8.7 mg/dL, ß2 microglobulina 2.9 μg/mL, IgA sieriche 2500 mg/dL, picco in ß2 all'elettroforesi proteica ed immunofissazione S/U con componente monoclonale IgA k e presenza di proteinuria di Bence Jones. L'aspirato midollare (AM) evidenzia un infiltrato di plasmacellule a clonalità k pari al 30%. Rx dello scheletro, RMN di colonna e bacino e PET/TC non presentano anomalie, confermando dunque la diagnosi precedente. A Ottobre 2012, per rachialgia il paziente esegue Rx e RMN evidenziando crollo somatico di L5, osteolisi a livello di C3, C6, L1, L5, della cresta iliaca destra, del III prossimale del femore destro. L'AM mostra infiltrato plasmacellulare del 15% e la biopsia osteomidollare il 35% della cellularità. Le IgA sono 3050 mg/dL, ß2 microglobulina 3.6 μg/mL, albumina, Hb, creatinina e calcio nella norma. Viene posta diagnosi di Mieloma sintomatico (Stadio Durie e Salomon IIA, ISS-2) e intrapresa terapia con Velcade, Melphelon, Prednisone per 9 cicli. Gli esami di rivalutazione evidenziano una risposta completa (ipo-γglobulinemia, IF siero e urine negative; IgA 100 mg/dL, normali i parametri ematochimici, assenza di nuove lesioni). Follow-up osservazionale fino all'Aprile 2015, quando una nuova RMN pone sospetto di progressione. Incremento della quota plasmacellulare (8%) nell'AM. Tuttavia, IEF siero e le IgA sono nella norma. Sospettando recidiva come micromolecolare vengono dosate le catene leggere libere (FLC) di tipo k (246.4 mg/L) e ratio k/λ 128.3. Si tenta reinduzione con Bortezomib e Desametasone da Giugno a Ottobre 2015 senza ottenere risposta: gli esami evidenziano progressione di malattia, con aumento delle FLCk (1301 mg/L e ratio k/λ 1016), delle plasmacellule fino al 33% e Hb 10 mg/dL. Si somministra terapia di III linea (Revlimid e Desametasone), con una risposta precoce (normalizzazione dei livelli di Hb), ma transitoria, monitorata dal dosaggio delle FLCk che si riducono a 37.1 mg/L per poi aumentare nuovamente (596.1 mg/L), fino a progressione franca con Hb 9.9 mg/dL. Attualmente il paziente segue terapia sperimentale con Carfilzomib.

P049

CATENE LIBERE LEGGERE NEL MONITORAGGIO DELLA TERAPIA CON RITUXIMAB IN PAZIENTI AFFETTI DA CRIOGLOBULINEMIA MISTA HCV CORRELATA

C. Napodano[1], K. Pocino[1], F. Gulli [1], G.L. Rapaccini[2], U. Basile[1]

[1]Dip. di Medicina di Laboratorio, Fondazione Policlinico Universitario "A. Gemelli", Roma

[2]Istituto di Medicina Interna, Fondazione Policlinico Universitario "A. Gemelli", Roma

Si riporta il caso di una paziente, donna di 53 anni, che si è presentata all'Istituto di Medicina Interna, con porpora agli arti inferiori e astenia, riferisce pregressa infezione da HCV, per cui si sospetta Vasculite crioglobulinemica. Per un corretto inquadramento diagnostico la paziente è stata sottoposta a prelievo venoso per Elettroforesi proteica, Immunofissazione, presenza di crioglobuline, C4 e Fattore reumatoide. I risultati hanno evidenziato ipogammaglobulinemia, presenza di componente monoclonale IgMk, criocrito pari al 2.1% di tipo II, bassi livelli di C4 e presenza di Fattore Reumatoide IgM, confermando il sospetto di vasculite crioglobulinemica associata ad HCV. Dai dati bioptici la paziente è risultata affetta da un linfoma a cellule B di basso grado. Essendo la nostra paziente ineleggibile alla terapia virale convenzionale si è deciso di misurare i livelli di Free Light Chains (FLCs) e la loro ratio poiché studi recenti hanno valutato l'uso delle FLC come marcatore di efficacia terapeutica, inoltre hanno dimostrato che nei pazienti con una ratio aumentata la terapia migliore è la somministrazione di Rituximab. Siccome la deplezione delle cellule B dal sangue periferico in questi pazienti riduce marcatamente le concentrazioni di FLCs, questo dato può costituire un marker valido nel monitoraggio dei pazienti che ricevono terapia anti-CD20.

La nostra paziente presentava una ratio k/λ di 1.89 (intervallo di riferimento 0.26-1.65), per cui si è optato per il Rituximab. Alla fine del ciclo terapeutico la paziente ha mostrato una risposta completa al farmaco con ratio k/λ di 0.94, la scomparsa delle crioglobuline e regressione del linfoma. Pertanto l'uso delle FLCs ci ha permesso di scegliere la terapia migliore e di predire la risposta a un trattamento costoso per i pazienti ineleggibili o refrattari alla terapia a base di Interferone. Il rapido turnover delle FLCs, particolarmente le k (meno di 6h) spiega perché possono essere considerate marker ideali di risposta al trattamento con Rituximab.

P050

A STRANGE CASE OF HYPOGONADISM : UTILITY OF SHBG ASSAY

G. Spaggiari[1], B. Madeo[1], L. Roli[2], M.C. De Santis[2], E. Baraldi[2]

[1]Unit of Endocrinology, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena

[2]Laboratory of Endocrinology, Department of Laboratory Medicine and Pathological Anatomy, NOCSAE, AUSL, Modena

Background: Androgens play a crucial role in the development and maintenance of male health. Low androgens levels in adulthood may cause reduced fertility, sexual dysfunction, decreased muscle formation and bone mineralization, disturbances of fat metabolism and cognitive dysfunction. Hypogonadism is diagnosed on the basis of persistent signs and symptoms related to androgen deficiency and assessment of consistently low testosterone levels (at least on two occasions) with a reliable method.

Case description: A 44-year-old Caucasian man presented with metastatic disease 12 years following a right adrenalectomy for androgens-secreting adrenocortical carcinoma. Blood tests and radiological findings showed metastases to right kidney and liver from androgens- and cortisol-secreting adrenocortical carcinoma. The patient was surgically treated, then he was started on mitotane monotherapy. This drug is cytotoxic on the adrenal cortical cells, although the exact mechanism of this action is actually unknown. After surgery and adjuvant therapy, there has been no evidence of recurrence of malignancy. During the follow-up, the patient complained hypogonadism symptoms, despite normal levels of total testosterone (TT).

Methods: Laboratory tests: TT, luteinizing hormone (LH) and sex hormone binding globulin (SHBG) levels were measured with routine CMIA assays Architect (Abbott GmbH & Co, Germany). Free testosterone (FT) and bioavailable testosterone (BT) were calculated with Vermeulen algorithm.

Results: Blood tests confirmed normal TT (6.3 ng/ml) with high levels of LH (42 mIU/ml [normal range 1-9]). SHBG was very high (>250 nmol/L [normal range 13.5-71.4]), so serum levels of FT and BT resulted undetectable.

Conclusions: High levels of SHBG in this patient can be explained by the concomitant mitotane therapy, able to stimulate the hepatic production of binding proteins. Iatrogenic hypogonadism diagnosis required androgen replacement therapy, with resolution of hypogonadism-related symptoms. In this case report, SHBG assay, although considered obsolete, facilitated clinical practice and improved patient care.

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P051
FRAZIONE IMMATURA DELLE PIASTRINE IN UN CASO DI PORPORA TROMBOTICA IMMUNE

B. Manenti, M. Seghezzi, P. Dominoni, S. Buoro

USC SMeL Generale di Base - Analisi Chimico Cliniche, Azienda Socio Sanitaria Territoriale Papa Giovanni XXIII

Presentiamo il caso di una donna di 35 anni che esegue un emocromo per astenia e metrorragia. L'emocromo, eseguito con Sysmex XN 9000, mette in evidenza una lieve piastrinopenia (116x109/L), con restanti parametri nella norma. Dopo 5 giorni, il conteggio delle piastrine scende a 91x109/L, con frazione delle piastrine immature (IPF) nella norma (4,2%, intervallo di riferimento 1,2-7,9).Dopo due settimane il conteggio è di 9x109/L piastrine,con IPF aumentata a 19,1%, con comparsa di petecchie al collo. Non vengono evidenziate alterazioni allo striscio di sangue periferico, vengono esclusi lupus eritematoso sistemico ed Epatite C. Viene eseguito aspirato midollare negativo per patologie maligne. Viene impostata terapia steroidea, con risoluzione della piastrinopenia (PLT 211x109/L, IPF 3,6%). La terapia viene scalata gradualmente, nell'arco di settimane. Alla sospensione si ha nuovamente riduzione del numero delle piastrine con aumento della IPF (PLT 46x109/L, IPF 9%). La paziente permane in osservazione senza terapia ed esegue regolarmente emocromi di controllo.

E' stata posta diagnosi di porpora trombotica immune (PTI), un disordine autoimmune caratterizzato da piastrinopenia isolata con distruzione immunomediata delle piastrine.

Questo caso, sebbene sia una presentazione classica di PTI, è adatto ad analizzare il ruolo diagnostico e di monitoraggio della IPF nella PTI: la IPF rappresenta la porzione di piastrine appena prodotte dal midollo, per cui, in caso di piastrinopenia, una sua diminuzione è indicativa di insufficiente produzione di piastrine a livello midollare. L' aumento della IPF, invece, può significare aumentata produzione di piastrine, in genere in risposta ad un processo di consumo o distruzione periferica. Nel nostro caso, è visibile come la IPF sia elevata in corrispondenza della discesa del numero delle piastrine. Non è possibile apprezzare il ruolo della IPF nel predire la risalita del conteggio piastrinico, perché gli emocromi sono stati eseguiti a distanza di troppo tempo l'uno dall'altro.

Diversi lavori hanno confermato il ruolo della determinazione della IPF in diagnostica, sia nella PTI che in altre patologie ematologiche, oltre al ruolo predittivo della ripresa della trombopoiesi dopo chemioterapia o dopo trapianto di midollo.

P052
UN CASO DI EMOFILIA ACQUISITA

C. Dodaro, M. Vitiritti, G.R. Greco, C. De Rosa , M. Miraglia , B. Mancuso, A. Amendola , S. Vaccarella

Laboratorio Analisi Cliniche, Biologia Molecolare e Genetica, Osp. Annunziata, Cosenza

L'emofilia acquista è una patologia rara (prevalenza: 1 caso/milione), caratterizzata dall'insorgenza di gravi manifestazioni emorragiche causate da autoanticorpi diretti contro il fattore VIII circolante. Le emorragie sono spesso gravi e interessano i tessuti molli e le mucose, mentre gli emartri sono più rari. La diagnosi si basa sull'evidenza di un aPTT prolungato (ratio 2,5), non corretto dalla miscela. Gli inibitori del fattore VIII sono tempo e temperatura dipendenti, quindi è necessario eseguire il test della miscela al tempo 0 e dopo 2h di incubazione. Il prolungamento del aPTT dopo 2h di incubazione è tipico della presenza di autoanticorpi contro il fattore VIII. La determinazione quantitativa degli autoanticorpi viene eseguita con il metodo Bethesda. La presenza di inibitori viene classificati a basso titolo (<5 UB/mL) e alto titolo (>5 UB/mL). Il caso clinico da noi studiato riguarda un pz di 82 anni ricoverato presso il P.O. di Cosenza con diagnosi di anemia grave in pz con IRC, con pregresso ictus cerebri e in terapia con dicumarolici. Veniva sospesa la terapia anticoagulante a base di dicumarolici ed eseguito uno studio approfondito della coagulazione dopo normalizzazione dell'INR. Il test della miscela eseguito senza incubazione non correggeva il prolungamento dell aPPT, deponendo per la presenza di inibitori. I dosaggi LAC e gli anticorpi anti beta2 glicoproteina 1 sono risultati negativi, mentre il dosaggio dei fattori della via intrinseca evidenziava una carenza del fattore VIII (6%). Il dosaggio degli inibitori acquisiti del fattore VIII eseguito con il metodo Bethesda modificato Nijmegen, ha invece dato esito positivo (4 UB/mL). Viene quindi diagnosticata una emofilia acquisita che richiede tempestività e competenza diagnostica. Nel caso del pz in esame evidentemente solo grazie al basso livello di inibitori e quindi alla persistenza di una residua attività di fattore VIII si è evitato l'aggravarsi della situazione anemica del pz. La gestione di tale patologie richiede un intervento tempestivo e competente. Pertanto, è auspicabile che vengano individuati centri regionali, clinici e di laboratorio, attivi h24, in grado di provvedere alla valutazione e alla diagnosi di casi selezionati.

Franchini M, Lippi G. Acquired hemophilia A. Adv Clin Chem 2011;54:71-80.

P053
INSUFFICIENZA RENALE ACUTA IN CORSO DI PARAPROTEINEMIA: ALLA RICERCA DEL COLPEVOLE!

S. Murgia, G. Quattrocchio, M. Ferro, G. Beltrame, G. Del Vecchio, R. Fenoglio, P. Mesiano, D. Roccatello

SCDU Nefrologia e Dialisi e Centro di Ricerche di Immunopatologia e Documentazione Malattie Rare, Ospedale San Giovanni Bosco e Università di Torino

L'interessamento renale in corso di paraproteinemia è frequente e può portare a danno d'organo irreversibile. Il dosaggio delle catene leggere libere (FLC), in particolare il rapporto k/λ, permette un'identificazione precoce dei soggetti a rischio ed è di grande ausilio nella definizione diagnostica e nell'orientamento terapeutico.
Descriviamo il caso di un paziente di 80 anni, con polipatologia ed insufficienza renale di grado moderato, giunto in DEA per edema, dolore all'arto inferiore destro, disuria ed oliguria. EDV: TVP femoropoplitea, con inizio di terapia anticoagulante. Ematochimici: creatinina=10 mg/dl. Ricovero in Nefrologia e avvio di trattamento sostitutivo dialitico per ulteriore incremento della creatinina. Non eseguibile biopsia renale per terapia anticoagulante. Elettroforesi sierica: marcata ipogammaglobulinemia, non picchi monoclonali. Dosaggio FLC: notevole incremento catene λ sieriche (15 g/l, ratio κ/λ<0,01) ed urinarie (2,5 g/24h). Rx sistematica ossea: non lesioni osteolitiche. Biopsia osteomidollare: 7% di plasmacellule monoclonali λ. L'elevata quota di FLC indirizzava fortemente verso una tubulopatia ostruttiva da verosimile precipitazione delle catene leggere e veniva avviata terapia citoriduttiva con desametasone 20 mg/die per 4 gg. Inoltre, si sottoponeva il paziente a 10 sedute di emodiafiltrazione con reinfusione endogena (HFR) con filtro ad elevata permeabilità. A fine ciclo: λ sieriche = 2 g/l, consensuale ripresa della funzionalità renale e sospensione del trattamento dialitico.
L'insufficienza renale acuta in corso di paraproteinemia può riconoscere diverse eziologie e quadri istologici polimorfi. L'accertamento bioptico è fondamentale per una corretta allocazione nosografica e la conseguente terapia. Nel caso descritto non è stato possibile effettuare la procedura bioptica ma il dosaggio FLC ha consentito una diagnosi di presunzione ed è stato determinante nell'indirizzare la terapia. Inoltre, i controlli sequenziali del livello di FLC in corso di HFR hanno mostrato una progressiva riduzione del "tossico endogeno" accompagnata dal consensuale miglioramento funzionale renale, a supporto dell'ipotesi diagnostica.
In conclusione, il dosaggio delle FLC rappresenta un indispensabile strumento diagnostico e un valido mezzo per valutare l'efficacia terapeutica.

P054
PHENOTYPIC CORRECTION OF ACTIVATED PROTEIN C (APC) RESISTANCE AFTER LIVER TRANSPLANTATION

C. Mandoj[1], M.G. D'Alessandro[1], M. Montalbano[2], L. Autullo[1], G.M. Ettorre[3], L. Conti[1]

[1]Clinical Pathology, Regina Elena National Cancer Institute, Rome
[2]Hepatology and Infectious Diseases Unit, National Institute for Infectious Diseases "L. Spallanzani", Rome
[3]Division of General Surgery and Liver Transplantation, San Camillo Hospital, Rome

Activated protein C (APC) resistance is a prominent risk factor for venous thrombosis. Over 90% of cases of APC resistance are associated with the factor V (FV) Arg506Gln mutation (FV Leiden), which impairs the APC-mediated inactivation of both FVa and factor VIIIa. Although APC resistance may occur in the absence of FV Leiden, the opposite is usually not true. Here we report a case of 59-year-old man, who underwent an orthotopic liver transplantation for HCV and alcohol related cirrhosis, who presented with thrombosis of piggy-back 15 days post liver transplant. Further questioning revealed that he had previously suffered a mesenteric-portal vein thrombosis. Laboratory findings for risk factors contributing to hypercoagulability showed normal plasma levels of fibrinogen and antithrombin, slightly low levels of protein C and protein S, and a normal activated protein C resistance test (2.76; reference range >2.60). A genotypic analysis from the patient's peripheral blood cells showed that the patient was heterozygous for FV Leiden. As the liver is the main site of factor V synthesis, this discrepancy between genotype and phenotype suggested that the normal APC resistance was acquired through liver transplantation from FV wild-type donor.
Several cases of heterozygous FV Leiden subjects with phenotypic correction of APC resistance by orthotopic liver transplantation have been reported (1-3). In some of them, the patients suffered thrombosis before or after liver transplantation, despite the phenotypic correction of APC resistance. These cases witness the difficulties of thrombophilia testing after liver transplantation and emphasize the need for evaluating both FV phenotype and genotype in order to accurately assess thrombotic risk in such patients.
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2. Estelles A, et al. Factor V Leiden in absence of activated protein C resistance after orthotopic liver transplantation in a patient without thrombosis but with familial thrombophilia. Haematologica 2000;85:111-12.
3. Ayala R, et al. Recipient and donor thrombophilia and the risk of portal venous thrombosis and hepatic artery thrombosis in liver recipients. BMC Gastroenterol 2011;11:130.

P055
CAN LAMBDA FREE LIGHT CHAIN INDEX BE A USEFUL PARAMETER FOR THE DIAGNOSIS OF MULTIPLE SCLEROSIS?

M.T. Dell'Abate, D. Lopergolo, T. De Michele, C. Zuppi, D. Scribano

Fondazione Policlinico Universitario A. Gemelli, Rome

Multiple Sclerosis (MS) is an inflammatory disease leading to demyelination and axonal damage. The diagnosis is based on Magnetic Resonance Imaging (MRI) and laboratory test. The detection of oligoclonal immunoglobulin bands (OCB) by mean isoelectric focusing (IEF) suggests an intrathecal production of immunoglobulins. Although OCB analysis requires time, manual expertize and qualified personnel for results interpretation, it is still considered the Gold Standard technique for diagnosing MS. Moreover, OCB analysis discloses interpretative limitations upon dubious OCB results. Recently, Free Light Chains (FLCs) have raised attention on their potential diagnostic value in the context of MS, especially κFLCs. Indeed, significance of λFLC values in MS have not been clarified yet, as only κFLCs have been found to be specific for MS. In fact, elevated λFLCs seem specific for acute central nervous system (CNS) infections. However, the single serum levels of FLCs were not taken into account for the assessment of a transudation process.

A 56 years old man presented with strength deficiency and lower left limb hyperreflexia, low white blood cell count and negative microbiological cerebrospinal fluid (CSF) and serum examinations. CSF analysis yielded negative IgG index, negative CSF/serum albumin ratio (5.3) and OCB analysis indicated a borderline aspect (1 single supernumerary band). FLCs were quantified in coupled CSF and serum samples using Freelite (The Binding Site Ltd, UK).

CSF κ and λ FLCs were respectively 0.64 and 0.70 mg/L; interestingly the κFLC index was 10.5 (normal reference values ≤5.9) and λFLC index was 25.4 (normal reference values ≤17). Serum FLCs were within the reference range.

The patient underwent MRI, visual evoked potentials, motor evoked potential and sensory evoked potentials. Results were indicative of clinically isolated syndrome (CIS).

The FLCs CSF test may be a valid tool in aid of MS diagnostic lab protocols especially upon dubious or unclear OCB results, in order to confirm or reject all suspicions. Moreover, our clinical case suggests that determination of λFLC index could be useful in diagnosis of demyelinating diseases of the CNS. Further studies are required to confirm this observation.

P056
AN INCREASED CDT CAMOUFLAGED A MONOCLONAL LIGHT CHAIN GAMMOPATHY: AN APPROACH FOR DIAGNOSIS

M. Barbaro, G. Passerini, M. Trbos, A. Soldarini, M. Locatelli

Servizio di Medicina di Laboratorio, Ospedale San Raffaele, Milano

Introduction: Carbohydrate-deficient transferrin (CDT) is the most reliable indicator for the detection of chronic alcohol consumption. Recently, we have investigated a clinical case in which a concomitant monoclonal light chain gammopathy mimicked an increase of this biomarker.

Materials and methods: A patient's serumwas routinely examined by capillary electrophoresis (CE) for evaluation of CDT, and it was subsequently analysed through high-performance liquid chromatography (HPLC) to confirm the referred result. Then, according to the patient's clinical history, we performed serum and urine immunofixation, together with k and λ free light chain measurement.

Results: The pathological CDT value obtained by CE agreed with the patient's previous data, but it was not confirmed by the HPLC. The patient's medical record revealed hypogammaglobulinaemia since 2006, which had been recently examined by a haematological visit. Serum and urine immunofixation revealed a light chain gammopathy, which had been suspected but never confirmed by laboratory assessment. The k and λ free light chain measurement completed the diagnostic process.

Conclusion: To the best of the authors' knowledge, this is the first study of its kind to report on a perfect camouflaging of a monoclonal light chain as disialo-transferrin. The importance of the careful examination of the patient's clinical history for the correct evaluation of laboratory results, thereby preventing misinterpretations, is also highlighted.

Barbaro M, Passerini G, Trbos M, et al. An increased CDT camouflaged a monoclonal light chain gammopathy: An approach for diagnosis. Clin Biochem 2016;49:835-8.

P057

A BETA-MIGRATING COMPONENT CO-MIGRATING WITH C3 PROTEIN: HEAVYLITE IS AN ESSENTIAL TOOL FOR ACCURATE MONOCLONAL COMPONENT QUANTIFICATION

L. Fringuelli[1], R. Buzzi[1], R. Carrozza[1], A. Fiorini[2], M. Montanaro[2], M.t. Muratore[1]

[1]U.O. Diagnostica Clinica PO Belcolle, Viterbo
[2]U.O.C. Ematologia

Background: Laboratory testing plays a crucial role in the diagnosis and monitoring of multiple myeloma patient. According to national and international guidelines, all serum tests must be performed, in order not to miss any patients. As of today, most guidelines (IMWG, NICE) recommend performing serum protein electrophoresis (SPE) and immunofixation (IFE), along with serum free light chain (sFLC) tests, in order to assess monoclonal component (MC) homogeneity and entity. Nevertheless, SPE may not be sensitive enough: thus, the Hevylite test, a novel assay enabling immunometric quantification of individual immunoglobulins may provide additional information in these clinical situations.
Patient and methods: We report a case of a 59 year old male subject, referring to our Hematology unit of Belcolle Hospital, Viterbo, in 2013. SPE and IFE (Interlab) were performed as routine analysis, along with free light chain (FLC) (The Binding Site) quantification.
Results: SPE analysis revealed a peak in the β1 region, not quantifiable by densitometric scanning due to the limited entity. IFE analysis confirmed the presence of an IgAλ component. SFLC tests were in range (FLCκ=10.33mg/L, FLCλ=13.53 mg/L, FLC ratio=0.76). Peak entity was so limited that was impossible to quantify it, the β1 region was within normal ranges (8.7%, n.v.= 6-9.8%); only the Hevylite test was able to accurately measure the MC, yielding precise numerical results (IgAκ=0.559 g/L, IgAλ=3.965* g/L;), HLC ratio= 0.14. Both IgAλ and HLC ratio were were out of range (n.v.: IgAκ=0.57-2.08 g/L, IgAλ=0.44-2.04 g/L, HLC ratio= 0.78-1.94), indicating an imbalanced production of monoclonal IgAλ component, accurately quantifiable by Hevylite assay only.
Conclusion: Accurate MC quantification is crucial for patient monitoring. SPE densitometric scanning in particular cases is not adequate (especially for beta-migrating components where co-migrating proteins may cause inaccurate estimates). Hevylite enables accurate intact Ig quantification of both involved and uninvolved Igs, and is therefore a valid alternative tool for accurate MC quantification in these particular situations.
Caldini A, Graziani MS, Basile U, e al. Il contributo della diagnostica proteica nella gestione delle gammopatie monoclonali. Biochim Clin 2014;38:47-53.

P058

HEAVYLITE IS AN ADDITIONAL TOOL FOR ACCURATE MONOCLONAL COMPONENT QUANTIFICATION: OUR EXPERIENCE - A CASE HISTORY

L. Fringuelli[1], M.t. Muratore[1], R. Carrozza[1], A. Fiorini[2], M. Montanaro[2], R. Buzzi[1]

[1]U.O. Diagnostica Clinica PO Belcolle, Viterbo
[2]U.O.C. Ematologia

Background: Laboratory testing plays a crucial role in the diagnosis and monitoring of multiple myeloma patient. Among laboratory tests, both national and international guidelines recommend serum protein electrophoresis (SPE) for monitoring the monoclonal component (MC) entity. Nevertheless, SPE is a semi-quantitative method with limited accuracy: restrictions to the technique may be due to components migrating in other zones of the electropherogram or MC of small entity, below linearity. The new Hevylite test, a polyclonal antibody-based immunoassay, may therefore offer a valid alternative, providing immunometric quantification of individual immunoglobulins and additional information in these clinical situations.
Patient and methods: We report a case of a woman aged 80, referring to our Hematology unit of Belcolle Hospital, Viterbo, with a C.M. IgAλ since 1990. In 2000 she was diagnosed with renal amyloidosis. SPE (Interlab) were performed as routine analysis, along with free light chain (FLC) and Hevylite (The Binding Site) quantification. SPE revealed a peak in the β1 region. Total Igs were also assessed (Siemens Healthcare).
Results: We repeated sFLC test and Hevylite test, in 2015 and in 2016. Single sFLC tests were out of range, despite FLC ratios were not in either determination (FLCκ=22.36 mg/l, FLCλ=34.48 mg/L, FLC ratio=0.65), (FLCκ=38.45 mg/L, FLCλ=37.31 mg/L, FLC ratio=1.03). Hevylite quantifications yielded the following: IgAκ=0.303 g/L, IgAλ=9.409 g/L, HLC ratio=0.03, IgAκ=0.06 g/L, IgAλ=10.25 g/L, HLC ratio=0.006. Both IgAλ and HLC ratios were out of range (n.v.: IgAκ=0.57-2.08 g/L, IgAλ=0.44-2.04 g/L, HLC ratio= 0.78-1.94). MC by SPE densitometric scan was 8.0 g/L and total IgA= 8,4 g/L initially, then 10 g/L by SPE and 13.3g/L total IgA.
Conclusion: SPE and accurate MC quantification are crucial for patient monitoring. IMWG indications suggest not to quantify β migrating peaks, and IgA values are affected by policlonal concentration; only the Hevylite test accurately quantifed the MC giving precise numerical values. Moreover in this case the sFLC ratio used for monitoring amyloidosis gave always normal values, while Hevylite showed an increase in the monoclonal isotype and suppression of the non monoclonal type.

P059
UN CASO DI MIELOMA MICROMOLECOLARE: RUOLO ESSENZIALE DEL LABORATORIO

F. Bottan, C. Maida, F. Pauselli, M. Gaudio

Azienda Ospedaliera San Giovanni Addolorata, Roma

Scopo del lavoro: Dimostrare, attraverso un caso clinico, il ruolo essenziale della diagnostica di laboratorio nelle discrasie plasmacellulari.
Materiali e metodi: Paziente di anni 81 viene ricoverata presso il DH Ematologico a causa di una anemia di ndd. L'esame morfologico del midollo osseo mostra la presenza del 20% di plasmacellule. Un campione di aspirato midollare viene inviato, con sospetta diagnosi di mieloma multiplo, presso la Patologia Clinica dell'A.O. San Giovanni Addolorata di Roma. E' stato eseguito lo studio immunofenotipico utilizzando la combinazione dei marker CD38 CD138 CD45 CD117 CD56 CD19, in un'unica provetta. I dati sono stati ricavati con tecnica citofluorimetrica a 6 colori (FACS CANTOII Bencton Dickinson) cui è seguita l'acquisizione tramite il FACSDiva Software. L'elettroforesi sierica è stata eseguita con Capillarys Sebia, l'immunofissazione con Hidrasys, i dosaggi nefelometrici con BNAII Siemens.
Risultati: Lo studio CFM dell'aspirato midollare ha evidenziato una popolazione omogenea di plasmacellule (20% circa) con immunofenotipo atipico CD38+ CD138+ CD56+ CD117+ CD19- CD45-. L'EF del siero ha evidenziato assenza di CM caratteristico del mieloma multiplo ma anche una spiccata ipogammaglobulinemia, confermata dalle basse concentrazioni delle immunoglobuline con i dosaggi nefelometrici. L'immunofissazione con antisieri standard e quella con antisieri anti IgD, anti IgE e anti catene lambda libere hanno evidenziato e confermato la presenza di catene leggere libere monoclonali di tipo lambda. La FLC ratio nel siero è 0.05 (range normale 0,26-1,65). Per un migliore e completo inquadramento diagnostico, seguirà lo studio sulle urine per la ricerca della Proteina di Bence-Jones.
Conclusioni: Fondamentale risulta essere l'apporto del laboratorio per un corretto inquadramento diagnostico delle discrasie plasmacellulari o malattia mielomatosa e per una valutazione nel tempo della risposta alla terapia come riportato dai criteri proposti dall'International Myeloma Working Group (IMWG) nel 2006 e aggiornati nel 2015.
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P060
LUPUS ANTICOAGULANT AND ORAL ANTICOAGULANT THERAPY (VITAMIN K ANTAGONISTS AND RIVAROXABAN): A DIAGNOSTIC DILEMMA

H. Hu[1], B. Montaruli[1], T. Bertero[2], D. Cosseddu[1], M. Migliardi[1]

[1]Laboratory Analysis Department, Umberto I Mauriziano Hospital, Torino
[2]Immunology Department, Umberto I Mauriziano Hospital, Torino

Case presentation: A 45-year-old white woman with Antiphospholipid syndrome (APS) came to our laboratory in march 2016 for Antiphospholipid Antibodies (aPL) laboratory screening: Lupus anticoagulant (LA), anticardiolipin antibodies (aCL) and anti Beta2-glycoprotein I antibodies (aB2GpI). In March 2004 the patient referred to Neurology Department with a cerebral venous sinus thrombosis. She received anticoagulant therapy (LMWH followed by warfarin) with a good recovery. She performed thrombophilia and autoimmune screening on vitamin K antagonist (VKA) therapy and she was found to be positive only for aPL [aB2GpI IgM = 33 U/ml (normal values <9.5 U/ml) and LA performed on a 1:1 mixture of normal pool because of VKA] and classified as APS with a recommended long term anticoagulation treatment. In april 2009 she moved to Belgium and continued her follow up at Rheumatology Centre of Utrecht. She was retested for aPL which resulted all negative and she stopped oral anticoagulation. In december 2015 she was admitted to Utrecht Hospital with a diagnosis of varicella zoster infection and a pulmonary embolism. During the hospital admission she received standard antiviral therapy and anticoagulation with tinzaparin sodium followed by rivaroxaban. At that time isolated LA positivity was found. In march 2016 she came back to Italy and was found to be positive by our laboratory for aB2GpI IgM (125 U/ml, normal values <9) and LA. Because of her PT ratio = 1.24 (normal values <1.20) the presence of Direct Oral Anticoagulants DOAC was suspected and an interpretative comment of possible false positive LA results in a DOAC treated patient was added in the report. APS was than redefined in this patient and it was again recommended a long-term anticoagulation.
Conclusion: This case provides evidence of the difficulties to perform and to obtain correct interpretation of LA assays on VKA and DOAC therapies. Interpretative comments might be particularly advisable when reporting results of LA testing given the large of potential issues that impact test results.

P061

CSF BIOMARKERS IN THE DIFFERENTIAL DIAGNOSIS OF SUBPIAL AND SUBARACHNOID HEMORRHAGE

G. Sancesario[1], F. De Lorenzo[2], A. Martorana[2], S. Bernardini[3]

[1]*Dept. of Clinical and Behavioural Neurology, Santa Lucia Foundation IRCCS, Rome*
[2]*Dept. of System Medicine, Tor Vergata University, Rome*
[3]*Dept. of Experimental Medicine and Surgery, Tor Vergata University, Rome*

Subpial hemorrhage (SPH) is a rare finding identified by blood along the cortical surface underneath the pia mater at magnetic resonance imaging (MRI) but without blood in the cerebrospinal fluid (CSF). Lumbar puncture (LP) can help identify patients with so called late-presenting subarachnoid hemorrhage (SAH), but traumatic tap with blood contamination makes problematic the differential diagnosis. We report that levels of ferritin clearly can differentiate the CSF of SPH from SAH.
We observed a case of a 55-year-old woman, presenting at the ER because of a strong headache with tingling. Two years before she had a breast cancer, treated with surgery and radiotherapy and thereafter with aromatase inhibitor. Blood screening excluded infections, coagulation disorders or autoimmune processes.
The basal Computed Tomography (CT) showed signs of intrasulcar and intrascissural bleeding at fronto-parietal level bilaterally, without spreading of bleeding in the subarachnoid cisterns of the base, suggesting a non-traumatic isolated cortical SPH. Angio-CT and MRI venography did not reveal presence of aneurysms, congenital abnormality, cerebral vasoconstrictions nor venous thrombosis.
LP was performed 6 day after the onset of symptoms. Unfortunately, a traumatic tap occurred with a predominantly red fluid in three sequential tubes. After centrifugation, the CSF was clear but the content of protein was high. To exclude the occurrence of an antecedent episode of SAH we assessed the content of CSF ferritin, testing 10.4 ng/ml (normal range <12 ng/ml). We compared this CSF examination with another performed at the 6th day from symptom onset in a woman presenting classical radiological signs of SAH (with blood also in the cisterns): in this case CSF showed the classical xantocromic aspect in all three specimens with high ferritin level (76 ng/ml). In our case, normal level of ferritin in CSF helps to exclude the presence of a previous SAH: indeed, ferritin levels take approximately 3 days to consistently increase and 7–10 days to reach over 1000 ng/mL [1] after SAH.
SPH should be considered in the case of isolated intrasulcal bleeding; CSF ferritin can help in the differential diagnosis of SPH and SAH. Distinguishing SAH from SPH have therapeutic and outcome differences.

1. Nagy K, Skagervik I, Tumani H, et al. Clin Chem Lab Med 2013;51:2073–86.

P062

CRYOGLOBULINEMIA TYPE III CONFUSED WITH IgM k MONOCLONAL GAMMOPATHY

A. Ombrato[3], F. Marciano[3], E. De Sisto[1], I. Migliaccio[4], L. Catalano[2], M. Savoia[1]

[1]*D.A.I. MedLab, A.O.U. Federico II, Napoli*
[2]*D.A.I. Ematologia, A.O.U. Federico II, Napoli*
[3]*Sc. Spec. Biochim. Clin., Univ. Studi di Napoli Federico II*
[4]*Sc. Spec. Ematologia, Univ. Studi di Napoli Federico II*

A 56 year old female patient was admitted at the Department of Hematology of AOU Federico II of Naples in May 2016, showing lab reports of Capillary zone electrophoresis (CZE) of serum proteins and immunosubtraction (ISE), carried out in an external lab, which suggested the presence of marked entity of monoclonal gammopathy IgM κ, not quantified because it is overlapped to the IgG. Moreover, haematochemical tests showed the alteration of the following parameters: PT=10.8 g/dL; ALB=3.3 g/dL; IgM= 2875 mg/dL and marked eosinophilia (37.6%). She underwent a mielobiopsia and the bone marrow aspirate showed no plasmacytosis and increase of eosinophils, not in agreement with the suspected diagnosis of monoclonal gammopathy; 18F-FDG CT/PET was negative. The hematologist contacted the laboratory of DAI MedLab, who proceed with a revaluation of the proteic profile of the patient, pointing out the polyclonal nature of the band by: CZE, increase of polyclonal immunoglobulins in γ zone, confirmed at ISE; serum immunofixation on agarose gel (IFEs), the gold standard technique, polyclonal band in M, κ and λ, milder in G; quantification of the serum free light chains (sFLC) with κ/λ ratio=1.33 (0.31 to 1.56). Finally, immunofixation urinary protein (IFEur) showed the absence of Bence Jones proteinuria. The serum, after a day at 4 °C, showed the start of cryoprecipitation, the presence of which was confirmed in the same conditions 6 days later. Crioprecipitate characterization on a sample at 37 °C showed positivity to polyclonal IgM and IgG, allowing classification of the patient as suffering from cryoglobulinemia type III (CRG III). The CRG III, consisting of polyclonal IgG and IgM, are not often diagnosed because it is necessary to perform targeted and accurate procedures for their identification. Now we are investigating about viral infections and immunological disorders CRG III correlated. Therefore, it is essential that the Laboratory: provides an interpretative and exhaustive lab report; uses the highest sensitivity proteic full panel (HRE, ISE, IFEs, IFEur, sFLC, IgG, IgA, IgM quantification); and establishes a close collaboration with clinician, in order to arrive at a correct diagnosis.

Passerini G, Basile U, et al. Ricerca, quantificazione e caratterizzazione delle crioglobuline: indicazioni per un protocollo condiviso. Biochim Clin 2010;34:218-22.

P063

AMIODARONE-INDUCED ACUTE HEPATITIS IN A PATIENTS TREATED WITH APIXABAN: A CASE REPORT

C.A.E. Novelli[1], S.L. Puricelli[2], L. Trolese[2], G.M. Gambino[2], B. Brando[1]

[1]Haematology Laboratory and Transfusion Center, ASST Ovest Milanese, Legnano

[2]Emergency Medicine Departement, ASST Ovest Milanese, Legnano

Acute hepatitis is a very rare, but potentially fatal, adverse effect of intravenous amiodarone. Furthermore Amiodarone is a drug with possible interaction with Apixaban, and the latter should be used with caution in patient with liver dysfuction, namely when AST and ALT levels >2x ULN or bilirubin levels >1.5x ULN.

We present a case of a 78-year-old woman with history of paroxystic AF in hypertensive cardiomyopathy and mitral valve prolapse and a HCV-related chronic liver disease. She was in therapy with Apixaban for two years and was admitted to our Emergency Medicine Departement with diagnosis of AF; we decided to treat the patient with intravenous amiodarone for the rhythm control. The day after the beginning of intravenous Amiodarone administration she developed an acute hepatitis (more than 100-fold elevation in alanine transaminase) and 25mL/min of CrCL. Both Amiodarone and Apixaban were withdrawn, but the patient needed anticogulant therapy to prevent stoke and systemic embolism. The PT was high (>2 Ratio) due to hepatopathy and possibly because of drug overdose due to the elimination route of Apixaban (#75% Hepatobiliary). We decided to monitor the anticogulation effect with anti-Xa test properly calibrated and to initiate the anticoagulation treatment not before the drug concentration fall within the normal range. Indeed test like PT or APTT are not recommended to assess the pharmacodynamic effects of Apixaban: only the specific anti-Xa test is raccomanded. The patient was switched from apixaban to LMWH with subsequent rapid resolution (eleven day after the admission) of laboratory abnormalities.

The case highlights the need for a close monitoring of hepatic function during amiodarone infusion, and also anti-Xa specific for Apixaban in order to identify any potential hepatotoxicity and to prevent potentially fatal complication.

Flaker G, Lopes RD, Hylek E, et al. Amiodarone in AF and anticoagulation. J Am Coll Cardiol 2014;64:1541-50.

P064

NUOVE FRONTIERE DI INDAGINE PER LA DIAGNOSI DI ANEMIA EMOLITICA CONGENITA: L'ANALISI TERMOGRAVIMETRICA ASSOCIATA ALLA CHEMIOMETRIA

R. Risoluti[1], S. Materazzi[1], M. Luciani[2], A. Amato[3], C. Bozzi[4], P. Caprari[4]

[1]Dipartimento di Chimica, "Sapienza" – Università di Roma, Roma

[2]U.O. Ematologia, Ospedale Pediatrico Bambino Gesù, Roma

[3]Associazione Nazionale lotta contro le Microcitemie in Italia, Roma

[4]Dipartimento di Ematologia, Oncologia e Medicina Molecolare, Istituto Superiore di Sanità, Roma

L'analisi termogravimetrica (TGA) associata alla chemiometria è stata recentemente proposta come strumento diagnostico per lo screening della talassemia [1]. In questo studio è stata applicata la TGA e l'analisi statistica multivariata dei profili termogravimetrici del sangue intero in un caso di anemia emolitica congenita di natura da determinare. Caso clinico: una bambina italiana di 6 anni che presentava anemia (Hb 8-9 g/ dL) emolitica cronica caratterizzata da iperbilirubinemia, milza aumentata, test di Coombs diretto e indiretto negativi, HbA2 e HbF nella norma, MCV diminuito, aumento di RDW, reticolociti, e LDH ed anomalie della morfologia eritrocitaria (ovalociti, sferociti e rari schizociti). Sono state effettuate le indagini specifiche per le anemie emolitiche congenite da difetto delle proteine di membrana e/o dei più comuni enzimi eritrocitari, ma sono risultate nella norma. L'analisi TGA è stata applicata su campioni di sangue intero (30 μL), prelevati con anticoagulante EDTA, che sono stati analizzati mediante la termobilancia TG7 (Perkin Elmer) e le curve risultanti dalle variazioni di massa in funzione della temperatura sono state confrontate con quelle di soggetti sani e talassemici. Il profilo termogravimetrico del sangue della paziente risultava ben distinto da quelli di soggetti sani e confrontabile con quello di soggetti talassemici dando una indicazione della presenza di un difetto emoglobinico. L'analisi molecolare dei geni globinici ha confermato il sospetto diagnostico dimostrando la presenza di una variante emoglobinica rara l'Hb Bibba (α-2-136Pro(H19)-ß2) dovuta ad una emoglobina instabile. L'applicazione dell'analisi termogravimetrica e successiva analisi chemiometrica fornisce un nuovo approccio per lo screening e lo studio delle anemie emolitiche congenite. Risulta particolarmente adatta in pazienti pediatrici, in quanto richiede piccoli volumi di campione, in caso di soggetti sottoposti a trasfusione, o in presenza di forme di anemia emolitica congenita di difficile diagnosi.

1. Risoluti R, Materazzi S, Sorrentino F, et al. Thermogravimetric analysis coupled with chemometrics as a powerful predictive tool for ß-thalassemia screening. Talanta 2016;159:425-32.

P065

MIELOMA MULTIPLO IgD LAMBDA: SWITCH ISOTIPICO IN CORSO DI TRAPIANTO AUTOLOGO

F. Piccolo[3], D. Ambrosino[3], E. Caracciolo[1], M. Di Perna[4], I. Zacheo[4], L. Catalano[2], M. Savoia[1]

[1]D.A.I. MedLab, A.O.U. Federico II, Napoli
[2]D.A.I. Ematologia, A.O.U. Federico II, Napoli
[3]Sc. Spec. Biochim. Clin., Univ. Studi di Napoli Federico II
[4]Sc. Spec. Ematologia, Univ. Studi di Napoli Federico II

Maschio di 38 a, in apparente stato di buona salute nel gennaio 2013 (Creatinina-CREA-0.93 mg/dL, Urea 37 mg/dL, Hb 15.3 g/dL), in giugno, in seguito a febbre persistente e malessere generalizzato, si reca in pronto soccorso dove rilevano: CREA 10.6 mg/dL; Urea 213 mg/dL; Hb 7.1 g/dL. Trasferito alla Nefrologia della AOU Federico II di Napoli si conferma diagnosi di insufficienza renale acuta (CREA 13,4 mg/dL, Urea 225 mg/dL ed Hb 6.4 g/dL), è sottoposto a trasfusione e sedute emodialitiche. Profilo proteico: proteinuria 388 mg/L, Bence Jones λ +; all'elettroforesi capillare sieroproteica(CZE) ipogammaglobulinemia policlonale e 2 componenti monoclonali(CM) in zona γ, 11% e 5%. All'immunosottrazione(ISE) standard(G,A,M,κ,λ), le CM risultavano positive solo per catene λ, deponendo per mieloma micromolecolare λ, escluso dall'immunofissazione sierica(IFEs) eseguita con anti D che identificava le CM come IgDλ e λ libera. Trasferito in Ematologia, diagnosi definitiva Mieloma Multiplo IgDλ, IIIB (infiltrato plasmacellulare midollare 62%; RMN rachide: multiple alterazioni ossee). Inizia terapia con bortezomib, talidomide, desametasone ed è sottoposto a doppio autotrapianto di midollo osseo, con risposta completa di durata breve. Il follow-up è caratterizzato da recidive multiple, trattate con diversi e ripetuti schemi polichemioterapici, seguiti da periodi di breve stabilità della risposta che non consentono l'avvio alla procedura trapiantologica allogenica. A seguito del doppio trapianto autologo si evidenzia uno switch isotipico IgGκ+IgGλ transitorio. In giugno 2016 si verifica ripresa aggressiva di malattia e il profilo proteico mostra: marcata ipo-γ-globulinemia (IgG 0.45, IgA<0.23, IgM<0.17g/L); CM 5% e 1% + comparsa in zona ßγ di CM D lieve; catene leggere libere del siero (sFLC) κ=0.304 e λ=709 mg/L. Il caso clinico rimarca il ruolo chiave del laboratorio nella gestione del paziente affetto da discrasie plasmacellulari: 1)alla diagnosi, il riscontro di CM IgD non deve essere escluso quando, impiegando procedure di tipizzazione standard, si osserva positività delle sole catene leggere; 2)nel follow-up, dove in corso di terapia e/o trapianto, oltre a variazioni quantitative possono manifestarsi switch isotipici.

Chepovetsky J, Chari A, Jagannath S, et al. Clin Chim Acta 2013;425:233–5.

P066

PSEUDO CHEDIAK-HIGASHI GRANULES AND OTHER UNUSUAL CYTOPLASMIC INCLUSIONS IN REFRACTORY ANAEMIA WITH EXCESS BLASTS-2

a. La Gioia[1], M. Bombara [2], F. Fiorini[1], P. Valentini [1], L. Francioni[1], M. Fiorini[2]

[1]Patologia Clinica Osp. Lotti, Pontedera
[2]U.O.C. Medicina di Laboratorio Ospedale di Livorno

Introduction: The word "dysgranulopoiesis" describes the morphologic anomalies of the granulocytes which can be observed in peripheral blood and in bone marrow. These features in a clinical and haematological contest suggesting a Myelodysplastic Syndrome (MDS) such as anemia and other peripheral cytopenia, represent a cardinal diagnostic criterion. Because a 10% cut off has been proposed as distinctive criterion for MDS classification, an unique descriptive classification for morphologic manifestation of dysplasia is encoded by the WHO2008 classification. For granulocytic series, are dysplastic features: small or unusual large size; nuclear hypolobulation; irregular hypersegmentation; decreased granules and/or agranularity; pseudo Chediak-Higashi granules; Auer rods.
We describe a case of MDS with Blasts excess-2 in which pseudo Chediak-Higashi granules and other unusual cytoplasmic inclusions were present.
Case description: A 75-years-old man with previous diagnosis of Refractory Anemia with Excess of blasts - 1 (RAEB-1) had an increase of both anemia and blast cells in peripheral blood (6%), Immature granulocytes 18%, erythroblasts 3/100 leukocytes, megalocytosis and dysgranulopoiesis were even observed. A bone marrow film showed increased granulocytes up to more than 80% with relevant dysgranulopoiesis. Blast cells containing azurophils granules but not Auer bodies were 13.0%. Dyserythropoiesis and dys-megakariocytopoiesis were even present. The conclusive diagnosis was RAEB-2.
The most important morphological features were represented by the presence of different unusual cytoplasmic inclusions in the immature granulocytes and blasts. In particular pseudo Chediak-Higashi granules and giant pseudo Chediak-Higashi granules; azurophilic rectangular tablets and, finally, multilocular vacuolar formations.
Conclusion: In a morphological contest suggesting a MDS, dysgranulopoiesis can be represented not only by "classic" features but even by other morphological changes that can be helpful to define the framework of dysplasia.
Regarding the diagnosis of RAEB-2, the last release of WHO classification (Arber et al. 2016) changed the previous nomenclature based on the type of cytopenia and replaced it with only the acronyms "MDS". So, this case must be classified now as "MDS with blast excess-2" (MDS-EB-2).

P067

POSTPARTUM ACQUIRED HEMOPHILIA: A PROMPT AND ACCURATE DIAGNOSIS DETERMINED THE CLINICAL OUTCOME

S. Vitale[1], M. Napolitano[2], L. Lo Coco[1], E. Zora[1], F. Bonura[1], M.T. Segreto[1], I.N. Sciortino[1], S. Siragusa[2], M. Ciaccio[1]

[1]U.O.C. Medicina di Laboratorio Corelab, A.O.U.P. "P. Giaccone", Palermo

[2]U.O.C. Ematologia, A.O.U.P. "P. Giaccone", Palermo

A 25 years old woman was admitted to our Reference Center from Castelvetrano E.R. with clinical suspicion of von Willebrand Disease (vWD) for sudden onset of spontaneous bruising 4 months after her third full-term pregnancy. She reported the appearance of small spontaneous ecchymoses not further investigated 2 mounths before. Our hemostatic screening showed PT and fibrinogen within normal limits but an aPTT> 300 seconds. In addiction to the protocol for VWS we performed that for Acquired Hemophilia (AH) and a thromboelastographic study (TEG). VWF:Rcof and Antigen, PFA100 and platelet aggregation performed with different inducers resulted within normal ranges, ruling out vWD. However the total lack of FVIII and a high titer of FVIII inhibitor (69.0 UB/mL) confirmed our suspicion of AHA. Moreover TEG showed an infinite R-segment, typical plot of patients with AHA. Within a few hours from hospital admission a diagnosis of postpartum AH was made and treatment immediately started. Antihemorrhagic therapy with rFVIIa and tranexamic acid and the immunosuppressive therapy with methylprednisolone, cyclophosphamide and rituximab allowed a rapid improvement of the clinical condition. Laboratory tests showed progressive normalization of aPTT, net reduction of FVIII inhibitor and a constantly growing FVIII. The TEG plots performed during clinical improvement provided key information on the kinetic of fibrin formation, critical to monitor therapy efficacy, also considering that inhibitor title and residual FVIII activity are not directly proportional to the severity of bleeding symptoms and mortality. The patient was discharged in 3 weeks in good general condition, chest and abdomen CT scans ruled out both the presence of blood collection in thoracic region and retroperitoneal mass related to cancer. Postpartum acquired hemophilia was thus considered idiopathic. No recurrence to date. A prompt and accurate diagnosis determined the clinical outcome, confirming that the management of this disease still needs a well experienced multidisciplinar team hardly available outside specialized centers.

P068

IL LABORATORIO DI COAGULAZIONE NELLA RISOLUZIONE DI UN CASO DI PAZIENTE AFFETTO DA DEFICIT DI FATTORE VON WILLEBRAND, RESISTENTE ALLA DESMOPRESSINA

C. Bellini[1], E. Milletti[1], D. Fineschi[2], E. Franceschini[2], A. Silvietti[2], L. Puccetti[3], A. Sicuranza[3], L. Terzuoli[1], D. Vannoni[1], R. Guerranti[1], C. Scapellato[2], R. Leoncini [1], P. Calzoni[2]

[1]Dip. Biotecnologie Mediche, Università di Siena / UOC Lab. di Patologia Clinica, AOU Siena

[2]UOC Lab. di Patologia Clinica, AOU Siena

[3]Centro Coagulopatie/Ematologia, AOU Siena

Premessa: La Malattia di von Willebrand è un difetto dell'emostasi relativamente raro determinato da deficit o alterata funzione del Fattore di von Willebrand (VWF). Gli Studi rilevano una prevalenza dell'1%; tuttavia la maggior parte dei pazienti presenta un quadro così lieve da non richiedere terapia. In questi casi la diagnosi non è semplice per le numerose variabili che influenzano i livelli di VWF, quali difficoltà nella standardizzazione dei test diagnostici e/o presenza di variabili fisiologiche quali gravidanza, esercizio fisico, età e gruppo sanguigno. Con un'adeguata organizzazione e un approccio multidisciplinare è altresì possibile risolvere casi complessi, come quello di un paziente affetto da deficit di VWF, risolto mediante l'attivazione di un percorso diagnostico terapeutico assistenziale (PDTA) specifico.
Case Report: MD, maschio di 32 anni, con storia di sanguinamenti post chirurgici, necessitava di sottoporsi ad un intervento al ginocchio in una struttura privata. Data la diatesi emorragica, lo specialista ortopedico, conoscendo il PDTA per le patologie coagulative attivo presso la AOUS, richiedeva la consulenza del Laboratorio di Patologia Clinica per una valutazione preoperatoria dell'assetto coagulativo. I test di base PT e aPTT, seguiti dai test miscela e dalla determinazione dei relativi fattori carenti hanno evidenziato un lieve deficit di VWF e di FVIII che ha indotto il Laboratorio ad indirizzare il paziente verso il PDTA specifico.
Risultati e Conclusioni: Il paziente è stato sottoposto al Test alla Desmopressina cui è risultato insensibile; infatti i test eseguiti prima e dopo il trattamento con il farmaco hanno evidenziato un incremento non significativo di FVIII e VWF che ha indotto l'ematologo a suggerire il trattamento con concentrati di VWF/FVIII in caso di intervento chirurgico maggiore. Poiché la struttura privata non era in grado di fornire tale terapia, il paziente, grazie alla condivisione del PDTA con le strutture territoriali, ha potuto eseguire l'intervento presso un ospedale periferico dell'Area Vasta Sud-Est con esito positivo.
Castaman G, Montgomery RR, Meschengieser SS, et al. von Willebrand's disease diagnosis and laboratory issues. Haemophilia 2010;16:67–73.

P069

IMPORTANCE OF FREE LIGHT CHAIN DETERMINATIONS IN THE NEW MULTIPLE MYELOMA DIAGNOSTIC CRITERIA: A CASE REPORT

L. Fringuelli[1], R. Buzzi[1], R. Carrozza[1], G. Trapè [2], M. Montanaro[2], M.T. Muratore[1]

[1]U.O. Diagnostica Clinica, P.O. Belcolle, Viterbo
[2]U.O.C. Ematologia, P.O. Belcolle, Viterbo

Backgound: In 2014 the International Myeloma Working Group (IMWG) updated the disease definition of Multiple Myeloma (MM), adding to the classic CRAB features (hypercalcaemia, renal failure, anaemia, and bone lesions) recently validated biomarkers associated with the near development of clinical symptoms, and recommended the implementation of these criteria in routine practice: these included the serum free light chain (sFLC) ratio >100 with an absolut level of the involved sFLC>100mg/L.
Patient and methods: We report the case of a man aged 68, referring to our Hematology Unit in March 2016 who was affected by MGUS since 2009 (monoclonal component 0,2g/dl). Serum protein electrophoresis (SPE) and immunofixation (IFE) (Interlab) were performed as routine analysis, along with sFLC (The Binding Site) quantification.
Results: IFE analysis showed two components: free K in the ß region and IgGK in the γ region. Despite the small peak entity (0,4g/dl) the k sFLC level was very high (3684 mg/L; range 3.3-19.40) with sFLC ratio abnormal (303,7; range 0.26-1.65). Due to this unexpected feature in sFLC ratio, a bone marrow aspiration was performed, resulting in a 10% plasmacell infiltration; total body CT scan showed no bone lytic lesions, and no bio-humoral signs of CRAB were detected: Hb 13 g/dl, serum calcium level 8.9 g/dl, normal renal function. In june 2016 all the analysis but the bone marrow aspiration were repeated, and again the peak entity was in the MGUS range (0,6g/dl) but the k sFLC were even higher than the first time (7468mg/L) and sFLC ratio too (639,93). The patient didn't show signs of CRAB features in Hb level (12.3g/dl), serum calcium level (9.0g/dl) and renal function (glomerular filtration rate according to EPI 91mL/min); a MRI of the skeleton showed the presence of several >5mm lytic lesions; thus, the patient was definitively diagnosed as an overt MM, and treatment was started.
Conclusions: This case underlines the crucial role of sFLC in the MM management and shows how sFLC ratio can lead to early diagnosis of MM, before the development of major organ damage; indeed, lytic lesions were not yet dangerous for bone integrity, while all other CRAB features were still negative.
Rajkumar SV, Dimopoulos MA, Palumbo A, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol 2014;15:e538-48.

P070

UTILITÀ DEL PERCORSO DIAGNOSTICO TERAPEUTICO ASSISTENZIALE NELLA RISOLUZIONE DI UN CASO DI PAZIENTE AFFETTA DA MALATTIA DI VON WILLEBRAND

D. Fineschi[2], C. Bellini[1], E. Milletti[1], E. Franceschini[2], F. Cinci[1], L. Puccetti[3], L. Aprile[3], D. Vannoni[1], L. Terzuoli[1], A. Silvietti[2], R. Leoncini[1], R. Guerranti[1], C. Scapellato[2], P. Calzoni[2]

[1]Dip. Biotecnologie Mediche, Università di Siena/UOC Lab. di Patologia Clinica, AOU Siena
[2]UOC Lab. di Patologia Clinica, AOU Siena
[3]Centro Coagulopatie/Ematologia, AOU Siena

Premessa: Nella AOUS è attivo un percorso integrato diagnostico clinico terapeutico per le patologie coagulative, secondo il modello PDTA. Il Paziente usufruisce della consulenza clinica e dei servizi diagnostici necessari mediante un approccio multidisciplinare, che pianifica, coordina e valuta le opzioni terapeutiche utili a raggiungere esiti di appropriatezza, qualità ed efficienza (Case Management). Descriviamo qui un esempio di applicazione di tale PDTA nella risoluzione di un caso clinico.
Case report: PS, femmina di 56 anni, proveniente da altra Area Vasta, affetta da Malattia di von Willebrand (VWD) e sindrome da defecazione ostruita, con rettocele ed invaginazione rettale. Il gastroenterologo richiede pancolonscopia ed eventuale polipectomia per sangue occulto positivo, ma la mancanza di una coordinata interazione tra gastroenterologo, ematologo e laboratorio nella struttura ospedaliera rende la procedura endoscopica altamente rischiosa. La paziente si presenta dunque presso la AOUS e, sulla base delle indicazioni cliniche ed anamnestiche, viene inserita nel PDTA per le patologie coagulative, dove il team specialistico identifica e applica la migliore sequenza di interventi.
Risultati e Conclusioni: La valutazione diagnostica pre-operatoria per diatesi emorragica conferma la Malattia di von Willebrand precedentemente diagnosticata. La determinazione dei FVIII e VWF (ACL TOP CTS 500; HemosIL-FVIII deficient plasma; HemosIL-VWF:RCo; HemosIL-von Willebrand Factor Antigen), effettuati prima e dopo trattamento con desmopressina, evidenzia un significativo incremento del complesso FVIII/VWF dopo trattamento, suggerendo all'ematologo la corretta terapia per effettuare in sicurezza la procedura endoscopica e garantendo così alla paziente le condizioni migliori per concludere l'iter diagnostico terapeutico.
Castaman G, Montgomery RR, Meschengieser SS, et al. von Willebrand's disease diagnosis and laboratory issues. Haemophilia 2010;16:67–73.

P071
SEPSI E PROTEINA C ANTICOAGULANTE, CASO CLINICO

A.M. Di Fabio, A. Pizzoferrato, U. Occhiuzzi

Lab. di Patologia Clinica, Osp. Civile di Avezzano, L'Aquila

La proteina C anticoagulante è una glicoproteina di 62 kD presente in circolo in forma inattiva e sintetizzata dal fegato in presenza di vitamina K. Tale glicoproteina per acquisire la sua funzione anticoagulante, deve essere trasformata in una proteasi serinica attiva (PC-attivata o APC)dal suo attivatore fisiologico che è la trombina,una proteina multifunzionale con importanti effetti omeostatici. In condizioni normali un certo numero di meccanismi di regolazione che comprendono gli inibitori naturali della coagulazione, gli attivatori della fibrinolisi e i mediatori antinfiammatori, mantengono uno stato di equilibrio (omeostasi) nella risposta endoteliale all'infiammazione o al trauma. Al contrario, in quelle situazioni in cui questo equilibrio viene perso,come ad esempio nella sepsi, e piu' in generale nell'infiammazione sistemica, il danno endoteliale prodotto è in grado di provocare allo stesso tempo sia un'attivazione della coagulazione che una inibizione della fibrinolisi con conseguente aumento del danno endoteliale stesso a livello del microcircolo che porta a disfunzione d'organo, shock e nei casi piu' gravi a morte. Le funzioni che conferiscono all'APC la capacità di compensare gli effetti di una infiammazione generalizzata sono molteplici; infatti essa: inibisce la produzione di TNFalfa da parte dei monociti; annulla gli effetti negativi sull'espressione di trombomodulina e EPCR (TNF-dipendenti); limita la produzione di TNF e quindi riduce l'espressione della selectina-E e di conseguenza l'attivazione e l'adesione dei neutrofili; riduce la produzione di trombina prevenendo tutti gli effetti protrombotici e proinfiammatori che l'attivazione trombinica comporta. A maggio 2014 giungeva alla ns. attenzione c/o Lab. Analisi la richiesta di striscio periferico di una paziente di 51 anni dopo sei ore dall'intervento chirurgico con crollo di WBC da 6,0 mmc a 1,0 mmc. All'osservazione microscopica non si evidenziavano atipie morfologiche. L'ipotesi piu' probabile era la marginazione dei leucociti relativa ad un'importante flogosi (sepsi) in atto.Trasferita nel reparto di rianimazione la paziente veniva trattata ogni due ore con concentrati standard di proteina C. Ciò ha permesso di ripristinare i livelli fisiologici di PC e normalizzare il valore dei WBC, nonchè scongiurare il pericoloso evento di CID.

P072
HEREDITARY BREAST CANCER AND GENE PANEL ANALYSIS – A CASE REPORT

M. Nunziato[1], M.V. Esposito[2], F. Starnone[2], M.A. Diroma[2], A. Calabrese[3], P. Buono[1], M. D'Aiuto[4], V. D'Argenio[5], F. Salvatore[5]

[1]CEINGE-Biotecnologie Avanzate and Department of Sport Sciences, University of Naples Parthenope, Naples

[2]CEINGE-Biotecnologie Avanzate, Naples

[3]CEINGE-Biotecnologie Avanzate and Department of Senology, Istituto Nazionale Tumori Pascale, Naples

[4]Istituto Nazionale Tumori – IRCCS Fondazione Pascale, Naples

[5]CEINGE-Biotecnologie Avanzate and Department of Molecular Medicine and Medical Biotechnologies, University Federico II, Naples

About 10% of all Breast Cancers (BCs) are hereditary and can be related to a germline predisposing-mutation in the high penetrance-genes BRCA1 and BRCA2. However, mutations in these genes are identified in only about 25% of the familial BCs. Consequently, it has been supposed that these familial cancers may be caused by germline mutations in other high-, moderate-, and low-penetrance cancer genes (1). To increase the diagnostic sensitivity of the molecular diagnosis for BC germline predisposition, we have validated a custom, NGS-based, multi-gene panel analysis for the simultaneous sequencing of 84 candidate genes in BRCA-negative BC patients.
We used the above mentioned method, to analyze a family with an history strongly suggestive for inherited predisposition to cancers. Our proband, now 39 years old, is a young woman that was affected by bilateral breast cancer and, during this molecular screening, developed also a lung cancer. Multiple cases of cancers, including 2 BCs (a mathernal aunt and the maternal grandmother) were present in her family. In particular, the patient's mother (now died) was diagnosed to have 2 primary cancers affecting respectively pancreas and lung. In this patient, we were able to identify a pathogenic mutation, c.734G>A (p.Gly245Asp), in the TP53 gene. This finding is compatible with patient's personal and familial history and strongly support the relevance of multi-gene testing for the identification of cancer-predisposing mutations in at risk families.
1. Melchor L, Benítez J. The complex genetic landscape of familial breast cancer. Hum Genet 2013;132:845-63.

P073
DETERMINAZIONE DELL'INDICE DI EMOLISI: CONFRONTO TRA METODI

G. Andreani[1], S. Lombardi[1], S. Cau[1], M.S. Parri[2], V. Zanetti[2], A. Baroni[2], S. De Vito[1], A. Coco[1], S. Storti[2]

[1]S.S.D. Analisi Chimico Cliniche e Immunoallergologia, Ospedale Apuane, Azienda U.S.L. Toscana Nord Ovest
[2]U.O. Medicina di Laboratorio, Fondazione Toscana G. Monasterio, Massa

L'emolisi in vitro è la principale causa di non idoneità dei campioni biologici. Da tempo gli analizzatori di biochimica clinica misurano gli indici di emolisi (HI) e ultimamente anche quelli di coagulazione hanno sviluppato metodi analoghi.
Scopo: Confrontare gli HI misurati con ACL TOP750 (IL, Werfen Company), DXC600 e AU680 (Beckman Coulter) con il dosaggio spettrofotometrico dell'emoglobina libera nel plasma (Hb libera).
Materiali e metodi: Dal 1 gennaio al 31 maggio 2016 l'HI è stato misurato su 306 campioni di plasma trisodio citrato (3.2%. 109 mmol/L) con emolisi visibile, pervenuti al Laboratorio del Nuovo Ospedale delle Apuane, con algoritmi di calcolo di proprietà di ciascuna Ditta basati sull'assorbanze a lunghezze d'onda diverse: 340, 410, 470, 600, 670 nm per il DXC600, 410, 480, 570, 600, 660, 800 nm per AU680, 405, 535 e 670 nm per ACL TOP750. L'Hb libera nel plasma è stata misurata poi con metodo spettrofotometrico presso il Laboratorio della Fondazione Monasterio di Massa secondo il protocollo di Fairbanks VF et al.
Risultati: Il metodo spettrofotometrico (Hb libera 85±58 mg/dl media±sdev, 24-405 mg/dl) è stato scelto come riferimento; la regressione lineare (software JMP) ha dato: con AU680 r2=0.853 (HI AU680=5.74+1.06 Hb libera); con DXC600 r2=0.893 (HI DXC600=-10.36+1.27 Hb libera); con ACL TOP750 r2=0.917 (HI TOP750=1.83+1.26 Hb libera). L'analisi multivariata ha dato come indici di correlazione: 0.958 per ACL TOP750, 0.945 per DXC600, 0.923 per AU680. Scegliendo come livello decisionale per non accettare i campioni la concentrazione di Hb libera ≥150 mg/dl, sono state valutate la sensibilità (sens), la specificità (sp) e l'accuratezza diagnostica (acc) dei metodi, ottenendo per ACL TOP750 sens=0.939, sp=0.934 e acc. 94%, per DXC600 sens=0.939, sp=0.927 e acc. 93%, per AU680 sens=0.970, sp=0.828 e acc. 84%.
Conclusioni: Tutti i metodi considerati nello studio hanno mostrato buona resa nel quantificare in automazione l'emolisi nei campioni di plasma citrato. Lo strumento ACL TOP750 ha mostrato la migliore sensibilità, specificità, accuratezza e correlazione con il metodo di riferimento, confermando di essere in coagulazione un ottimo strumento per la valutazione dell'indice di emolisi.
Fairbanks VF, Ziesmer SC, O'Brien PC, et al. Clin Chem 1992;38:132-40.

P074
VALUTAZIONE DEL GRADO DI EMOLISI NEL LABORATORIO DI COAGULAZIONE: CONFRONTO TRA ISPEZIONE VISIVA E MISURAZIONE AUTOMATICA SU ACL TOP750

S. Storti[1], S. Lombardi[2], S. Cau[2], E. Battipaglia[1], R. Lombardi[1], M.S. Parri[1], E. Giuliani[1], L. D'Errico[1], A. Coco[2], G. Andreani[2]

[1]U.O. Medicina di Laboratorio, Fondazione Toscana G. Monasterio, Massa
[2]S.S.D. Analisi Chimico Cliniche e Immunoallergologia, Ospedale Apuane, Azienda U.S.L. Toscana Nord Ovest

Nel laboratorio di coagulazione il grado di emolisi (HI) è valutato con ispezione visiva soggettiva del campione, ma di recente gli strumenti hanno sviluppato sistemi per la misura in automazione.
Scopo: Caratterizzare i campioni emolisati pervenuti al settore di coagulazione e confrontare l'HI ottenuto in base alla scala visiva riportata nel documento Sibioc per la rilevazione dei campioni emolisati con l'HI su ACL TOP750 (IL, Werfen Company).
Materiali e metodi: Su 306 campioni di plasma trisodio citrato (3.2%, 109 mmol/L) con emolisi visibile, provenienti da reparti ospedalieri di urgenza (pronto soccorso, medicina d'urgenza e terapia intensiva) e degenza medica, da centri prelievi territoriali e da prelievi a domicilio è stato misurato l'HI con un algoritmo di calcolo (proprietà Ditta IL) dall'assorbanza a 405, 535 e 670 nm.
Risultati: I campioni con emolisi di 116 maschi (38%) e 190 femmine (62%), età media 74±18 (5-101 anni), provenivano: il 30% dai reparti d'urgenza, il 13% dai reparti di degenza, il 27% dai centri prelievi territoriali, il 30% dai prelievi domiciliari. La regressione lineare fra HI visivo e HI ACL TOP750 ha dato r2=0.648 (HI visivo=-102.3+3.22 HI TOP750). HI=150 mg/dl è stato scelto come cut-off sopra cui un campione non è conforme: usando l'HI ACL TOP750 sarebbero stati scartati 49 campioni (16%) mentre con la scala visiva i non conformi sarebbero stati 150 (49%) e solo 48/150 (32%) lo sarebbero stati anche secondo HI TOP750. L'ANOVA per dati appaiati non ha mostrato associazione fra HI ≥150 mg/dl e sesso o età dei pazienti, mentre esiste una correlazione con la provenienza dei campioni ($p < 0.05$).
Conclusioni: La maggiore prevalenza di campioni emolisati dai reparti di urgenza è in linea con la letteratura. Dei campioni provenienti da pazienti non ospedalizzati (57%) una parte significativa (89%) è di pazienti in terapia con anticoagulanti orali: l'accesso venoso difficile e il trattamento durante il trasporto dei prelievi a domicilio potrebbero aumentare l'HI. La scarsa correlazione tra HI visivo e su ACL TOP750 e l'alto numero di prelievi (49%) considerati non conformi sulla base della sola ispezione visiva indicano la necessità di avere metodi strumentali standardizzati oggettivi.
Lippi G, Caputo M, Banfi G, et al. Biochim Clin 2011;35:481-90.

P075

TRANEXAMIC ACID: DURATION OF POST-OPERATIVE FIBRINOLYSIS AND PROTHROMBOTIC RISK AFTER TOTAL HIP OR KNEE REPLACEMENT

L. Erroi[1], B. Montaruli[1], V. Camarda[2], P. Sivera[3], M. Bruzzone[2], R. Rossi[2], M. Migliardi[1]

[1]*Laboratory Analysis Department, Umberto I Mauriziano Hospital, Torino*
[2]*Orthopedic Department, Umberto I Mauriziano Hospital, Torino*
[3]*Haematology Department, Umberto I Mauriziano Hospital, Torino*

Background: Hyperfibrinolysis, leading to increase bleeding, is observed during and immediately after major orthopedic surgery. Strong evidences indicate that Tranexamic Acid (TXA), a synthetic analog of lysine that acts as antifibrinolytic agent, reduces blood loss and blood transfusion requirements in orthopedic surgery. However, safety is still a controversial issue because TXA may increase thromboembolic risk. Conflicting results on the effect of TXA on coagulation prothrombotic markers are reported in the literature. Aim of our study was to quantify the duration of postoperative fibrinolysis and to assess the impact of TXA administration after total hip replacement (THR) and total knee replacement (TKR).
Materials and methods: 15 patients undergoing THR and 10 patients undergoing TKR were included in this study. Among these patients 13 THR and 8 TKR received TXA (two intra-operative doses of 10 mg/Kg given three hours apart). All patients gave written informed consent before the surgical procedure. D-dimers (DD) and thrombin generation time (TGT) were measured prior to surgery as well as 3, 6, 24 and 72 hours (h) after.
Results: No statistically significant difference in DD was observed between patients treated and not treated with TXA, even if DD increased postoperatively more in patients not receiving TXA (median = 14070 μg/L FEU; range: 5397-16135) than in patients receiving TXA (median = 3932 μg/L FEU; range: 2231-6036) as measured at 6h. No significant changes in TGT between patients treated and not treated with TXA was observed peak thrombin and endogenous thrombin potential (ETP).
Conclusions: We observed in all patients a peak of fibrynolisis 6h after surgery with a subsequent decrease at 72h postoperatively as evidenced by an increase in DD. Moreover it looks like that TXA limits postoperative fibrinolysis after THR and TKR, as evidenced by a lesser increase in DD in patients receiving TXA than in patients not receiving it. Finally, we observed no increase in peak of thrombin and ETP by TGT analysis in both patients receiving and not receiving TXA suggesting that TXA infusion did not increase biological thrombotic risk. In conclusion in our study TXA administration limited post-operative fibrinolysis with no increase in prothrombotic risk.

P076

COAGULATION TESTING INAPPROPRIATENESS IN DIRECT ORAL ANTICOAGULANT ERA

B. Montaruli, I. Canta, N. Renzi, O. Ruggia, D. Cosseddu, M. Migliardi

Laboratory Analysis Department, Umberto I Mauriziano Hospital, Torino

Background: The reporting of incorrect or inappropriate test results in coagulation occurs even frequently as in the past in direct oral anticoagulant era. Aim of our study was to assess test ordering behaviour for second level coagulation tests (hemorrhagic and trombophilic diathesis) in order to try to prevent or minimize error in the interpretation of test results due to anticoagulant therapy interferences.
Materials and methods: We reviewed all second level coagulation test request received by our department during a two months period. Nurses for outpatients or clinicians for hospitalized patients had to fill out a specific paper form containing therapy informations.
Results: On a total of 926 second level coagulation test requests, we estimated 159 as inappropriate (17.2%). 98 out of 159 (61.6%) due to therapy interferences: 82/159 (51.5%) anticoagulant therapy [59 Vitamin K Antagonist (VKA) and 23 direct oral anticoagulants (DOA)] and 18/59 (11%) hormone replacement/oral contraceptive therapy. The remaining inappropriateness were: 28/159 (17.6%) hemorrhagic tests request in thrombophilic patients and vice-versa; 17 out of 159 (10.6%) genetic test already requested; 14 out of 159 (8.8%) coagulative factor V and or factor II test requests instead of genetic polymorphisms and 2/159 (1.2%) thrombophilic test requests in pregnancy patients.
Conclusions: Errors related to second level haemostasis assays are very serious and cause adverse consequences for patients and healthcare system. Hormone replacement/oral contraceptive therapy as well as anticoagulant therapy (VKA, DOA, heparin) may produce false positive or false negative results leading to inappropriate or lack of appropriate treatment. The introduction of DOA has increased inappropriateness in specialized haemostasis tests; moreover, especially for Rivaroxban and Apixaban, is very difficult by using coagulation global tests (Prothrombin Time and Activated Partial Thrombin Time) to identify treated patients. In conclusion, we think that it is mandatory for haemostasis laboratories to identify anticoagulant therapy interferences by using paper or informatics forms and to introduce comments on specialized haemostasis assays in the laboratory report.

P077

CAN ADAMTS-13 ACTIVITY MONITORING PREDICT EARLY TREATMENT RESPONSE OR DISEASE RELAPSE IN THROMBOTIC THROMBOCYTOPENIC PURPURA?

M. Napolitano[1], S. Siragusa[1], S. Vitale[2], S. Raso[1], I.N. Sciortino[2], F. Bonura[2], E. Zora[2], M. Ciaccio[2], L. LoCoco[2]

[1]UOC Ematologia, Centro di Riferimento Regionale per le emocoagulopatie, Policlinico "P. Giaccone", Palermo

[2]Dipartimento di Medicina di Laboratorio CORELAB, Settore Emostasi e Trombosi, Policlinico "P. Giaccone", Palermo

Thrombotic thrombocytopenic purpura (TTP) is a rare but life-threatening disease. Therapeutic plasma exchange (PEX) in combination with steroids is the standard frontline treatment of TTP. ADAMTS13 activity cutoff values <5 % establish the laboratory diagnosis of TTP. ADAMTS13 activity is also proposed as a prognostic marker for relapse(1),however, the role for ADAMTS13 testing during initial treatment of TTP is less clear and ADAMTS13 assay is still not commonly available. We here report a case of TTP in which serial measurements of ADAMTS13 activity proved useful in guiding treatment .A 33-year-old Caucasian male with no significant previous medical history, was referred to our Center for hematuria, without neurologic symptoms. Laboratory results showed severe thrombocytopenia (PLT 7 x 10/L), anemia (Hgb=11g/dL) and hemolysis (LDH=4187U/L, indirect bilirubin=3 mg/dL). Peripheral blood smear revealed a pronounced schistocytosis, thus clinical diagnosis of TTP was performed. TTP was confirmed by ADAMTS13 activity of 2% (assay result available the day after Hospital admission).The test was performed in ELISA (Alifax®). Daily treatment with PEX and methylprednisolone was started with initial clinical improvement. However, standard laboratory parameters remained quite stable for the first week: PLT and LDH levels normalization was completely achieved only after 11 days. At 4 days, ADAMTS13 activity reached 55%.After one week, platelet count slightly decreased (PLT=80 x 10/L), without increased LDH levels (400 U/L).ADAMTS13 assay was repeated and it resulted 2%. Early relapse was suspected and Rituximab was administered at that time. Since then, ADAMTS-13 activity determination was adopted to drive treatment duration and evaluate clinical outcome, as an earlier and more reliable marker of disease relapse and treatment response. PEX was continued for additional ten days.The patient clinically recovered and he was discharged in good health (ADAMTS13=60 %). Serial ADAMTS13 testing proved useful in monitoring TTP, better than commonly adopted laboratory parameters. Availability of ADAMTS13 activity assay together with anti-ADAMTS13 antibodies should be actively promoted.

1. George JN. Measuring ADAMTS13 activity in patients with suspected thrombotic thrombocytopenic purpura: when, how, and why? Transfusion 2015;55:11-3.

P078

ANALYTICAL PERFORMANCE OF THE POINT-OF-CARE DEVICE XPRECIA STRIDE FOR PROTHROMBIN TIME-INTERNATIONAL NORMALIZED RATIO (PT-INR) DETERMINATION

A.R. Corno[1], A. Longhi[1], A. Masarin[1], T.M. Caimi[2], L. Grassi[1]

[1]SC Analisi Chimico-Cliniche e Microbiologia, ASST Grande Ospedale Metropolitano, Niguarda

[2]SS Emostasi e Trombosi, SC Ematologia, ASST Grande Ospedale Metropolitano, Niguarda

Background: The Prothrombin Time-International Normalized Ratio (PT-INR) test is used for Oral Anticoagulant Therapy (OAT) monitoring. In recent years Point-of-Care (POC) devices have been developed. We compared the analytical performance of the POC device Xprecia Stride (Siemens, Germany) with the coagulometer CS-5100 (Siemens) in PT-INR determination.

Materials and Methods: Xprecia repeatability was verified with lyophilized controls. INR values from Xprecia were compared with CS-5100 by testing capillary and venous blood samples, respectively, from 611 patients in OAT (79.5% INR results were in the 2.0-4.5 range), and 18 normal subjects. Analytical accuracy was verified according to ISO 17593:2007. Diagnostic accuracy was measured on a subgroup of patients (n=111), with known OAT therapeutic range, by expanded and narrow agreement, and by determining the percentage of differences ≥15%.

Results: Xprecia repeatability was consistent with manufacturer's declaration (CV=3.6/2.1% vs. CV=3.98/2.86% for normal/pathological controls, respectively). Xprecia INR results were significantly correlated (P <0.0001) with those provided by CS-5100. Deming and PassingεBablok regressions resulted in the following equations: Y=1.105X-0.274 (95%CI, slope: 1.066;1.143; intercept: -0.355;-0.193); Y=1.190X-0.493 (95%CI, slope: 1.139;1.242; intercept: -0.625;-0.391), respectively, showing the presence of systematic and proportional errors. The mean difference between Xprecia and CS-5100 was -0.010 (95%CI: -0.0035;0.016). For INR values <2.0, 98.6% diffferences were within ±0.5; in the 2.0-4.5 INR range the mean difference was 0.006 with 96.9% differences within ±30% (ISO requirements: 90%; ±0.3 INR; 90%, respectively). Expanded and narrow agreement was 82.0% and 79.3%, respectively; 81.7% differences were ≥15%.

Conclusions: Xprecia Stride met the analytical accuracy acceptance criteria of ISO 17593:2007; however, method comparison showed the presence of differences between Xprecia and CS-5100, which could be explained by analytical and extra-analytical variables (e.g. population, thromboplastin origin and sensitivity). These differences, which may affect OAT management, will be further investigated by comparing PT-INR values below and above the therapeutic range.

ISO 17593:2007 Clinical laboratory testing and in vitro medical devices. 1st ed. 2007-04-15.

P079
METRICA SIGMA E MEDX CHART NORMALIZZATE DI 25 ANALITI SU DUE ANALIZZATORI ABBOTT ARCHITECT CI16000

D. Frattolillo, S. Peruzzi, C. Chiodino, U. Sartori, S. Storchi Incerti, R. Colla

U.O. Lab. Analisi, Osp. Civile di Guastalla, AUSL di Reggio Emilia

Lo scopo del lavoro è stato calcolare la metrica sigma (S) del sistema in uso nel nostro Laboratorio per 25 analiti utilizzando la variabilità biologica (VB) per calcolare l'errore totale accettabile (TEa) (1). Nella gerarchia dei traguardi analitici (TA) è preferibile scegliere quelli basati su outcome clinici; quando non disponibili, l'approccio della VB, se praticabile, è centrato sul Paziente e permette di scegliere tra più livelli di TEa. Abbiamo monitorato per 8 mesi 25 analiti: acido urico (UA), albumina (ALB), ALP, ALT, amilasi (AM), AST, bilirubina diretta e totale (BD/BT), Ca, CK, Cl, colesterolo totale (CO), creatinina (CR), PCR, Fe, GGT, glucosio (GL), K, LD, lipasi (LI), Mg, Na, P, proteine totali (TP) e trigliceridi (TG) su due Architect ci16000 (Abbott) con controlli di qualità allargati di terza parte (QCT) Multichem S (Tecnopath) su tre livelli; abbiamo calcolato per ogni livello la media globale ponderata per numero di determinazioni/strumento, il bias rispetto alla media del gruppo omogeneo cumulativa e la DS comune tenendo conto della variabilità interserie ed interstrumentale. I TA in termini di TEa sono stati scelti utilizzando il cutoff desiderabile della VB. Abbiamo calcolato la metrica S=(TEa% - |Bias%|)/CV e plottato i rapporti bias/TEa e imprecisione/TEa in una MEDx Chart normalizzata (2).
17 analiti hanno su tutti i livelli in esame S>3 (adeguato); di questi AM, P, UA, LI, CO hanno S>4; AST S>5; ALT, GGT, BT, TG, Fe, BD, CK, PCR S>6 su 3 livelli. Alcuni analiti hanno S<3: Na ha S=1; ALB, Ca, Mg e Cl S tra 1 e 2; TP e ALP S tra 2 e 3; LD S>4 su 2 livelli e S=2 sul livello 1. Il basso valore S del Na è giustificato dal fatto che con il modello della VB il TEa di 0,88% è molto stringente anche con test precisi (errore totale, ET 1,3-1,9%, CV 0,68-0,87%). Anche per TP, ALB, Ca, Cl e Mg il TEa è basso a causa della bassa VB: tra loro l'ET più alto è 5% sul Mg (CV 2,7%). LD ha S<3 su un valore di nullo significato clinico. ALP risente di aumento dell'attività dopo apertura del flacone di CQ, che, seppur limitato rispetto ad altri QCT, aumenta il CV. Complessivamente, le performance del sistema sono a nostro avviso molto buone, con un terzo dei test con performance 'world class'.

1. Ottomano C, et al. Biochim Clin 2008;32:102-21.
2. Supak Smolcic V, et al. Clin Chem Lab Med 2013;51:e99-101.

P080
A NEW RECOMBINANT CALPROTECTIN ANTIGEN FOR INTERNAL QUALITY ASSESSMENT OF FECAL CALPROTECTIN

A. Padoan, M. La Malfa, M. Razetti, D. Basso, M. Plebani

Department of Laboratory Medicine, University-Hospital of Padova

Objectives: Fecal calprotectin (fCal) allows the diagnosis and monitoring of inflammatory bowel diseases (IBD). For measuring fCal, a pre-analytical phase (fecal samples weighting and dilution) is coupled to ELISA, and the monitoring of the overall variability is achieved by internal and external quality schemes by stool samples analyses, since no independent standard material is actually available.
Aims: The aim of this study was to verify weather a purified recombinant calprotectin antigen (PRCA) (DiaSorin Inc., USA) is suitable for fCal imprecision assessment.
Methods: PRCA solutions were prepared in a buffer containing 50 mM Tris pH 8, 150 nM NaCl, 2.5 mM $CaCL_2$, 3% BSA, 0.05% Tween 20, added or not with polyethylenimine (PEI). MALDI-TOF/MS and PhiCal Calprotectin ELISA (Immundiagnostik, Germany) were used for the analyses.
Results: MALDI-TOF/MS analyses confirmed PRCA purity, showing S100A8 and S100A9 peaks and the resulting hetero-dimer (calprotectin). PRCA solutions at 1400 and 700 ng/mL, prepared with or without adding 0.025% PEI, confirmed that PEI allowed to obtain results closer to the expected concentrations (1220 and 469 ng/mL instead of 720 and 232 ng/mL) and that the biases (-27% for high and -28% for low PRCA concentrations) were not affected by PEI at 0.03%, 0.035%, 0.04% and 0.05%. PRCA was dissolved with 0.025% PEI at the final concentration of 840, 420 and 210 ng/μL. 15 μL of each solutions were further diluted 1:25 in stool extraction buffer, to reproduce the entire pre-analytical and analytical processes. The expected ELISA results (336, 168 and 84 ng/mL) chosen were close to the 300, 150 and 50 μg/g diagnostic cut-offs. Biases were: -1.2%, -4% and +6.6% from two replicated measures for the 336, 168 and 84 ng/mL targets respectively. Inter-assay imprecisions (11 independent assays) were: mean=331.84 ng/mL, CV=6%; mean=175.7 ng/mL, CV=9%; mean=100.3, ng/mL, CV=21%. In parallel, two fecal samples were analyzed, being inter-assay CV 24% (mean=73.8 ng/mL) and 6% (mean=221.7 ng/mL).
Conclusions: PRCA is suitable as internal quality control for fCal assay and commutable with fecal samples.

Ikhtaire S, et al. Fecal calprotectin: its scope and utility in the management of inflammatory bowel disease. J Gastroenterol 2016;51:434-46.

P081

EVALUATION OF LONG-TERM IMPRECISION OF AUTOMATED COMPLETE BLOOD CELL COUNT PERFORMED ON SYSMEX XN-9000 PLATFORM

S. Birindelli, A. Carnevale, E. Aloisio, M. Panteghini

Clinical Pathology Unit, 'Luigi Sacco' University Hospital, and Department of Biomedical and Clinical Sciences, University of Milan, Milan

Background: Blood cell counters are an undeniable component of total laboratory automation (TLA) systems. Beside the sophisticated technology now available, which improves the throughput and decrease the turnaround time, the analytical performance continues to cover a central role in assuring the quality of results. Here we present data on long-term imprecision obtained on three Sysmex XN-9000 platforms for the complete blood cell count parameters (WBC, RBC, Hb, Hct, MCV, MCH, MCHC and PLT), working in parallel in our core-lab TLA.
Methods: By using the Bio-Rad Liquichek Hematology-16 Control Normal Level material, we collected daily data from April to December 2015. Monthly and cumulative CVs were calculated and compared with the performance specifications derived from biological variability (desirable and minimum level of quality).
Results: A total of 195, 194 and 205 measurements were performed on the three instruments, respectively, in a 9-month period. Overall, the three platforms performed with desirable levels of imprecision. MCH and MCHC represented exceptions, fulfilling with some difficulties the minimum quality level. Particularly, MCHC failed to reach this goal (i.e., a CV ≤0.8%) in 50% of the 26 evaluated working months. It is worth to mention that average values for all parameters obtained on the three platforms were equivalent, confirming their perfect interchangeability.
Conclusions: Daily collection of internal quality control data performed for 9 months on three identical platforms shows that the XN-9000 analyser can guarantee a high throughput without affecting the analytical quality performance. An improvement in MCH and MCHC estimates is however desirable.

1. Sandberg S, Fraser C, Horvath AR, et al. Defining analytical performance specifications: Consensus Statement from the 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine. Clin Chem Lab Med 2015;53:833-5.
2. Buttarello M. Quality specification in haematology: the automated blood cell count. Chim Clin Acta 2004;346:45-54.

P082

EVALUATION OF THE TRUENESS OF SERUM ALKALINE PHOSPHATASE (ALP) MEASUREMENT IN A GROUP OF ITALIAN LABORATORIES

E. Frusciante[1], F. Braga[1], I. Infusino[1], E. Aloisio[1], E. Guerra[2], F. Ceriotti[2], M. Panteghini[1]

[1]Centro di Ricerca per la Riferibilità Metrologica in Medicina di Laboratorio (CIRME), Università degli Studi, Milano
[2]Servizio di Medicina di Laboratorio, Osp. San Raffaele, Milano

The reference measurement procedure for ALP published in 2011 by the IFCC allowed to define the metrological traceability chain for the standardization of ALP measurement. This study reports the results of an EQA experiment conducted to evaluate the level of ALP standardization among different Italian laboratories enrolled for a scientific project with the final aim to derive ALP traceable reference intervals for pediatric population. Three frozen serum pools with a target value assigned by the IFCC reference procedure were distributed to 13 centers and analyzed in triplicates for 3 different days. Only 3 laboratories averagely fulfilled the desirable goal of bias (≤±5.5%) at all 3 concentrations (59.9 U/L, 186.9 U/L and 401.5 U/L), but only one provided data with a dispersion always within the uncertainty of the target result. The different ability to meet the goal clearly depended on the analytical system used. Focusing on the two most used analytical platforms, the Cobas systems (Roche Diagnostics) underestimated the ALP values, while the AU systems (Beckman Coulter) overestimated them. The regression parameters between the average values obtained by participating laboratories and the target values indicate that it would be possible to correct the results of all analytical systems and make them unbiased by a simple recalibration approach. The analysis of the commercial calibrator package inserts of the IVD companies involved in this study showed that, with the exception of Roche still aligned to the old Tietz method published in 1983, all companies offer at least two options, sometimes (e.g., Beckman AU) both not in line with the recommended standardization approach.

Infusino I, Ceriotti F, Panteghini M. Standardization in clinical enzymology: a challenge for the theory of metrological traceability. Biochim Clin 2010;34:96-102.

P083
ISO15189 ACCREDITATION: VERIFICATION OF NEWLY INTRODUCED EXAMINATION PROCEDURES

G. Antonelli[1], A. Padoan[1], A. Aita[1], L. Sciacovelli[2], M. Plebani[1]

[1]Dept. of Medicine, University of Padova
[2]Dept. of Laboratory Medicine, University-Hospital, Padova

Introduction: The International Standard ISO 15189 describes the requirements for quality and competence for medical laboratories (ML). The verification of examination procedures (EP) is a crucial step for accreditation. Validated EP used without modification shall be subjected to independent verification by ML before being introduced into routine practice. The analytical performances claims have to be confirmed through objective evidence.
Aim of this study is to propose an operative flow to verify the EP performances for all the methods typologies that can be introduced in a medical laboratory.
Methods: After the analysis of the available scientific documents, the following steps have been considered for the verification procedure: a) the typology of EP; b) performance characteristics to consider; c) the workflow; d) criterion for the results acceptability; e) the feasibility.
Results: Quantitative, semi-quantitative and qualitative EP were individuated. For quantitative EP, imprecision and trueness are evaluated in terms of CV% and bias%, respectively. The precision verification study consisted of three parts: 1) repeated measurements over five days on patient samples; 2) calculations of repeatability and within-laboratory precision; 3) assessment of consistency with the claims. Trueness is estimated by analysing materials with known concentration and comparing the results to the target values. For qualitative EP, diagnostic accuracy is evaluated, in terms of sensibility and specificity with true negative samples and true positive patient samples. For semi-quantitative EP, imprecision and diagnostic accuracy are assessed.
Conclusions: A model for the verification of newly introduced examination procedures is suggested, considering all kind of methods commonly carried out in a routine medical laboratory.
ISO 15189:2012. Medical laboratories- requirements for quality and competence. Geneva, Switzerland: ISO, 2012.
Clinical and laboratory Standard Institute. User verification of precision and estimation of bias; approved guideline-third edition. CLSI document EP15-A3. Wayne (PA), 2014.

P084
STUDIO OSSERVAZIONALE DELLE PROCEDURE DI PRELIEVO VENOSO IN SARDEGNA

E. Sorrentino[1], P. Chirra[1], M. Boi[1], L. Tarquini[1], V. Ventura[1], P. Mele[1], A. Zinellu[1], C. Carru[2]

[1]Dipartimento di Scienze Biomediche, Università di Sassari
[2]Servizio Controllo di Qualità, AOU Sassari

Negli ultimi anni numerosi studi hanno focalizzato l'attenzione sul processo di armonizzazione della produzione del dato analitico. La fase preanalitica viene considerata una delle fasi più complesse e la raccolta dei campione biologico rappresenta forse quella più critica. Al fine di poter valutare nelle principali realtà sanitarie della Regione Sardegna, attraverso una indagine osservazionale in singolo cieco, il grado di aderenza alle linee guida del campionamento venoso, è stata predisposta una Check list di 27 domande in conformità con le linee guida CSLI GP41-A6 [1].
La rilevazione è stata effettuata presso i centri prelievo delle seguenti strutture sanitarie regionali (AOUSS Policlinico Universitario, Sassari; ASL1 Poliambulatori e Osp. Ss. Annunziata, Sassari, Osp. Civile di Alghero; ASL3 Osp. Zonchello, Nuoro; ASL4 Osp. N.S. della Mercede, Lanusei; ASL5 Osp. Civile San Martino, Oristano; ASL6 Osp. S. Gavino Monreale; ASL8 Osp. S. Giovanni di Dio, Cagliari; ASL8 Osp. Ss. Trinità, Cagliari). Sono stati seguiti un totale di 30 operatori, ciascun operatore per 10 prelievi consecutivi, per un totale di 300 schede.
I risultati dell'analisi hanno mostrato una variabilità minima, tra le varie strutture, sostanzialmente operatore dipendente. Le principali criticità sull'aderenza alla linee guida hanno riguardato i seguenti punti: Procedura d'identificazione del paziente (66%); Rispetto dell'asepsi nell'igiene delle mani (15%); Evitare di toccare la sede del prelievo dopo la disinfezione (49%); Utilizzo di guanti non sterili nuovi (16%); Valutazione delle condizioni del paziente (34%); Rimozione del laccio emostatico successivamente all'inizio del flusso di sangue (34%); Registrazione dell'identità dell'operatore (20%).
I risultati ottenuti hanno fatto emergere per molti casi scarsa aderenza alle linee guida, evidenziando diversi elementi verosimilmente critici sia per i pazienti che per i prelevatori. Tali dati, tramite i responsabili di settore, sono stati condivisi con gli operatori.
È inoltre prevista una fase conclusiva dello studio, in cui si valuterà l'outcome degli operatori stessi rispetto alle informazioni fornite alla fine della prima parte dello studio.
1. Simundic AM, Church S, Cornes MP, et al. Clin Chem Lab Med 2015;53:1321-31.

P085

VALUTAZIONE DEL GRADO DI RISCHIO CLINICO E STRATEGIA MIGLIORATIVA DI CQ

D. Vandini[1], G. Bravi[1], G. Bianchi[1], M.G. Rauseo[1], A. Bianchi[1], P. Riccobene[2], O. Tiberi[1]

[1]U.O.C. Diagnostica Di Laboratorio, ASUR AV 1 Urbino
[2]Bio-Rad Laboratories S.r.l.

In linea con gli standard ISO e la linea guida EP23A, la qualità delle prestazioni erogate è divenuta l'obiettivo principale delle attività di laboratorio. Scopo dello studio è stato individuare e quantificare con Expected QC Event E(QCE), il grado di rischio di ottenere risultati inaccurati su N° pazienti Expected number final E(Nuf) ed Expected number correctable E(Nuc) e di individuare strategie di CQ per ridurlo e se possibile eliminarlo.
L'analisi è stata condotta su 6 analiti (Colesterolo, Potassio, Creatinina, ALP, CEA e Troponina-I) usando in modo alternato un livello di CQ indipendente Bio-Rad Multiqual 1, 2, 3 e Bio-Rad Tumor Marker Plus e un livello di CQ Beckman al dì, su strumenti Beckman AU680 e DXI. Attraverso il software Mission Control (Bio-Rad Laboratories) e i relativi moduli Risk Calculator e QC Designer, si è valutata la realtà del momento usando Media, DS, CV, ETa, Sigma, Regole di Westgard (1-2s, 1-3s, 2-2s, R4s, 7-T), livelli di CQ eseguiti/dì e N° Pazienti/ dì.
L'impostazione dei dati nella sezione Risk Calculator ha suggerito l'uso simultaneo di 2 livelli di controllo indipendente e un livello di CQ Beckman sia per chimica che immunometria per strumento al dì. L'aumento del n ° di livelli di CQI/dì e l'applicazione della sola regola 1-3s hanno evidenziato su tutti i test, tranne ALP, un basso o nullo E(Nuf), un accettabile E(Nuc), un basso E(QCE), conseguendo così una maggiore stabilità del sistema di CQI. Si è monitorato per 4 mesi l'andamento dei CV: solo per Troponina-I si è notato un lieve incremento sul livello 1 (da 4.15% a 4.45%); i CV degli altri test sono migliorati. Rilevare gli errori nella prima fase del processo ed incrementare le procedure di controllo attraverso l'analisi del rischio sul paziente è il migliore approccio al CQ. Mission Control ha permesso di valutare il nostro livello di confidenza per situazioni di CQ fuori controllo anche a seguito di lievi scostamenti sistematici. Aumentando l'esecuzione di livelli di controllo del CQ per chimica clinica, sono diminuite le ripetizioni di CQ e calibrazioni legate a segnalazioni di falsi allarmi, determinando inoltre un risparmio economico.
Yago M, Alcover S. Selecting statistical procedures for quality control planning based on risk management. Clin Chem 2016;62:959-65.

P086

A MODEL FOR ESTIMATING MEASUREMENT UNCERTAINTY IN MEDICAL LABORATORIES FOR ISO 15189 ACCREDITATION

A. Padoan, G. Antonelli, A. Aita, L. Sciacovelli, M. Plebani

Department of Laboratory Medicine, University-Hospital of Padova

Objectives: Measurement uncertainty (MU) is a requirement to comply for the achievement of ISO 15189:2012 accreditation. However, ISO 15189 does not specify how to estimate the MU, while several guidelines and manuscripts are available in literature proposing different theoretical approaches.
Aims: Aim of this work is to describe the model used to estimate MU for several tests, included in the different fields of our laboratory: clinical biochemistry, haematology and coagulation, clinical molecular biology and diagnostic immunology.
Methods: The following steps have been followed to define the model: a) analysis of available literature; b) definition of contributions of different sources or uncertainty in MU, such as random and systematic errors; c) classification of tests in relation to their goal; d) definition of models to estimate the MU in relation to the scope of the test; e) association between test and model to estimate the MU.
Results: Two models of MU estimation were identified: a) first model, only the imprecision component of MU has been calculated for the test results used mainly for comparison to previous results, (e.g. tumour markers); b) second model, MU was calculated by combining imprecision (from internal quality controls data, IQC), bias and the bias-associated error (from external quality assessment data, EQA). Further, MU was calculated at different concentration levels for those measurement procedures where a significant trend was observed between imprecision and/or bias and analyte concentrations.
Conclusions: The model proposed was found to comply with the requirement of ISO 15189. Moreover, it is a feasible and reliable procedure, thanks to the availability of IQC and EQA data, and significant in relation to the test purpose and the clinical needs to produce effective information in order to improve patients outcome.
NORDTEST. Handbook for calculation of measurement uncertainty in environmental laboratories. (NT TR 537 - Edition 3.1), 11-2012.
Tate JR, Plebani M. Measurement uncertainty - a revised understanding of its calculation and use. Clin Chem Lab Med 2016.

P087
GESTIONE DEL RISCHIO TRASFUSIONALE: DALLE NORMATIVE ALLA SICUREZZA DEL PAZIENTE

M.R. Cavallo[1], C. Vella[1], V. Granero[1], L. Calosso[2], P. Ottone[2]

[1]S.C. Lab. Analisi, O.R. Pinerolo ASL TO3
[2]S.I.M.T. Pinerolo ASL TO3

Introduzione-scopo: Il monitoraggio degli eventi sentinella è un'importante azione di sanità pubblica ed uno strumento di governance garante del diritto di salute dei cittadini. La Raccomandazione 5/2008 identifica le attività esposte a fattori potenzialmente a rischio, il DM 69 2 novembre 2015 implementa le indicazioni per prevenire le reazioni avverse.
Per applicare le normative vigenti sono state pianificate verifiche ispettive interne nelle strutture dell'ASLTO3. L'obiettivo degli audit è di evidenziare le non conformità dalla richiesta del gruppo sanguigno alla trasfusione, valutando eventuali scostamenti al fine di pianificare le azioni correttive (AC).
Materiali e metodi: Metodologia lavoro: audit nei tre Presidi Ospedalieri (12 strutture coinvolte). Standard: Raccomandazione 5/2008, DM 69 2 novembre 2015. Intervistati: Direttori, Medici, Coordinatori, Professionisti Sanitari. Periodo: Novembre 2015-Marzo 2016.
Evidenze/comportamenti ricercati: documentazione aziendale, cartelle cliniche ed infermieristiche, consenso informato conforme, modalità prelievo standardizzate, tracciabilità informatica del campione, predisposizione al cambiamento.
Risultati: Clima: 1/12 non collaborante, 3/12 collaboranti passivi, 8/12 collaboranti proattivi. Criticità: documentazione aziendale non presente e/o non aggiornata, scarsa integrazione tra cartella clinica ed infermieristica, etichetta campione e tracciabilità informatica incompleta, raccolta consenso informato non conforme, attività formative non documentate, scarsa cultura dell'errore, comunicazione inefficiente, carenza di risorse umane.
Conclusioni: Le AC pianificate sono legate: al cambiamento culturale, supporto informatico, procedure nell'identificazione del paziente, controllo incrociato tra dati paziente-cartella e clinica-emocomponente. I risultati degli audit e le AC pianificate sono stati condivisi con la Direzione Sanitaria ed il Comitato per il Buon uso del sangue per attuare un'azione sinergica ed efficace al fine di migliorare l'intero processo.

P088
EVALUATION OF A CLIA AUTOMATED ASSAY SYSTEM FOR THE DETERMINATION OF CARBOHYDRATE ANTIGEN 19-9

C. Di Segni, G. Gennarini, S. Zarrillo, E. Anastasi, A. Angeloni

Department of Molecular Medicine

Purpose: The Carbohydrate Antigen 19-9 (Ca 19-9) is an high molecular weight glycoproteic compless used in the clinical setting of pancreatic, biliary and gastrointestinal cancer. Its determination is relevant in the surgical and pharmacological pre-treatment evaluation and for early identification of relapses. Many studies demonstrated the lack of harmonization between the results obtained from different assays.
The aim of this work was to compare the analytical characteristics between two different methods, in particular we compared an automated chemiluminescent immunoassay (CLIA, Maglumi Snibe) versus a radioimmunometric assay (RIA, CisBio) already in use in our laboratory.
Materials and methods: There were evaluated 185 blood samples that were sent to our Laboratory of Tumor Markers (Policlinico Umberto I, Sapienza) enrolled consecutively (87 males and 98 females, aged 22-89 and 29-89 respectively). For both assays were considered a cut-off of normality of 37 U/mL.
Results: Comparison between assays was analyzed using Passing–Bablok regression which showed an elevated interassay correlation (R=0.926, Slope 0.6966, Intercept 0.3360). Mean intra-assay coefficient of variation (CV) was 17.6% (range: 6.1-50.7%) with RIA (n.10 samples) and 5.2% (range: 0.4-17.0%) with CLIA assay (n. 26 samples).
Conclusions: In conclusion this study showed that Maglumi has a good reliability on all samples analyzed and it should be preferred to RIA with the aim to decrease costs, to have more standardization and to produce a more harmonized results.

P089
INDICATORI DI QUALITA' DELLA FASE ANALITICA BASATI SUL RAGGIUNGIMENTO DELLE SPECIFICHE DI QUALITA'

C. Bellini, F. Cinci, R. Guerranti, R. Leoncini, E. Milletti, C. Scapellato

UOC Patologia Clinica – AOU Senese, Policlinico Santa Maria alle Scotte, Siena

Obiettivo: I Sistemi di Gestione della Qualità prevedono l'adozione di indicatori per il controllo di processo. Relativamente alla fase analitica gli indicatori sono spesso ricavati dai Controlli di qualità e dalle VEQ. Tuttavia la misura delle prestazioni analitiche di un metodo non è utilizzabile di per sé come indicatore ma è necessario confrontarla con specifici traguardi rappresentati dall'Errore Totale accettabile (ETa). L'indicatore analizzato è la "percentuale di analiti che rispettano il traguardo" considerandolo raggiunto quando l'Errore Totale Sperimentale (ETs) risulta inferiore all'ETa. Per calcolare la componente sistematica dell'ETs si deve utilizzare un valore di consenso ricavabile dai confronti interlaboratorio, VEQ o CQA. Il nostro obiettivo è verificare la concordanza dei giudizi sul rispetto dei traguardi ottenuta da VEQ e CQA.
Metodologia: Abbiamo analizzato gli ETs di 30 analiti di chimica clinica ottenuti dalle VEQ e dal CQA 2015. Per determinare il rispetto dei traguardi, gli ETs ricavati dalla VEQ e dal CQA sono stati confrontati con le specifiche di qualità scelte dal Laboratorio e sono stati correlati tramite regressione lineare. Il grado di concordanza del giudizio sul raggiungimento dei traguardi è stato analizzato tramite tabelle di contingenza.
Risultati: Utilizzando l'ETs ricavato dal CQA il 93% degli analiti rispetta il traguardo mentre con l'ETs ricavato dalla VEQ la percentuale è del 87%. Dall'analisi dei giudizi tramite tabella di contingenza si ottiene una concordanza generale dell'80%, mentre 4 analiti raggiungono il traguardo con il CQA ma non con la VEQ e 2 lo raggiungono con la VEQ ma non con il CQA. Con la regressione lineare si evidenzia una scarsa correlazione tra gli ETs calcolati con i due sistemi.
Conclusioni: Ai fini del controllo della fase analitica il monitoraggio della "percentuale di analiti che rispettano i traguardi" rappresenta un valido indicatore sia utilizzando i dati ricavati dal CQA che dalla VEQ. Le differenze riscontrate sono attribuibili alle diverse basi dati dei due sistemi. Ciononostante quando i giudizi concordano il monitoraggio risulta migliorato.
Ottomano C, Ceriotti F, Galeazzi M, et al. Linee guida per la gestione dei programmi di Controllo di Qualità Interno. Biochim Clin 2008;32:102-21.

P090
UNITA' DI MISURA NELL'ESPRESSIONE DEI RISULTATI PER LE PROTEINE: STATO DELL'ARTE PRIMA DELL'AUSPICATO "PASSAGGIO AL LITRO" COME INDICATO DALLA 'EFLM CAMPAIGN FOR THE HARMONIZATION OF THE UNITS OF MEASUREMENT'

S. Secchiero, M. Baldon, L. Sciacovelli, M. Pebani

Centro di Ricerca Biomedica (CRB), Azienda Ospedaliera - Università di Padova

Introduzione: Armonizzare le unità nel referto è una sfida per il laboratorio clinico per evitare gli attuali rischi di confusione e di incomprensione. L'EFLM Working Group on Harmonization of Total Testing Project (WG-H) ha iniziato una campagna, articolata in vari step, per promuovere tale armonizzazione.
In particolare, relativamente alle Proteine, il WG-H invita tutti i laboratori ad abbandonare il "dL" a favore del "L", entro il 31 ottobre 2016.
Scopo: Valutare lo stato dell'arte delle unità di misura utilizzate dai laboratori per esprimere i risultati delle proteine.
Metodi: Sono state analizzate le unità di misura attualmente in uso dai 90 Partecipanti al Programma di VEQ del CRB. Il Programma prevede infatti che il singolo partecipante esprima i risultati con le stesse unità utilizzate nel referto. I risultati sono quindi trasformati dal CRB nelle unità SI, applicate nell'elaborazione e riportate nel rapporto periodico.
Risultati: Proteine Tot: g/L 22,1%, g/dL 77,9%; Albumina: g/L 28,9%, g/dL 63,2%, mg/dL 7,9%; IgA, IgG e IgM: g/L 26,1%, mg/dL 73,9%; C3 e C4: g/L 29,9%, mg/dL 70,1%; α1-Antitripsina: g/L 36,1%, mg/dL 63,9%; α1-Glicoproteina ac.: g/L 26,8%, mg/dL 73,2%; α2-Macroglobulina: g/L 33,3%, mg/dL 66,7%; Ceruloplasmina: g/L 27,3%, mg/dL 69,7%, mg/L 3,0%; PCR: mg/L 47,6%, mg/dL 51,2%, g/L 1,2%; Prealbumina: g/L 25,0%, mg/dL 68,8%, mg/L 6,3%; Aptoglobina: g/L 27,1%, mg/dL 72,9%; ß2-Microglobulina: mg/L: 82,4%, mg/dL 7,8%, ng/mL 2,0%, μg/mL 7,8%; Transferrina: g/L 22,7%, mg/dL 77,3%; TLC: g/L 61,4%, mg/dL 37,1%, mg/L 1,4%; FLC: mg/L 100%; RBP: mg/L 90,9%, mg/dL 9,1%.
Discussione: Per Proteine Tot. e Albumina la maggior parte dei laboratori utilizza ancora i g/dL; per FLC e ß2-Microglobulina sono utilizzate prevalentemente le unità raccomandate (mg/L), per la PCR mg/L e mg/dL sono utilizzate in egual misura; per tutte le altre proteine la maggior parte dei laboratori (~70%, range 63,9% -77,3%) utilizza ancora i mg/dL.
Conclusioni: Lo stato dell'arte evidenzia, ad eccezione delle FLC, la presenza di almeno 2 unità di misura. Evidenzia inoltre che la maggior parte dei laboratori deve operare il cambiamento da dL a L per uniformare le unità, e successivamente iniziare il processo di armonizzazione degli IR, a beneficio del paziente. E' tempo di agire!

P091

COMPARISON BETWEEN TWO APPROACHES TO INTERNAL QUALITY CONTROL SYSTEM: WESTGARD RULES AND EXPANDED UNCERTAINTY DERIVED ALARM ZONES

D. Brugnoni[1], S. Mattioli[2], P. Iandolo[3], C. Ottomano[4], F. Ceriotti[5]

[1]*Laboratorio Analisi Chimico Cliniche, Spedali Civili di Brescia, Brescia*

[2]*Laboratorio di Patologia Clinica, Presidio Ospedaliero di Esine, Esine (BS)*

[3]*Laboratorio Analisi, Ospedale Civile di Lavagna, Lavagna (GE)*

[4]*Centro Analisi Monza, Monza (MI)*

[5]*Servizio di Medicina di Laboratorio, Ospedale San Raffaele, Milano*

Background: The aim of an Internal Quality Control (IQC) system is to provide alarms when the risk of reporting results affected by an error exceeding a tolerable level becomes significant. This risk derives from the relationship between the performance observed for the analytical method and the allowable Total Error (TEa) for the selected measurand (Sigma value). The number of possible defective results (i.e, the number of results out of TEa) can be predicted in terms of defects per million opportunities (DPMO). To monitor this risk, Westgard proposed an alarm system based on statistical rules, selected through a σ-metric QC selection tool (1); recently, we proposed an alternative IQC alarm system based on TEa reduced on either sides by “alarm zones” derived from expanded uncertainty (U) estimation (2). Scope of our work is to compare the two approaches.

Materials and Methods: IQC results obtained during a 12 months period for glucose, creatinine and total cholesterol were collected from 4 clinical laboratories using 8 analyzers. For each measurand biological variation derived TEa were used; Westgard rules were selected according to the σ-metric calculated from a 6 months planning period and U was calculated from the imprecision of the IQC results during the same period and from the calibrator’s uncertainty. Based on these settings, the number of “out of control” alarms occurred in the following 6 months with the implemented Westgard’s rules were compared with the number of QC results falling within the “alarm zones”.

Results: The IQC performance was evaluated from the relationship between the alarm frequency and the DPMO of the method derived from the σ value estimated during the testing period. This relationship resulted much higher with our IQC procedure (Spearman’s rs = 0.83; 95%CI = 0.62 to 0.93; p <0.0001) in comparison to the Westgard’s procedure (Spearman’s rs = 0.36; 95%CI = -0.07 to 0.68; p = ns).

Conclusions: These results suggest that our IQC procedure seems more prone to identify results possibly falling outside TEa, while Westgard’s procedure is more focused on monitoring the analytical stability of the method, not always related to the risk of providing unacceptable results.

P092

COMPARISON BETWEEN HBA1C RESULTS OBTAINED BY TWO INSTRUMENTS VARIANT II AND D-10

L. Loiodice, A. Fontana, R. Lovero , L. Nisi , M. Pasculli, T. Calabria, A. Colacicco, G. Ferrara, A. Lopello, S. Marsico, A.M. Rutigliano, F. Di Serio

U.O. Patologia Clinica I, Osp. Policlinico, Bari

Introduction: In order to improve and optimize the technicalization in the execution of the glycosylated hemoglobin, to speed up and to update also the software for actual necessity, a study has been started before the change from the D-10 (Biorad) to Variant II (Bio-Rad), comparing the relative HbA1c assays obtained.

Materials and methods: It has been selected, random, a number of samples of a group of patients referred to our Operative Unit of Clinical Pathology I (31 M and 18 F) with average age between 34 and 82 aa, the results of HbA1c, performed on D-10 (Biorad) and Variant II (Biorad), has been compared and processed using statistical functions of the MedCalc program.

Results: The comparison has been performed according to the linear regression Passing & Bablok (P = 0.42); with the indipendent sample t test, a significant difference hasn't been found between the results of the two instruments because the P value is 0.8, confermed with the Welch test whose P value is 0.5. The comparison has been also executed with Bland & Altmann whose elaboration has demonstrated that the average difference of HbA1c dosages is in the range ± 1.96.

Discussion and conclusions: The statistics confirmed the overlap of the results of the D-10 and Variant II, together with the assessment of comparative clinical diagnostics data for the effectiveness.

The Variant II has been considereted available for performance and efficiency: so the change has been made.

P093

IL LABORATORIO COME STRUMENTO DI PROMOZIONE SALUTE: 10 ANNI DI GIORNATE IN PIAZZA PER LA PREVENZIONE DEL DIABETE

M.R. Cavallo[1], V. Granero[1], E. Richetta[1], A. Cosola[2]

[1]*S.C. Lab. Analisi, O.R. Pinerolo ASL TO3*

[2]*S.S. Promozione Salute, ASL TO3*

Introduzione: Dal 2006 il Laboratorio Analisi di Pinerolo (LAB) e le Associazioni FAND di Pinerolo e AMD (Associazione Nazionale Medici Diabetologi) hanno organizzato oltre 40 manifestazioni denominate “Giornata contro il Diabete” per sensibilizzare l’utenza del Pinerolese alla prevenzione della Sindrome metabolica e del diabete (DBT).

Il LAB è stato coinvolto nella gestione/archiviazione informatica dei dati anagrafici, anamnestici e diagnostici, nella valutazione preliminare delle performances dei dispositivi per l’esecuzione della glicemia capillare e nel supporto tecnico e di consulenza durante lo svolgimento della giornata.

Scopo del lavoro: Utilizzare i dati ottenuti per definire/ ridefinire nuove strategie aziendali d’intervento sul territorio utili alla prevenzione del DBT nonché a promuovere stili di vita più sani.

Materiali e metodi: Ai partecipanti è stata offerta la possibilità di misurare gratuitamente la glicemia (capillare), la pressione arteriosa, l’indice di massa corporea (BMI) e sono stati proposti 2 questionari: uno per la valutazione del rischio di sviluppare il DBT e l’altro sulle abitudini alimentari.

Le misurazioni della glicemia capillare sono state eseguite con Glucometri ACCU-CHECK Aviva Roche (Glucosio deidrogenasi).

Risultati: Sono stati valutati complessivamente 8564 cittadini, dei quali il 38% maschi e il 62% femmine. L’età mediana era di 55 anni.

Il 9% dei cittadini è risultato avere un’alterata glicemia a digiuno, con una probabilità di rischio di Diabete superiore al 35% dovuta soprattutto alla mancanza di attività fisica quotidiana e per un’alimentazione scorretta.

Conclusioni: L’analisi dei dati ha consentito il confronto con la prevalenza ufficiale dichiarata (7.5 % Vs 5.5%) permettendo anche una valutazione complessiva dello stato di salute, dei rischi e sulle abitudini alimentari dei partecipanti.

È stato rivaluto il PDTA DBT e sono state proposte nuove iniziative di sensibilizzazione per la popolazione (giornate aperte in teatri e scuole, corsi di nordic- e fit- walking) in cui il LAB è sempre coinvolto e che ben si presta come strumento di promozione salute, con professionisti che nel tempo hanno incrementato conoscenze e competenze sviluppando una nuova medicina di LAB.

P094

EFFECTIVENESS OF CITRATE BUFFER-FLUORIDE MIXTURE IN SARSTEDT S-MONOVETTE GLUCOEXACT TUBES AS AN INHIBITOR OF IN VITRO GLYCOLYSIS

G. Bonetti[1], M. Montagnana[2], C. Lo Cascio[3], M. Carta[4]

[1]*Clinical Chemistry Laboratory, ASST- Spedali Civili, Brescia*

[2]*Clinical Biochemistry Section, University of Verona, Verona*

[3]*Clinical Laboratory, AOUI Verona, Verona,*

[4]*Clinical Chemistry and Haematology Laboratory, St. Bortolo Hospital, Vicenza*

Introduction: Since glycolysis affects glucose determination in vitro NACB and ADA recommend to immediately place sample tubes in ice-water slurry and to separate plasma within 30 min or to use an effective glucose stabilizer.

Aim of the study: To evaluate glucose concentration in different Sarstedt S-Monovettes maintained at room temperature (R.T.) for 2 h, compared to reference glucose according to NACB-ADA guidelines.

Materials and methods: Blood from 113 volunteers (36 M, 77 F), was collected into lithium heparin (LH), NaF/ Na2EDTA (NaF) and NaF/citrate buffer (GlucoEXACT) tubes. GlucoEXACT tubes contain a liquid additive and requires a proper tube filling and the use of a correction factor (1.16). Reference plasma glucose was determined in LH tube placed in an ice-water slurry, centrifuged at 4°C with plasma separation from the cells within 30 min. Samples were maintained at RT for 2 h after drawing. Glucose testing of all samples of the same subject was performed in duplicate in the same analytical run using an hexokinase method.

Results: Median glucose concentrations were 5.10 (IQR: 4.90-5.50) mmol/L in reference tubes, 5.20 (IQR: 4.92-5.52) mmol/L in GlucoEXACT tubes and 4.61 (IQR: 4.40-5.10) mmol/L in NaF tubes after 2 hours at RT. Mean absolute bias was +1.12% (95% CI: 0.65-1.58%) for glucoEXACT tubes and -8.11% (95% CI: -8.80 - -7.42%) for NaF tubes. Mean bias was within the desirable analytical goal (<±1.8%) in 56.6% of glucoEXACT and in 3.5% of NaF tubes. ANOVA has shown statistically significant difference between glucose concentration in reference tube, NaF and glucoEXACT tube (P <0.0001 and P=0.0001, respectively).

Conclusions: GlucoEXACT tube showed to be superior to NaF alone in glucose stabilization after 2 h at RT. Tubes with only enolase inhibitors, such as NaF, should not be used to prevent glycolysis. If the NACB-ADA recommended treatment of blood sample is not possible the use of an acidified tube, such as glucoEXACT, is needed.

Sacks DB, Arnold M, Bakris GI, et al. Guidelines and recommendation for laboratory analysis in the diagnosis and management of diabetes mellitus. Clin Chem 2011;57:e1-47.

P095

PERSISTENCE OF SERUM FREE LIGHT CHAINS (sFLC): A CLINICAL CASE OF NEGATIVE SERUM IMMUNOFIXATION (IF) AT RELAPSE, WITH sFLC POSITIVITY ONLY

E. Rivolti[1], L. Tognazzi[1], M. Quaresima[1], B. Gamberi[1], E. Bellesia[2], F. Merli[1]

[1]Haematology Unit, Oncology Department, Azienda Ospedaliera Arcispedale Santa Maria Nuova, IRCCS, Reggio Emilia

[2]LACCE, Azienda Ospedaliera Arcispedale Santa Maria Nuova, IRCCS, Reggio Emilia

Background: In Multiple Myeloma (MM) an apparent long-lasting response, with both negative serum immunofixation (IF) and serum protein electrophoresis (SPEP), can cover up a persistence of sFLC. About 20% of Micromolecular myeloma produces only light chain which can be quantified in urine by urine protein ekectrophoresis (UPEP) and, more recently, in blood by sFLC. Micromolecular myeloma, that is mainly located in urine, show up a difficult and underhand identification.
Clinical case: A 63-year old female was diagnosed with K chain micromolecular MM, ISS stage III in October 2012. She presented a SPEP showing a small monoclonal component, IF positive for k chains, elevated sFLC k chains (30600 mg/L), FLC ratio 3445, bone marrow (BM) plasma cells (PCs) 33%, with acute renal failure; patient was treated with 8 dialysis sessions and started therapy: 7 cycles of Bortezomib/dexamethasone/thalidomide (VTD) lead patient to a complete remission of MM. At relapse, in August 2015 patient presented, MM localization in spine MRI, band thickening in SPEP, negative IF, positive Bence Jones (BJ) and a continuous significant increase of sFLC k chains (3190 mg/L) with FLC ratio of 234, that prompted us to perform a BM aspirate that showed a 18% of PCs, thus identifying a significant MM relapse, that needed salvage re-treatment with 8 cycles of VTD. Disease re-evaluation at the end of therapy provide the achievement of a complete remission, with normal sFLC ratio.
Discussion: This clinical case shows the importance of detection of sFLC in every phase of MM disease, in particular during the follow up with no therapy administration. In this patient IF, BJ and SPEP persisted always negative, while sFLC gradually increased. We can conclude that sFLC detection permits to promptly evaluate a relapse that need salvage therapy and could lead to a deep remission of MM disease.

P096

THE IMPORTANCE OF SERUM FREE LIGHT CHAINS (sFLC) DETECTION IN DIAGNONIS OF PROGRESSION IN PLASMACELL DYSCRASIAS

L. Tognazzi[1], E. Rivolti[1], M. Quaresima[1], B. Gamberi[1], E. Bellesia[2], F. Merli[1]

[1]Haematology Unit, Oncology Department, Azienda Ospedaliera Arcispedale Santa Maria Nuova, IRCCS, Reggio Emilia

[2]LACCE, Azienda Ospedaliera Arcispedale Santa Maria Nuova, IRCCS, Reggio Emilia

Background: About 20% of Multiple Myeloma (MM) does not produce intact immunoglobulin chains, but only the light chains which can be detected in the urine as Bence Jones (BJ) protein or by urine protein electrophoresis (UPEP). In blood we can demonstrate light chain by immunofixation (IF), but only sFLC assay can quantify them. sFLC assay has recently been recognized by International Myeloma Working Group (IMWG) as one of the biomarkers of malignancy which can identify patients affected by Smouldering Myeloma with an imminent risk of progression to overt Myeloma.
Clinical report: A 66-year old male, with diabetes mellitus type 2, in hypoglycaemic agents treatment, was diagnosed with IgG k Monoclonal Gammopathy of undetermined significance (MGUS) in November 2013. Patient was monitored by serum protein electrophoresis (SPEP) only, while sFLC were not evaluated. In March 2016 he was hospitalized due to worsening nephrotic proteinuria (10 g/24 h) and health conditions decline, together with detection of acute renal failure, creatininemia (4,1 mg/dl), anaemia, prot total protein (9,6 g/dl), hypercalcaemia (12,1 mg/dl), double monoclonal component at SPEP (1.2+1.69 g/dl), IF positive for k light chains, with exorbitant dosage of serum k chains (616 000 mg/l , k/λ ratio 49677). Bone marrow biopsy showed 52% of plasma cells, confirming the clinical suspicion of MM, and renal biopsy showed a "cast nephropathy". Haemodialysis with "high cut-off" filters for the removal of FLC, and treatments with Bortezomib-dexamethasone and thalidomide, were immediately started. After 1st cycle of therapy, SPEP and IF were negative, with a normal sFLC ratio.
Discussion: This patient was diagnosed with MM at a late stage, with renal failure requiring dialysis. During MGUS follow-up, sFLC assay was never performed. IMWG updated criteria for the diagnosis of MM, identify sFLC as an important tool to diagnose MM, in order to prevent end-organ damage, in particular acute renal failure. In this case, an early and careful assessment of sFLC could have permitted a precocious suspicion of progression from MGUS to MM, avoiding the onset of renal failure.

P097
CASO CLINICO DI ANOMALIA DI MAY-HEGGLIN

G. Covili Faggioli[1], T. D'Atena[1], E. Di Somma[1], A. Frazzoni[1], M.A. Laudadio[1], C. Melotti[1], T.P. Papillo [1], E. Gnudi[2], M. Maglione[2], R. Mancini[1]

[1]U. O. Laboratorio Unico Metropolitano, Azienda USL Bologna
[2]Scuola di Specializzazione in Patologia Clinica, Università degli Studi di Bologna

L'anomalia di May-Hegglin appartiene al gruppo delle 'sindromi MYH9', che comprendono altre tre varianti alleliche ad espressione fenotipica simile: le sindromi di Sebastian, di Fechtner e di Epstein. La prevalenza dell'anomalia di May-Hegglin non è nota. Queste sindromi sono caratterizzate da macrotrombocitopenia, di solito grave, ma paradossalmente paucisintomatica o addirittura asintomatica. La macrotrombocitopenia è caratterizzata da piastrine giganti, con un diametro superiore o uguale a quello del globulo rosso. L'anomalia di May-Hegglin, come la sindrome di Sebastian, è una forma ematologica pura di sindrome MYH9, dovuta a mutazione del gene che codifica per la catena pesante della miosina non muscolare IIA. E' caratterizzata da inclusioni citoplasmatiche nelle cellule della linea granulocitaria. Le inclusioni citoplasmatiche tipiche dell'anomalia di May-Hegglin sono ovalari, di colore blu alla colorazione May-Grümwald-Giemsa (MGG) e sono spesso definite come pseudocorpi di Döhle. La tendenza alle emorragie è spesso minima e circa la metà dei pazienti sono asintomatici. Tuttavia, il 40% dei malati presenta emorragie significative (porpora trombocitopenica: epistassi, menoraggie, ecchimosi). La maggior parte dei pazienti non va incontro a emorragie significative e pertanto non è di regola necessario nessun trattamento specifico. Tuttavia, nel caso di interventi chirurgici possono essere utili le trasfusioni piastriniche. L'aspettativa di vita è normale. Nel maggio 2012, in seguito ad un controllo dell'esame emocromocitometrico, torna alla nostra attenzione il caso di una paziente in cui precedentemente era stata posta diagnosi di Anomalia di May- Hegglin. Il conteggio piastrinico, eseguito con il contatore automatico, evidenziava un valore di 12000/ uL. Lo striscio di sangue periferico, però, rilevava la presenza di un discreto numero di megatrombociti. Il mese successivo, ad un ulteriore controllo, il conteggio piastrinico evidenziava un valore attorno a 9000/uL. Allo striscio di sangue periferico, esse apparivano comunque in numero maggiore ed inoltre si rilevava la presenza costante di piastrine giganti. Per questo motivo, si richiedeva la conta in citofluorimetria ed il risultato del conteggio piastrinico indicava un valore di 31000/uL.

P098
THE EYE IS STILL THE DIFFERENCE!

M. Monari[1], I. Timofeva[2], L. Castagna[2], A. Montanelli[3], R. Assandri[1], F. Maura[1], A. Santoro[2]

[1]Humanitas Clinical and Research Center, Rozzano (Mi)
[2]Humanitas Cancer Center, Humanitas Clinical and Research Center, Milan
[3]Clinical Investigation Laboratory, Diagnostic Department, Spedali Civili, Brescia

Introduction: a 48 year old man presents to the emergency room of our hospital for malaise, are sent to the laboratory routine investigations including blood counts that the preliminary assessment suggests a framework compatible with acute leukemia.
methods: the CBC runs on the instrument Beckman Coulter LH780. Immediately has been analyzed the scatter of WBC, it was clear that there is an abnormal distribution of cells but it was necessary to examine the smear blood to define the type and amount of blast. We have colored with traditionally May-Grumwald Giemsa. It has been done the flow cytometry with the Backman Coulter FC 500.
Results: the CBC provided the following parameters: white blood cells (WBC) 10 ^3 cells/mm3, hemoglobin 10.1 g/dL and 10 X 10^ 3/mm^3 platelets; by the scatter we didn't found a predominant population of lymphatic or myeloid population. The smear blood showed a carpet colored with compatible with acute leukemia highly undifferentiated. All it has done in 3 hours. The flow cytometry showed, the day after, 93% of blasts CD45+, CD34-, HLA-DR-, CD33+, CD38+, CD117-, CD13-, MPO+, CD15-, CD4-, CD19-, CD10-, CyCD3-, CyCD22-, CyCD79a-. In the population of Lymfocites it showed that CD19+ on amount of 17% and result a polyclonal B lymphocyte population (ratio Kappa/ Lambda = 1.8), lymphocyte CD3+ (75%) had a ratio of T4/ T8 = 5.2 (aumented). It not documented an incremented ratio of Natural killer. The analysis is compatible with a finding of immunophenotypic acute myeloid leukemia. This result confirmed the very slow differentiation of this leukemia.
Conclusion: as in this case of emergency, despite the sophisticated instruments available, the blood smear and its reading by experienced staff is a fundamental step of diagnostic process of patient: it has a very reduce turnaround time. It guarantees still valid, rapid and certainly effective indication for taking charge of the patient. The hope is that, in ever shorter, the counting instrument has the specific markers that are helpful to identify the different populations of blast, pending the outcome of the result of cytofluorymetry.
Rabizadeh E, Pickholtz I, Barak M, et al. Acute leukemia detection rate by automated blood count parameters and peripheral smear review. Int J Lab Hematol 2015;37:44-9.

P099
CASE OF MYELOID LEUKEMIA IN TRANSITION FROM CHRONIC TO ACUTE IDENTIFIED BY EXPERT SYSTEM HEMALINK

S.T. Caravetta[1], M. De Palo[1], C. Fermi[1], F. Ghisoni[2], L. Ippolito[1], A. Rapalli[1], A. Turini[1], D. Vallisa[3], G. Pedà[1]

[1]U.O. di Patologia Clinica, Lab. Analisi, Ospedale di Vaio, Fidenza
[2]U.O.C. Cure Palliative, Ospedale di Vaio, Fidenza
[3]U.O. Ematologia e Centro Trapianti, AUSL Piacenza

Introduzione: Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder of hematopoietic stem cells. In this type of leukemia is present a rearrangement BCR-ABL characterizing the chromosome Philadelphia (ph). Bone marrow derived mesenchymal stem cells (MSCs) are pluripotent stem cells that can differentiate into several mesenchymal tissues. Usually the Chronic myeloid leukemia can be characterized or severe leukocytosis or a very high increase in leukocytes; analizing the smear of perferic blood you notice an increase in neutrophils, basophils and immature myeloid precursors, as promyelocytes, myelocytes, metamyelocytes, which they are not normally present in the peripheral blood. In patients with this disease it is present in most cases of splenomegaly. This chronic disorder can develop into acute and morphologically disorder we can see, in patients in the acute transformation, the rise of undifferentiated cellular elements with the characteristics of immaturte cells (blasts) as in the typical acute leukemias.
Materials and methods: We report here the clinical case of a 72 year old man came to our attention because of the presence of a high number of leucocytes in blood count examination, this examination was required now in routine by the family doctor. Subsequent to our report, abdominal ultrasound revealed a marked splenomegaly.
Results: The peripheral smear has detected the presence of immature cells of the myeloid line of emopatia characteristics. It was also found to contain erythroblasts. Additional diagnostic investigations led to the diagnosis of chronic myeloid leukemia characterized by rearrangement BCR-ABL transformation in acute phase.
Conclusions: The reported case shows how a careful analysis of blood count indices may be essential in supporting the diagnosis of rare and aggressive diseases such as chronic myeloid leukemia. It is important to know how to properly use this instrument in the lab and set it with special alarms that can bring the staff to indicate the presence of significant anomalies. The patient was immediately sent to oncohematology to be treated as soon as possible with uncertain prognosis, but at the moment seems to be stable.

P100
STUDIO DEGLI EFFETTI DEL BORTEZOMIB SU PROGENITORI DELLA LINEA MESANGIOBLASTICA NEL MIDOLLO OSSEO NORMALE E NEL MIELOMA MULTIPLO

L. Macchia, M. Montali, S. Pacini, S. Barachini, A. Azzarà, M. Petrini

Laboratorio di Ematologia, Osp. S. Chiara, Pisa

Introduzione: Il microambiente midollare gioca un ruolo cruciale nella patogensi del Mieloma Multiplo. In esso si distinguono le Mesenchymal Stromal Cells (MSCs), capaci di produrre tessuto osseo, adiposo e cartilagineo. Sono state inoltre isolate i loro precursori, le Mesodermal Progenitor Cells (MPCs), con caratteristiche molecolari, morfologiche e immunofenotipiche proprie.
Scopo: Le MPCs state studiate sia isolate da midolli ematologicamente sani che ottenute da midolli con Mieloma Multiplo valutandone le differenze in termini di frequenza e di capacità differenziativa. Lo studio di farmaci che interferiscono sui momenti principali della patogenesi del Mieloma Multiplo, ricopre notevole interesse. In questo lavoro è stata indagata l'azione del Bortezomib (1) sulle MPCs in midolli sani e patologici, confrontandone gli effetti.
Gli aspirati midollari sono stati ottenuti da 23 donatori "sani" e da 34 donatori con Mieloma Multiplo alla diagnosi o in recidiva dopo almeno 2 anni liberi da terapia. E' stata calcolata la frequenza delle MPCs isolate. È stato valutato l'effetto del Metodi: Bortezomib sul differenziamento delle MPCs, misurando la proliferazione cellulare proporzionale alla percentuale di Alamar Blue ridotta.
Risultati: Per quanto riguarda frequenza numerica e capacità differenziativa delle MPCs non sono emerse differenze signicative tra casi e controlli. Nei midolli sani il Bortezomib ha un effetto diretto stimolante sul differenziamento mesenchimale delle MPCs alla concentrazione di 2 nM, che non si osserva alla dose di 3 nM. Nei midolli patologici, al contrario, il Bortezomib svolgerebbe un effetto inibitorio dose dipendente sul differenziamento mesenchimale
Discussione: L'effetto finale del Bortezomib di "spinta" osteogenica nel Mieloma Multiplo, potrebbe derivare da un suo effetto sulle plasmacellule patologiche. Queste, per effetto del farmaco, interromperebbero la produzione di MPCs "patologiche", mentre le MPCs "sane" verrebbero stimolate all'osteogenesi. Questi risultati rafforzano l'idea che le MPCs siano protagoniste nell'evoluzione patogenetica della malattia Mielomatosa.
1. Giuliani N, et al. The proteosome inhibitor Bortezomib affects osteoblast differentiation in vitro and in vivo in multiple myeloma patients. Blood 2007;110:334-8.

P101
MONOCYTES SUBSETS IN PATIENTS WITH PROBABLE SYSTEMIC INFECTION

M. Greco, A. Mazzei

Clinical Pathology Laboratory, "Vito Fazzi" Hospital, ASL Lecce, Lecce

Background: Monocytes are critical effectors in the immune response and regulators of inflammation; an heterogeneous population with subset-specific phenotype and function. Differential expression of LPS receptor, CD14, and FcγIIIR, CD16, differentiates classical monocytes as CD14++CD16-, non-classical as CD14+CD16++ and intermediate as CD14++CD16+. Classical monocytes show antimicrobial potential, while CD16-positive monocytes are involved in Ag processing and presentation function as well as transendothelial migration (Blood 2011; 118:e50-e61).
Aim: The present study was aimed to evaluate changes in blood monocytes subsets composition in patients with probable systemic infection compared with control patients and, in particular, to evaluate whether the shift in CD16-positive monocytes reported in many inflammatory disease could be related to selective increase of CD14++CD16+.
Methods: We studied 50 patients with high procalcitonin level (>0.5 ng/mL), as indirect evaluation of infection presence, and 50 control patients by flow-cytometrical analysis of CD14 and CD16 surface expression in blood monocytes by fluorescent labeled monoclonal antibodies and BD FACS CANTO II.
Results: Intermediate monocytes CD14++CD16+ showed significant increase in patients with high procalcitonin level compared to control patients ($p < 0.001$), while classical monocytes CD14++CD16- were significantly reduced. In agreement with the known proinflammatory role of CD16-positive monocytes, we found a positive correlation trend between procalcitonin level and both intermediate and non-classical monocytes; while classical monocytes showed an opposite trend of correlation with the increase of procalcitonin.
Conclusions: Possible presence of systemic infection appears correlated with significant increase of the specific subset of intermediate monocytes CD14++CD16+; this subpopulation, which is characterized by higher MHC II complex expression and higher tumor necrosis factor production, could support, at least in part, some systemic manifestations of infection and trigger the intervention of acquired immune system cells. Further analysis are ongoing in order to match up results to blood colture positivity and to confirm our preliminary data by analyzing a larger number of patients.

P102
URINARY ALBUMIN TO CREATININE RATIO IN DIAGNOSIS AND RISK STRATIFICATION OF RENAL AL AMYLOIDOSIS

G. Palladini[1], P. Milani[1], M. Basset[1], F. Russo[1], M. Nuvolone[1], F. Lavatelli[1], T. Bosoni[2], L. Pirolini[2], A. Foli[1], R. Albertii[2], G. Merlini[3]

[1]Centro per lo Studio e la Cura delle Amiloidosi Sistemiche, Fondazione IRCCS Policlinico San Matteo, Dip. di Medicina Molecolare, Università di Pavia, Pavia
[2]Lab. Analisi Chimico Cliniche, Fondazione IRCCS Policlinico San Matteo, Pavia
[3]Direzione Scientifica, Fondazione IRCCS Policlinico San Matteo, Pavia

In AL amyloidosis, the measurement of 24 hour proteinuria (24hUP) is fundamental for the assessment of renal involvement but it is cumbersome and prone to errors. The urinary albumin to creatinine ratio (UACR) has been proposed as an alternative to measure urine protein loss. We evaluated the performance of UACR in diagnosing renal involvement and predicting renal outcome in 224 newly diagnosed (2013-2015) patients with AL amyloidosis. Correlation between UACR and 24hUP was assessed by Pearson's r. ROC analyses were used to identify UACR cutoffs. Patients who died off-dialysis were censored. Sixty-four percent of patients had kidney involvement [24hUP >0.5 g/24h (predominantly albumin)]. Renal stage (Palladini et al. Blood 2014) based on 24hUP (cutoff 5 g/24h) and estimated glomerular filtration rate (eGFR, cutoff 50 mL/min per 1.73 m2) was I in 48% of cases, II in 37%, and III in 15%. Median (interquartile range) 24hUP and UACR were 1.7 g/24h (0.3-6.3 g/24h) and 1312 mg/g (98-6188 mg/g), respectively. There was a good correlation between 24hUP and UACR (Pearson's r = 0.90, 95%CI: 0.87-0.92). The best UACR cutoff for the diagnosis of renal involvement (defined as 24hUP >0.5 g/24h) was 500 mg/g (area under the ROC curve 0.94, 95%CI: 0.90-0.97; sensitivity 89%; specificity 97%). The definition of renal involvement with 24hUP and UACR was concordant in 92% of cases (95%CI: 88-95%). 16 patients (7%) required dialysis. The UACR cutoff best discriminating patients who required dialysis at 6 months was 3600 mg/g. This was used to substitute the 24hUP cutoff in the renal staging system. There was a 90% concordance in renal staging with the 24hUP and the UACR based staging systems. Both staging systems discriminated 3 groups with significantly different rate of progression to dialysis. A >20% decrease in UACR best predicted a >30% change in 24hUP, which is used to define renal response in the absence of >25% decreases in eGFR. Although the difference did not reach statistical significance, all progressions to dialysis occurred in non-responders defined either per standard criteria or using the novel UACR criteria. These data indicate that UACR can be used to identify renal involvement, predict renal outcome and possibly assess renal response in AL amyloidosis.

P103

BLOOD COUNT IMPORTANT IN THE DIAGNOSIS OF ACUTE LEUKEMIA WITH MYELOID ALTERATIONS RELATED TO MYELODYSPLASIA

G. Pedà[1], S.T. Caravetta[1], L. Craviotto[2], M. Crugnola[2], M. De Palo[1], C. Fermi[1], G. Grandi[1], M. Monica[1], F. Maradini[1], A. Rapalli[1], A. Turini[1], G. Lippi[3], L. Ippolito[1]

[1]U.O. di Patologia Clinica, Lab. Analisi, Ospedale di Vaio, Fidenza

[2]Ematologia e Centro trapianti midollo osseo (Ctmo), Azienda Ospedaliero-Universitaria di Parma, Parma

[3]Lab. Chimica Clinica ed Ematologia, Azienda Ospedaliera Universitaria integrata di Verona, Verona

Introduction: The acute myeloid leukemia (AML) is one of two main forms of the family of acute leukemias and is described as a tumor clonal myeloid progenitors who live in the bone marrow. Clonal hematopoietic cells, once the bone marrow occupied will dominate the activity and determine the failure justifying the whole series of symptoms that characterize the clinical history of the patient with AML.
Material and methods: We present here the clinical case of a 70 year old man came to the hospital with a note of pallor and shortness of breath, come to our attention the blood count showed marked reduction in red blood cell count, drastic decrease in hemoglobin and hematocrit values, pronounced thrombocytopenia. In addition the expert system Hemalink signaled a major presence of blasts and atypias.
Results: After an initial analysis of the peripheral blood smear that showed the presence of numerous blasts and neutrophils hypersegmented, flow cytometric investigation of the marrow showed abundant cellularity, represented 65% of blastic elements myeloid appearance of uneven size, with round nucleus, cytoplasm often abundant, basophilic, frequently vacuolated and partly containing granules, with frequent blebs. Myeloid series maturing markedly hypoplastic and mainly represented by mature granulocytes. Megakaryocytic series markedly hypoplastic and dysplastic. Mild plasma cell hyperplasia. Subsequently 'cytogenetic examination revealed the presence of two clones, one majority (50%) in complex karyotype and one minority (20%) hypotriploid to 67-68 chromosomes. The picture was found compatible with Acute Myeloid Leukemia with disorders related to myelodysplasia.
Conclusions: Cytometric exam confirmed blasts previously detected by the alarm blasts of expert system Hemalink and displayed cytomorphological laboratory exam. Careful examination of the blood counts scoresheet through alarms set in Hemalink system allowed an early diagnosis of a rare and complex disease, allowing early pharmacological approach. The patient is being treated with decitabine and seem to respond to treatment.

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MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE IN A WELL-DEFINED GEOGRAPHIC AREA IN SARDINIA

G. Serra, S. Caria, G. Demuro, F.B. Ronchi

Clinical Pathology Service, P.O. N. S. di Bonaria, San Gavino M.le, ASL Sanluri

Introduction: Monoclonal Gammopathy of Undetermined Significance (MGUS) is a common premalignant plasma-cell disorder, precursor of myeloma. MGUS is defined by the presence of a serum monoclonal protein (M-protein) at a concentration <3 g/dL and through the absence of organ damage as anemia, hypercalcemia, renal insufficiency and lytic bone lesions. We decided to evaluate the prevalence of MGUS in a well-defined geographic area of Sardinia, Italy and compare it with literature.
Methods: Serum samples were obtained from all patients afferent to laboratory of San Gavino M.le Hospital from 1 January to 31 December 2015. We perfomed all samples by sensitive and high-resolution fused-silica capillary zone electrophoresis (Capillarys™ 2, Sebia). For each serum sample that showed a discrete, well separated and localized band in the electrophoretic patterns, it was performed immunofixation to characterize and quantify monoclonal immunoglobulin.
Results: Serum samples were obtained from 11090 patients. MGUS was identified in 155 patients and the prevalence was 1.42%. The percentage of isotype of the monoclonal immunoglobulin in 155 patient with MGUS was IgG 68.9 % (IgGκ 66%,IgGλ 34%), IgM 16.6% (IgMκ 69%, IgMλ 31%), IgA 9.6% (IgAκ 60%, IgAλ 40%) and biclonal 4.6%. The monoclonal immunoglobulin concentration was less than 1.00 g/dL in 140 patients (90.3%), from 1.00 to 1.49 g/dL in 3 patients (1.9%), from 1.50 to 1.99 g/dL in 6 patients (3.9%) and greater than 2.00 g/dL in 6 patients (3.9%). The prevalence of MGUS in the 155 patients was higher in men than in women (58,7% men versus 41.3% women).
Conclusions: This study defines the prevalence of MGUS in general population of our provincial area. Our results were consistent with the data reported in literature. This study provides a useful and updated epidemiological tool for the MGUS management and could lay the basis for a future screening study. We propose to evaluate the possible progression to a malignant condition by the monitoring of monoclonal component of our patients with MGUS.
Kyle RA, Therneau TM, Rajkumar SV, et al. Prevalence of monoclonal gammopathy of undetermined significance. N Engl J Med 2006;354:1362-9.

P105
A NEW TOOL FOR THE ASSESSMENT OF OPERATORS AGREEMENT IN HAEMATOLOGY MORPHOLOGY

F. Tosato, L. Sciacovelli, E. Piva, M. Plebani

Department of Laboratory Medicine, Padua University School of Medicine, Padua

Background: In the field of clinical risk and patient safety in Laboratory Medicine, the accuracy in morphological interpretation and classification of leucocytes and red blood cells depends upon both blood pathology knowledge and experience of the laboratory scientists using the microscope. The hematology laboratory should provide harmonization and quality assurance in morphology results.
Methods: On the basis of results obtained in EQA program of Haematology Morphology, managed of Center of Biomedical Research of Veneto Region, we have assessed the morphologists agreement for the microscopic evaluation of the differential white blood cells count and the peripheral blood smear features. All the morphologists have a personal and confidential username and password for participation so that each operator could evaluate the blood smear and individually insert the results. Subsequently, all data have been compared and the agreement among the operators evaluated. The evaluation result has been shared with all operators and possible incoherence discussed in order to assure their resolution.
Results: Considering as reference values the mean percentage and standard deviations of each white blood cells population provided by the two most experienced morphologists, we reanalyzed the results provided by other operators. We also checked the agreement in the diagnostic hypothesis formulated by the operators. In a case of anaemia, the reference interval calculated as mean +/- 1sd were: neutrophils 64.79-66.21%, lymphocytes 15.17-20.83%, monocytes 4.79-6.21%, eosinophils 7.59-10.41%, basophils 0.59-3.41%. Among 15 morphologists, if an incorrect result had been provided by the operators (10% in this exercise), a review of the blood smear was performed and recorded by one of the two experts. The diagnostic hypothesis formulated by the operator was the same.
Conclusions: The creation of a tool to evaluate the agreement among operators that provide morphological evaluation could represent an important step forward patient safety and quality assurance in Laboratory Medicine. This way assures harmonization for a correct diagnosis and patient management.

P106
CATENE LEGGERE LIBERE SIERICHE (sFLC): CONFRONTO TRA METODI

C. Bernini[1], R. Buzzi[1], R. Carrozza[1], A. Fiorini[2], M. Montanaro[2], M.T. Muratore[1]

[1]U.O.Diagnostica Clinica, PO Belcolle, Viterbo
[2]U.O.C. Ematologia

Razionale: La determinazione della concentrazione delle sFLC è utile a livello diagnostico, prognostico e nel follow up di discrasie plasmacellulari. Oggetto dello studio è la comparazione di due metodi per il dosaggio delle sFLC: il test policlonale Freelite The Binding Site ed il test monoclonale N Latex FLC Siemens. I valori assoluti ottenuti con i due test sono significativamente differenti; poiché il Freelite é disponibile da più tempo (2001) le linee guida internazionali si basano su tale metodo e non sono applicabili all'altro test. L'esigenza di questo lavoro nasce da motivazioni pratiche: essendo la diagnostica di laboratorio soggetta a gare d'appalto e quindi suscettibile a variazione anche il test per le FLC potrebbe cambiare con conseguente difficoltà per i clinici nel follow up già avviato dei pazienti.
Pazienti e metodi: Nell'arco di 13 mesi, abbiamo dosato le sFLC su 60 campioni, di 46 pazienti con componente monoclonale (27 maschi, 52–88 anni; 19 femmine, 45-83 anni) di cui 23 con mieloma (8 IgGK, 2 IgGλ, 1 IgAK, 2 IgAλ,1 IgDλ, 3 micromolecolari K, 3 micromolecolari λ, 1 oligosecernente, 1 non secernente K ed 1 smoldering K), 10 con amiloidosi AL (7 K, 2λ), 1 con leucemia linfatica cronica, 1 con malattia da deposito di catene leggere k e 8 con MGUS (4 IgGK, 1 IgGλ, 3 IgAλ). Le determinazioni sono state effettuate in parallelo con la metodica Binding Site sul turbidimetro SPAPLUS e con la metodica Siemens sul nefelometro BNII.
Risultati: 33 campioni con rapporto kλ alterato e 27 non alterato con test Freelite (V.N.0,26-1,65); 38 campioni con rapporto Kλ alterato e 22 non alterato con test N Latex FLC (V.N. 0,31-1,56); concordanti con entrambi i test 49/60 (82%), discordanti 11/60 (18%). In 10 determinazioni si sono riscontrati rapporti delle catene concordanti nell'alterazione ma profondamente diversi nell'entità, valore medio 48,5 (17-98) vs 364,4 (118-1024).
Conclusioni: Poiché uno dei criteri decisionali IMWG del 2014 per trattare i pazienti con smoldering mieloma è il rapporto delle FLC coinvolte e non coinvolte >100 dai nostri dati sembra assolutamente necessario definire un nuovo cut-off per il test monoclonale per questa categoria.
Rajkumar SV, Dimopoulos MA, Palumbo A, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol 2014;15:e538-48.

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POTENZIALI APPLICAZIONI CLINICHE DEL PARAMETRO IPF (IMMATURE PLATELET FRACTION) NELLO STUDIO TROMBOFILICO

G. Vitale, M. Diquattro, S. De Michele, G.M. Calabrò, M. Imburgia, A. Aiosa, G. Maggiore, A. Iuculano, F. Roccamatisi, C. Di Maio, M.G. Consolo, E. Mandalà, B. Palma

U.O.C. di Patologia Clinica, ARNAS Ospedale Civico, Palermo

Scopo dello Studio: valutare se il parametro IPF possa essere utilizzato nello screening trombofilico per evidenziare variazioni qualitative delle piastrine in presenza di condizioni protrombotiche. Materiali e Metodi: sono stati studiati n.43 paz. (26 M e 17 F) in età adulta, positivi allo studio trombofilico, con anamnesi positiva per eventi trombotici, con conteggio PLT nel range di riferimento. 28/43 presentavano una singola anomalia protrombotica, 12/43 una doppia, 2/43 una tripla e 1/43 quattro anomalie; il 28% mut. protrombotiche. Due gruppi di controllo: n.149 soggetti sani in età adulta con parametri ematologici nei limiti di riferimento e n.39 pazienti negativi allo screening trombofilico, con anamnesi positiva per trombosi e sovrapponibili per provenienza al gruppo dei positivi. Il conteggio piastrinico e i parametri piastrinici [MPV fL, P-LCR%, PDW %, IPF % e IPF#] sono stati determinati entro un'ora dal prelievo, con analizzatore Xe2100, Sysmex. Risultati: applicabile statistica (t Test), incremento significativo di IPF% nei pazienti positivi (mediana 1.8%, range 0.7-7.3; p 0.001) e in quelli negativi (mediana 1.9 %, 0.5-7.5; p 0.005) vs il gruppo di controllo (mediana 1.5%, range 0.4-3.1), i valori più alti in presenza di eterozigosi per mutaz. del Fattore II, differenza di IPF# nel gruppo positivi (4.9x109/L, range 1.4-14.7) e nel gruppo negativi (4.4x109/L, range 2.3-15.8) vs gruppo di controllo (3.6x109/L, range 1.10-9.20). I parametri piastrinici MPV, PDW, P-LCR non differenziavano i due gruppi dai controlli sani. Nessuno dei parametri piastrinici studiati differenziava il gruppo dei positivi dai negativi. Conclusioni: i soggetti positivi presentavano variazioni qualitative della popolazione piastrinica, espressione di incrementato turnover e potenzialità emostatica. I soggetti negativi si differenziavano dai controlli ma non dai positivi, questo riscontro potrebbe essere spiegato dalla presenza anche in questi pazienti di un'anamnesi positiva per trombosi e/o dalla presenza di patologie che comportano un consumo subclinico delle PLT. Il parametro IPF potrebbe contribuire alla stratificazione del rischio trombotico, tuttavia sono necessari ulteriori studi per supportare l'evidenza che modificando l'approccio terapeutico in pazienti con incrementati valori di IPF vi sia una riduzione degli eventi trombotici.

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ARMONIZZAZIONE NELLA LETTURA E INTERPRETAZIONE DELLE REVISIONI MICROSCOPICHE IN EMATOLOGIA: UN PERCORSO DI AREA VASTA 2

C. Micucci[1], E. Tili[1], S. Secondini[1], L. Petrolati[3], G. Carta[3], A. Politi[2], G. Ghiandoni[2], R. Vannucci[4], P. Pauri[1]

[1]U.O.C. Patologia Clinica, Ospedale di Jesi (AN), AV2, ASUR Marche

[2]U.O.C. Patologia Clinica, Ospedale di Osimo/Loreto (AN), AV2, ASUR Marche

[3]U.O.C. Patologia Clinica, Ospedale di Senigallia (AN), AV2, ASUR Marche

[4]U.O.C. Patologia Clinica, Ospedale di Fabriano (AN), AV2, ASUR Marche

Scopo del lavoro: Negli ultimi anni, il concetto di armonizzazione si è esteso a tutta la Medicina di Laboratorio, nell'ambito di un percorso di miglioramento continuo della qualità delle prestazioni nella fase analitica ed extra-analitiche, verso la sicurezza del dato (e quindi del paziente) e di una migliore comprensione del referto. Nell'ambito del progetto di integrazione fra i laboratori dell'Area Vasta 2 Marche (Jesi, Fabriano, Senigallia, Osimo/Loreto), abbiamo intrapreso dal 2015 un percorso di armonizzazione all'interno di vari settori (biochimica clinica, ematologia, coagulazione, urine) tra i diversi laboratori. In ambito ematologico, in particolare, si è lavorato per valutare la concordanza fra lettori e per andare verso un'interpretazione armonica e comune delle revisioni microscopiche.
Metodologia: Il progetto ha previsto la revisione microscopica di 46 campioni ematologici, colorati con metodo May-Grunwald-Giemsa, da parte dei Dirigenti delle 4 sedi AV2:
-in una prima fase tali letture sono state rapportate con i citogrammi ottenuti dallo strumento ADVIA 2120 (Siemens) in uso presso la sede di Jesi;
-nella seconda fase il confronto è stato effettuato con un operatore esterno di pluriennale esperienza (gold standard), membro del GdS di Ematologia SIBioC.
Le conte ottenute sono state, poi, analizzate attraverso l'indice di Pearson (o correlazione lineare) e la Kappa di Cohen.
Risultati: La correlazione di Pearson ha evidenziato che le migliori concordanze si sono avute per le popolazioni dei linfociti e dei neutrofili, le peggiori per i monociti e gli eosinofili. L'analisi attraverso la Kappa di Cohen ha mostrato letture abbastanza discordanti tra gli operatori per le popolazioni immature considerate (IG, Blasti).
Conclusioni: I risultati ottenuti hanno evidenziato alcune difformità fra gli operatori e performance non ottimali che hanno richiesto l'organizzazione di audit, anche con i clinici, che si sono mostrati molto coinvolgenti ed utili. Un ulteriore ciclo di confronto prevede l'introduzione di griglie comuni di blocco, di ottimizzare il referto attraverso l'adozione delle unità di misura internazionali e l'inserimento di commenti comuni ed appropriati, secondo le indicazioni del GdS di Diagnostica Ematologia SIBioC.

P109

IMAGES IN HEMATOLOGY. METASTATIC INVOLVEMENT AND REPLACEMENT OF BONE MARROW IS NOT AN INFREQUENT MICROSCOPIC OBSERVATION IN CLINICAL PRACTICE: FOUR CASES DESCRIPTION

A. La Gioia[1], M. Bombara [2], R. Incensati[3], F. Fiorini[1], P. Valentini [1], M. Fiorini[2]

[1]*Patologia Clinica, Osp. Lotti, Pontedera*
[2]*U.O.C. Medicina di Laboratorio, Ospedale di Livorno*
[3]*U.O.C. Anatomia Patologica, Ospedale di Livorno*

Introduction: Bone marrow have been described as metastatic niche for disseminated tumor cells from solid tumors whose cells can be detected in bone marrow in approximately 30 % of cases (Shyozawa, 2015; Fontanella, 2015). Starting from this place, tumor cells can spread to other organs but in some cases the metastatic tumor grows right in the bone marrow. Depending on the timing as well as on the degree of bone morrow involvement metastatic invasion can causes organ failure until the myelophthisis. We report morphologic aspects that have been observed in bone marrow aspirate of four cases of they metastatic involvement.
Cases description:
1. A 67-year-old woman who had previous diagnosis of left lobular breast cancer. Bone marrow aspirate was performed because of pancytopenia. The bone marrow film showed the disappearance of the normal myelopoietic cellularity and the prevalent presence of metastatic cells that had a single nucleus and a large non-homogeneous cytoplasm often containing an oval or round large purple inclusion. In most cases 3 or more cells formed a cluster with non-distinct cellular border (syncytia).
2. A 79-year-old man without previous history of cancer. Even in this cases pancytopenia was present and bone marrow aspirate was performed. Due to the prevalence of small cells from a lung microcytoma previously undiagnosed, an aspect mimicking leukaemia it was observed.
3. A 69-year-old man whit previous diagnosis of squamous cell carcinoma of the penis who had a pancytopenia after radio- and chemo therapy. Bone marrow aspiration revealed numerous isolated cells and syncytia of epithelioid cells.
4. A 44-year-old woman without previous history of cancer had a sudden and rapid decrease of both haemoglobin and platelets. Just as the previous case, numerous isolated cells and syncytia were observed but the origin of the primary tumor remains unknown.
Conclusions: The microscopic observation of smears is a sensitive tool for metastatic cells detection in bone marrow. Their systematic search is mandatory in cases of unexplained peripheral cytopenia and in all cases in which a solid tumors was diagnosed previously. A preventive and prolonged observation by using lower microscopic magnification can improve the detection ratio.

P110

IMPACT OF THE NEW INTERFERON-FREE ANTIVIRAL REGIMENS FOR HCV ON PERIPHERAL BLOOD LYMPHOCYTE SUBSETS: A FLOW CYTOMETRIC APPROACH

A. Schiavinato[1], G. Pantano[1], F. Tosato[1], M. Nabergoj[2], P. Fogar[1], E. Piva[1], M. Plebani[1]

[1]*Department of Laboratory Medicine, Padua University School of Medicine*
[2]*Department of Hematology, Padua University School of Medicine*

An estimated 130-150 million persons are infected with the hepatitis C virus (HCV) and approximately 500000 people die each year from hepatitis C infection worldwide. About 15-45% of infected persons spontaneously clear the virus within 6 months of infection without any treatment. The remaining 55-85% of persons will develop chronic HCV infection. Besides liver disease, HCV chronic infection can be associated with extrahepatic manifestation such as cryoglobulinaemia and other lymphoproliferative disorders, specifically B-cell non-Hodgkin lymphoma (NHL). Several studies reported an epidemiological association between HCV infection and NHL (odds ratios between 2 and 3 on average), but the molecular mechanisms are still unclear. Clinical studies reported antiviral treatment based on interferon-α to be efficacious for treatment of HCV-associated lymphomas (1). Recently, direct acting antiviral agents (DAAs) have dramatically altered the treatment landscape for HCV chronic disease with impressive cure rates (>90%) and low rate of adverse effects. To gain new insights on this topic, in our laboratory we have studied by flow cytometry peripheral blood of HCV patients who underwent DAAs treatment and generated data about dynamic changes in lymphocytes number and subsets distribution in regard to HCV-RNA load. We also studied the effect of DAAs on peripheral lymphocytes from patients affected by HCV-related lymphoproliferative disorders: interestingly, we could observe the persistence of clonal populations even in patients that obtained a sustained viral response. To our best knowledge, this is the first report of a flow cytometry-based study of the impact of DAAs on normal and pathologic peripheral blood lymphocytes in HCV patients. We propose flow cytometry as a useful laboratory method for monitoring the efficacy of antiviral therapy on extrahepatic HCV-related hematologic disorders and conclude that our findings can improve our understanding of lymphomagenesis and HCV immunity.
1. Hermine O, Lefrere F, Bronowicki JP, et al. Regression of splenic lymphoma with villous lymphocytes after treatment of hepatitis C virus infection. N Engl J Med 2002;347:89–94.

P111
PROGRAMMA DI SCREENING NEONATALE DELL'IPOTIROIDISMO CONGENITO: DEFINIZIONE DEGLI INTERVALLI DI RIFERIMENTO PER TSH IN DIFFERENTI ETA' DI PRELIEVO

C. Corbetta[1], L. Alberti[1], A. Arenzi[1], E. Manzoni[1], T. Mariani[1], S. De Angelis[2], D. Rotondi[2], A. Olivieri[2]

[1]Lab. di Riferimento Reg.le per lo Screening Neonatale, Osp. dei Bambini V. Buzzi, ASST Fatebenefratelli Sacco, Milano
[2]Unità Metabolismo e Endocrinologia, Dip. Biologia Cellulare e Neuroscienze, Istituto Superiore di Sanità, Roma

Introduzione: Linee guida internazionali (1) raccomandano la misura della Tireotropina (TSH) come test di screening neonatale (2-4 gg di vita) a maggiore sensibilità per individuare i soggetti a rischio di Ipotiroidismo Congenito (IC). Le linee guida raccomandano anche un secondo prelievo all'età di 2 settimane in categorie di neonati a rischio: pretermine (<37 settimane), gemelli, ricoverati in Terapia Intensiva Neonatale, neonati con primo prelievo ad età <48 ore. Non esistono attualmente specifiche indicazioni circa la soglia decisionale del TSH (cutoff) da applicare a 2 settimane di età, né sono disponibili gli intervalli di riferimento in questa finestra temporale di prelievo. Scopo di questo studio è la definizione degli intervalli di riferimento per TSH all'età di 2-4 e 14-16 gg di vita, in neonati non affetti da IC.
Metodi: Sono stati esaminati i campioni ematici (sangue capillare assorbito e disidratato su speciale carta da filtro) di neonati a termine (>37 settimane), nati e residenti in Lombardia nel 2015. La misura del TSH è stata eseguita con metodo fluoroimmunometrico a tempo risolto (TR-FIA) su piattaforma analitica GSP Perkin Elmer® in 70962 campioni prelevati a 2-4 gg e in 8225 campioni prelevati a 14-16gg. Il calcolo dei percentili per TSH (P2.5, P50, P97.5, P99, P99.5) è stata eseguita con software STATA 11.0®.
Risultati: Non si evidenziano differenze significative per il P2.5 di TSH misurato a 2-4 gg (0.59 mU/L; 95% CI: 0.59-0.60) e a 14-16gg di vita (0.58 mU/L; 95% CI: 0.56-0.59), mentre il P50 è risultato significativamente più ridotto a 14-16gg (1.52 mU/L; 95% CI: 1.51-1.54) rispetto al valore misurato a 2-4gg (2.06 mU/L; 95% CI: 2.05-2.07); analoga differenza si osserva per il P97.5 (4.04 mU/L; 95% CI: 3.91-4.21 vs 6.24 mU/L; 95% CI: 6.19-6.29), P99 (5.10 mU/L; 95% CI: 4.80-5.64 vs 7.60 mU/L; 95% CI:7.49-7.73), e P99.5 (6.64 mU/L; 95% CI: 6.11-7.55 vs 8.80 mU/L; 95% CI: 8.65-9.00).
Conclusioni: I limiti di riferimento ottenuti in questo studio dimostrano che i livelli ematici di TSH subiscono modifiche significative nelle prime 2 settimane di vita. Tali risultati hanno importanti ricadute per la corretta selezione dei valori di cutoff per TSH, da differenziare in funzione delle finestre temporali di prelievo (2-4 e 14-16 gg).
1. Léger J, Olivieri A, Donaldson M, et al. J Clin Endocrinol Metab 2014;99:363-84.

P112
PHARMACOGENETICS OF POSACONAZOLE: EFFECT OF SINGLE NUCLEOTIDE POLYMORPHISMS ON PLASMA EXPOSURE

S. Allegra, G. Fatiguso, A. Arduino, F. Favata, J. Cusato, F.G. De Rosa, G. Di Perri, A. D'Avolio

Lab. di Farmacologia Clinica e Farmacogenetrica, Osp. Amedeo Di Savoia, Università di Torino, Dip. Scienze Mediche

Aim: Posaconazole is a triazole antifungal agent with a broad spectrum of activity. Both in antifungal treatment and prophylaxis, drug blood trough levels are associated with clinical outcome; moreover, a significant pharmacokinetic variability has been reported. The aim of this work was to evaluate posaconazole plasma exposure according to single nucleotide polymorphisms (SNPs) in genes involved in drugs metabolism and elimination.
Material/methods: Adult patients treated with posaconazole (oral administration) were enrolled. Allelic discrimination for MDR1 3435 C>T (rs1045642), 1236 C>T (rs1128503), 2677 G>T (rs2032582) and 1199 G>A (rs2229109), OATP1B1 521 T>C (rs4149056), PXR 63396 C>T (rs2472677), BSEP rs228762 T>C, MRP2 1249 G<A (rs2273697), BCRP1 rs13120400 T>C, MRP2 24 G>A (rs717620), OAT1 453 G>A (rs4149170) and SLCO3A1 rs8027174 T>G SNPs was performed by real-time PCR. Trough plasma concentrations (Ctrough) were measured using HPLC-MS validated methods. Linear regression analysis was performed including age, gender, Body Mass Index (BMI), ethnicity and genetic factors.
Results: Seventy-two patients were enrolled. 35 (58.6%) were males and 69 (95.8%) Caucasians. Median age was 52 years old (interval of confidence at 95%, IC 95%, 47.52-52.82 years old) and median BMI was 23.94 Kg/m^2 (IC95% 23.14-25.0 ^{2}). Median posaconazole Ctrough was 1357.50 ng/mL (IC 95%: 1496.14-2490.88 ng/mL).
We found a significant association between MDR1 2677 TT genotype and posaconazole Ctrough (p=0.009): patients with GG/GT genotypes had drug concentrations lower (median: 1215.50 ng/mL; IC95% 1078.41-2080.05 ng/mL) than TT carriers (median: 2729.50 ng/mL; IC95% 1890.07-2901.50 ng/mL). This genotype was retained in multivariate liner regression analysis as drug Ctrough positive predictive factor (p=0.002; ß=0.357, IC95% 620.105-2732.608).
Conclusions: Pharmacogenomics and therapeutic drug monitoring could contribute to enhance the antifungal therapy optimization on the basis of inter-individual genetic variability. This study suggests the usefulness of genetic-based posaconazole therapy and highlights the needed of therapy personalization. However, further studies are required to confirm this data and to clarify the role of the evaluated SNPs in drug pharmacokinetics

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INVOLVEMENT OF ABCB1 AND ABCB11 GENE POLYMORPHISMS IN THE PREDICTION OF NEW ANTI HEPATITIS C VIRUS DRUG PLASMA CONCENTRATIONS

J. Cusato, A. De Nicolo', L. Boglione, A. Ariaudo, F. Favata, S. Mornese Pinna, C. Carcieri, G. Cariti, G. Di Perri, A. D'Avolio

Unit of Infectious Diseases, University of Turin, Department of Medical Sciences, Amedeo di Savoia Hospital, Turin

P- glycoprotein (P-gp, ABCB1 gene) is an efflux pump which transports many types of molecules, including drugs, across cell membranes. It is extensively expressed in human cells of different organs like liver, kidney, central nervous system, small intestine and lymphoid tissues. The bile salt export pump (BSEP, ABCB11 gene), also known as “sister of P-gp”, is an ATP-binding cassette transporter predominantly expressed in liver on the apical membrane of the hepatocytes and mediates the biliary excretion of numerous conjugated bile salts into the bile canaliculus. Since all the new anti hepatitis C virus drugs actually in use (simeprevir, dacalatasvir, sofosbuvir, dasabuvir, ledipasvir, ombitasvir, paritaprevir) are substrates of P-gp (Esposito et al., 2015), we aimed to analyze single nucleotide polymorphisms (SNPs) in ABCB1 gene, relating them to drugs plasma levels at different timings (1/3 days, 1/2 weeks and 1/2/3/4 months of therapy).
Moreover, we evaluated if ABCB11 gene could have a role in predicting drug exposure, since its connection with ABCB1 gene.
We enrolled 118 patients (13 ombitasvir/paritaprevir, 11 dasabuvir, 22 daclatasvir, 49 sofosbuvir, 23 simeprevir) within our "Kineti-C" clinical multicentric study. Allelic discrimination has been performed through real-time PCR. Drug plasma concentrations were analyzed with a validated HUPLC-MS/MS method.
We observed that ABCB1 3435 CT/TT genotypes were associated with sofosbuvir metabolite plasma levels at 1 month of therapy ($p < 0.001$); ABCB1 1236 CT/TT with sofosbuvir metabolite plasma levels at 1 month ($p = 0.028$) and daclatasvir at 2 weeks ($p = 0.012$), whereas ABCB1 1236 TT genotype was related to simeprevir plasma exposure at 1 day ($p = 0.042$).
ABCB11 1131 CC genotype influenced simeprevir plasma concentrations at 3 days ($p = 0.011$), 1 week ($p = 0.003$) and at 3 months ($p = 0.030$) of treatment and daclatasvir levels at 1 week ($p < 0.001$) and at 1 month ($p = 0.020$).
No associations were suggested for ABCB1 2677 G>T SNP.
This is a preliminary analysis focusing on new anti hepatitis C virus drugs pharmacogenetics, showing that ABCB1 SNPs could be used as markers of prediction of drugs plasma levels and that ABCB11 gene SNPs could have a role in this treatment, suggesting a possible involvement of BSEP in its biliary transport and elimination.

P114

ROLE OF CYP2D6 POLYMORPHISMS IN THE OUTCOME OF POSTOPERATIVE PAIN TREATMENT

S.A. Santini[1], P. Latina[2], M. Savino[2], A.P. Gallo[2], U. Basile[1], D. Seripa[2]

[1]Area Medicina di Laboratorio, Fondazione Policlinico Gemelli, Roma
[2]Unità di Ricerca Geriatria e Gerontologia, IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo (FG)

Objective: To investigate the role of CYP2D6 phenotype in the outcome of postoperative (PO) pain (POP) treatment.
Design: Longitudinal cohort study. Open-label trial with post hoc analysis.
Setting: General Hospital Surgery and Recovery Units.
Patients: Ninety unrelated Caucasians submitted to abdominal/thoracic surgery.
Interventions: Standard multimodal POP treatment including opioids (tramadol) and nonsteroidal anti-inflammatory drugs (ketoprofen) at different dosages and infusion rates according to the predicted mild, moderate, or severe POP.
Outcome Measures: Pain (Numeric Rating Scale—NRS) and sedation (Ramsay Sedation Scale—RSS) up to 24 hours after surgery. By genotyping 16 CYP2D6 alleles, the four CYP2D6 phenotypes poor metabolizer (PM), intermediate metabolizers (IM), extensive metabolizers (EM) and ultrarapid metabolizers (UM) were predicted.
Results: As compared with the CYP2D6-EM phenotype, in the early PO time (30 min) a higher RSS mean score in IM was observed ($P = 0.035$). A suggestion towards higher mean score in PM ($P = 0.091$) and a minor mean score in UM ($P = 0.091$) was also detected. No difference in the outcome of pain across the CYP2D6 phenotypes was observed.
Conclusions: In respect to the normal CYP2D6 phenotype, our results suggested that slowly metabolizers (IMs and PMs) might have a major sedation, whereas more rapid metabolizers (UM) a minor sedation, in the early time after surgery. A minor role of CYP2D6 phenotype in PO analgesia may be suggested

P115

A UHPLC MS/MS METHOD COUPLED WITH AUTOMATED ON-LINE SPE FOR QUANTIFICATION OF TACROLIMUS AND EVEROLIMUS IN PERIPHERAL BLOOD MONONUCLEAR CELLS: APPLICATION ON SAMPLES FROM CO-TREATED PEDIATRIC PATIENTS

D. Pensi[1], A. De Nicolò[1], M. Pinon[2], C. Pisciotta[1], P.L. Calvo[2], A. Nonnato[4], R. Romagnoli[3], F. Tandoi[3], G. Di Perri[1], A. D'Avolio[1]

[1]Unit of Infectious Diseases, University of Turin, Dep. of Medical Sciences, Amedeo di Savoia Hospital, Turin

[2]Unit of Pediatric Gastroenterology and Hepatology, University of Turin, "Città della Salute e della Scienza" Hospital, Turin

[3]Liver Transplantation Center and General Surgery 2U, "Città della Salute e della Scienza" Hospital, University of Turin, Turin

[4]Clinical Biochemistry Unit, Dept. Diagnostic Laboratory, A.O.U. "Città della Salute e della Scienza" Hospital, Turin

Background: Tacrolimus and everolimus are immunosuppressors used to treat pediatric patients undergoing liver transplantation. Their hematic TDM by liquid chromatography became standard practice. However, it does not always reflect concentrations at their active site. Our aim was to develop and validate a new method for the simultaneous tacrolimus and everolimus quantification into target cells: Peripheral-Blood-Mononuclear-Cells (PBMCs) (Ghareeb and Akhlaghi, "Alternative matrices for therapeutic drug monitoring of immunosuppressive agents using LC–MS/MS", 2015).
Methods: PBMCs were collected using Cell-Preparation-Tubes; cells number and MCV were evaluated by an automatic cell counter. Tacrolimus and everolimus were quantified using UHPLC-MS/MS coupled with an automated on-line SPE platform. Chromatographic run was performed on an Acquity UPLC® BEH C18 1,7 µm (2,1x50 mm) column at 45°C, for 6 minutes at 0.5 mL/min. Mobile phases were water and methanol, both with 2 mM ammonium acetate and 1 mL/L formic acid). XBridge® C8 10µm (1x10 mm) SPE cartridges were used and the internal standard was ascomycin.
Results: Following FDA guidelines, method validation resulted in high sensitivity and specificity. Calibration curves were linear (r2=0.998) and intra- and inter-day imprecision and inaccuracy were <15%. A contained and stable matrix effect was observed, with a good recovery for all compounds. Drug amounts in 15 "real" PBMCs samples from 5 pediatric patients in co-treatment resulted within the calibration range (0.039-5ng). Concentrations from each patient were standardized using their evaluated MCV: intra-PBMCs concentration was meanly 19.23 and 218.61 times higher than the hematic one for TAC and EVE, respectively.
Conclusion: This method might be useful in clinical routine, giving more reliable data on drugs concentration at the active site.

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DEVELOPMENT AND VALIDATION OF UHPLC-MS/MS METHODS FOR THE QUANTIFICATION OF COLISTIN IN PLASMA AND DRIED PLASMA SPOTS

G. Cangemi[1], B. Sebastiano[1], E. Castagnola[1], G. Tripodi[1], F. Favata[1], A. D'Avolio[1]

[1]Clinical Pathology Laboratory Unit , Istituto Giannina Gaslini, Genoa

[2]Infectious Disease Unit, Istituto Giannina Gaslini, Genoa

[3]Department of Medical Sciences, University of Turin, Laboratory of Clinical Pharmacology and Pharmacogenetics, Amedeo di Savoia Hospital, Turin

In the last 15 years, the emergence of multi-drug resistant gram negative bacilli and the few novelties in the development of antibiotic drugs have led to return and increasingly worldwide the use of colistin. The bactericidal effect of colistin is concentration-dependent and it shows a post-antibiotic effect against many bugs. Therapeutic drug monitoring (TDM) of colistin could be useful in clinical practice to improve outcome especially in especially in special patients' population. Nevertheless, TDM of colistin is still limited probably for the low number of laboratories which perform this analysis and for high shipment costs. A valid alternative is dried plasma spots (DPS). DPS technique allows to collect and to store samples in simple envelope bags that can be shipped as a mail. We developed and validated new UHPLC-MS/MS methods to quantify colistin in plasma and in dried plasma spots (DPS) collected on dried sample spots devices (DSSD). Mass spectrometric detection was performed using a Thermo Scientific TSQ Quantum Access MAX triple quadrupole system. Colistin A, Colistin B and polimixin B, used as internal standard, were detected using multiple reaction monitoring (MRM) of the following transitions: 585.5 —> 534.9; 576, 578.5—>527.9; 568.9 and 602.5 —> 100.9, 551.9, 592.8, respectively. Colistin A and B were extracted from plasma using protein precipitation and from DSSD using an extraction basic solution. Methods were validated following international guidelines, and the mean intra and inter-day accuracies and precisions were all 85-115% and < 15% respectively. The linear regression fit for the calibration curves was achieved with R2 >0.9985 in the range 0.1-26 and 0.2-54 µg/mL for colistin A and B respectively. Colistin in DPS was found to be stable for 30 days at 20-25°C. A statistically significant linear correlation was found between colistin extracted from plasma and from DPS [r2 0.9864 (P <0.0001, 95% CI 0,9699 to 0,9939) for colistin A and 0.9695 (P<0.0001, 95% CI 0,9310 to 0,9866) for colistin B, respectively]. DPS represents a safe and cheap strategy to store and ship plasma samples. Thus, it is suited for pharmacokinetic studies and therapeutic drug monitoring of colistin.

P118

STUDIO RETROSPETTIVO SULLE INDAGINI DI LABORATORIO PER L'ASSOLVIMENTO DEGLI OBBLIGHI DI LEGGE DERIVANTI DALL'APPLICAZIONE DEL CODICE DELLA STRADA Art.186/187

P. Bucchioni, P. Franceschini, G. Petricciani, I.M. Sbarbaro

Lab. Tossicologia Levante Ligure Osp. San Bartolomeo, Sarzana

Scopi e obbiettivi: Dal 2011 il nostro Servizio ha sottoscritto, condiviso e reso operativo con la Prefettura di La Spezia un "Protocollo tossicologico-codice stradale" con la finalità di disciplinare e razionalizzare la materia in oggetto, in piena osservanza della normativa vigente. Ciò ci ha permesso di seguire tutte le procedure previste: dall'attivazione della "catena di custodia" fino alla conferma dei risultati con indagini di "secondo livello" in spettrometria di massa, allo scopo di produrre un referto finale conforme e sostenibile in sede amministrativa e/ o giudiziaria. Ed è proprio questo il punto più critico e al contempo più qualificante: superare il valore indiziario degli esami di screening (I livello) su urine, per raggiungere il valore probatorio delle conferme (II livello) su sangue.
Materiali e metodi: Le urine sono state sottoposte ad un test di screening (metodica CEDIA) ed a un test di conferma in LC-MS/MS
Il sangue è stato direttamente analizzato con metodologia di conferma LC-MS/MS per le droghe, mentre per l'etanolo è stato eseguito un test immunochimico e successivamente la conferma in GC-MS "spazio di testa".
Strumentazione utilizzata:
• ILAB650 (per test di screening etanolo su plasma e droghe su urine)
• Agilent GC-MS + campionatore Gerstel MPS2 per spazio di testa (per conferma etanolo su sangue intero)
• 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).
Pacifici R, Gori P, Martucci L, et al. Considerazioni sulle matrici biologiche idonee alla valutazione dell'"attualità d'uso di sostanze illecite" ai fini degli articoli 186 e 187 del nuovo Codice della Strada. Biochim Clin 2014;38:27-31.

P119

ECHINOCANDINS PLASMA CONCENTRATIONS IN IMMUNOCOMPROMISED PAEDIATRIC PATIENTS

S. De Gregori[1], M. Ventura [1], N. Decembrino[2], M. Zecca[2], M. Molinaro[1]

[1]Farmacocinetica Clinica e Sperimentale, Fondazione IRCCS Policlinico San Matteo, Pavia
[2]Oncoematologia Pediatrica, Fondazione IRCCS Policlinico S. Matteo, Pavia

Invasive fungal disease (IFD) causes significant morbidity and mortality in immunocompromised children with hematologic cancer or undergoing hematopoietic stem cell transplantation (HSCT). Antifungal prophylaxis is recommended for these patients so, routinely, four broad classes of systemic antifungal agents are employed: polyenes, triazoles, pyrimidine analogues and echinocandins (anidulafungin-ANF, caspofungin-CASPO and micafungin-MICA). Working by a distinct cell wall-specific mechanism of action, the echinocandins are characterized by excellent antifungal activity against Candida spp. and Aspergillus spp., low toxicity, few or negligible drug-drug interactions and pharmacokinetic independence of renal (and mostly hepatic) function. Therefore the echinocandins rapidly became established in guidelines and clinical practice as primary treatment options for prophylaxis and in management of invasive fungal infection. Best practices recommend low doses daily administered: loading dose 3 mg/kg and 1.5 mg/ kg/day for AN; loading dose 70 mg/m2 and 50 mg/ m2/day for CASPO; 1 mg/kg/day for MICA. Between February 2015 and March 2016, we analyzed plasma samples from 160 unscheduled withdrawals (104 MICA; 14 ANF; 42 CASPO) covering the time range 0-24h after infusion, from pediatric cancer patients, in order to support the physicians clinical practice and minimize the number of samples obtained from each child. The administered dose, trough and max concentrations (when available) were in accordance with data from literature for ANF (1 patient only) and CASPO (6 patients. Concentration range: Ctrough 1.261-5.439 µg/mL, CMAX 3.656-22.882 µg/mL). MICA dosing range was 0.71-2.8 mg/kg/day, Ctrough and CMAX were within 0.588-40.504 and 3.656-22.882 µg/mL, respectively. Analysis were performed by a validated, simple, selective, sensitive and fast (protein precipitation) HPLC-MS/MS analytical method for simultaneous detection of echinocandins in small volumes of plasma (100 µL), as required for pediatric population. During the next months we would like to investigate the potential of alternate day prophylactic administration, performing a PK study of micafungin (3 mg/kg), in young children undergoing HSCT.
Bochennek K, Balan A, Müller-Scholden L, et al. J Antimicrob Chemother 2015;70:1527-30.

P120

DEVELOPMENT AND VALIDATION OF ELISA FOR ANTI-THYMOCYTE GLOBULIN DETERMINATION AND PHARMACOKINETIC APPLICATION ON PATIENTS AFFECTED BY THALASSEMIA MAJOR RECEIVING HEMATOPOIETIC STEM CELL TRANSPLANTATION

M. Montagna[1], M.E. Bernardo[4], M.A. Avanzini[2], M. Regazzi[1], F. Locatelli[3]

[1]Laboratorio di Farmacocinetica Clinica e Sperimentale, IRCCS Fondazione Policlinico San Matteo, Pavia
[2]Laboratorio di Immunologia e Trapianti, IRCCS Fondazione Policlinico San Matteo, Pavia
[3]Dip. Onco–ematologia Pediatrica e Medicina Trasfusionale, Ospedale Pediatrico Bambino Gesù, Roma
[4]Unità Operativa di Immunoematologia Pediatrica, Ospedale San Raffaele, Milano

The use of anti-thymocyte (ATG) modulate the alloreactivity of T lymphocytes, reducing the risk of immunological complications post-transplantation, in particular rejection and GVHD. A new ELISA was developed and validated, to assay serum levels of ATG and evaluate the pharmacokinetics (PK) in paediatric population with ß-Thalassemia, receiving HCST. (Seidel, 2005). Diluted serum samples were allowed to react with Goat anti-Rabbit IgG antibody coated on a microtiter plate and with Goat anti-Human IgG labelled with horseradish peroxidase. After incubation and washings, substrate solution was added and absorbance was read at 492 nm. ATG concentrations in samples were determined by interpolation from a standard curve (range: 200-0.095 ng/ mL) prepared diluting known amount of ATG into PBS. Low, medium and high quality controls concentrations were 1.56, 6.25 and 25 ng/mL, respectively. Serum samples unknown were diluted 1/5000. This method is highly sensitive accurate and precise, with all parameters within the acceptance criteria in compliance with the Guidance for Industry guidelines: the sensitivity of method was 0.095 ng/mL. QCs were analysed with each analytical run and showed a coefficient of variation for precision and accuracy of <15%. We analyzed serum samples of 14 patients ß-Thalassemia who received ATG Fresenius at dose of 10 mg/kg administered as an intravenous (IV) infusion on day -5, -4 and -3 before day of HSCT (day 0). Blood sampling for PK evaluation was performed: on day -5, -4 and -3 before and after IV infusion; and then on day-2, -1, 0, +3, +5, +7, +14, +21, +28, +42 and +56. The median ATG levels pre and post IV were: 0 µg/mL and 118 µg/mL on day -5; 85.9 µg/mL and 199.2 µg/mL on day -4; 153 µg/mL and 270.9 µg/mL on day -3, respectively. The median PK values of CL was 0.0756 L/days (range 0.0297-0.1881 L/days), V_d was 1.46 L (range 0.48-6.74 L) AUC median 10334 µg*days / mL (range 10174-535 µg*days /mL) and $T_{1/2}$ was 20.2 days (range 5.8-50.23 days). Given the marked inter-individual variability of ATG disposition, the development of a validated ELISA and therefore the possibility to evaluate the main pharmacokinetic parameters in paediatric patients affected by ß-Thalassemia receiving HCST, is an essential step to optimize the therapeutic regimens.

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THE RELEVANCE OF LEVAMISOLE IN ILLECIT TRAFFICKING COCAINE SEIZED: A ONE YEAR STUDY

S. Pignalosa[1], S. Martello[2], M. Pieri[1], C. Ialongo[1], G. Noce[2], F. Vernich[2], C. Russo[1], F. Mineo[2], S. Bernardini[1], L.T. Marsella[2]

[1]Department of Experimental Medicine and Surgery, "Tor Vergata" University Hospital, Rome
[2]Department of Biomedicine and Prevention, "Tor Vergata" University Hospital, Rome

Trial design: Cocaine use is increasing around the world and the drug purity is frequently altered through dilution, substitution, contamination and adulteration. Usually, cocaine is adulterated with local anesthetics, phenacetin, sugars, talc, flour or boric acid as well as pharmacologically active adulterants. Levamisole is used as cocaine adulterant because is an odorless powder with physical properties similar to cocaine, as well as it has a reasonable cost and availability being widely used in veterinary. Our aim is to show the prevalence of main adulterants present in seized cocaine samples in order to evaluate their relevance with respect to cocaine purity. Particularly, our investigations focuses on levamisole due to its recognized human toxicity.
Methods: Seized material was assayed underivatized through GC-FID and GC-MS in order to assess the purity level and to quantify the amount of drug, using an internal standard, and to investigate the presence and the nature of some contaminants.
Results: We analyzed 324 illicit samples, including 88 related to cocaine, associated with 235 seizures. The cocaine analyzed showed an average purity of 55% and the most of samples were laced with adulterants. Adulterants we identified were: levamisole (31,8%), caffeine (6,8%), lidocaine (2,3%), acetaminophen (2,3%) and phenacetin (1,1%).
Conclusions: Levamisole might have an enhancing effect on cocaine's euphoria because it is metabolized to aminorex, producing a synergic effect with that of cocaine itself. The chronic use of levamisole-adulterated cocaine induces several side effects including dermal vasculopathy, leukoencephalopathy, leukopenia, agranulocytosis, pulmonary haemorrhage, multiple emboli and several other effects. Moreover, aminorex causes idiopathic pulmonary hypertension, so that this could be another harmful and mostly lethal side effect due to cocaine cut with levamisole. In conclusion, levamisole and its metabolite aminorex determination should be performed in routine toxicological analysis in the cocaine abusing population and in deaths due to cocaine use.
Cole C, Jones L, McVeigh J, et al. Adulterants in illicit drugs: a review of empirical evidence. Drug Test Anal 2011;3:89-96.

P122

CLINICAL APPLICATION OF A FAST AND SENSITIVE HPLC METHOD WITH FLUORIMETRIC DETECTION TO MEASURE PLASMA LEVELS OF VITAMIN A IN PRETERM INFANTS

A.N. Bartoli[1], M. Broglia[1], F. Garofoli[2], I. Mazzucchelli[3], P. Villani[1], L. Decembrino[2]

[1]Unit of Clinical and Experimental Pharmacokinetics, IRCCS Foundation Policlinico San Matteo, Pavia

[2]Neonatal Unit and Neonatal Intensive Care Unit, IRCCS Foundation Policlinico San Matteo, Pavia

[3]Department of Internal Medicine and Therapeutics, University of Pavia

Background: Vitamin A (retinol) is one of the most important micronutrients affecting the health of children. In the developing world, vitamin A supplementation programmes significantly reduce infant mortality as well as the incidence of xerophthalmia, respiratory infection and morbidity from gastrointestinal disease. Term infants are well supplied with vitamin A in utero, at the expense of maternal stores, and human milk provides adequate amounts of vitamin A in the first six months. Preterm infants, particularly those of low birth weight, (≤1500 g), have low plasma concentrations of both retinol and retinol binding protein at birth compared with term infants because vitamin A is transferred across the placenta mainly during the third trimester. Newborn preterms with plasma levels <200 ng/mL could be at risk for chronic respiratory problems and retinopathy. We have developed and validated a sensitive HPLC assay with fluorimetric detection (excitation and emission wavelengths: 325 nm and 470 nm respectively) that requires a small plasma volume (100 μL) and can be a valuable tool for measuring low concentrations of retinol in preterm infants. Methods: Vitamin A plasma concentrations were measured in 41 preterm infants receiving oral supplementation of 3000 IU of vitamin A from day 2~4, when enteral feeds were tolerated. Sample preparation was performed in one step and involved precipitation of protein and extraction of lipid with ethanol–chloroform mixture (3:1). Validation of analytical method was performed according to the EMA Guideline (July, 2011). Results: A linear correlation was found in the range from 75 to 1200 ng/mL. The non zero standards were within ± 15% of their nominal value. The intra-interday accuracy of the assay was within 85%-115% for each group of samples (QC, LLOQ), while precision data showed a coefficient of variation ≤ 10.2 % for both intra- inter-day runs. Retinol mean plasma concentration at birth, on days 14 and 28 were 175.95 ± 73.37; 175.75 ± 86.84, 155.42 ± 69.56 ng/mL respectively. Conclusions: We developed a very simple and rapid method for measuring low concentrations of vitamin A in preterm infants with sensitivity, precision and accuracy.

Taibi G, Nicotra CM, et al. J Chromatogr B Analyt Technol Biomed Life Sci 2002;780:261-7.

P123

DEVELOPMENT OF AN UHPLC-MS/MS METHOD FOR THE THERAPEUTIC DRUG MONITORING OF ANTIHYPERTENSIVE DRUGS IN HUMAN PLASMA: VALIDATION ON PATIENTS WITH "RESISTANT" HYPERTENSION

V. Avataneo[1], A. De Nicolò[1], G. Bonifacio[1], F. Rabbia[2], E. Perlo[2], E. Berra[2], C. Fulcheri[2], P. Mulatero[2], F. Veglio[2], G. Di Perri[1], A. D'Avolio[1]

[1]Unit of Infectious Diseases, University of Turin, Department of Medical Sciences, Amedeo di Savoia Hospital, Turin

[2]Division of Internal Medicine and Hypertension Unit, University of Turin, Department of Medical Sciences, AOU "Città della Salute e della Scienza", Turin

Aim: Managing resistant hypertension (RH) is becoming increasingly difficult and one of the main issues is to discriminate "true" RH patients from cases of poor medication adherence, as called pseudoresistant hypertension. A useful tool could be the Therapeutic Drug Monitoring (TDM) of antihypertensive drugs in plasma samples. It has been hence validated an UHPLC-MS/MS method for simultaneous TDM of ten currently used antihypertensive drugs: amlodipine, atenolol, clonidine, chlortalidone, doxazosin, hydrochlorothiazide, nifedipine, olmesartan, ramipril and telmisartan.

Methods: The proposed method has been validated according to FDA guidelines. Samples were prepared as below: 200 µL of plasma sample, standard or quality control were added with 40 µL of internal standard (IS, 6,7-dimethyl-2,3-di(2-pyridyl)quinoxaline) in amber PTFE tubes and underwent a protein precipitation protocol with 1 ml of acetonitrile. Samples were then vortex-mixed for 10 sec and centrifuged at 21000 x g at 4 °C for 10 min. After a drying step (50 °C for about 1,5 hs), extracts were resuspended in 200 µL of water:acetonitrile 90:10 (v:v) and then analyzed through a Shimadzu Nexera X2® UHPLC system coupled with a LCMS-8050® tandem mass detector. The validated method was tested on real samples from patients with RH/pseudo-RH, enrolled in the SEAL study (protocol CS/504 03/09/2015), all giving informed consent.

Results: Accuracy, intra-day and inter-day precision, recovery and matrix effect fitted FDA guidelines for all analytes. LOD and LLOQ successfully fitted literature-reported expected concentrations. 36 patients have been enrolled. On the basis of preliminary data, 28% of patients resulted partially non-adherent and 17% were totally non-adherent. Continuing the TDM of those patients, after a period of tight control, two of them became adherent.

Conclusions: The simple and cheap extraction procedure makes this method eligible for a clinical routine use. From the clinical point of view we obtained encouraging results: we managed to discriminate some cases of poor adherence to the therapy preserving those patients from an invasive and expensive therapeutic approach, promoting adherence.

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ACCERTAMENTI TOSSICOLOGICI RICHIESTI DALLE COMMISSIONI MEDICO LOCALI: LA NOSTRA ESPERIENZA E LE CRITICITA' RILEVATE

S. Martello[2], G. Noce[2], S. Pignalosa[1], C. Russo[1], M. Pieri[1], S. Bernardini[1], L.T. Marsella[2]

[1]Dipartimento di Medicina Sperimentale e Chirurgia, Ospedale Universitario "Tor Vergata", Roma
[2]Dipartimento di Biomedicina e Prevenzione, Ospedale Universitario "Tor Vergata", Roma

L'art. 119 del Codice della Strada (C.d.S.) definisce i "Requisiti fisici e psichici per il conseguimento della patente di guida", mentre l'art. 320 del Regolamento attuativo del C.d.S., modificato dall'art.223 del D.L.vo 59/2011, prevede l'esclusione dell'idoneità alla guida per conducenti che presentino uno stato di dipendenza da alcol e/o sostanze stupefacenti e psicotrope, o anche un abuso o uso abituale di medicinali determinanti disabilità alla guida, come benzodiazepine e barbiturici.
Pertanto, gli accertamenti tossicologici richiesti dalle Commissioni Medico Locali hanno un ruolo fondamentale nell'emissione del certificato di idoneità e devono essere eseguiti da Laboratori di Tossicologia Forense specializzati nel rispetto della privacy dell'utente seguendo una idonea procedura di catena di custodia. Le classi di sostanze che le Commissioni Medico Locali richiedono di ricercare su matrice urinaria e/o cheratinica sono: cocaina, amfetamine, ecstasy, cannabinoidi, oppiacei, metadone, barbiturici, benzodiazepine e alcol; su matrice ematica emocromo e alcol etilico; su siero CDT, gamma-GT, AST e ALT. Da settembre 2015 a giugno 2016 sono stati effettuati accertamenti tossicologici su 708 soggetti sottoposti a revisione o rinnovo della patente dopo la contestazione di condotte di guida in stato di ebrezza e/o sotto effetto di sostanze stupefacenti. Dei 708 soggetti esaminati, un 8% è risultato positivo a sostanze stupefacenti, un 2% a CDT e uno 0,8% sia a sostanze stupefacenti sia a CDT. Gli accertamenti tossicologici che vengono richiesti dalle Commissioni Medico Locali presentano la criticità di non considerare le Nuove Sostanze Psicoattive (NSP), problema ormai molto sentito a livello nazionale nel campo della Tossicologia Forense. E' infatti concreto il rischio che, a seguito di assunzione di tali sostanze, si manifestino effetti non noti e inattesi soprattutto sullo stato di coscienza o su performance psico-fisiche. E' auspicabile che tali sostanze, come ad esempio i cannabinoidi sintetici e le sostanze amfetamino simili, siano prese in considerazione in tali accertamenti.

Favretto D, Pascali JP, Tagliaro F. New challenges and innovation in forensic toxicology: focus on the "New Psychoactive Substances". J Chromatogr A 2013;1287:84-95.

P125
FUNDAMENTAL ROLE OF THERAPEUTIC DRUG MONITORING IN VORICONAZOLE DOSE ADMINISTRATION IN CRITICALLY ILL PATIENTS: A PHARMACOKINETIC DESCRIPTIVE STUDY

G. Fatiguso, S. Allegra, F. Favata, L. Baietto, G. Di Perri, A. D'Avolio

Department of Medical Sciences, Unit of Infectious Diseases, University of Turin, Amedeo di Savoia Hospital, Turin

Background: invasive fungal infections are a serious problem in critically ill immunosupressed patients. Different classes of antifungal drugs are used either prophylactically or for targeted therapy, offering a broader coverage. In the last decades new antifungal agents have been approved, but azole compounds remain a primary necessity. Voriconazole belongs to this class and it inhibits 14a-demethylase, a key enzyme in the ergosterol biosynthesis of yeasts and molds. Different studies described a substantial intra- and interindividual variation in pharmacokinetics: therapeutic antifungal monitoring could be an important tool in clinical practice because it integrates pharmacokinetic and pharmacodynamic knowledge considering each patient individually.
Methods and results: a fully validated chromatographic method was used to quantify voriconazole trough concentrations in plasma samples (1). 386 patients were enrolled: they were 278 men (72%), only 43 (11.1%) followed a prophylaxis strategy and 13 (3.4 %) were characterized by sepsis outcome. Median age was 56 years [95% CI: 53.52-56.59], while median body mass index (BMI)and voriconazole trough plasma concentration were 23.31 Kg/m^2 [95% CI: 22.82-23.80] and 2141.00 ng/mL [95% CI: 2656.49-3199.88], respectively.
Associations between variables were tested using Pearson test and, concerning pharmacokinetic parameters, an higher inter-individual variability was shown. A positive and significant correlation (r=0,063; p=0,000) was found between voriconazole trough concentrations and patients age.
Conclusions: these results represent a preliminary study; in further analyses we aim to increment our cohort sample size. Evidence of a possible role for therapeutic drug monitoring of voriconazole first emerged in 2005 and has since continually grown up; this important approach results very useful to optimize antifungal therapy and to reduce patients hospitalization costs.

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DEVELOPMENT AND VALIDATION OF AN UHPLC-MS/MS METHOD FOR THE INTRACELLULAR QUANTIFICATION OF NEW ANTI-HCV DRUGS AND EVALUATION OF THE INFLUENCE OF GENETIC POLIMORPHISMS

A. Ariaudo[1], F. Favata[1], M. Simiele[2], E. Gontero[1], J. Cusato[1], A. De Nicolò[1], G. Di Perri[1], A. D'Avolio[1]

[1]Laboratory of Clinical Pharmacology and Pharmacogenetics, Department of Medical Sciences, University of Turin, Amedeo di Savoia Hospital, Turin
[2]Coqualab, Accademic spin-off of Turin University

Background: In the last few years a lot of new drugs sich as sofosbuvir, simeprevir, daclatasvir and ledipasvir and the new formulation Viekirax (paritaprevir, ombitasvir (OMB) and Ritonavir) together with dasabuvir have been produced and approved for the treatment of HCV. Few informations about new DAA's plasma concentrations in real patients and possibly correlation with therapeutic response and toxicity are available (Ref). Since these drugs explicate their activity inside cells, it could be useful to evaluate their concentration in the intracellular compartment. PBMC may represent a valid and easier available surrogate to hepatocyte cells.
Aim of the study: Develop and validate a new UPLC-MS-MS method for the intracellular quantification of new HCV drugs and analysis of genetic polymorphisms that may affect drugs concentrations.
Method: A gradient of Ammonium-acetate 5mM ph9,5 and Acetonitrile at flow rate of 0.4 ml/min was used for the chromatographic separation. Detection was carry out with a Triple-quadrupole-tandem mass spectrometric coupled with electrospray ionization (ESI). PMBC samples were treated with acid phosphatase for GS331007 analysis. Calibration curve and quality control were prepared using PBMC from healthy donors spotted with standards. Quinoxalina, DAC-D8, OMB-D6 were used as internal standards.
Results and Discussion: Accuracy and precision inter and intraday were below 15% as required by FDA guidelines. Recovery was above the 50% for all drugs. The correlation coefficient of the curve was always 0.990 and the range of calibration for all drugs covered the expected intracellular concentrations. Each drug was monitored at two different transitions: the first for quantification whereas the second for confirmation. Mean corpuscular volume of each PBMC sample was used to calculate the intracellular concentration of drugs. We analyzed genetic polymorphisms of genes ABCB1, ABCBG2, OCT1, CYP2D6 OATPB1 OATPB3, ABCC2,CYP2C19 that may have an influence on the intracellular concentrations. Data about intracellular concentration and the correlation with genetic polymorphisms could lead to a greater knowledge of HCV drugs pharmacokinetic and pharmacodinamic, helping in the management of hcv therapy.
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P127

THERAPEUTIC DRUG MONITORING OF IMMUNOSUPPRESSANT: USING RISK MANAGEMENT TOOLS TO IMPROVE ANALYTICAL QUALITY

V. Fulgido, A. Bandinelli , A. Casalucci, L. Centola, N. Cini, A. De Stefano, O. Gioia, F. Luceri, P. Pezzati

Lab. Generale, Dip Servizi, AOU Careggi, Firenze

Cyclosporine A, Tacrolimus, Sirolimus and Everolimus are immunosuppressive drugs used mainly in organ transplantation and Therapeutic drug monitoring (TDM) is routinely performed. Liquid Chromatography methods coupled with mass spectrometry (LC-MS) are more specific and sensitive than immunological methods and present the advantage of simultaneous measurements of several drugs. Since 2010 the Laboratorio Generale AOU Careggi (Florence Italy) adopted a LC-MS 4000 Q Trap, (ABSciex Framingham MA USA) for immunosuppressant TDM. Aim of the present work is to analyse this specific testing process according to clinical risk management procedure (1). Failure Mode Errors Analysis (FMEA) steps were used: 1) Process study: we recorded phases and activities. 2) Hazard analysis: we listed activity-related failure modes and their effects; we described control measures; we assigned severity, occurrence and detection scores for each failure mode and we calculated the risk priority index (IPR) by multiplying the 3 scores. We analysed failure modes causes, made recommendations and planned new control measures according to the Plan Do Check Act (PDCA) methodology; 3) Monitoring: two years after the first analysis, we performed a new FMEA to verify numbers and typology of failures and we evaluated Internal Quality Control measure of dispersion, to verify any change in analytical quality. The new FMEA shows that failures with a higher IPR such as "vials wrong positioned on autosampler rack" are still occurring, but, after corrective actions, failures about pre-analytical phase, such as "making a wrong working solution" occurs just episodically and post-analytical phase errors are completely avoided, due to implementation of a middleware. The analytical quality shows a major improvement for every immunosuppressant, over a two years period, as indicated by the decrease of Internal Quality Control CV. The magnitude of the effect varied according to molecule and concentrations; the effect was higher for Tacrolimus (12.7 CV% vs. 7.2 CV%) and lower for Cyclosporine (7.5 CV% vs. 6.3 CV%). Employing project management control measures allows to obtain improvement of analytical quality and to reduce patient's clinical risk.
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P128
PROGETTARE IL LABORATORIO DEL FUTURO: COME RIDURRE I COSTI OPERATIVI (EFFICIENZA) MIGLIORANDO LA QUALITA' (EFFICACIA)

M. Plebani[1], M. Zaninotto[1], D. Faggian[1], L. Sciacovelli[1], R. Rossoni[2], A. Villa[2], E. Magnani[2], M. Ariseanu[2]

[1]Dip. Strutturale Medicina di Laboratorio, Azienda Ospedaliera di Padova
[2]Direzione Lab Consultancy & Project Management, Roche Diagnostics Italia

Rispondere efficacemente alla costante riduzione del budget sanitario mantenendo e incrementando il valore dei servizi offerti al Paziente costituisce una delle più grandi sfide che si trovano a dover affrontare oggigiorno i reparti di Medicina di Laboratorio.
A tale scopo, con il supporto dei servizi di consulenza erogati da Roche Diagnostics, è stato realizzato, presso l'UOC Medicina di Laboratorio dell'Azienda Ospedaliera Universitaria di Padova una consulenza strategica volta all'individuazione di un percorso evolutivo sostenibile che consenta, attraverso l'ottimizzazione dei processi interni, di raggiungere una riduzione dei costi operativi mantenendo al contempo elevati standard di qualità e sicurezza del dato analitico.
Il percorso strutturato di consulenza, attraverso l'applicazione dei principali concetti e strumenti relativi alla metodologia Lean, ha consentito di definire gli obiettivi strategici dell'UOC Medicina di Laboratorio su quattro indicatori di performance: costi, qualità, tempi e persone. Successivamente, la Value Stream Analysis ha permesso di individuare le aree in cui, l'applicazione della cella Lean per il loro miglioramento, avrebbe contribuito sostanzialmente al raggiungimento degli obiettivi strategici.
La realizzazione di tre eventi di miglioramento rapido basati sulla metodologia Lean in tali aree, ha supportato UOC Medicina di Laboratorio nell'ottimizzare e razionalizzare i relativi processi, consentendo di raggiungere, e in alcuni casi superare, gli obiettivi strategici prefissati.
Fra i principali benefici identificati attraverso questa attività di consulenza risulta una riduzione dei costi operativi dell'UOC Medicina di Laboratorio pari al 10%.
Questa esperienza conferma che il percorso strutturato di consulenza strategica basata sulla metodologia Lean, permette di allineare i processi operativi delle specifiche aree agli obiettivi strategici di evoluzione sostenibile, dimostrando quindi di essere un supporto concreto per il decisore sanitario nello sviluppo di una strategia evolutiva che comprenda una gestione ottimale delle risorse a disposizione.

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ANALISI DELLE COMUNICAZIONI DEI VALORI CRITICI DI LABORATORIO DAL 2012 AD OGGI

A. Modenese, M.G. Piovan, A. Rivolta, S. Ricca

Lab. di Biochimica Clinica e Microbiologia, Ist. Clinico Città Studi, Milano

Al fine di garantire uno dei requisiti di sicurezza del paziente, il laboratorio analisi dell'Istituto Clinico Città Studi ha redatto una Procedura per la corretta e tempestiva comunicazione telefonica, ai reparti di degenza, dei valori critici di laboratorio. Le soglie di criticità, definite secondo le indicazioni della SIBIoC (1),sono state condivise con i Medici della Struttura. Dal 2012 ad oggi si opera un controllo crociato fra le comunicazioni effettuate e registrate dal laboratorio e quelle registrate dai reparti. Le comunicazioni vengono tracciate su identiche schede di raccolta dati; dall'incongruenza delle informazioni si rilevano le non conformità.
Risultati: le segnalazioni effettuate e registrate dal laboratorio nel 2012, 2013, 2014, 2015 e 2016 sono state rispettivamente il 95.9%, 98.1%, 99.7%, 98.0% e 97.2% del totale; le comunicazioni registrate nello stesso periodo dai reparti, sono state il 94.6%, 95.6%, 88.1%, 88.8%, 88.9% del totale. Verificando la completezza delle segnalazioni registrate nei 5 anni, per il laboratorio è risultata del 98.9%, 96.1%, 100%, 100% e 100%, mentre per i reparti le comunicazioni registrate in modo completo erano il 94.6%, 85.4%, 93.8%, 97.7% e 100% del totale. L'errore più frequente non riguardava il valore critico, ma l'incompletezza dell'anagrafica del paziente.
Conclusione: i nostri dati dimostrano una buona, ma non ancora completa, aderenza alla Procedura. Le segnalazioni non tracciate dai reparti (circa il 10%), non hanno portato a conseguenze per i pazienti. Ipotizziamo che ciò sia attribuibile alla immediata visibilità in reparto del dato validato, anche se non registrato; inoltre, i valori di panico vengono sempre comunicati dal Laureato del laboratorio direttamente al Medico. Tali considerazioni, possono forse tranquillizzarci anche per i valori che sfuggono alla registrazione sia del laboratorio che del reparto, al momento non quantificabile da sistemi di confronto. Ulteriori studi mirano a valutare se, alla comunicazione, vi è una pronta risposta del Clinico. I primi dati, relativi agli emocromi, evidenziano che, in giornata, segue sempre un esame di approfondimento/ricontrollo e spesso, una richiesta di Emocomponenti.
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LA GESTIONE REMOTA DEGLI EMOGAS NELL'AZIENDA USL DI LIVORNO (AREA NORDOVEST)

F. Antonelli[1], A. Canapicchi[1], I. Curreli[1], A. Bernardini[2], P. Masi[2], U. Paoletti[2], C. Quintavalle[2], E. Agarini[3], S. Casu[3], S. Anselmi[4], M. Chelli[4], D. Michela[4], M. Pennisi[4], G. Turelli[4]

[1]U.O.S. Lab. Medicina d'urgenza, Osp. Cecina, Livorno
[2]U.O.S. Lab. Medicina d'urgenza, Osp. Piombino, Livorno
[3]U.O.S. Lab. Medicina d'urgenza, Osp. Portoferraio, Livorno
[4]U.O.C. Patologia clinica, Osp. Livorno

Nell'Azienda USL di Livorno sono installati 22 emogas GEM 3500 e 4000 Werfen in dotazione a reparti. Nell'ottica delle principali Linee guida di gestione POCT il laboratorioha creato in accordo alla Direzione Sanitaria un gruppo di lavoro di TLSB con la funzione di verificare il corretto funzionamento degli strumenti attraverso la valutazione giornaliera dei CQ (IQM) delle azioni correttive effettuate dallo strumento garantendo la qualità del risultato. Il gruppo di lavoro è organizzato nelle sedi di Livorno, Cecina, Piombino, Portoferraio, in ogni laboratorio è presente una postazione dedicata per il controllo remoto degli strumenti, attraverso un server dove è installato un programma gestionale GEM WEBPlus, questo permette il controllo di tutti gli strumenti, ogni gruppo gestisce i propri ma interagisce se necessario sugli altri. Le attività di verifica sono registrate in un programma di gestione presente nel sito interno di laboratorio, l'attività svolta con le segnalazioni opportune sia per i reparti sia per il coordinatore del gruppo di lavoro sono disponibili on line, nell'ottica delle normative Regionali d'Accreditamento dei reparti Ospedalieri è stato attivato un percorso dedicato nel sito di laboratorio POCT dove con accesso personalizzato i reparti verificano il controllo effettuato sul proprio strumento. L'attività del gruppo di controllo in un anno di gestione giornaliera ha permesso di :a) migliorare l'affidabilità degli strumenti intervenendo con la competenza e professionalità del TSLB; b) migliorare la gestione economica in base agli esami effettati indicando la cartuccia reagente più idonea; c) segnalato il ripetersi di errori preanalitici permettendo di formare il personale utilizzatore sia nella gestione dello strumento che del campione; d) di gestire la formazione del personale per l'introduzione della corretta anagrafica del paz. attraverso l'utilizzo del Lis di Laboratorio garantendo la tracciabilità del paz e l'archiviazione dei dati analitici riducendo notevolmente sui reparti più critici l'anonimato degli esami effettuati; e) di garantire la qualità del risultato grazie all' integrazione del gruppo di controllo remoto che è in grado di intervenire su tutte le zone; f) di attivare il servizio di comunicazione rapida di anomalie tramite l'attivazione della e-mail su strumento.

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RICHIESTA E REFERTAZIONE ESAMI DI LABORATORIO IN PRESIDIO SPECIALISTICO: REVISIONE DEL PROCESSO SECONDO LA "LEAN ORGANIZATION"

E. Battipaglia[1], M.S. Parri[1], A. Baroni [1], R. Lombardi[1], V. Zanetti[1], D. Chicchi[1], E. Giuliani[1], L. D'Errico[1], S. Banti[2], S. Borghetti[2], S. Bertanelli[2], S. Storti[1]

[1]U.O. Medicina di Laboratorio, Fondazione Toscana G. Monasterio Massa
[2]Dipartimento Cardiotoracico, Ospedale del Cuore, Fondazione Toscana G. Monasterio Massa

L'Ospedale del Cuore di Massa è un centro specialistico cardiologico. Il laboratorio riceve campioni prevalentemente dai reparti della struttura, con flusso continuo 12 ore/365 giorni. I risultati sono visibili in cartella clinica elettronica entro un'ora dall'arrivo del campione urgente e due ore per la routine.
Obiettivo: Rivedere secondo la metodologia Lean Thinking il processo dalla richiesta di un esame di laboratorio alla refertazione del risultato, per ridurre le azioni non a valore garantendo la qualità del percorso analitico.
Metodo: Le figure professionali coinvolte (infermiere, biologo, tecnico di laboratorio, operatore socio-sanitario, medico) hanno definito una mappa di flusso a valore (value stream map) del processo attuale, individuandone le fasi; stimato il tempo totale dalla richiesta del medico fino alla refertazione dell'analisi ne è stato poi calcolato il rapporto con il tempo effettivo del processo (indice di flusso). Per l'analisi FMEA e FMECA sono state definite le scale per indicizzare la probabilità, la gravità e la rilevabilità dell'errore con cui è stato calcolato l'indice di priorità di rischio (IPR).
Risultati: L'indice di flusso del processo in essere è 8,38. Dall'analisi FMECA avevano IPR più alto: la fase dell'inserimento della richiesta sul programma di laboratorio (appropriatezza della richiesta), la fase di preparazione del materiale del prelievo, compreso l'etichettatura delle provette e il controllo della corrispondenza fra anagrafica su etichetta e identità del paziente (qualità e sicurezza) e la fase di consegna del campione al laboratorio (tempo di processo).
L'indice di flusso del processo rivisto è sceso a 1,84. Le azioni di miglioramento hanno riguardato soprattutto la riorganizzazione del sistema di trasporto interno e la riduzione delle non conformità, tramite l'acquisizione di una maggiore consapevolezza del personale infermieristico del loro impatto clinico derivata anche da corsi di formazione interni.
Conclusione: Tramite l'applicazione dei principi della Lean Organization è stato possibile individuare chiaramente le fasi del processo e intervenire su quelle considerate maggiormente a rischio di errore e sui tempi non a valore, ottenendo risultati più che soddisfacenti per la riduzione dello spreco.
Lawal AK, Rotter T, Kinsman L, et al. Syst Rev 2014;3:103.

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NEXT GENERATION LABORATORY MEDICINE: RIEQUILIBRARE MENTE, CUORE E PROFESSIONALITÀ

M.R. Cavallo[1], C. Vella[1], V. Granero[1], E. Richetta[1], D. Fortunato[1], R. Sacco[2]

[1]S.C. Lab. Analisi O.R. Pinerolo, ASL TO3
[2]Direzione Sanitaria O.R. Pinerolo, ASL TO3

Introduzione - scopo: L'evoluzione del concetto "Salute" e l'aumento delle patologie croniche richiedono un cambiamento nella medicina di Laboratorio (LAB) e dei suoi professionisti. Quest'ultimi devono essere in grado di analizzare il problema secondo un pensiero salutogenico che permette di promuovere la salute con un processo dinamico incentrato sul paziente.
Il nostro LAB ha sperimentato un percorso di formazione sul campo (FSC) basato sul confronto con altre professionalità ed altre realtà condividendo emozioni, risorse psichiche e professionali, favorendo la resilienza ed il senso di coerenza. In questo "viaggio" fondamentale è stato il supporto dello Psicologo dell'area Promozione Salute.
La FSC si è posta l'obiettivo di condividere criticità e paure al fine di favorire la capacità di rivederle dal punto di vista analitico ricercando le risorse per un nuovo equilibrio professionale, emotivo e culturale.
Materiali e metodi: Sono stati progettati due corsi di FSC:
• "HUB-SPOKE: il laboratorio che cambia"; dal 9/03/2016 al 23/05/2016 con 7 "pionieri" e 1 narratore.
• "I professionisti del laboratorio analisi nel cambiamento"; dal 17/03/2016 al 27/05/2016 con 15 professionisti coinvolti (medici, biologi, tecnici di laboratorio).
Metodologia di lavoro: gruppi di lavoro, seminari con esperti, plenarie, tavole rotonde tra pari.
Strutture coinvolte: S.C. Ricerca e Formazione, S.S. Promozione Salute, S.C. Laboratorio Analisi ASLTO3-ASLTO4-ASLTO5-ASLCN1.
Conclusioni: I percorsi di FSC hanno permesso di guardare ai cambiamenti come "opportunità" per sviluppare sinergie all'interno del proprio team e con gruppi di lavoro esterni multi-professionali.
A tal proposito, abbiamo acquisito consapevolezza dell'apporto professionale e culturale che possiamo dare nel promuovere salute attraverso lo sviluppo delle nostre risorse interne e con il metodo della condivisione.
Con la riscoperta di queste risorse abbiamo delineato concretamente delle attività rilevanti nella prevenzione e nello sviluppo dei PDTA.

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LA GESTIONE REMOTA DEI POCT EMOGASANALIZZATORI E L'ACCREDITAMENTO DEI REPARTI DI CURA

F. Antonelli[1], C. Quintavalle[2], P. Masi[2], A. Bernardini[2], U. Paoletti[2], R. Pastorella [2], G. Turelli[4], M. Chelli[4], D. Michela[1], S. Anselmi[4], M. Pennisi[1], E. Agarini[3], S. Casu[3], A. Canapicchi[1], I. Curreli[1]

[1]U.O.S. Medicina di Laboratorio d'urgenza, Cecina (LI)
[2]U.O.S. Medicina di Laboratorio d'urgenza, Piombino (LI)
[3]U.O.S. Medicina di Laboratorio d'urgenza, Portoferraio (LI)
[4]U.O.C. Medicina di Laboratorio, Livorno

Nella Azienda Usl di Livorno, parte dell'area Nordovest, sono installati 22 emogas GEM 3500 E 4000 (Werfen) in dotazione ai reparti di cura, collegati in rete ad un server GEM WEB PLUS, su indicazione della Direzione Sanitaria è stato creato un gruppo di lavoro formato da TSLB coordinati da un Dirigente, distribuito sulle 4 strutture di Laboratorio esistenti per ogni P.O. Livorno Cecina Piombino Portoferraio, aventi il compito di garantire attraverso il controllo remoto la qualità del dato analitico emesso. Il gruppo di lavoro con l'esperienza e la professionalità che lo distingue da un operatore sanitario, semplice utilizzatore di un Poct, garantisce i requisiti di qualità, precisione e accuratezza del risultato, ed uno strumento controllato e visionato nelle sue funzioni quotidianamente, il tutto attraverso la gestione del software GEM WEB PLUS che raccoglie tutti gli emogasanalizzatori. L'attività svolta oltre a soddisfare parte dei requisiti presenti nelle principali linee guida sui POCT, permette ai reparti di cura di adempiere a quanto richiesto nella documentazione di accreditamento istituzionale DRG 1141/2014 "Assicurazione della qualità o garanzia della qualità Tutte le attività pianificate e sistematiche, attuate nell'ambito del sistema qualità e di cui, per quanto occorre, viene data dimostrazione, messe in atto per dare adeguata confidenza che un'entità (prodotto/servizio) soddisferà determinati requisiti di qualità" Tutte le attività di verifica di qualità (IQM), Non conformità (azioni correttive) e quanto altro necessario a garantire il corretto funzionamento dello strumento viene registrato su un data base all'interno del sito intranet (Dipartimento dei servizi). Tutti i dati raccolti oltre ad essere disponibili al gruppo di lavoro per le azioni dovute è accessibile per la parte di competenza ai Responsabili dei reparti dotati di emogas, che con accesso controllato da login e password sul sito di laboratorio verificano il corretto funzionamento del loro strumento e le azioni intraprese a conferma di quanto richiesto del DRG 1141/2014 con ricezione di report di attività elaborati per non conformità e quanto indicato nei requisiti richiesti per l'utilizzo del POCT.

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THE ISO 15189 ACCREDITATION: THE STAFF COMPETENCE FIRST OF ALL

A. Aita[1], A. Padoan[1], G. Antonelli[1], L. Sciacovelli[2], M. Plebani[1]

[1]*Department of Medicine, University of Padova*
[2]*Department of Laboratory Medicine, University-Hospital, Padova*

Introduction: The ISO 15189 (IS) Accreditation is an important tool in Laboratory Medicine to guarantee: a quality system that supports the quality assurance procedures; the attaining of a competence level of staff that assures the identification and prevention of errors; the patient safety.
Aim of this work is to describe the criteria used to achieve a full awareness of the laboratory staff in the path to IS Accreditation.
Methods: The following steps have been implemented: a) Training course for all staff on requirements of IS; b) set up of working groups on specific issues, by involving the different professional categories. The purposes are: to verify the compliance of internal operative instruction (OI) with the requirements of IS and to identify the new OI to be implemented; c) implementation of OI and revision of internal documents; d) Internal audit to check the suitability of the system. Specific criteria and procedures have been implemented to assure the effectiveness of each step.
Results: The results demonstrate the effectiveness of the proposed model and the achievement of the defined objective. In fact: all staff during the evaluation visit demonstrated: a) good awareness of the issues included in the IS and the close linked to the operative flow; b) improvement of the competence in particular about the new matters such as verification and validation of examination procedures or uncertainty measurement; c) new enthusiasm, new zest and new dynamism due to the achievement of a very ambitious and difficult objective.
Conclusion: The pursuit of IS Accreditation process has allowed achieving an objective much more ambitious than the Accreditation certificate. In fact in every organization the excellent level of quality can be achieved only if the staff is competent and has the full awareness of the importance to work according to procedures approved at an international level and in a well-structured QS. The implementation of IS Accreditation process has to begin with the knowledge sharing and the stimulating of the enthusiasm in order to achieve a high level of quality of patient safety.
Plebani M, Sciacovelli L, Chiozza ML, et al. Once upon a time: a tale of ISO 15189 accreditation. Clin Chem Lab Med 2015;53:1127-9.

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INTRODUCING AUTOMATION IN A CLINICAL LABORATORY: A COST EVALUATION

C. Archetti[1], A. Montanelli[2], L. Caimi[3], E. Garrafa[3]

[1]*Dept. of Economics and Management, University of Brescia*
[2]*Unique Laboratory, ASST Spedali Civili, Brescia*
[3]*Dept. of Molecular and Translational Medicine, University of Brescia*

The aim of our work is to describe the strategy adopted by the Hospital “Spedali Civili” where the management has recognized on one side the importance of introducing automation to increase the efficiency of the service, on the other side, their aim was also to contain as much as possible the cost increase charged to the hospital structure when turning to automation using the same analyzers and contracts of four pre exiting labs converged into an unique laboratory. We also analyze how the introduction of automation impacts on staff costs and compared the pre-automation situation with the new setting and with similar Italian realities. We note that, to the best of our knowledge, there is no such study in the literature. In fact most of the studies focus on the performance of automated labs in terms of quality of service, that of course is very important, without considering how introducing automation in clinical labs impacts on costs. We considered the costs charged to the cost centre corresponding to the laboratories, both direct and indirect. As far as direct costs, we considered two terms: equipment and staff costs. The equipment costs are given by the monthly renting rates, while the staff costs are the monthly salary. The indirect costs are formed by two components. The first one, which is called “indirect costs”, is a fixed percentage of the sum of the equipment and staff costs and is equal to 4.6%. This term takes into account all minor costs related to the management of the laboratories. The second term, called “general costs”, is 13.95% of the sum of direct and indirect costs as described above. This term amounts to the part of general managing and fixed cost of the hospital charged to the single centre of costs. The introduction of the automated chain lead to a slight increase in equipment costs which is highly compensated by a remarkably decrease in staff costs. As a consequence, total costs decreased by 12.55%. The strategy adopted by the management, which was based on re-using the available equipment and staff when merging the pre-existing laboratories, has reached its goal: introducing automation while minimizing the costs.

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SYSMEX XN-9000 BODY FLUID MODE: PERFORMANCE OF NUCLEATED CELL COUNTING IN SYNOVIAL FLUID

M. Seghezzi[1], B. Manenti[1], P. Dominoni[1], R. Ferrari[2], G. Zappalà[2], A. Crippa[1], S. Buoro[1]

[1]Chimical Chemistry Laboratory, ASST Papa Giovanni XXII, Bergamo
[2]Departments of Orthopedics and Traumatology, ASST Papa Giovanni XXIII, Bergamo

Introduction: Synovial fluid (SF) analysis is a clinical laboratory test useful in the diagnose of joint inflammation (eg arthritis). Because of its viscosity, SF is usually analyzed after pre-treatment with Hyaluronidase (HY). Sysmex XN-9000 is an automated hematology analyzer with a dedicated module for body fluid analysis (XN-BF) that provides the following parameters: total nucleated cells (TC-BF), leukocytes (WBC-BF) polymorphonuclear (PMN#; PMN%) and mononuclear (MN#; MN%) cells. Aim of this study was to evaluate the application of XN-BF in SF analysis, according to CLSI document H56-A (1).
Methods: 88 SF samples with a TC-BF ranging from 33 to 161333x10^6/L collected in K_3EDTA tubes (Becton Dickinson) were simultaneously analyzed after HY pre-treatment (Sigma) with XN-BF and Manual Microscopy (OM) performed in a Burker chamber. The agreement between XN-BF and OM was assessed with Pearson's correlation coefficient (r), Passing Bablok (PB) regression and Bland-Altman analysis. Limit of Blank (LoB), Detection (LoD) and Quantitation (LoQ) were assessed according to CLSI EP17-A2 (2). Carry Over (CO) and Linearity were assessed according to CLSI H56-A (1) and EP06-A3 (3) documents, respectively. Statistical analysis was carried out with Analyse-it software 3.90.5 (Leeds, UK).
Results: For TC-BF the comparison between XN-BF and OM showed a PB regression of y=0.99-12.64 (r value=1.0, p <0.0001) and a Bias of -24.1x10^6/L. For WBC-BF, PB regression was y=0.98x-6.50 (r value=1.0, p <0.0001) with a Bias of -67.8x10^6/L. LoB, LoD and LoQ were 0.9,1.9 and 2.6x10^6/L respectively for TC-BF and 0.6, 1.6 and 2.6x10^6/L for WBC-BF. Linearity was good in the whole range evaluated (from 44 to 43936x10^6/L) and CO was absent (0.00%) for both TC-BF and WBC-BF.
Conclusion: In the analysis of SF pre-treated with Hyaluronidase, XN-BF offers excellent performance and rapid and accurate cell count in clinically relevant concentration ranges, replacing the counting chamber for most samples.
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2. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures. CLSI document EP17-A2, 2012.
3. Evaluation of the linearity of quantitative measurement procedures: a statistical approach. CLSI document EP06-A, 2003.

P137
SERUM PROTEIN ELECTROPHORESIS AND PRESCRICTIVE APPROPRIATENESS

P. Natali, G. Patelli, D. Carra, M. Varani, S. Tagliavini, T. Trenti

S.C. Medicina di Laboratorio, NOCSAE Modena

Introduction: The diagnostic objective of the request of serum protein electrophoresis (EF) is the detection of monoclonal components in serum and their monitoring. However, is common EF prescription for a medical general checkup in patients without particular clinical indications. Except for patients in active therapy for plasma cell dyscrasias, the scientific evidence highlights the futility of recurrent EF before a 60 day period, but sometimes the requests of this exam are more frequent. We adopted strategies to limit the request of EF before 60 days after the last run. The purpose of this study was to evaluate the impact of this approach.
Methods: The Corelab NOCSAE Modena, has adopted informatic systems allowing addressing of adequate EF requests. A computer alert appears every time that the EF request from a hospital department is made if the exam was already carried out during the previous 60 days. The EF is scheduled but the analysis is not performed, and the report automatically shows the results of the last EF executed with a note: “Not performed analysis: the result is related to the dosage of the day dd/mm/yy. Scientific evidence considers it inappropriate for the EF application before 60 days since the last time". Oncology, haematology and nephrology departments are excluded from this rule.
For outpatients whose the medical history is not always known, EF is performed, but accompanied by a note: "Previous analysis performed on dd/mm/yy. Scientific evidence considers it inappropriate for the EF application before 60 days since the last time".
Results: Our Laboratory in 2015 received 154,719 EF requests both from the hospital departments (33,697=21.8%) and blood collection centers (121,022=78.2%). The requests subject to the rule were 8,468 (5.4%) in total, specifically 4,939 (14.7%) coming from the inpatients and then not carried out, while 3,529 (2.9%) coming from the outpatients.
Conclusions: The information technology rule introduced allowed to limit the number of EF performed and to increase the appropriateness of the requests. It represents an important means of professional support and improvement of prescribing practices.
Ludwig H, Miguel JS, Dimopoulos MA, et al. International Myeloma Working Group recommendations for global myeloma care. Leukemia 2014;28:981-92.

P138
BENCE JONES PROTEIN AND PRESCRIPTIVE APPROPRIATENESS

P. Natali, G. Patelli, S. Tagliavini, D. Carra, M. Varani, T. Trenti

S.C. Medicina di Laboratorio, NOCSAE Modena

Introduction: The Bence Jones protein (BJP) is a marker for the management of patients with plasma cell dyscrasias. However, the prescription of BJP in patients that need to undergo an examination requiring contrast medium is common. There are a lot of documents about the inappropriateness of this kind of procedure, as also recommended by the SIRM-SIBIOC consensus document of 2014, which highlights that for renal damage prevention the examination of the serum creatinine and eGFR measure is adequate. The request of BJP with the indication for tests with contrast medium may be considered a sign of prescriptive inappropriateness.
The aim of this study is to evaluate what is the percentage of patients undergoing assessment of BJP before the administration of contrast medium in 2015.
Methods: We assessed the requests for BJP received by the Corelab NOCSAE Modena in 2015. We counted how many requests had been reported indications for contrast medium divided by inpatients and outpatients.
Results: During 2015, the laboratory received 12,789 urine samples with a request for BJP. These were divided as follows: 3,430 (26.8%) coming from inpatients, of which only 37 (1.1%) with a clinical request of contrast medium. The remaining 9,359 (73.2%) of samples were from outpatients, 2,401 (25.7%) of these with a clinical indication for a contrast medium.
Conclusions: From the hospital departments we found a small number of requests for BJP in patients requiring examination with contrast medium, but it is not known if this is due to a correct prescription, rather than the absence of diagnostic indication. The situation is different for the patients from the blood collection centres where 1 request of BJP out of 4, still reach the laboratory with the indication for contrast medium. The data suggest that it is necessary to continue the information-training to the physician, and to this end, the laboratory plays a key role as interlocutor with the prescribers.
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P139
REGOLE TEMPORALI DI APPROPRIATEZZA PRESCRITTIVA APPLICATE ALL'ASSETTO LIPIDICO: UN ANNO DI ESPERIENZA

M. Varani, S. Tagliavini, P. Natali, L. Giampaolo, D. Carra, C. Canali, A.R. Soliera , T. Trenti

S.C. Medicina di Laboratorio NOCSAE, Modena

Introduzione: L'appropriatezza prescrittiva è importante presupposto per il corretto inquadramento diagnostico e importante valore per i professionisti di laboratorio. Regole temporali sono proposte nel decreto ministeriale sull'appropriatezza delle prestazioni di laboratorio (per l'assetto lipidico controllo ogni 5 anni in assenza di fattori di rischio). Nella provincia di Modena (AUSL e AOU) regole temporali di appropriatezza sono state implementate per molti test da dicembre 2014, condivise dal Collegio di Direzione Aziendale, basate su indicazioni ed evidenze scientifiche.
Scopo del lavoro: Verificare la ricaduta della applicazione di regole temporali di appropriatezza per l'assetto lipidico di base (colesterolo, HDL, LDL, trigliceridi) in pazienti interni ed esterni sul totale delle prestazioni eseguite.
Materiali e metodi: La valutazione è stata effettuata nella S.S. Corelab del Dipartimento di Medicina di Laboratorio AUSL ove una personalizzazione del sistema informatico di laboratorio blocca la esecuzione degli analiti citati nei pazienti interni se eseguiti nei 60 giorni antecedenti (riportando sul referto data e risultato precedente); la mantiene nei pazienti esterni con "warning" alla refertazione.
Risultati: Nel 2015 sono stati eseguiti 290.536 colesteroli (9% int-91% est), 237.727 HDL (6% int-94% est), 161.897 LDL (8% int-92% est), 27.9455 (8% int-92% est) trigliceridi. Inappropriatezze temporali: 11.550 per colesterolo (21.5% int-2.3% est), 5.989 per HDL (14% int-1.8% est), 4.113 per LDL (11% int-1.8% est), 9.841 per trigliceridi (19% int-2.2% est).
Conclusioni: Il supporto informatico consente l'applicazione di percorsi di appropriatezza con ricadute significative nei pazienti interni che per facilitazione informatica vedono prescrizioni ripetute di esami in assenza di evidenze scientifiche o reali necessità cliniche. Per richieste motivate il laboratorio è sempre disponibile alla violazione delle regole (stima violazioni <0.5%) ma nella quasi totalità dei casi riportare il precedente risultato appare azione utile a soddisfare la richiesta clinica. Nei pazienti esterni il percorso è volto a sensibilizzare i medici sulle corrette tempistiche di prescrivibilità degli esami.

P140
APPLICATION OF FMEA ANALYSIS AND SCORING TO MONITOR AND LOWER LABORATORY CLINICAL RISK

L. Bartoloni[1], M. Favarato[2], A. Ramuscello[1], G. Romagnosi[1]

[1]UOSD Lab. Analisi Venezia, ULSS12 Veneziana, Venezia
[2]UOC Lab. Analisi, ULSS12 Veneziana, Zelarino (VE)

Failure Mode and Effect Analysis (FMEA) is an excellent technique to assess and monitor failures in a system. We applied this method to each and every diagnostic sector of our laboratory (pre-analytical, cytogenetics, molecular biology, toxicology, chemistry, haematology, coagulation). The FMEA analysis is divided in three steps and it was performed as collaborative work of all the actors involved in the process. At first risks were identified: each step of each process was described and possible risks, both rare and frequent, discussed and listed. Afterwards, for each possible error, the frequency of occurrence and the consequences on the patient were evaluated and scored. The sum of all these scores gave us a measure of the total risk in each sector. The goal of the FMEA analysis is to lower the risk, therefore we analysed the high scored errors (very damaging, or very frequent, or not frequent but damaging errors) and developed strategies to improve the most risky steps of the processes and ways to monitor the effect of these changes. After an observation time different for each diagnostic sector and depending on the number of samples and tests, the FMEA analysis was re-evaluated and the new score assessed. For each diagnostic area the FMEA risk score was lowered and therefore the FMEA analysis is now routinely used to monitor the laboratory processes and to individuate error prone passages and to prioritise the corrective actions that need to be taken.
As example, in the Cytogenetics sector the assessed risk during the first FMEA analysis was 525, with major issues in labelling of culture plates and slides and typing and transcription errors in reports. Barcodes for culture plates, along with a printing system for slides were introduced. Moreover double check of reports was stressed and therefore errors were strongly reduced. Once the new process was implemented, the FMEA analysis was repeated and the risk score was assessed as 114, with a five fold reduction.
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P141
ALGORITMO PER LA STIMA INDIRETTA DEGLI INTERVALLI DI RIFERIMENTO

E. Milletti[1], F. Cinci[1], C. Bellini[1], R. Guerranti[1], M. Quercioli[3], V. Vallerignani[2], C. Scapellato[4], G. Cevenini[2], R. Leoncini[1]

[1]Dip. Biotecnologie Mediche, Università di Siena / UOC Lab. di Patologia Clinica, AOU Siena
[2]Dip. Biotecnologie Mediche, Università di Siena
[3]Centro Regionale di Riferimento per la Sicurezza di Qualità – AOU Careggi (Firenze)
[4]UOC Lab. di Patologia Clinica, AOU S. Maria alle Scotte (Siena)

Introduzione: L'intervallo di riferimento (IR) è uno degli strumenti decisionali medici più utilizzato. Come tale, è di vitale importanza che i suoi valori siano correttamente stabiliti e regolarmente rivisti nel laboratorio clinico.
La loro determinazione con metodi diretti è lunga, difficile e economicamente molto dispendiosa principalmente a causa della necessità di selezionare un sufficiente numero di soggetti di riferimento rispondenti a ben precisi criteri di selezione. I dati provenienti da grandi centri ospedalieri, al contrario, sono numerosi e facilmente disponibili (con gli attuali sistemi di immagazzinamento dati informatizzati). Anche se tutte le informazioni necessarie per la determinazione diretta dell'IR non sono generalmente disponibili, questi dati contengono informazioni che possono essere analizzate per ottenere come minimo IR grezzi.
Materiali e metodi: In questo lavoro abbiamo cercato di elaborare un metodo di stima indiretto degli IR. Il metodo si basa su alcune assunzioni concernenti la distribuzione. Questa elaborazione è una evoluzione del metodo di Concordet (1) che permette di stabilizzare la soluzione consistente nel separare in modo ottimale una popolazione di "sani" rispetto a una o più distribuzioni di soggetti patologici. Inoltre l'algoritmo è in grado di rilevare eventuali differenze di genere e la presenza di andamenti legati all'età.
Per verificare le performance dell'algoritmo abbiamo utilizzato dati relativi alla Creatininemia estratti dalla banca dati della UOC Laboratorio di Patologia Clinica (Policlinico Le Scotte – Siena) e relativi all'anno 2015. I dati sono stati selezionati su alcuni criteri di inclusione quali per esempio l'unicità del dosaggio all'interno dell'anno solare in esame.
Risultati e discussione: L'algoritmo si è dimostrato molto versatile e robusto nell'analizzare una grande quantità di dati, riportando parametri coerenti con quelli riportati dalle linee guida IFCC e confermando le differenze di genere e l'andamento correlato con l'età.
Le soddisfacenti performance dell'algoritmo ci incoraggiano a metterlo quanto prima in utilizzo per la stima indiretta di IR nei casi in cui la procedura raccomandata si riveli di difficile applicazione.
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P142
ANTIDEPRESSANT DRUG MONITORING IN NEONATES AFTER IN UTERO EXPOSURE TO SELECTIVE SEROTONIN REUPTAKE INHIBITORS (SSRIs)

M. Molinaro[1], M. Broglia[1], R. Butera[2], G. Eleftheriou[2]

[1]Clinical and Experimental Pharmacokinetics Unit, Foundation IRCCS Policlinico San Matteo, Pavia
[2]Poison Control Center and Teratology Information Service, Papa Giovanni XXIII Hospital, Bergamo

Depression and psychiatric disorders are common during pregnancy and the untreated or undertreated depression may be associated with different perinatal complications.
Poor neonatal adaptation syndrome is a well-known constellation of signs and symptoms due to prenatal maternal use of drug once the placental access to the substance is no longer available. However, insufficient evidence exists to support serotonin toxicity or neonatal abstinence syndrome (NAS).
In this view, a proper method for quantitative analysis of trace concentrations of SSRIs drugs and metabolites needs to be chosen.
We evaluated 19 neonates (one twin) admitted in the neonatal ICU for symptoms initially attributed to NAS or because the mothers were under SSRIs drug therapy. All the 18 mothers were in treatment with SSRIs and/or benzodiazepines along the last trimester of pregnancy until delivery and no maternal use of alcohol, tobacco or drugs of abuse during pregnancy. Infant blood was collected on the first 24h after delivery to enable measurement of SSRIs concentrations that were performed by a validated HPLC-MS/MS method including isotopically deuterated internal standards.
9/19 neonates were symptomatic at birth with neuromuscular symptoms, hypoglycemia and respiratory distress. The mean gestational age and weight at birth were 37 wk and 2.67 kg, respectively. SSRIs maternal therapy included 8 sertraline, 3 paroxetine, 6 citalopram and 1 duloxetine. SSRIs/metabolite serum concentration were <LOQ in 4/19 neonate, but we found 5 cases with sertraline+ N-desmethylsertraline ranging from 18.2-63.4 ng/mL, 3 with citalopram at 37.5, 54.8 and 55.4 ng/mL, 2 with escitalopram at 14.5 and 16.6 ng/mL, and in 5 cases sertraline, paroxetine and duloxetine were found in traces.
The onset and severity of the symptoms were not correlate with the drug levels, but in all symptomatic babies the drug was present, although in low concentrations.
We conclude that, if neonatal symptoms at birth after in utero exposure to SSRIs and detectable drug concentrations are present, with an improvement of symptoms and with the decline of drug concentrations, this leads to a diagnosis of serotonin toxicity rather than withdrawal syndrome.
Boucher N, Bairam A, Beaulac-Baillargeon L. J Clin Psychopharmacol 2008;28:334-9.

P144
ORMONE ANTIMULLERIANO (AMH): STUDIO RETROSPETTIVO SU 649 PAZIENTI MARCHIGIANE

M. Brugia, A. Calcinari, P. Caprini, A. Ceka, M. Galeazzi

Laboratorio Biochimica Clinica e Microbiologia, Azienda Ospedali Riuniti, Ancona

Introduzione: L'ormone anti mulleriano(AMH)è un ormone glicoproteico secreto nelle donne dalle cellule della granulosa dell'ovaia che riflette con accuratezza la riserva follicolare ovarica;ha grande importanza nel predire il successo dei cicli di procreazione assistita; può essere utilizzato nella diagnosi e nel follow-up di donne affette da tumori delle cellule della granulosa; può essere utilizzato nella diagnosi della sindrome dell'ovaio micropolicistico. A differenza di altri test di riserva ovarica, come l'FSH e l'estradiolo, l'AMH rimane costante durante le fasi del ciclo mestruale, e in gravidanza, potendosi quindi misurare in qualunque momento. AMH è più precoce e affidabile dei tradizionali markers per la valutazione quantitativa e qualitativa della riserva ovarica. Scopo del presente lavoro è stato quello di identificare la percentuale di donne in età fertile con bassi valori dell'ormone AMH.
Materiali e metodi: Sono state studiate retrospettivamente dal 02/01/2015 al 30/03/2016 649 donne marchigiane con problemi di infertilità (età media 38,2±6,9, range 16-60 anni) afferenti al Laboratorio Analisi degli Ospedali Riuniti di Ancona che fa da centro di riferimento per tale test. Il dosaggio dell'AMH è stato effettuato in chemiluminescenza (Cobas 6000 Roche sensibilità 0.01 ng/ml). I valori di riferimento usati: 13-45 anni 0,9-9,5 ng/ml, >45 anni: fino 1 ng/ml. Le pazienti sono state divise in fasce di età di 5 anni, e per ogni fascia calcolate media e DS.
Risultati e conclusioni: Nelle fasce di età studiate le medie ottenute mostrano un andamento sovrapponibile a quello presente in letteratura,aumentano gradatamente fino a 35 anni e decrescono in età più avanzata. Nelle 649 donne studiate, 355 (54.6%) avevano valori di AMH molto bassi al di sotto di 0,9 ng/ml. Ad oggi, escludendo le condizioni di sterilità assoluta, non esistono indici universalmente riconosciuti che valutano il grado di fertilità ovarica; è invece possibile, grazie anche al dosaggio del AMH, ottenere informazioni, con buona approssimazione, sul probabile stato di fertilità e sulla previsione di risposta ai trattamenti farmacologici di stimolazione dell'ovulazione.
Khadum I, Ranieri M, Serhal P. Antral follicle count, anti-mullerian hormone and inhibin B: predictors of ovarian response in assisted reproductive technology? Bjog 2005;112:1384-90.

P145
ROLE OF NURSE CASE MANAGER OPENNESS AND MANAGEMENT COMBINED WITH LABORATORY ANALYSIS AMBULATORY INFECTIOUS DISEASES FOR PREGNANT PATIENTS

G. Naranjo Munoz, M. Locatelli

Servizio di Medicina di Laboratorio, Ospedale San Raffaele, Milano

Objectives: Birth is more frequent care intervention (526,729 live births according Cedap 2007). Very differents are the origin of the mothers: 25.6% Africa; 26.1% European Countries; 19.9% non-European countries; 17.3% Asia and South America 9.8% Among Italian women 3.6% performed 1st visit after the 12th week pregnancy (WP) and among foreign women made 16.2% 1st visit after the 12th PW. There are few women who begin pregnancy having performed the pre-conception screening. We would like to open a clinic al nursing laboratory coordinated by nurse-case managers with the primary of the laboratory in order to reduce the time of taking charge of the patients in case of infections during pregnancy.
Methods: this clinical nursing laboratory has free access from Monday to Saturday to pregnant women and the nurse monitors the sierological and infectivologicals exams to mapping individual risks of all them. In case of an unknown reactivity he facilitates the path with infectious disease specialist and gynecologist continuing with any addictional exams discussed with the chief of the laboratory and clinical colleagues contacted directly.
Results: this approach shows a preliminary reduction in direct and indirect costs, according to the Spending Review (Legge 189/2012) in order of 20% of expenditure in place and a very big
Reduction in the timing of reporting order to prevent the possible damage to the fetus
accelerating any treatments.
Conclusions: Observing as the check point of the optimization of the territory assistance and the implementation of the rapidest patients' accompaniment during the entire course of clinical-care study, nurse case manager with the support of the laboratory chief can reduce the direct costs by implementing an innovative management of exams in pregnancy designed on the guidelines and safeguarding the reduction of expenditure in accordance with the Regional and Ministerial directives.

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CONFRONTO TRA PCR E PRESEPSINA COME MARCATORI DI SEPSI NEONATALE

G. Bragato[1], M.M. Mion[1], M. Zaninotto[1], D. Faggian[1], F. Leone[2], B. Dalla Barba[2], E. Baraldi[2], M. Plebani[1]

[1]U.O.C. Medicina di Laboratorio, Azienda Ospedaliera-Università di Padova, Padova
[2]Dipartimento di Salute della Donna e del Bambino, Azienda Ospedaliera-Università di Padova, Padova

Scopo: La sepsi neonatale è un'importante causa di morbilità e mortalità, la cui gestione e cura dipendono dalle possibilità di una diagnosi ed intervento terapeutico tempestivi. Scopo dello studio è individuare un marcatore di infezione neonatale più precoce e specifico rispetto alla Proteina-C-Reattiva (PCR) identificando nella Presepsina (PSP) un possibile complemento diagnostico. Metodi. Dal 23/11/2015 al 23/04/2016 sono stati arruolati 75 neonati accolti presso l'Assistenza Neonatale di Padova, con sospetto di sepsi neonatale e richiesta di PCR urgente; il campione destinato alla determinazione della PCR (plasma da litio eparina) è stato utilizzato anche per la determinazione della PSP. I risultati ottenuti sono stati posti in relazione tra loro, con i dati clinici, l'esito degli esami microbiologici, la scelta di intraprendere una terapia antibiotica (AB) e il decorso clinico.
Risultati: Caratteristiche generali neonati: 56% M, 44% F; età gestazionale e peso alla nascita (range, media): 33-41, 38 settimane gestazionali, 1135-4445, 3280 grammi; parto (%): eutocico/vaginale con ventosa/ cesareo 50/3/47. Clinica madre (si/no/non noto %): tampone vaginale (TV) negativo 49/22/29; febbre 9/91/0, corioamnionite 17/51/32, liquido amniotico (LA) tinto: 17/83/0. Clinica neonati (si/no %): febbre 49/51; irritabilità 21/79; tremori 16/84; ipertono muscolare 23/77; alterazioni colorito 5/95; sintomi respiratori 29/71, terapia AB 27/73, emocoltura pos 2/98. PCR (mg/L) <=6 (negativa)/>6 (positiva) in %: 65/35; range, media: 0.61-70.94, 7.74. PSP (pg/L) <300 (negativa)/300-1000 (positiva)/>1000 (molto positiva) in %: 8/81/11; range, media: 225-4295, 694. Correlazioni: PCR neg/PSP neg 9.5%; PCR neg/PSP pos 78.6%; PCR neg/PSP molto pos 11.9%; PCR pos/PSP neg 4.6%; PCR pos/PSP pos 86.4%, PCR pos/PSP molto pos 9.0%.
Conclusioni. Nella maggior parte dei casi in cui la PCR era negativa, la PSP risultava positiva o molto positiva (90.5%), informazione potenzialmente utile ai fini della decisione terapeutica. La PSP, in questo studio valutata solo retrospettivamente, potrebbe essere impiegata come biomarcatore di scelta rispetto a quelli già in uso nella pratica clinica per la gestione della sepsi neonatale.

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RICERCA DEL PAPILLOMAVIRUS IN BIOLOGIA MOLECOLARE: QUALE TEST DI SCREENING?

R. Marozzi[1], G. Catanoso[2]

[1]*ASST Franciacorta, Lab. Analisi P.O. di Iseo*
[2]*ASST Franciacorta, Lab. Analisi P.O. di Chiari*

L'infezione da Papillomavirus umano (HPV) rappresenta la patologia sessualmente trasmessa più diffusa nel mondo. Ne esistono circa 200 tipi, con un elevato tropismo per il tessuto epiteliale.
Il virus nell'epitelio può rimanere silente, indurre forme vegetative o integrandosi produrre gravi lesioni cellulari. Sulla base della patologia i ceppi HPV sono distinti per grado di malignità. Lo IARC ha classificato i genotipi sulla base del potere oncogeno per l'uomo.
Sono pertanto attuati progetti di screening e l'esecuzione della vaccinazione verso alcuni ceppi di HPV.
I principali programmi di screening sono basati sull'impiego del Pap test. Sono stati inoltre sperimentati programmi basati sulla ricerca del DNA di HPV.
E' noto che il Pap test, impiegato anche nel territorio dell'ASST, non rileva la presenza del virus, ma le anomalie cellulari.
Nel corso dell'ultimo anno sono stati sperimentati due metodi di ricerca del DNA, di cui uno già in uso per programmi di screening, applicandoli alla rivalutazione di casi con Pap test anomalo.
Confrontando i risultati dei due metodi, uno basato sulla ricerca mirata di vari genotipi ad alto rischio e l'altro per la ricerca generale e la successiva tipizzazione di HPV, il risultato è stato che la concordanza assoluta si è ottenuta per 36 campioni (sul totale di 64), concordanza parziale per 8/64 e non concordanza per 20/64.
In particolare il metodo per la ricerca generale ha individuato la presenza di HPV nei 20 casi non concordi (31,3% del totale dei campioni), che il test mirato avrebbe dato come negativi.
Pur su una casistica limitata, si deduce che la scelta del metodo molecolare da utilizzare per le campagne di screening deve essere adeguatamente robusto nell'individuare comunque HPV e che scelte di test mirati potrebbero avere un alto numero di falsi negativi. Il tutto anche nell'ottica dei flussi di nuove popolazioni nelle quali le prevalenze dei ceppi di HPV si discostano in modo significativo da quelle degli studi epidemiologici del territorio nazionale.

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INCIDENZA DELLE INFEZIONI OCCULTE DA HBV (OBI) IN UNA POPOLAZIONE "PRIVILEGIATA", I DONATORI - INDAGINE RETROSPETTIVA

F. Camilli[1], M.P. D'Errico[2]

[1]*U.O.S. Biologia Molecolare, Dipartimento di Medicina Trasfusionale, Osp."A.Perrino", Brindisi*
[2]*U.O.C. Patologia Clinica, Osp."A.Perrino", Brindisi*

L'introduzione di tecniche di biologia molecolare altamente sensibili nei servizi di Medicina Trasfusionale ha permesso di individuare l'esistenza di numerose infezioni occulte da Epatite B, con l'evidenza di concentrazioni molto basse di DNA virale in assenza di HBsAg.
Questo studio pone come obiettivo la valutazione dell'incidenza delle OBI nella popolazione di donatori al fine di migliorare il quadro reale della diffusione di tale fenomeno.
Materiali e metodi: Per lo screening NAT (Cobas TaqScreen MPX test, Roche Diagnostics,v. 1.0, sensibilità su singolo per HBV 3.8 UI/ml,da aprile 2013 v. 2.0 con sensibilità 2.8 UI/ml) sono stati testati da novembre 2008 a maggio 2016, 98.789 campioni; i positivi sottoposti a test quantitativo HBV DNA (AmpliPrep/Cobas TaqMan HBV test v.2.0 con sensibilità 20 UI/ml, Roche Diagnostics)
Il test HBsAg è stato eseguito con kit Vitros HBsAg ES Assay, Ortho-Clinical Diagnostics, con tecnica CLIA potenziata per la rivelazione di 37 possibili mutazioni "escape"; il quadro dei marcatori sierologici è stato completato su strumentazione Modular E170 Hitachi con kit "Elecsys and cobas e analyzers", Roche Diagnostics.
Risultati: Complessivamente sono stati osservati 26 casi di donatori OBI con concentrazioni di HBV DNA ai limiti della sensibilità (<20 U/ml): età media 55 anni (range 37-67), prevalenza di soggetti maschi (22/26), nessun paziente vaccinato o coinfettato con HCV.
Tali casi rappresentano lo 0.026% del totale delle donazioni, ma la percentuale sarebbe maggiore se si quantificasse il numero reale dei donatori che afferiscono al SIMT (più del 50% è stato rivalutato diverse volte).
Il quadro sierologico è variegato: 3 pz presentano solo anti-HBc, 8 anti-HBc e anti-HBe, 7 anti-HBc e anti HBs, 2 sieronegativi, 6 del tutto sieropositivi; 13 pz (50%) hanno livelli significativi di anti-HBs (range 16-256 UI/ml, 1 pz >1000 UI/ml).
Come già descritto in letteratura (1), anche nel nostro studio i soggetti OBI, sottoposti più volte ai test NAT, hanno registrato basse viremie e un profilo fluttuante delle stesse.
Conclusioni: Lo screening molecolare sui donatori si è rivelato una fonte notevole di informazione su un fenomeno quale l'infezione occulta da HBV certamente sottostimato.
1. Raimondo G, Caccamo G, Filomia R, et al. Occult HBV infection. Semin Immunopathol 2013;35:39-52.

P149
INCIDENZA DELLE INFEZIONI DA LEGIONELLA RILEVATE PRESSO IL P.O. "ANNA RIZZOLI" (ISCHIA) DAL 2008 AL 2015: ESPERIENZA DI UN SINGOLO CENTRO

R.M. Mendella, M.C. Monti, G. Caruso, M.C. Stanziola, G. Mattera, A. Castagliuolo

Lab. di Patologia Clinica, Osp. A.Rizzoli, Lacco Ameno d' Ischia

Le infezioni sostenute da Legionella spp. rappresentano un problema di sanità pubblica per la frequente presenza del microrganismo nelle acque degli impianti idraulici di case, alberghi, centri sportivi, ospedali, case di riposo, oltre che nelle torri di raffreddamento degli impianti di condizionamento di grandi edifici e in ogni situazione in cui l'acqua ristagna a temperatura di almeno 25 °C.
Tale infezioni rappresentano delle nuove emergenze nel campo delle malattie infettive, sono presenti nel nostro paese a livello endemico, e costituiscono un pericolo costante nella realtà turistica dell'isola d'Ischia.
I dati raccolti dal 2008 al 2015 presso il P.O. "Anna Rizzoli" mostrano una positività per l'antigene solubile urinario di Legionella pneumophila sierogruppo-1 e anche la valutazione di criteri di laboratorio correlati ai dati clinico-laboratoristici e radiologici di pazienti afferenti a tale struttura, utili nella diagnosi differenziale della polmonite da legionella.
In totale sono stati diagnosticati 21 casi di legionellosi correlati alla permanenza in strutture turistico-ricettive.
I dati in particolare rilevano una sostanziale concordanza con l'incidenza della legionellosi in Italia e in Europa sia per quanto riguarda l'andamento mensile dei casi, sia per quanto attiene la distribuzione per età e genere.
Il numero dei casi accertati e notificati risulta significativo pur essendo limitato rispetto alle effettive presenze turistiche annuali sull'isola e ciò dimostra la reale intensificazione dei controlli e la gestione mirata del rischio di legionellosi negli ambienti che maggiormente favoriscono la crescita di questo organismo.
In conclusione, attualmente risulta necessaria una sorveglianza clinica specifica per la diagnosi precoce della malattia attraverso un'attenta sorveglianza ambientale con idonei piani di valutazione del rischio e programmi di autocontrollo nelle strutture turistico-ricettive e in quelle a rischio di contaminazione allo scopo di poter cristallizzare una migliore strategia operativa nella prevenzione di tale terribile infezione multi-sistemica.

P150
INFEZIONE OCCULTA/LATENTE DA VIRUS DELL'EPATITE B (OBI) IN NAPOLI E PROVINCIA

M. Caputo, E. Assentato, A. Del Viscovo, G. Longo, W. Utech

U.O.S.D. Biologia Molecolare di Virologia, Centro NAT e Immunodiagnostica dei Trapianti, Dipartimento dei Servizi, A.O. "A. Cardarelli", Napoli

Premessa: Alcuni soggetti, anche se asintomatici, presentano una particolare forma d'infezione da HBV definita occulta/latente (OBI); essi risultano HBsAg- e HBV DNA+ a bassa carica, non sempre rilevabile. L'infezione occulta/latente è correlata al caratteristico ciclo vitale del virus e diversi fattori possono determinarla. Scopo dello studio è evidenziare i casi di infezione HBV occulta (Diagnosis and Management of Occult Hepatitis B Virus Infection: A Short Review, 2015) riscontrati in una popolazione di presunti soggetti sani (donatori di sangue di Napoli e Provincia).
Materiali e Metodi. Sono stati testati 341.556 campioni di sangue di Donatori afferiti al nostro Centro dal 2012 al 2015. Per la rivelazione dell'HBV DNA è stato utilizzato il test COBAS TaqScreen MPX v2.0 (Roche Diagnostics). I markers sierologici sono stati effettuati con metodica CMIA su piattaforma Architect (Abbott).
Risultati: Sono stati riscontrati 51 campioni HBV-DNA positivi con una media di 13 casi l'anno. Il numero maggiore di casi (n=20) è stato riscontrato nel 2012 mantenendosi stabile negli anni successivi. I suddetti casi, 47 italiani e 4 stranieri (età media 52 anni), erano 36 maschi e 15 femmine. Tutti avevano una viremia <20 UI/mL ed erano HBsAg-/HBcAb+. Di questi, 16 erano HBeAb +. La gran parte presentava un titolo HBsAb non protettivo e valori di ALT nella norma. Non sono stati riscontrati casi di coinfezioni.
Conclusioni: L'implementazione di test molecolari molto sensibili ha permesso di identificare 51 casi di positività al virus dell'epatite B in soggetti che risultavano essere in uno stato apparente di buona salute ma che sono risultati tutti HBcAb+, segno di un'esposizione attiva al virus. Ciò sottolinea quanto sia sfumata, da un punto di vista clinico, questa particolare forma d'infezione e quanto sia importante identificarla dal momento che l'infezione nella sua forma occulta/latente può determinare la classica forma di malattia epatica cronica. Il sequenziamento genico potrebbe essere un valido supporto per il riscontro di nuove mutazioni in grado di inibire l'espressione di HBsAg o cambiarne le caratteristiche antigeniche rendendo impossibile la sua rilevazione da parte dei comuni saggi sierologici commerciali.

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INFECTION BIOMARKERS' ACCURACY BASED ON THE NEW CRITERIA OF CLINICAL DIAGNOSIS OF SEPSIS PROPOSED IN "THE THIRD INTERNATIONAL CONSENSUS DEFINITIONS FOR SEPSIS AND SEPTIC SHOCK (SEPSIS-3)"

V. Sargentini[1], D. Collepardo[1], M. D'Alessandro[1], G. Ceccarelli [2], F. Alessandri[3], G. Petralito[1], A. Morelli[3], A. Angeloni[4], M. Venditti[2], A. Bachetoni[1]

[1]Lab. di Patologia Clinica, Dip. di Medicina Sperimentale, Sapienza Università di Roma

[2]Dip. di Salute Pubblica e Malattie Infettive, Sapienza Università di Roma

[3]Dip. di Anestesiologia e Terapia Intensiva, Sapienza Università di Roma

[4]Dip. di Medicina Molecolare, Sapienza Univ. di Roma

Objective: Sepsis and septic shock are main healthcare problems in intensive care units (UTI). Improvements in diagnostic procedures are required for effective management of infectious diseases. Many sepsis biomarkers have not been thoroughly tested due to their low specificity. The aim of the study is to evaluate presepsin (PRE-S) and procalcitonin (PCT) contribution, alone or in combination, for an accurate diagnosis of sepsis. Data were analyzed considering new criteria of severity and clinical diagnosis recommended by the 2106 Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3).
Methods: 35 patients admitted to UTI were recruited according to clinical diagnosis and severity of illness. Specimens were collected from different clinical cases: patients without signs of infection (no-sep), sepsis and septic shock. 30 healthy subjects were assessed as controls (ctr). Serum PRE-S and PCT were measured using PATHFAST® and KRYPTOR® analysers.
Results: Median (IQR) concentrations of PRE-S (pg/mL) were 497.6 (312-584.5) in ctr group, 622.5 (322-934) in no-sep group, 1046 (429-2344) and 1739 (937-3235) in septic and septic shock patients respectively. PRE-S values were significantly lower in ctr compared to sepsis ($p=0.03$) and septic shock ($p <0.0001$); and in no-sep patients compared to septic shock ($p=0.001$). Median (IQR) concentrations of PCT (μg/L) were 0.1 (0.0-0.1) in ctr group, 0.2 (0.1-0.3) in no-sep group, 1.4 (0.8-3.5) and 1.6 (0.1-3.7) in septic and septic shock patients respectively. PCT values were significantly lower in ctr compared to no-sep patients, sepsis and septic shock ($p <0.0001$), and in no-sep patients compared to sepsis and septic shock ($p <0.0001$). Ctr and no-sep patients were well distinguished from infection group using as cut off values: 650 for PRE-S and 0.5 for PCT. Areas under the curve were 0.83 for PRE-S and 0.98 for PCT. The combined used of the two sepsis biomarkers improve specificity (%) from 71.88 for PRE-S and 92.19 for PCT to 98.44.
Conclusions: Study confirms the importance of sepsis biomarkers' combined use to improve diagnostic accuracy, increasing the success of therapeutic interventions and preventing the onset of antibiotic resistance.

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VALUTAZIONE DELLA VITAMINA D E DELLA PRESEPSINA NEI PAZIENTI CON SEPSI. NOSTRA ESPERIENZA

L. Varraso[1], R. Lovero[1], A. Mileti[1], M. Pepe[1], L. Santacroce[2], G. Miragliotta[2], L. Nisi[1], A. Schirinzi[1], E. Mascolo[1], F. Di Serio[1]

[1]U.O.C. Patologia Clinica Ospedaliera, Azienda Ospedaliero-Universitaria Consorziale Policlinico Bari

[2]U.O.C. Microbiologia e Virologia, Azienda Ospedaliero-Universitaria Consorziale Policlinico Bari

La diagnosi precoce di sepsi grave e di shock settico, che trova nella emocoltura il suo gold standard, rappresenta il principale obbiettivo da perseguire per migliorare la sopravvivenza dei pazienti. Numerosi studi hanno dimostrato che la presepsina (glicoproteina espressa sulla superficie di membrana dei monociti e macrofagi, con ruolo di recettore per i complessi di lipopolisaccaridi (LPS)) è significativamente aumentata in pazienti settici. La vitamina D svolge un ruolo importante nella difesa dell'ospite contro le infezioni. La carenza di vitamina D è stata associata a un aumentato rischio di infezioni. Scopo di questo studio è stato quello di valutate se nei paziente con emocolture positive esiste una correlazione tra le concentrazioni della vitamina D e della Presepsina.
Materiali e metodi: nel periodo compreso tra marzo e aprile 2016 sono stati selezionati 22 pazienti con emocolture positive (12 maschi e 10 femmine) (età mediana per i maschi 76 anni, per le donne 68 anni). Le determinazioni della presepsina (plasma litio-eparina - PathFast®) e della vitamina D (siero - DiaSorin Liaison XL) sono state effettuate per tre giorni consecutivi. L'analisi statistica è stata ottenuta mediante l'utilizzo del programma statistico MedCalc.
Risultati: retta di regressione: $y= 26.48 – 4.98 \log(x)$; intercetta 26.48, (95% CI =19.42 a 33.55 $P <0.0001$); slope = -4.98 (95% CI =-7.09 a -2.88 $P <0.0001$). Coefficiente di correlazione $R= -0.52$ (95% CI=-0.68 a -0.31 $P <0.0001$) Il coefficiente di correlazione mostra una correlazione inversa statisticamente significativa.
Conclusioni: l'aumento delle concentrazioni della vitamina D, soprattutto in terza giornata, nei pazienti con sepsi associata ad una riduzione della presepsina può essere indicativo di un miglioramento dei situazione clinica del paziente settico. Ulteriori studi potranno documentare l'utilità del dosaggio della vitamina D nel management del paziente con sepsi o shock settico.

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CIRCULATING MICRORNAS: NEW POTENTIAL PLAYERS IN ACUTE MYOCARDIAL INFARCTION

M.A. Perrone[1], C. Russo[1], M. Pieri[1], P. Casalino[2], S. Bernardini[1]

[1]Cattedra di Biochimica Clinica e Biologia Molecolare Clinica, Università Tor Vergata, Roma
[2]Dipartimento di Medicina di Laboratorio, Policlinico Tor Vergata, Roma

Background: Circulating microRNAs (miRNAs) are reported to be present in the blood of humans and have been increasingly suggested as novel biomarkers for various pathological processes in the heart, including myocardial infarction, myocardial remodeling and progression to heart failure. Previous study demonstrated that cardio-enriched miRNAs were released into bloodstream from injured myocardium in cardiovascular diseases. However, the dynamic change of circulating miR levels in patients with acute myocardial infarction (AMI) is still unclear. We aim to determine the potential of cardiac-specific miRNAs in circulation as biomarkers for acute myocardial infarction (AMI).
Methods and results: 30 AMI patients and 30 control subjects were enrolled to investigate the expression levels of circulating cardio-enriched miR-133a and miR-1. The plasma samples from AMI patients were obtained at 12 and 24 hours after first symptoms. Plasma miR levels of participants were examined by real-time quantitative PCR. Plasma cardiac troponin I (cTnI) concentrations were also measured using an electrochemiluminescence-based method. Both miRs here analyzed resulted to be increased in AMI patients compared to control group with miR-133a being the most expressed (#120 fold change at 12h after infarction). Furthermore circulating miR-133a levels positively correlated with plasma cTnI.
Conclusions: Our findings confirm increased levels of cardio-enriched miRNAs in the blood of AMI patients. Circulating miR-133a and miR-1 may be novel biomarkers for acute myocardial infarction and may have a potential as diagnostic tools, enabling an earlier diagnosis of cardiac damage.
Ahlin F, Arfvidsson J, Vargas KG, et al. MicroRNAs as circulating biomarkers in acute coronary syndromes: A review. Vascul Pharmacol 2016;81:15-21.

P154
ATTIVITA' DI CATECOL-O-METILTRANSFERASI DURANTE EVENTI APNEICI IN PAZIENTI CON SINDROME DELLE APNEE OSTRUTTIVE NEL SONNO

L. Brugnolo[1], C. Artusi[1], G. Ceolotto[2], V. Bisogni[2], M. Pengo[2], G. Maiolino[2], G. Rossi[2], M. Plebani[1]

[1]U.O.C. Medicina di Laboratorio, Azienda Ospedaliera-Università degli Studi, Padova
[2]Dipartimento di Medicina, Azienda Ospedaliera-Università degli Studi, Padova

Introduzione: La Sindrome delle Apnee Ostruttive nel Sonno (OSAS) è uno dei più diffusi disturbi del sonno caratterizzata da ricorrenti episodi di ostruzione totale o parziale delle vie aeree superiori. E' un fattore di rischio per lo sviluppo di eventi cardiovascolari e ipertensione. In questi pazienti, l'ipossia intermittente induce iperattivazione del sistema simpatico che aumenta pressione arteriosa e frequenza cardiaca. Catecol-O-metiltransferasi (COMT) è un enzima chiave nel catabolismo delle catecolamine, i principali neurotrasmettitori del sistema nervoso simpatico.
Obiettivo: Sviluppare un metodo HPLC-ECD per misurare in vivo l'attività eritrocitaria della COMT in pazienti con OSAS durante Drug Induced Sleep Endoscopy (DISE) attraverso la determinazione dell'acido vanillico e dell'acido isovanillico prodotti dell'attività di COMT a partire da una catecolamina sintetica: acido diidrossibenzoico (DHBAC).
Materiali e metodi: Sono stati arruolati 8 pazienti ipertesi affetti da OSAS. Durante DISE, sono stati effettuati a tempi prestabiliti i prelievi ematici (K2-EDTA) per il dosaggio dell'attività di COMT. La validazione del metodo HPLC-ECD consiste in prove di linearità, stabilità e imprecisione.
Risultati: Il metodo è lineare nell'intervallo di concentrazioni di acido vanillico e isovanillico valutato (0.05-2 μM, r^2> 0.998); l'imprecisione calcolata su due campioni a diversa attività enzimatica (62 e 181 pmol/min/mg prot) risulta essere rispettivamente del 7.4% e 13.0%. La stabilità dei prodotti di reazione è stata misurata in diversi momenti in un intervallo di 30 giorni e la loro concentrazione (vanillico 0.77μM, isovanillico 0.13μM) non risulta significativamente modificata. Rispetto all'attività enzimatica misurata nel preoperatorio (100±14 pmol/min/mg prot) si riscontrano due picchi: il primo in corrispondenza dell'apnea centrale con un aumento fino a 169±24 pmol/min/mg prot (p <0.01) e il secondo due minuti dopo l'apnea ostruttiva con un aumento fino a 140±13 pmol/min/mg prot.
Conclusioni: In questo primo studio in pazienti OSAS sottoposti a DISE il metodo sviluppato e validato permette di misurare l'attività della COMT negli eritrociti e di associarla ad eventi di apnea, indicando così un possibile ruolo di questo enzima in eventi cardiovascolari.

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RISCHIO GENETICO E RISCHIO TRADIZIONALE IN PAZIENTI CON SINDROME CORONARICA ACUTA

R. Rolla[1], A. Lupi[2], P. Pergolini[1], A. Appiani[1], G. Bauce[1], A. Rognoni[2], L. Rossi[2], R. Rosso[2], A.S. Bongo[2], G. Bellomo[1]

[1]Department of Health Sciences, Amedeo Avogadro University of Eastern Piedmont, Clinical Chemistry Laboratory, Maggiore della Carità Hospital, Novara
[2]Hospital Cardiology, "Maggiore della Carità" Hospital, Novara

La malattia coronarica aterosclerotica (CAD) e le sue manifestazioni cliniche sono tra le principali cause di morbilità e mortalità nel mondo occidentale ma non tutti i casi clinici sono riconducibili ai tradizionali fattori di rischio cardiovascolare (FRCV).
Lo scopo del nostro studio è stato paragonare, in pazienti con sindrome coronarica acuta (SCA), il rischio CV (RCV) ottenuto dall'analisi dei FRCV tradizionali (FRCVT) con il RCV predetto attraverso l'analisi genetica di 11 polimorfismi (1) risultati associati alla CAD in studi "genome-wide association" (1).
71 pazienti consecutivi ricoverati presso l'Unità Coronarica per SCA sono stati sottoposti all'ingresso ad un prelievo di sangue intero/EDTA per la ricerca di 11 single nucleotide polymorphism (SNP) notoriamente correlati ad un aumentato RCV indipendentemente dai FRCVT (Cardio-kit, NLM AC097, Nuclear Laser Medicine S.r.l., Milano, Italy) ed è stato calcolato il rischio di eventi CV a 5 anni mediante un software dedicato (# race, https://www.fondazioneinuit.it/race). In questi pazienti il rischio predetto geneticamente è stato paragonato al rischio predetto impiegando i FRCVT.
La prevalenza di soggetti considerati a RCV medio (RCV a 5 anni fra 20-e 30%) e severo (RCV a 5 anni >30%) stimata con l'analisi dei FRCVT (software # race) è rispettivamente del 16% (11 pz su 71) e del 13% (9 pz su 71), mentre quella ricavata dall'analisi genetica è rispettivamente del 37% (26/71) e del 32% (23/71). Da tali dati emerge come il paziente con SCA abbia un profilo di RCV elevato in una minima parte di soggetti se calcolato secondo i FRCVT, mentre l'analisi genetica permette di riclassificare a rischio medio/severo un numero di soggetti significativamente più elevato (P <0.0001).
In una popolazione di pazienti con SCA, la prevalenza di pazienti con RCV elevato predetto dai FRCVT risulta significativamente più bassa di quella calcolata sulla base dell'analisi genetica (Cardio-kit). Un impiego in prevenzione primaria di questo nuovo test genetico potrebbe predire l'occorrenza di un numero significativamente più elevato di eventi CV avversi.
1. Schunkert H, Erdmann J, Samani NJ. Genetics of myocardial infarction: a progress report. Eur Heart J 2010;31:918-25.

P156
CHEST PAIN AND TROPONIN I ASSAY IN EMERGENCY ROOM

R. Morgagni[1], M. Perrone[1], A. Intorcia [1], M. Pieri[2], S. Bernardini[2], F. Romeo[1]

[1]U.O.C. di Cardiologia e Cardiologia Interventistica, Policlinico Tor Vergata, Roma
[2]Biochimica Clinica, Policlinico Tor Vergata, Roma

Background: Acute coronary syndromes (ACS) are classified in two groups: ST elevation myocardial infarction (STEMI) and non ST myocardial infarction (NSTEMI)/unstable angina (UA). Diagnosis and classification are based on the presence of two of the following criteria: 1) chest pain 2) ST-T abnormalities on ECG 3) time variations of cardiac markers concentrations. European Society of Cardiology (ESC), American College of Cardiology (ACC), American Heart Association (AHA) guidelines on ACS and myocardial infarction defines an elevated troponin level as a measurement exceeding the 99th percentile of a reference control group with an imprecision ≤10% coefficient of variation (CV). The new cardiac Troponins assays can detect very small elevations until now undetectable: sensitivity for detecting Acute Coronary Syndromes (ACS) including MI has thus been improved, moreover some non cardiac conditions may now also cause an elevated Tn level. Aim of the study: appropriateness of the Tn I request.
Materials and Methods: We have evaluated Tn I values (analytical sensitivity: 0.015 ng/ml, normal range: 0-0.045 ng/ml) during one month (August 2015), in 450 patients presenting to our emergency room with typical or atypical chest pain. All patients underwent a complete cardiological evaluation, ECG, exercise test or cardiac CT. Patient at high risk with tipical chest pain were sent to our cathlab (63 on 321). All STEMI were sent to the cathlab without waiting for Tn I results.
Results: Among 450 patients 321 (72%) showed Tn I values under 99th percentile, 129 (28%) showed pathological values. Of the latter only 15 (3%), were admitted for ACS (STEMI and NSTEMI).
Conclusions: These findings require a re-evaluation of the current cut-off values of Tn I and a greater request appropriateness in order to reduce the number of false positives and non-coronary-related myocardial injury. The detection of elevated cTn levels alone is not sufficient for a diagnosis of acute MI, and requires an in-depth assessment of clinical presentation to determine the source and severity of myocardial damage.

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TROPONINA CARDIACA I IN PRONTO SOCCORSO: CONFRONTO TRA POCT AQT90 FLEX E ANALIZZATORE DIMENSION VISTA

M.M. Mion[1], A. Casarotti[1], G. Bragato[1], M. Zaninotto[1], G. Vettore[2], F. Tosato[2], M. Plebani[1]

[1]U.O.C. Medicina di Laboratorio, Azienda Ospedaliera-Università di Padova, Padova
[2]Pronto Soccorso Centrale, Azienda Ospedaliera di Padova, Padova

Scopo: La troponina cardiaca (cTnI o T) rappresenta il marcatore di scelta nella diagnosi di infarto miocardico acuto (IMA). Sono state valutate l'imprecisione analitica di AQT90 FLEX e l'efficienza diagnostica di AQT90 FLEX in confronto all'analizzatore Dimension Vista. Metodi: Studio dell'imprecisione (1): è stato condotto impiegando n=2 lotti su n=3 livelli di CQI. Confronto tra metodi (2): sono stati confrontati i risultati ottenuti con i due sistemi analitici sui campioni dello studio. Studio clinico (3): dal 15/09 al 16/12/2014 sono stati arruolati n=786 pazienti (pz) in pronto soccorso (PS) con dolore toracico e sospetto di sindrome coronarica acuta (SCA). La determinazione della cTnI (μg/L) é stata condotta all'ammissione in PS e dopo 3 e/o 6 ore utilizzando AQT90 FLEX (sangue intero-EDTAK2) e Dimension Vista (plasma-litio-eparina). L'accuratezza diagnostica è stata valutata con l'analisi delle curve ROC in base alle diagnosi di dimissione.
Risultati: (1): range-concentrazioni: 0.033-1.260; range-CV%=2.81-7.56%. (2): correlazione statisticamente significativa (regressione lineare, R2=0.90, p <0,0001, range di concentrazioni AQT90 FLEX 0.00-6.10 μg/L; Dimension Vista 0.00-82.00 μg/L); bias negativo statisticamente significativo (Bias:-0.234; 95%CI:-0.422/-0.046, p=0.0150); differenza significativa tra le concentrazioni medie (Wilcoxon test, p <0,0001). (3): l'analisi delle curve ROC ha evidenziato prestazioni diagnostiche confrontabili tra AQT90 FLEX e Dimension Vista risultando non statisticamente significativa la differenza tra AUC sia per la diagnosi di SCA (p=0.1589) che di IMA (p=0.9319); per AQT90 FLEX la concentrazione di cTnI associata alla migliore sensibilità e specificità (0.79 e 0.93) per la diagnosi di IMA é risultata 0.014 μg/L, inferiore a quella dichiarata dal produttore (0.023, CV<10%).
Conclusioni: L'Imprecisione osservata é risultata confrontabile a quella dichiarata dal produttore. Entrambi i metodi hanno dimostrato un'elevata sensibilità e specificità per la diagnosi di IMA e SCA. La valutazione dell'imprecisione al cut-off della curva ROC (0.014 μg/L) consentirà di verificare se a questa concentrazione, la cTnI AQT90 FLEX potrà essere classificata come metodo "guideline accetable" (CV <10%) o "clinically usable" (CV <20%).
Apple FS. Clin Chem 2009;55:1303-6.

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UTILIZZO DI UN NUOVO METODO AD ELEVATA SENSIBILITA' PER LA DETERMINAZIONE DELLA TROPONINA CARDIACA IN PRONTO SOCCORSO: RISULTATI ATTESI?

M.M. Mion[1], G. Bragato[1], S. Vigolo[2], M. Zaninotto[1], G. Vettore[2], F. Tosato[2], M. Plebani[1]

[1]U.O.C. Medicina di Laboratorio, Azienda Ospedaliera-Università di Padova, Padova
[2]Pronto Soccorso Centrale, Azienda Ospedaliera di Padova, Padova

Scopo. Sono state valutate le prestazioni diagnostiche di un metodo ad elevata sensibilità per la misura della troponina cardiaca I (cTnI) (Architect STAT high sensitive troponin I, Abbott=metodo A, ng/L) in confronto a quelle del metodo in uso in urgenza fino al 21/12/2015 (Dimension troponin I, Siemens=metodo B, ug/L). Metodi. A vs B: LOD=2 vs 0.017; livello decisionale per danno miocardico (99° percentile=cut-off) = 16 (F) o 34 (M) vs 0.045. Nel periodo 15-21/09/2015 sono state determinate le concentrazioni di cTnI con i metodi A e B in pazienti (pz) afferenti al Pronto Soccorso (PS) con richiesta di determinazione di cTnI. Risultati. Pz arruolati n=135 M (51%)+132 F (49%); età (media, range)=71, 15-98 anni; 4 gruppi (GR) (cTnI >cut-off=POS; cTnI <cut-off=NEG): A vs B: GR1=NEG/NEG=210 (79%); GR2=POS/POS=32 (12%); GR3=POS/NEG=5 (2%); GR4=NEG/POS=20 (7%). Concordanza (GR1+2) vs discordanza (GR3+4) tra i metodi A e B: 91% vs 9%. In GR3 (3M+2F) le concentrazioni di cTnI sono risultate prossime al livello decisionale sia col metodo A (8-34) che col metodo B (0.047-0.099); in GR4 (4M+16F) 11 pz sono stati ricoverati (A vs B range=16-427 vs 0.020-0.044) e 9 dimessi (A vs B range=16-74 vs <0.017-0.044). Confronto gennaio+febbraio 2015 vs 2016: accessi per dolore toracico in PS (1289 vs 1230); esito accessi per dolore toracico in PS (ricoveri + dimissioni = 300+962 vs 280+937); tempo di permanenza in PS (ore) (dimessi: 6.3 vs 5.1, ricoverati: 4.7 vs 4.6); follow-up dei pz con dolore toracico a 30 gg: nessun pz dimesso è rientrato in PS per un evento cardiovascolare acuto/ episodio di dolore toracico. Conclusioni. Il metodo A è risultato: efficace quanto il metodo B nell'identificazione dei pazienti con SCA/IMA o danno miocardico e nella stratificazione del rischio dei pz con dolore toracico; maggiormente efficace nell'identificazione precoce del danno miocardico; maggiormente sensibile, con una cinetica d'incremento che consente un più rapido rule out dei pz con dolore toracico a basso/intermedio rischio di SCA/IMA. Il cut-off differenziato per sesso sembra rendere questo biomarcatore più sensibile. Sarà utile in futuro valutare l'ulteriore adeguamento dei cut-off per età.
Thygesen K, Alpert JS, Jaffe AS, et al. Third universal definition of myocardial infarction. Eur Hearth J 2012;33:2551-67.

P159
"ULTRA" SENSITIVE TROPONIN I: CHEST PAIN ALGORHITM IN EMERGENCY CARE UNIT

D. Cosseddu[1], P. Balzaretti[2], S. Bongioanni[3], L. Erroi[1], B. Montaruli[1], M. Giacometti[4], A. Vitale[4], M.R. Conte[3], A. Cottino[2], M. Migliardi[1]

[1]*SC Laboratorio Analisi, AO Ordine Mauriziano, Torino*
[2]*SC Medicina d'Urgenza, AO Ordine Mauriziano, Torino*
[3]*SC Cardiologia, AO Ordine Mauriziano, Torino*
[4]*SC Direzione Medica di Presidio, AO Ordine Mauriziano, Torino*

Background: Introduction of high-sensitivity cardiac troponin (hs-cTnI) assays in the early diagnosis of acute myocardial infarction (AMI) has led to a revision of rule-in and rule-out patients' algorithms of AMI in Emergency Care Units. Aim of our study was to develop an algorithm for the chest pain management in our hospital.
Methods: In November 2015 we introduced Abbott Architect immunoassay for the measurement of hs-cTnI in our hospital with sex specific diagnostic thresholds: men 34 ng/L and women 16 ng/L (manufacturer's 99th percentiles).
Discussion: As reported in literature Architect hs-cTnI has a limit of detection (LOD) of 1.1-1.9 ng/L, a limit of quantification (LOQ) of 4.2 ng/L and allows measurement of cardiac troponin in the majority of apparently illness-free individuals (more than 95%). Clinicians and laboratorists collaboration gave us the possibility to develop an algorithm using hs-cTnI measurement for early rule out and rule in of AMI in our hospital.
In brief, for rule-out of AMI the criteria were defined as either a baseline hs-cTnI level of less than 2 ng/L (indicative for LOD) and blood drawn was beyond 6 hours from onset of chest pain or baseline hs-cTnI less than 2 ng/L or less than normal sex values, chest pain onset less than 6 hours and absolute change within 3 hours of less than 5 ng/L. For rule-in of AMI, the criteria were defined as either baseline hs-cTnI above normal sex values and absolute change within 3 hours of more than 20% or baseline hs-cTnI 5 times above normal sex values (170 ng/L for men and 80 ng/L for women). Patients fulfilling neither the above criterion for rule-out or rule-in were classified in a third group called "observational zone" in order to exclude other clinical causes. In this group were included: patients with baseline hs-cTnI less than 2 ng/L or less than normal sex values, chest pain onset less than 6 hours and absolute change within 3 hours of more than 5 ng/L; patients with baseline hs-cTnI above normal sex values, absolute change whitin 3 hours of less than 20%.

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IMPACT OF THE INTRODUCTION OF A HIGH-SENSITIVITY TROPONIN ASSAY ON THE MANAGEMENT OF PATIENTS WITH CHEST PAIN IN THE EMERGENCY DEPARTMENT

A. Garnero [1], P.L. Balzaretti[2], C. Berlingò[1], L. Erroi[3], D. Cosseddu[3], M. Migliardi[3], A. Cottino[2]

[1]*School of Medicine, University of Turin*
[2]*Emergency Department, Ordine Mauriziano Hospital, Turin*
[3]*Laboratory Medicine Department, Ordine Mauriziano Hospital, Turin*

The new cardiac troponin assays are characterized by increased sensitivity but reduced specificity, which may contribute to an increase in false positive results. In this study we aimed to evaluate the impact of their adoption on the management patients with chest pain, in terms of use of resources and length of stay in the Emergency Department (ED).
The study was retrospective with a "before-after" design. Consecutive adult patients presenting to the ED of the Ordine Mauriziano Hospital in Turin complaining of chest pain with at least one assay of cardiac troponin I performed were included, before and after the introduction of a highly sensitive assay (hs-Tn). Patients with a diagnosis of myocardial infarction with ST-segment elevation or with only one cardiac troponin ordered (followed by direct discharge) were excluded.
On 11/02/15, the method ST AIA-PACK cTnI 3rd Gen (Tosoh) was replaced by the high sensitivity assay Abbott Architect STAT hs-cTnI. Complying with international guidelines (1), the time interval between serial determinations was reduced from 6 to 3 hours. The primary outcome was the length of stay in ED; secondary outcomes were rates of NSTEMI diagnoses and use of procedures.
Charts regarding 1432 ED visits occurred between 10/11/14 and 05/25/15 were screened obtaining the 405 visits forming the study population (202 in pre-adoption and 203 in the post-adoption group). The clinical characteristics were similar between the two groups except for higher prevalence of hypertension in the post-adoption group. The median ED length of stay was 539 minutes (IIQ 2.5% - 97.5% 123-2375) in the pre-adoption group and 457 minutes (IIQ 2.5% - 97.5% 123-3778) in the post-adoption group (p=0.039). There were no statistically significant differences in the rates of NSTEMI diagnoses (13,37% [95% C.I. 8,68 - 18,06] in pre-switching vs. 13,79% [95% C.I. 9,05 – 18,54] in the post-switching group) and use of provocative testing, emergency coronary angiography and myocardial revascularization.
The adoption of hs-Tn is associated with a reduced length of stay in the ED without significant changes in the use of diagnostic resources or NSTEMI diagnoses in patients with chest pain at high risk of acute coronary syndrome.
1. Roffi M, et al. Eur Heart J 2015;36:2921.

P161

BENEFICIAL EFFECT OF HELPER-DEPENDENT ADENOVIRAL VECTORS EXPRESSING AN LDL RECEPTOR/TRANSFERRIN CHIMERIC PROTEIN IN MOUSE MODEL OF FAMILIAL HYPERCHOLESTEROLEMIA

E. Leggiero[1], G. Labruna[2], L. Iaffaldano[1], J. Chiaro[3], L. Sacchetti[1], L. Pastore[1]

[1]*CEINGE-Biotecnologie Avanzate, Napoli*

[2]*IRCCS SDN, Istituto di Ricerca Diagnostica e Nucleare, Napoli*

[3]*Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università di Napoli Federico II, Napoli*

Familial hypercholesterolemia (FH) is a well-characterized genetic hyperlipidemia due in most of the cases to mutations in the LDL receptor (LDLR) gene; FH is characterized by elevated concentration of plasma LDL cholesterol (LDL-C) with consequent deposition of LDL-C in tendons, skin and arteries. Statins can lower cholesterol levels but are not effective in all patients whose prognosis is still quite poor. In the past, we have developed safe and effective gene-therapy strategies for hepatocytes transduction and consequent expression of anti-atherogenic proteins using PEGylated helper-dependent adenoviral (HD-Ad) vectors. We have recently devised a therapeutic strategy for reducing LDL using a secreted protein that can potentially be expressed in non-hepatic tissues used as bioreactors. At this aim, we developed an HD-Ad vector for the expression of the soluble form of the extracellular portion of the human LDLR fused with transferrin (LDLR/Tf). We evaluated the efficacy of LDLR/Tf in cellular models such as CHOldla7 in which we restored the cell ability to uptake of labeled LDL; subsequently, we administered intravenously 1X10E11 vp/kg of the HD-Ad vector expressing LDLR/Tf in LDLR-deficient mice and demonstrated the efficacy of the above-mentioned vector in reducing total and LDL cholesterol levels; in addition, expression of LDLR/Tf significantly reduced aortic atherosclerotic lesions in treated LDLR-deficient mice compared to controls. We therefore demonstrated the efficacy of serum secretion of LDLR/Tf in reducing aortic atherosclerosis in FH mice. Moreover, we administered in VivoTag 750S-Labeled LDL to study biodistribution in LDLR-deficient mice treated with either HD-AdLDLR/Tf or control vector and we found a strong signal in the heart, liver and intestin after Fluorescence molecular tomography analysis. These results will allow evaluation of HD-Ad vector-mediated expression of LDLR/Tf in non-hepatic tissues using alternative routes of administration in order to develop safer gene transfer protocol more compatible with clinical applications.

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GALECTIN 3 IS RELEASED AFTER ACUTE MYOCARDIAL INFARCTION AND IT IS CORRELATED TO hsTnI ON ADMISSION

C. Bellia[1], B. Lo Sasso[1], G. Bivona[1], C. Scazzone[1], L. Agnello[1], A. Pivetti[1], C. Galli[3], G. Novo[4], M. Ciaccio[1]

[1]*Sezione Biochimica Clinica e Medicina Molecolare, Dipartimento di Biopatologia e Biotecnologie Mediche, Università degli Studi di Palermo*

[2]*U.O.C. Medicina di Laboratorio – Corelab, AOUP Policlinico P. Giaccone, Palermo*

[3]*Medical Scientific Liaison Europe, Abbott Diagnostics, Roma*

[4]*Cattedra di Cardiologia, AOUP Policlinico P. Giaccone, Palermo*

Introduction: Galectin 3 has been proposed as a marker of fibrosis in heart failure. Some evidences showed that both Galectin-3 mRNA and protein are specifically expressed in left ventricle after ischemic injury in mice (1), and that it participates in the AMI initial stage repairing process regulating macrophage infiltration and fibrosis in the AMI area (2). Nevertheless, the plasma levels of Galectin 3 have not been explored yet in relation to specific myocardial tissue damage due to ischemia in humans.
The aim of the study was to evaluate the kinetics of Galectin-3 release in plasma after AMI and its association to hsTnI.
Methods: two hundred and fifteen consecutive patients admitted to “P. Giaccone” hospital for AMI were included in the study. Complete medical history and a blood sample were collected for each patients on admission. A second blood sample was collected after 4.5 ± 0.8 days (T5). Galectin-3 and hsTnI were measured by Architect i-100SR (Abbott).
Results: The mean age of patients included in the study was 65.4 ± 13.8 years, 74% were males and 58% had a STEMI. Galectin-3 was correlated to hsTnI on admission ($r=0.2$; $P<0.001$). At T5, Galectin-3 level decreased with respect to basal sample (18 [14.2 – 25] ng/ml vs 16.8 [12.7 – 23.4] ng/ml; $P=0.006$) and was no more correlated to hsTn ($r=0.06$; $P=0.40$). Interestingly, Galectin 3 on admission was correlated to eGFR, evaluated by the CKD-EPI formula ($r=-0.25$; $P<0.001$).
Discussion: Galectin 3 is released very early after an ischemic injury and it decreased after about 5 days from the ischemic event. The correlation of Galectin-3 to hsTn on admission demonstrated that the release of Galectin 3 during the ischemic injury is linked specifically to the ischemic event. The lack of correlation at T5 suggests that the two markers are released with different kinetics.
1. Hashmi S, Al-Salam S. Galectin-3 is expressed in the myocardium very early post-myocardial infarction. Cardiovascular pathology 2015;24:213-23.
2. Gonzalez GE, et al. Galectin-3 is essential for early wound healing and ventricular remodeling after myocardial infarction in mice. Int J Cardiol 2014;176:1423-5.

P163
CORRELATION BETWEEN ST2 AND GALECTIN 3 IN PATIENTS WITH HEART FAILURE

G. Bivona[1], B. Lo Sasso[1], C. Bellia[1], S. Milano[2], A. Pivetti[1], L. Agnello[1], C. Scazzone[1], S. Novo[3], M. Ciaccio[1]

[1]Sezione Biochimica Clinica e Medicina Molecolare, Dipartimento di Biopatologia e Biotecnologie Mediche, Università degli Studi di Palermo
[2]U.O.C. Medicina di Laboratorio – Corelab, AOUP Policlinico P. Giaccone, Palermo
[3]Cattedra di Cardiologia, AOUP Policlinico P. Giaccone, Palermo

Introduction: soluble ST2, an IL-33 receptor family member and Galectin 3, a β- galactoside binding-lectin, are known to play a role in remodeling process and fibrosis at several sites. The physiopathology of Heart Failure (HF) is characterized by remodeling processes and fibrosis. ST2 and Gal3 are deemed to be promising biomarkers in HF, for having a good performance in predicting major cardiac events in these patients; however, even if many evidences are available about Gal 3 in HF patients, ST2 has been less investigated. The aim of this study is to analyze ST2 levels and that of Galectin 3 in HF patients.
Methods: 27 HF patients admitted to Cardiology Unit of Palermo School of Medicine were enrolled; those reporting inflammatory pathologies or recent surgery were excluded to avoid interferences on Galectin 3 levels. Serum ST2 was determined by ELISA (Critical Diagnostics Presage ST2 Assay); serum Galectin-3 was measured by Architect i-100SR (Abbott).
Results: our data show a significant correlation between ST2 concentrations and Galectin-3 levels (r=0.63; P=0.003); moreover, by linear regression analysis ST2 was found to predict those of Galectin 3 (r^2=0.4; P=0.003). An association with male gender was also reported given the higher levels of ST2 in comparison to women (75.1 ± 46.9 ng/ml vs 38.8 ± 24.4 ng/ml; P=0.043). No significant associations between ST2 and hypertension, ejection fraction (EF) and glomerular filtrate rate (GFR) were found.
Discussion: although the sample size is limited, our preliminary results showed ST2 to be a potential reliable biomarker for HF, as well as Galectin 3. Failure in correlating ST2 to other clinical variables may refer to the small sample size and need further investigations.

P164
miR-21 AND miR-375 IN URINARY PELLET AS BIOMARKERS FOR PROSTATE CANCER DETECTION AND PROGNOSIS

X. Filella[1], L. Foj[1], F. Ferrer[2], M. Serra[3], A. Arevalo[3], M. Gavagnach[3], N. Giménez[4]

[1]Dep Biochemistry and Molecular Genetics, CDB, Hospital Clínic, IDIBAPS, Barcelona
[2]Dep. Radiotherapy, Institut Català d'Oncologia, L'Hospitalet de Llobregat
[3]CAP Valldoreix, Sant Cugat del Vallès, Mútua de Terrassa
[4]Unity of Research, Fundació de Recerca, Mútua de Terrassa

miRNAs are non coding RNA involved in the post-transcriptional regulation of gene expression. Cumulating data suggest miRNAs as new promising biomarkers for prostate cancer (PCa) (1). In this study we analyzed 5 miRNAs in urine to explore their potential as non-invasive biomarkers for PCa. Urine samples were collected after prostate massage in 10 healthy males and in 38 patients with non treated PCa. These patients were classified according to the D'Amico risk criteria: 3 patients with low risk PCa (cT1–cT2a, Gleason < 7 and PSA ≤ 10 μg/L), 17 with intermediate risk PCa (cT2b or Gleason = 7 or PSA 10-20 μg/L) and 18 with high risk PCa (cT2c or PSA > 20 μg/L or Gleason >7). Urine samples were centrifuged at 2000g, 20 min. RNA was isolated using the miRNeasy reagent (Qiagen). RNA concentration was measured with nanodrop ND1000. We studied the expression of 5 miRNAs (miR-21-5p, miR-375, miR-141-3p, let-7c-5p, miR-214) (TaqMan® miRNA assays) by qRT-PCR (AbiPrism 7300) after preamplificacion (TaqMan® Preamp Mastermix). The results were normalized using cel-miR-39. Relative expression was calculated for every miRNA by the method 2-##Ct. We found significant differences in the overexpression of miR-21 and miR-375 (p=0.002 and p=0.003, respectively) comparing healthy subjects and PCa patients. The mean relative expression for these miRNAs comparing both groups was 6.39 and 3.23, respectively. No significant differences were found for the other biomarkers, although differences were near to statistical significance for miR-141 (p=0.059). We found significant differences for miR-21 (p=0.017) and miR-375 (p=0.02) comparing PCa high risk PCa patients with low/intermediate risk PCa patients according to D'Amico criteria. When we compared PCa patients according to Gleason score (6, 7, >7) we found that miR-375 was overexpressed in Gleason score >7 (p=0.026), while miR-21 was overexpressed (p=0.046) in advanced tumors when patients were compared according to clinical stage (t1-t2a versus t2b-t3). These preliminary results showed that miR-21 and miR-375 in the urine obtained after prostate massage can be useful biomarkers for the detection and prognosis of PCa.
1. Filella X, Foj L. miRNAs as novel biomarkers in the management of prostate cancer. Clin Chem Lab Med 2016 Jan 9. doi: 10.1515/cclm-2015-1073. [Epub ahead of print]

P165

MicroRNAs IN SERUM MICROVESICLES AS NON-INVASIVE POTENTIAL BIOMARKERS FOR THE DIAGNOSIS AND PROGNOSIS OF GLIOBLASTOMA

A. Santangelo[1], P. Imbrucè[2], S. Munari[1], P. Prandini[1], L. Provezza[1], A. Tamanini[1], F. Sala[2], G. Pinna[2], M. Meglio[2], A. Scarpa[3], G. Lippi[4], G. Cabrini[1], M.C. Dechecchi[1]

[1]Lab. of Molecular Pathology, Dept of Pathology and Diagnostics, University Hospital of Verona, Verona

[2]Inst.of Neurosurgery; Dept of Neurosciences, University Hospital of Verona; Dept of Neurosciences, Biomedicine and Movement Sciences, Univ. of Verona

[3]ARC-NET University and Hospital Trust, Verona

[4]Lab. of Molecular Pathology, Dept of Pathology and Diagnostics, University Hospital of Verona; Section of Clinical Biochemistry, Univ. of Verona

Glioblastoma multiforme (GBM) is the most aggressive primary brain tumor, discovered by imaging and confirmed by pathology, after surgical resection. Considering that the new (2016) WHO classification of CNS tumors proposes an integrated diagnostic approach combining histologic and molecular features, the identification of relevant diagnostic, prognostic and predictive biomarkers is a compelling need. Blood-derived biomarkers would hence appear particularly useful as minimally invasive indices to refine classification and improving prediction of therapy response and outcome. However, unlike other tumors, circulating biomarkers for GBM are in their infancy and not yet used in clinical practice. Primary cells obtained from GBM and cultured in vitro actively release microvesicles, which provide a rich source of selectively packaged molecules, including microRNAs (miRNAs), small non coding RNA that are relevant tumor regulators (Skog J, 2008; Westphal M and Lamszus K, 2015). This study (MiR2015, #44535, 29/09/2015) was aimed to develop a non-invasive, clinically viable biomarker assay, based on circulating miRNA analysis to support diagnosis and enabling monitoring of tumor growth and responsiveness to treatment in patients with GBM. Exosomes were purified by "ExoQuick-TC" exosome precipitation solution from serum obtained from 27 healthy controls and 40 patients with GBM both before and after surgery. The expression of miR-21, 222 and 124-3p in circulating exosomes was analyzed with TaqMan probes and normalized to snRNAU6. The expression levels of miR-21, miR-222 and miR-124-3p in exosomes isolated from serum of patients with GBM were significantly higher than in healthy controls, thus indicating that exosomes are enriched sources of biomarkers playing an important role in glioma diagnosis. Notably, their expression was found to be significantly decreased in serum exosomes obtained after surgery, which strongly suggests that they may derive from dissemination of microparticles from brain tumor to the blood stream. A longitudinal analysis during follow-up of patients affected by GBM will be essential to ascertain if these miRNAs will be ready for prime time for diagnosis, early detection of recurrence and prognostic assessment.

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EVALUATION OF DIAGNOSTIC PERFORMANCES OF FIBULIN-3 COMPARED TO MESOTHELIN IN PLEURAL EFFUSIONS OF MALIGNANT MESOTHELIOMA

M.G. Vivaldi[1], E. Battolla[1], P.A. Canessa[2], P. Ferro[3], M.C. Franceschini[3], A. Vigani[4], P. Dessanti[3], F. Fedeli[3], R. Cocilovo[1], G. Masini[3], R. Lanzoni[1], V. Fontana[5], M.P. Pistillo[6], S. Roncella[3]

[1]U.O. Patologia Clinica, Osp. S. Andrea, La Spezia

[2]U.O. Pneumologia, Osp. S. Bartolomeo, Sarzana (SP)

[3]U.O. Anatomia ed Istologia Patologica e Citodiagnostica, Osp. S. Andrea, La Spezia

[4]U.O. Oncologia, Osp. S. Andrea, La Spezia

[5]U.O. Epidemiologia e Biostatistica, IRCCS AOU San Martino-IST, Genova

[6]U.O. Epigenetica dei Tumori, IRCCS AOU San Martino-IST, Genova

Objective: Pleural effusion (PE) is often the primary manifestation of malignant pleural mesothelioma (MPM). However, PE is a common event in clinical practice being present in a large variety of neoplastic and benign diseases. Among the biomarkers, soluble mesothelin-related peptide (SMRP) and fibulin-3 (FBL-3) have received considerable attention. However, while the level of SMRP in serum and effusion has shown good performances and has been approved by the U.S.A Food and Drug Administration, the value of FBL-3 in the diagnosis and monitoring of MPM, has to be confirmed because conflicting data exist in the literature. In the present study, we compared the diagnostic performances of SMRP and FBL-3 detection in the same effusions taken at the time of diagnosis.

Methods: The study was performed in 33 MPM, 64 pleural benign lesions (BNG) and 23 non-MPM pleural metastases (MTS). The study was approved by the Liguria Regional Ethics Committee. The written informed consent was obtained from all patients. SMRP levels were detected by the ELISA Assay Kit "MesoMark" (Cis-Bio International Gif/Yvette; France) and FBL-3 was detected by the ELISA Assay Kit "Fibulin-3" (USCN Life Science Inc. Houston, Texas, USA) according to manufacturers' instructions. All pleural effusion samples were tested in duplicate. Statistical analyses were performed using Stata (StataCorp. Stata Statistical Software Release 11.2 Stata Corporation, College Station, TX, 2007).

Results: Patients with MPM were found to have higher SMRP-median levels in effusion than patients with other pathologies ($P < 0.001$). In contrast, the FBL-3 levels in MPM were similar to the levels in non-MPM patients ($P = 0.174$). The diagnostic accuracy of SMRP and FBL-3 biomarkers by using the ROC analysis showed that SMRP outperformed FBL-3. When MPM and MTS+BNG patients were compared, an AUC=0.79 ($P < 0.001$) was found in contrast to FBL-3 that showed an AUC=0.42 ($P = 0.889$).

Conclusions: Our results showed that FBLN-3 detection in PE was unable to discriminate MPM. In addition, FBL-3 does not add any information to the determination of the SMRP in effusion. However, at the moment, SMRP remains the best clinical marker for MPM PE diagnosis.

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BLOOD EXPRESSION OF MATRIX METALLOPROTEINASES 8 AND 9 AND OF THEIR INDUCERS S100A8 AND S100A9 SUPPORTS DIAGNOSIS AND PROGNOSIS OF PDAC-ASSOCIATED DIABETES MELLITUS

S. Moz[1], D. Basso[2], A. Padoan[1], D. Bozzato[1], P. Fogar[2], C. Zambon[1], M. Pelloso[1], C. Sperti[3], S. Vigili de Kreutzenberg[1], C. Pasquali[3], S. Pedrazzoli[4], A. Avogaro[1], M. Plebani[2]

[1]Department of Medicine - DIMED, University of Padova, Padova
[2]Department of Laboratory Medicine, University Hospital of Padova, Padova
[3]Department of Surgical, Oncological and Gastroenterological Sciences- DISCOG, University of Padova, Padova
[4]Associazione Wirsung-onlus, Padova

Background: Based on the knowledge that matrix metalloproteinases (MMPs) and S100A8/A9 synergistically work in causing PDAC-associated type 2 diabetes mellitus (T2DM), we verified whether tissue and blood MMP8, MMP9, S100A8 and S100A9 expression might help in distinguishing PDAC among diabetics.
Methods: Relative quantification of MMP8, MMP9, S100A8 and S100A9 mRNA was performed in tissues obtained from 8 PDAC, 4 chronic pancreatitis (ChrPa), 4 non-PDAC tumors and in PBMCs obtained from 30 controls, 43 T2DM, 41 ChrPa, 91 PDAC and 33 pancreatic-biliary tract tumors.
Results: T2DM was observed in PDAC (66%), in pancreatic-biliary tract tumors (64%) and in ChrPa (70%). In diabetics, with or without PDAC, MMP9 tissue expression was increased ($p<0.05$). Both MMPs increased in PDAC and MMP9 increased also in pancreatic-biliary tract tumors PBMCs. In diabetics, MMP9 was independently associated with PDAC ($p=0.025$), but failed to enhance CA 19-9 discriminant efficacy. A highly reduced S100A9 expression, found in 7 PDAC, was significantly correlated with a reduced overall survival ($p=0.015$).
Conclusions: an increased expression of tissue and blood MMP9 reflects the presence of PDAC-associated diabetes mellitus. This finding fits with the hypothesized role of MMPs as part of the complex network linking cancer to diabetes.

Huang H, Dong X, Kang MX, et al. Novel blood biomarkers of pancreatic cancer-associated diabetes mellitus identified by peripheral blood-based gene expression profiles. Am J Gastroenterol 2010;105:1661–9.

P168

DJ-1 CONCENTRATIONS IN SERUM OF PATIENTS AFFECTED BY ENDOMETRIAL CANCER

M. Benati[1], M. Montagnana[1], E. Danese[1], E. Paviati[1], O. Ruzzenente[1], S. Giudici[2], M. Franchi[2], G. Lippi[1]

[1]Dep. Neurosciences, Biomedicine and Movement Sciences, Clinical Biochemistry Section, University of Verona
[2]Dep. Surgical Sciences, Dentistry, Gynecology and Pediatrics University of Verona

Aim: The number of non-invasive diagnostic tool for early detection of endometrial cancer (EC) remains limited. To date, human epididymis protein 4 (HE4) has been intensively studied, but its diagnostic value in EC remains controversial. DJ-1 is an oncoprotein secreted by cancer cells and recently identified as a potential diagnostic biomarker for melanoma, breast and pancreatic cancers. The aim of this study was comparing the diagnostic performance of both DJ-1 and HE4 in EC.
Methods: 45 patients (63.9±12.0 years) with EC and 29 healthy controls (HC) (63.2±13.3 years) were enrolled. Serum concentration of DJ-1 and HE4 was measured by ELISA kits from R&D (Minneapolis, USA) and Fujirebio Diagnostic (Malvern, PA, USA), respectively. The differences between EC patients and HC were evaluated by with Mann-Whitney test and correlations were tested by Spearman's test. Diagnostic performance was evaluated by mean of receiver operating characteristics (ROC) curves analysis.
Results: DJ-1 concentrations was resulted significantly higher in EC patients than in HC (9533.6 vs. 1988.5, $p <0.0001$). The area under the receiver-operating curve (ROC-AUC) was 0.95 (95% CI: 0.91-0.99, $p <0.0001$). At the a cut-ff of 3654 pg/mL, the sensitivity and specificity were 88.9% and 89.7% respectively. HE4 serum levels were higher in EC patients than in HC (75.3 vs. 67.4 pmol/L, $p=0.019$). The AUC resulted 0.66 (95% CI: 0.54-0.78, $p <0.019$). No significant correlations were observed between values of HE4 and DJ-1 concentrations.
Conclusion: Serum DJ-1 levels are associated with EC and that could hence be considered a potentially useful biomarker for diagnosing of EC.

Morelli M, et al. DJ-1 in endometrial cancer: a possible biomarker to improve differential diagnosis between subtypes. Int J Gynecol Cancer 2014;24:649-58.

P169
HUMAN EPIDIDYMIS PROTEIN 4 E NUOVE PROSPETTIVE NELLA DIAGNOSI DEL TUMORE OVARICO

A. Pocognoli[1], M. Brugia[1], A. Ceka[1], G. Goteri[2], M. Galeazzi[1]

[1]Laboratorio di Biochimica Clinica e Microbiologia, Azienda Ospedali Riuniti, Ancona
[2]Anatomia Patologica, Azienda Ospedaliero-Universitaria Ospedali Riuniti, Ancona

Introduzione: Il carcinoma ovarico rappresenta nella popolazione femminile la prima patologia ginecologica oncologica nei paesi industrializzati. La sintomatologia, quando presente è aspecifica e complica spesso il processo diagnostico di questo tumore caratterizzato da elevata mortalità. Scopo del presente lavoro è stato quello di valutare il dosaggio di HE4 come utile marker nell'individuazione precoce dei tumori ovarici e nel suo utilizzo nelle donne con massa ovarica.
Materiali e metodi: Dal 1/1/2012 al 31/8/2015 sono state studiate in dettaglio 461 donne, 345 in pre-menopausa e 116 in post-menopausa, seguite presso gli ambulatori o i reparti di ginecologia dell'Azienda Ospedali Riuniti di Ancona. La diagnosi di carcinoma ovarico è stata posta dopo laparoscopia o intervento chirurgico a cielo aperto ed esame istologico, in accordo con i criteri proposti dalla International Federation of Gynecology and Obstetrics. La determinazione delle concentrazioni degli antigeni HE4 e CA125 è stata eseguita con dosaggio chemiluminescente (CMIA) su piattaforma ARCHITECT i1000 (Abbott Diagnostics). Il Risk of Ovarian Malignancy Algorithm (ROMA) è stato eseguito con software dedicato e considerato positivo lo score ≥7.5 per le donne in pre-menopausa e ≥25,3 per le donne in menopausa.
Risultati e conclusioni: Delle 345 donne in pre-menopausa studiate, solo 130 avevano ROMA positivo e di queste, 62 non sono state operate ma incluse in follow-up e 68 sono state sottoposte ad intervento chirurgico. Secondo il referto istologico, 54 avevano un tumore benigno e 14 un tumore maligno. Sono stati identificati 9 tumori ovarici di vario grado, 2 borderline e 3 uterini. Delle 116 donne in post-menopausa studiate, 31 avevano ROMA positivo e di queste 5 non sono state operate, 26 sono state sottoposte ad intervento chirurgico. 5 avevano un tumore benigno e 21 un tumore maligno. Sono stati identificati 16 tumori ovarici di vario grado, 2 borderline e 3 uterini. Nella casistica studiata si è dimostrato che la proteina HE4 già di per sé, ha un'efficacia diagnostica superiore al CA125 in quanto è risultata elevata nell'88,5% dei tumori maligni identificati contro il 71,4% del CA125. Il calcolo del ROMA ha invece permesso di identificare il 100% dei tumori maligni.

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MIRNA-135B CONTRIBUTES TO TRIPLE NEGATIVE BREAST CANCER MOLECULAR HETEROGENEITY: DIFFERENT EXPRESSION PROFILE IN BASAL-LIKE VERSUS NON-BASAL-LIKE

G. Pira[1], A. Angius [2], P. Uva[3], P. Cossu Rocca[4], F. Sanges[4], F. Loi [5], S. Ena[1], L. Murgia[4], C. Carru[1], M.R. Muroni[4], M.R. De Miglio[1]

[1]Dept. of Biomedical Sciences, Univ. of Sassari
[2]Istituto di Ricerca Genetica e Biomedica (IRGB), CNR, Monserrato (CA)
[3]CRS4, Science and Technology Park Polaris, Piscina Manna, Pula, CA
[4]Dept. of Clinical and Experimental Medicine, Univ. of Sassari
[5]Osservatorio Epidemiologico Veterinario Regionale, OEVR, Cagliari

Triple Negative Breast Cancer (TNBC), accounts for 12-24% of BC, are heterogeneous group of tumors with different clinical-pathologic features, genetic-molecular alterations which might require multiple therapeutic approaches. Distinct TNBC subtypes was identified by transcriptional analysis showing unique biology, including two basal-like, an immunomodulatory, a claudin-low-enriched-mesenchymal, a mesenchymal-stem-like, and a luminal-androgen-receptor subtypes. The potential clinical utility of assessing molecular TNBC subtypes was determined displaying different pCR rates after chemotherapy. Therefore, the treatment of TNBC patients has been demanding due to the heterogeneity of the disease and the absence of unambiguous molecular targets. The BC molecular biology has entered in the era of miRNAs, a class of endogenous small non-coding RNAs, which control complicated pathways, govern cell cycle, cell proliferation, differentiation, apoptosis, etc., whose deregulation contributes to the tumour development and correlates miRNAs functions with cancer. The aims of our study were to investigate miRNAs expression profiling in TNBC basal-like (BLBC) and non-Basal-like (QNBC), to study the molecular mechanisms of tumorigenesis between these TNBCs subcategories; to identify prognostic biomarkers and therapeutic strategies against a highly aggressive BC variants. 106 TNBCs were included in the study, characterized by ER, PR, and HER2 negativity, and proliferation-index from 6%-95% of neoplastic cells. IHC surrogate analysis was performed to define BLBC and QNBC using basal markers as EGFR and CK5/6. miRNAs differential expression in BLBC and QNBC was executed by TaqMan Low Density Array. SAM analysis showed miRNA-135b differentially expressed between BLBC and QNBC samples (p=0.011), these results were confirmed by qRT-PCR. Our study identifies for the first time a miRNA associated with specific molecular TNBC subtypes, contributing to define the complexity of TNBC category. Recently, miRNA-135b expression was found to be inversely correlated with ER and AR expression in BC; moreover, it regulates HIF1a and LATS2 mRNA involved in proliferation and growth cell pathways, indicating that miRNA-135b might represent a potential therapeutic target for TNBC.

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NGAL CAN BE USED AS PROGNOSTIC AND DIAGNOSTIC BIOMARKER IN HUMAN CANCER?

V. Pecoraro[1], L. Roli[2], T. Trenti[1]

[1]Laboratorio Tossicologia, Dipartimento interaziendale ad attività integrata Medicina di Laboratorio e Anatomia Patologica, Nuovo Ospedale Civile Sant'Agostino Estense, Modena

[2]Laboratorio Endocrinologia, Dipartimento interaziendale ad attività integrata Medicina di Laboratorio e Anatomia Patologica, Nuovo Ospedale Civile Sant'Agostino Estense, Modena

Background: Some studies have reported differentially altered NGAL levels in several malignancies. In cancer cells, NGAL function ranges from inhibiting apoptosis (thyroid), invasion and angiogenesis (pancreas), to increasing proliferation and metastasis (breast, colon)[1]. We evaluate NGAL, measured in plasma or urine, as both a prognostic and diagnostic marker for different types of human tumours.
Methods: We performed systematic electronic searches in Medline and Embase, as well as a list of reference literature for papers published before April 2016. Studies were included if they evaluated NGAL as a prognostic or diagnostic marker for human cancers. The selection of the studies, screening of the full texts and data extraction were conducted independently by two authors. We used the random effects models for the meta-analyses. All analyses were performed using Stata 11, Meta-Disc and RevMan5 software.
Results: We included 29 studies dedicated to colorectal, pancreas, breast, thyroid, gastric, kidney, endometrial, brain, liver, lung, oesophageal, oral and ovarian cancers. Our meta-analyses showed that, in patients with colorectal or breast cancer, positive NGAL expression was associated with a decrease of disease free survival (HR 2.27, 95%CI 1.54-3.36; HR 1.07, 95%CI 1.04-1.10, respectively). NGAL was a negative prognostic marker of overall survival in colorectal (HR 2.37, 95%CI 1.68-3.34) and endometrial (HR 4.38, 95%CI 1.9-10.12) cancers. Discriminative power of NGAL between cancer patients and control was moderate in colorectal cancer (AUC 0.6; pooled Sensitivity was 0.56; pooled Specificity was 0.72), acceptable in pancreatic cancer (AUC 0.8; pooled Sensitivity was 0.6; pooled Specificity was 0.8) and good in thyroid cancer (AUC 0.13; pooled Sensitivity was 0.85; pooled Specificity was 0.96).
Conclusions: NGAL is differently expressed in several human cancers. Its determination in plasma and urine could be useful in the prognosis of colorectal and breast cancer, but its prognostic accuracy remains uncertain for other human tumours.
1. Candido S, et al. Roles of neutrophil gelatinase-associated lipocalin (NGAL) in human cancer. Oncotarget 2014;5:1576-94.

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LIVELLI SIERICI DI GALECTINA-3 E CORRELAZIONE CON IMMAGINI CECT IN PAZIENTI AFFETTE DA CARCINOMA OVARICO.

M. Santulli[1], S. Tudini[2], L. Manganaro[3], S. Gigli[3], E. Anastasi[2], A. Angeloni[3]

[1]Dipartimento Medicina Sperimentale
[2]Dipartimento Medicina Molecolare
[3]Dipartimento Scienze Radiologiche Anatomo Patologiche e Oncologiche

Il carcinoma ovarico (CO) è la principale causa di morte per neoplasia ginecologica. La combinazione di indagini strumentali e valutazione dei biomarcatori è lo strumento migliore per la diagnosi. Studi recenti considerano la galectina -3 (Gal-3), una lectina legante la β-galattosidasi come un nuovo biomarcatore utile per diagnosi, prognosi e trattamento di alcune neoplasie. Obiettivo di questo studio è stato quello di a) valutare i livelli circolanti di Gal-3 in donne affette da CO alla diagnosi e durante il follow-up b) correlarli con le immagini di tomografia computerizzata di contrasto potenziato (CECT). Sono stati studiati i seguenti gruppi: 1) n.22 donne sane (età media 53 aa); 2) n.28 donne con recente diagnosi di CO (età media 68 aa); 3) n.15 donne in follow-up con CO ricorrente (età media 65 aa); 4) n.15 donne in follow-up con malattia stabile (età media 61 aa). Le concentrazioni sieriche di Gal-3 sono state determinate con metodo ELISA (BMG Galactin-3). Il valore soglia è stato considerato <95 ng/mL. Sono stati riscontrati livelli normali di Gal-3 in 22/22 (100%) delle donne del gruppo 1. Alti livelli di Gal-3 sono stati osservati in 22/28 (79%) delle donne del gruppo 2 (p <0,001). Lo studio di follow-up ha mostrato alti livelli di Gal-3 in 15/15 (100%) delle donne con CO ricorrente, mentre si è osservato un ritorno nell' intervallo di normalità in 15/15 (100%) delle donne del gruppo 4 (p <0,001). E' stata inoltre riscontrata nelle donne con CO ricorrente, mediante tecnica CECT, una correlazione statisticamente significativa tra alti livelli di Gal-3 e presenza di carcinomatosi 14/15 (93%), linfadenopatia 13/15 (87%) e metastasi 10/15 (67%) (p <0,001). I risultati di questo studio suggeriscono un ruolo della Gal-3 come potenziale indicatore di CO e di ripresa di malattia. Supponiamo che l'entità della sua espressione dipenda dall'invasività e dal potenziale metastatico della neoplasia. In futuro, il dosaggio della Gal-3 potrebbe completare il quadro analitico nella diagnosi, trattamento e prognosi del carcinoma ovarico.

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AUTOIMMUNE CONDITION OR EBV-RELATED CROSS-REACTIVITY? METHODOLOGICAL QUESTIONS AND CLINICAL IMPACT

R. Assandri[1], A. Montanelli[2], F. Maura[1], F.P. Leone[1], M. Monari[1]

[1]*Humanitas Clinical and Rersearch Center, Rozzano (Mi)*
[2]*Clinical Investigation Laboratory, Diagnostic Department, Spedali Civili, Brescia*

Background: Epstein-Barr virus (EBV) has been suspected of involvement in the pathogenesis of various chronic autoimmune diseases (AD). Previous experimental data directly link EBV to humoral autoimmunity, showing that common human antigenic targets, Sm and SSA/Ro60 KDa cross-react with sequences from the Epstein-Barr virus nuclear antigen. A recent approach to improve the sensitivity and specificity of assays that detect SS-A/Ro antibodies is to employ the technique of EBV vector-transfection. This approach has been utilized in the development of an indirect immunofluorescence (IIF) substrate that utilizes HEp-2 cells transfected with a full-length human 60 kDa SS-A/Ro cDNA (HEp-2000® ANA Test System, Immunoconcept).
Methods: We have evaluated the specificity of HEp-2000® ANA Test System by the use of consecutive patients sera (1460) with AD and high titer of EBV VCA IgG. The IIF Ro-60 positive sera (IIFRo60+) were confirmed by solid phase enzyme immunoassay (ELISA Orgentec) and Line-immuassay (LIA, Euroimmun).
Results: Of 630 sera (43.1%) with antinuclear antibodies (ANA), 48 of this samples were also positive for anti-SS-A/Ro antibodies. 43 samples were positive for anti-SS-A/Ro60 antibodies both in IIF-HEp-2000 and ELISA/LIA. 5 samples were considered positive only for anti-SS-A/Ro antibodies in IIF-HEp-2000. Clinical features or diagnoses of these patients included autoimmune hepatitis (AIH, 2/5), SLE (2/5) and liver disease (1/5).
Discussion: The pathogenesis of autoimmune diseases includes a combination of genetic factors and environmental exposures. EBV is a candidate in the pathogenesis of autoimmune disease because of its persistency throughout life in the host's B lymphocytes and its ability to alter the host's immune response. It's possible that five patients sera could recognized sequences of DNA-SSA/Ro60 transfetcing vector and they might be considered false positive. However EBV appears to initiate anti-Ro-AutoAb formation in all of the anti-Ro-positive SLE patients and all our patients had a AD diagnosis. It is necessary to investigate whether unusual SSA/Ro60 staining pattern is causally related with AD or it's a cross-reactivity EBV-related.
Schulte-Pelkum J, Fritzler M, Mahler M, et al. Latest update on the Ro/SS-A autoantibody system. Autoimmun Rev 2009;8:632-7.

P174
CORRELATION BETWEEN AUTOANTIBODIES PROFILE AND HLA HAPLOTYPES IN CHILDREN WITH TYPE I DIABETES MELLITUS AT THE MOMENT OF FIRST DIAGNOSIS

R. Merli[1], S. Manni[1], A. Zanetti[1], C. Bonaguri[2], R. Troiano[1], S. Giuliodori[1], V. Uliana[1], R. Aloe[2], P. Zanelli[1]

[1]*Immunogenetics Laboratory, Unit of Medical Genetics, Parma University Hospital*
[2]*Laboratory of Clinical Chemistry and Hematology, Parma University Hospital*

Aim: Type I diabetes mellitus (DM1) is an immune mediated disease leading to chronic insulin deficiency due to extensive beta cell destruction in subject with increased genetic susceptibility. The HLA genes on the short arm of the chromosome 6 are the major determinant of the genetic predisposition to DM1. The aim of this preliminary study was to investigate, at the moment of the diagnosis, the correlation between auto-antibody profile and HLA haplotypes.
Materials and methods: The possible relation between HLA DRB1-DQB1 haplotypes and level of auto-antibodies associated with DM1 was assessed by examining HLA - DRB1 and HLA - DQB1 alleles and antibodies to islet cells (ICA), glutamic acid decarboxylase 65 (GAD65) and the protein tyrosine phosphatase-related islet antigen 2 (IA-2) in 44 unrelated children with IDDM. The study population included 25 males and 19 females, median age 8 yr ± 4,3 yr.
Results: We observed that 19 patients had the DRB1*03-DQB1*02:01 haplotype, 5 the DRB1*04-DQB3*03:02 haplotype and 8 the two haplotypes DRB1*03-DQB1*02:01/DRB1*04-DQB1*03:02. The remaining 12 patients didn't have any DM1 related haplotypes. Between the 19 patients with the DRB1*03-DQB1*02:01 haplotype, 11 (57%) showed increased level of GAD65, whereas between the 5 with DRB1*04-DQB1*03:02 haplotype, 4 (80%) showed increased level of IA2. In the 87,5% (7/8) of patients with both at risk haplotype, we found also increased levels of both auto antibodies. No association was found between ICA and HLA haplotypes. In the group without at risk haplotypes, 17% (2/12) had GAD65 antibodies only and 17% (2/12) had GAD65 and IA-2 antibodies.
Conclusions: Considering these preliminary data it can be speculated that DRB1*03-DQB1*02:01 haplotype is characterized by high level of GAD65 and DRB1*04-DQB1-*03:02 haplotype is associated to increased value of IA2. As expected, when both the haplotypes are presents, also the auto- antibodies are jointly increased. However, to strengthen this conclusion we need to test other patients and possibly complete the study with the analysis of other kinds of auto-antibodies.

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ANTICORPI ANTI DS-DNA: QUALI FATTORI POSSONO INFLUENZARE LA POSITIVITA' DELLA CRITHIDIA LUCILIAE

A. Gubbiotti, L. Rosengart, A. Malgrande, G. Piccone

U.O.C Patologia Clinica, sez. Allergologia ed Autoimmunità, Az.Osp. S. Camillo-Forlanini, Roma

Scopo: La positività su Crithidia L. in assenza di LES a cosa è dovuta? Sono stati valutati due pazienti con positività per Abα-dsDNA su Crithidia L. (IFI) e con ANA negativi o a basso titolo. L'elevato grado di positività in IFI non trovava conferma con la ricerca di Abα-dsDNA con altre metodiche (CLIA, ELISA, BLOTTING). Gli Abα-cromatina erano negativi. Le indagini sierologiche presentavano una diffusa positività per virosi di varia natura, in particolare per Epstein Barr virus (EBNA IgG, EBV-VCA IgG e IgM.) Sulla base dei sintomi viene formulata diagnosi di mononucleosi ed esclusione di patologia autoimmune. In uno dei due pazienti si ripete il prelievo a distanza di un mese e si osserva sieroconversione (IgM versus IgG) per EBV e negativizzazione della Crithidia.
Materiali e metodi: ANA: cellule Hep2, Hep2-select (INOVA), Hep2000 (ALIFAX). AbαCromatina: QUANTA lite Chromatin(INOVA). AbαdsDNA:IFI Crithidia L. (INOVA; ALIFAX), CLIA (QUANTAFlash-dsDNA), ELISA (QUANTAlite-dsDNA), immunoblotting (ANAImmunoblotting 6 /12 antigeni ALIFAX).
Risultati: l'elevato grado di positività degli Abα-dsDNA su Crithidia L. viene confermata su più linee cellulari. Con metodiche alternative (CLIA; ELISA; IMMUNOBLOTTING) risultavano negativi.
Conclusioni: L'elevata positività della Crithidia L. in IFI in pazienti che presentavano una sintomatologia simile (artralgie diffusei, edemi articolari, linfoadenopatia, aftosi, astenia, affanno, dermatite di tipo orticarioide) non trovavano riscontro in indagini per Abα-dsDNA con metodi alternativi e non correlava con presenza di ANA. La diffusa positività invece delle ricerche virologiche (Cytomegalovirus IgG, IgM; Coxsackievirus A-B IgM, Adenovirus IgG, IgM Mycoplasma P. IgM) lascia spazio ai quesiti: la positività della Crithidia è così specifica per LES o può essere influenzata da fattori virali di natura varia? Se il siero dei pazienti ricco di anticorpi di varie classi fosse testato su cellule Hep2 con coniugato polivalente (IgG, IgA, IgM) come potrebbe cambiare il quadro fluoroscopico? In ogni caso si ritiene opportuno rivalutare i test autoimmuni e le indagini sierologiche per osservare se la risoluzione della malattia di partenza produce variazioni sulle indagini in immunofluorescenza.

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CROSS-SECTIONAL AND LONGITUDINAL EVALUATION OF ALPHA-FETOPROTEIN (AFP) AND PROTEIN INDUCED BY VITAMIN K ABSENCE OR ANTAGONIST II (PIVKA-II) IN PATIENTS WITH CIRRHOSIS OF VIRAL ETIOLOGY UNDER SURVEILLANCE FOR HEPATOCELLULAR CARCINOMA (HCC)

G.P. Caviglia[1], M.L. Abate[1], C. Bosco[1], S. Gaia[2], A. Olivero[1], C. Rosso[1], P. Carucci[2], A. Ciancio[1], E. Bugianesi[1], G.M. Saracco[3], A. Smedile[1]

[1]Dept. of Medical Sciences, University of Turin, Turin
[2]Dept. of Gastroenterology and Hepatology, Città della Salute e della Scienza - Molinette Hospital, Turin
[3]Dept. of Oncology, University of Turin, Turin

Background and aim: We investigated AFP and PIVKA-II diagnostic accuracy for HCC detection and prediction of HCC development in patients with cirrhosis of viral etiology under surveillance.
Patients and methods: A total of 254 patients, 127 patients with cirrhosis (78M/47F, median age 57 [33-82] years, 96HCV/31HBV) and 127 patients with HCC (106M/21F, median age 66 [31-89] years, 94HCV/33HBV) were consecutively enrolled (Cross-sectional cohort). Serial serum samples of 27 patients (21M/6F, median age 67 [53-78], 14 HCV and 13 HBV) who developed HCC during surveillance, were analyzed (Longitudinal cohort). AFP and PIVKA-II serum levels were measured with a fully automated chemiluminescent enzyme immunoassays on Lumipulse-G600-System (Fujirebio Inc, Tokyo, Japan) at single time-point in Cross-sectional cohort and at HCC diagnosis (T0), 6-9 months (T-1) and 12-18 months (T-2) before HCC diagnosis in Longitudinal cohort.
Results: Median AFP and PIVKA-II serum levels were significantly different between patients with cirrhosis and those with HCC ($p < 0.0001$). AFP was correlated with PIVKA-II ($r=0.325$, $p < 0.0001$) and with Barcelona Clinic Liver Cancer staging system ($r=0.237$, $p=0.007$). Area under ROC curve (AUC) was 0.725 and 0.807 for AFP and PIVKA-II, respectively, for the discrimination between patients with cirrhosis and those with HCC (AFP vs. PIVKA-II, $\Delta AUC=0.082$, $p=0.034$). No significant improvement in HCC detection was observed from the combination of AFP and PIVKA-II (AUC=0.818) compared to PIVKA-II used alone ($\Delta AUC=0.011$, $p=0.609$). In the Longitudinal cohort, a significant variation was found for both biomarkers from time T(-2) to T(0) (Friedman test, $p=0.002$ for both AFP and PIVKA-II). Multivariate Cox regression analysis, adjusted for age and gender, showed that only PIVKA-II (>55 mAU/mL) could significantly and independently predict HCC development up to 18 months before diagnosis (Hazard Ratio=3.71, 95% Confidence Interval 1.65-8.31, $p=0.002$; Logrank test, $p < 0.001$).
Conclusions: PIVKA-II diagnostic accuracy was significantly higher than AFP and the combination of both biomarkers was not superior than PIVKA-II alone for HCC detection. In cirrhotic patients undergoing surveillance for HCC, serum PIVKA-II levels >55 mAU/mL may allow the identification of those patients at higher risk of HCC development that could benefit from a closer monitoring.

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HEPATITIS B CORE-RELATED ANTIGEN LEVELS DURING NUCLEOS(T)IDE ANALOGUES OR PEGYLATED-INTERFERON TREATMENT IN PATIENTS WITH CHRONIC HBV-GENOTYPE D INFECTION

G.P. Caviglia[1], M.L. Abate[1], A. Olivero[1], C. Rosso[1], G. Troshina[1], A. Ciancio[1], G.M. Saracco[2], M. Rizzetto[1], A. Smedile[1]

[1]Department of Medical Sciences, University of Turin, Turin
[2]Department of Oncology, University of Turin, Turin

Background and aims: Hepatitis B core-related antigen (HBcrAg) is a combined serum HBV biomarker that simultaneously detects hepatitis B core antigen, hepatitis B e antigen (HBeAg) and a 22 kDa pre-core protein. We analyzed HBcrAg correlation with HBV DNA and hepatitis B surface antigen (HBsAg) levels and investigated HBcrAg kinetic during nucleos(t)ide analogues (NAs) or pegylated-interferon (PEG-IFN) treatment in a cohort of e-minus chronic hepatitis B (CHB) patients.
Patients and methods: One-hundred-thirty-eight sequential serum samples were collected from 28 HBeAg-negative CHB-genotype-D patients (20M/8F; median age 54 [47-58] years), who underwent NAs (n=20) or PEG-IFN (n=8) treatment. Serum HBcrAg levels were determined by chemiluminescent enzyme immunoassay (dynamic range 2-7 LogU/mL) (Fujirebio Europe, Gent, Belgium). HBV DNA and HBsAg were assessed with standard assay. Longitudinal analysis was performed at 6, 12, 24 and 36 months after NAs treatment initiation and at 6, 12, 18 months and at follow-up month-6 (M6-FU) after PEG-IFN administration.
Results: Basal HBcrAg levels were 4.7±1.8LogU/mL and 3.3±1.6LogU/mL in NAs and PEG-IFN-treated patients, respectively. HBcrAg showed a moderate correlation with HBV DNA (r=0.498, p <0.0001) and no correlation with HBsAg (r=0.192, p=0.0669). In PEG-IFN treated patients, a better correlation was found between HBcrAg and HBV DNA compared to NAs-treated group (r=0.787, p <0.0001 vs. r=0.496, p <0.0001, respectively; z-statistics=2.666, p=0.008).
In serial serum samples, a significant HBcrAg reduction was observed only in patients receiving NAs (p=0.019). In such patients, we observed a group (n=12) experiencing an early HBcrAg decline to <2LogU/mL between months 6-12, while the other group (n=8) had still detectable HBcrAg at month 36 (4.4±0.6LogU/mL), independently from HBV DNA and HBsAg kinetics.
Conclusions. Serum HBcrAg correlates with HBV DNA levels, most likely expression of viral replication activity. Further studies are needed to elucidate if HBcrAg kinetics reflect different intrahepatic virological status and if HBcrAg may represent a marker for safely discontinuing NAs in CHB-genotype D patients.
Matsumoto A, et al. Low serum level of hepatitis B core-related antigen indicates unlikely reactivation of hepatitis after cessation of lamivudine therapy. Hepatol Res 2007;37:661-6.

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TEST PIVKA II: INITIAL EVALUATIONS ON ITS USE

M. Brugia, A. Calcinari, F. Balducci, M. Galeazzi

Clinical Biochemistry and Microbiology Laboratory Hospital of Ancona

Introduction: Hepatocellular carcinoma (HCC) is an important cause of death worldwide. Most common causes are alcohol consumption, hepatitis C and B viruses and chronic inflammatory hepatic disease. Ultrasound examination of the liver and detection of AFP level in serum are commonly used to screen for liver cancer. Although detection of AFP level shows less sensitivity, since elevation in AFP level is common in patients with chronic liver disease. Ultrasound is better, but is more expensive, operator dependent and less reliable in the presence of cirrhosis. Thus, new markers with high sensitivity and specificity are required. Prothrombin induced by vitamin K absence-II (PIVKA-II) is also known as Des-gamma carboxyprothrombin (DCP) is an abnormal prothrombin protein that is increased in the sera of patients with HCC. The present study was designed to investigate the potential role of PIVKA-II as a diagnostic, non-invasive marker for HCC at its early stages and to assess its sensitivity and specificity as compared with the usual recommended marker AFP.
Patients and methods: This study was conducted on 80 patients (90 samples) admitted to Ancona hospital (Azienda Ospedali Riuniti) between 1/2-31/052016. Group I consisted of 22 patients with HCC, group II included 40 patients with chronic hepatitis, group III 8 cancer patients with liver metastases and 10 blood donors apparently healthy subjects. Serum was collected and stored at -20 °C until assayed. Level of serum AFP was detected using Vista (Siemens) and PIVKA-II level in the plasma using Architect (Abbott). Adopted reference values: < 6 ng/ml per AFP, <40 mAUml per PIVKA II. 26 samples of group I have average values of AFP 401.51 ng/ml e PIVKA di 1339.89 mAU/ml and only 15 (57.7%) have values of PIVKA above the cut-off. 40 samples of group II have values above the cut-off for AFP, but only 28 samples (66.6%) have elevated values of PIVKA. The average values of PIVKA in the group III is 27.47 mAU/ml.
Conclusions: Preliminary results show that the assay PIVKA, introduced in the surveillance of the population at risk of developing HCC and in the follow-up of patients already treated for this type of tumor, it could be used in combination with AFP.

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DETERMINATION OF GALLSTONE BIOCHEMICAL COMPOSITION BY INFRARED SPECTROSCOPY

A. Primiano, S. Persichilli, A. Schiattarella, C. Zuppi, A. Urbani, J. Gervasoni

Laboratorio Analisi I, Università Cattolica del Sacro Cuore, Fondazione Policlinico A. Gemelli, Roma

Introduction: Gallstones constitute a significant health problem in developed societies and it is the most common biliary disorders in adults. It is usually asymptomatic but may become symptomatic when stones migrate to the bile ducts. Gallstones are often caused by conditions such as obesity, type 2 diabetes, dyslipidaemia, hyperinsulinaemia, chronic hemolysis, intestinal malabsorption syndromes and various biliary tract diseases. Their pathogenesis is multifactorial and current research suggests that to know exactly chemical composition is important to identify the mechanism of formation. The biochemical composition of gallstones has often been investigated with chemical methods using conventional reagents, labor expensive and inadequate to give a complete accurate determination. The aim of our work it is to clarify the nature and type of calculus encountered in our daily practice by determining the biochemical composition of gallstones by infrared spectrometry (FT-IR).
Material and methods: The IR spectrum originates from the vibrational motion of the molecules. The vibrational frequencies are a kind of fingerprint of the compounds. The identification of chemical compounds was done by matching wavenumbers with the existing literature data to improve the accuracy. We analyzed 15 gallstones (GS), from our digestive endoscopic surgery department.
Results and discussion: Ten gallstones (66.7%) analyzed were composed only of single compound; the IR spectroscopy quantification reported 100% of cholesterol in 4 stones and of 100% of calcium bilirubinate in 6 stones. Instead 4 GS (26.6%) were composed with single compound and trace of another component; the analysis reveals that these stones contained 100% calcium bilirubinate or cholesterol and substances in trace. Only 1 (6.7%) GS was composed of multiple chemical compounds (35% calcium bilirubinate phosphate and 35% cholesterol and 30% of palmitic acid). FT-IR, recognized the gold standard to determinate the biochemical composition of urinary stones, can be very useful also to define accurate composition of gallstone, that is a prerequisite for gallstone formation research and for future therapeutic and preventive therapies.

P180
ANALISI DEL GENE MUTYH IN PAZIENTI CON FAP

G. D'Elia[1], G. Caliendo[1], V. De Stefano[1], M. Cioffi[2], A.M. Molinari[2], M.T. Vietri[3]

[1]Patologia clinica Dipartimento di Biochimica, Biofisica e Patologia generale, Seconda Università degli Studi di Napoli
[2]U.O.C. Patologia clinica e molecolare, A.O.U. Seconda Università degli Studi di Napoli
[3]U.O.C. Patologia clinica e molecolare Diagnostica molecolare - Tumori ereditari, Seconda Università degli Studi di Napoli

La poliposi adenomatosa familiare (FAP) è una patologia ereditaria rara, con un'incidenza di 1/10.000, dovuta nel 70-90% dei casi a mutazioni monoalleliche nel gene APC e nel 10-30% dei casi a mutazioni bialleliche nel gene MUTYH. Mutazioni in MUTYH sono associate ad una forma attenuata di poliposi definita MAP (poliposi MUTYH-associata). Il fenotipo della MAP è relativamente mite, caratterizzato dalla presenza di un numero di adenomi inferiore a 100 e dall'insorgenza, a volte, di manifestazioni extracoloniche.
Obiettivo del nostro studio è stato determinare la presenza di mutazioni di MUTYH in pazienti affetti da FAP, negativi per mutazioni in APC.
Sono stati reclutati 10 pazienti (6F e 4M) affetti da FAP, 5 presentavano una mutazione in APC. I 5 pazienti APC negativi (2F e 3M) sono stati sottoposti ad analisi mutazionale di MUTYH.
Un paziente su 5 (20%) presenta la mutazione patogenetica G396D (c.1187G>A) in omozigosi, mentre 3/5 (60%) riportano la VUS Q324H (c.1014G>C). Uno dei 3 pazienti presenta tale variante in omozigosi, inoltre riporta altre 2 variazioni di sequenza, IVS2+30 A>G (c.157+30A>G) e D271D (c.813C>T).
La mutazione G396D (c.1187G>A) comporta un cambiamento aminoacidico in una regione altamente conservata della proteina ed è responsabile di una riduzione della capacità di legame e di riparazione del DNA. Tale mutazione in omozigosi predispone all'insorgenza della MAP.
La variante Q324H (c.1014G>C) è stata analizzata in diversi studi con analisi in silico e riportata come benigna, mentre studi funzionali hanno evidenziato che tale sostituzione aminoacidica comporta una riduzione del 36% dell'attività proteica. La mancanza di uniformità dei dati rende opportuno considerarla una VUS.
In conclusione, abbiamo individuato una mutazione in MUTYH nel 20% dei pazienti analizzati. Ulteriori studi condotti sulla variante Q324H (c.1014G>C) potrebbero chiarire un eventuale ruolo nel determinare il fenotipo clinico dei pazienti.
L'individuazione di mutazioni in APC e MUTYH è utile non solo per un diverso approccio clinico nei pazienti affetti da FAP, ma anche per l'attuazione di piani di sorveglianza nei soggetti sani portatori di mutazioni.
Leoz ML, Carballal S, Moreira L, et al. The genetic basis of familial adenomatous polyposis and its implications for clinical practice and risk management. Appl Clin Genet 2015;8:95-107.

P181
CEREBROSPINAL FLUID OSMOLALITY: DETERMINANTS AND CORRELATION WITH PLASMA OSMOLALITY

G. Bellomo, B. Bardone, A. Mora, V. Castellina, E. Beltrami, E. Mairate, I. Crespi

Lab.di Ricerche Chimico Cliniche, A.O.U. Maggiore della Carità di Novara, Novara

Osmolality of cerebrospinal fluid (CSFosm) is one of the major determinants of neuronal swelling and shrinking in pathological conditions. Measure of CSFosm is, however, not routinely performed and its relation to plasma osmolality and osmolites has not been extensively investigated.

CSF and plasma concentrations of Na^+, K^+, Cl^-, were measured by direct potentiometry; glucose (Gluc) and urea nitrogen (UN) by conventional spectrophotometric assay and osmolality by depression of freezing point. Three algorithms were employed to estimate osmolality: OsmC1: 2*Na^++ Gluc/18 + UN/2.8; OsmC2: (1.86*Na^++ Gluc/18 + UN/2.8) + 9; OsmC3: (1.86*Na^++ Gluc/18 + UN/2.8)*1.09. All the assays were performed in 88 unselected consecutive patients undergoing spinal puncture.

Measured CSF osmolality (294±8.9 mOsm/Kg) correlated with CSF Na^+(R^2=0.6809) but not with glucose (R^2=0.0635) or urea nitrogen (R^2=0.1059). CSF measured osmolality exhibited a strong correlation with calculated osmolalities measured with the three different algorithms (R^2 always higher than 0.7000). The measured-calculated gap was higher for OsmC2 (13±14.5 mOsm/Kg) as compared to OsmC1 (2±14.1 mOsm/Kg) and OsmC3 (-3 ±14.0 mOsm/Kg). CSF measured and calculated osmolalities showed a significant correlation with plasma (R^2 always greater than 0.4600), whereas no correlations were found between the osmolality gaps obtained from the three different algorithms. The CSF-plasma differences in measured osmolality slightly (R^2=0.2328) correlated with the CSF-plasma differences in Na^+ but not in glucose or urea nitrogen (R^2 <0.0800).

These data suggest that neither plasma osmolality nor the concentrations of plasma Na^+, glucose and urea nitrogen can be efficiently employed for the indirect estimation of CSF osmolality. However, at least two of the employed algorithms (based on CSF Na, glucose and urea nitrogen) can be used for indirect calculation of osmolality.

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P182
COMBINED USE OF KAPPA FREE LIGHT CHAIN INDEX AND ISOELECTROFOCUSING OF CEREBROSPINAL FLUID IN DIAGNOSING MULTIPLE SCLEROSIS: PERFORMANCES AND COSTS

I. Crespi[1], M. Sulas[1], R. Mora[1], P. Naldi[2], D. Vecchio[2], C. Comi[2], R. Cantello[2], G. Bellomo[1]

[1]Lab. di Ricerche Chimico Cliniche, A.O.U. Maggiore della Carità di Novara, Novara

[2]Neurologia, Università Piemonte Orientale, Novara

Introduction: Although isoelectrofocusing (IEF) to detect oligoclonal bands (OBCs) in cerebrospinal fluid (CSF) is the gold standard for evaluating intrathecal immunoglobulin synthesis in multiple sclerosis (MS), the kappa free light chain index (KFLCi) is emerging as an alternative marker, and the combined or sequential use of IEF and KFLCi have never been challenged.

Methods: KFLCi has been measured on serum and CSF by nephelometry (Siemens); OCBs were evaluated by immunofixation (Hydragel9 CSF, SEBIA) in 150 consecutive patients: 48 with MS, 32 with other neurological inflammatory diseases (NID), 62 with neurological non-inflammatory diseases (NNID) and 8 without any detectable neurological disease (NND).

Results: we compared KLFCi to markers eshablished in MS work-up: the Link index (a ratio between the immunoglobulin G quotient and the albumin quotient) and IEF. IEF and KFLCi turn out as superior to Link index concerning sensitivity, positive and negative predictive values and number needed to diagnosis. This analysis was performed after the identification of a cut-off of 5 for KFLCi in the ROC analysis (AUC=0.948). KFLCi was comparable and superior to IEF. Addressing laboratory approximate costs of MS diagnosis, we evaluate 4 possible scenarios. Method A was performing exclusively IEF in all patients: the estimated cost for 150 patients amounted to 6072,00 euros. Method B was performing only KFLCi: cost decreased to 2277,00 euros (-62 %). Method C, both IEF and KFLCi performed contemporarily in all patients raised costs to 8349,00 (+37,5 %). Method D was a sequential approach with KFLCi as a first-line test, followed by IEF as confirmatory test in patients with elevated KFLCi: this would lead to an overall cost of 4790,00 euros (-21,1% compared to the first approach).

Conclusion: The high sensitivity and specificity associated with the lower cost of KFLCi suggested to use this test firstly, followed by IEF as a confirmative procedure. The “sequential testing” using KFLCi followed by IEF in MS may represent an optimal procedure with accurate performance and lower costs.

Reiber H, Peter JB. Cerebrospinal fluid analysis: disease-related data patterns and evaluation programs. J Neurol Sci 2001;184:101-22.

P183
K INDEX IN CEREBROSPINAL FLUID: A VALID AID TO THE MULTIPLE SCLEROSIS DIAGNOSIS

M. Pieri[1], M. Storto[3], S. Pignalosa[1], R. Zenobi[1], D. Centonze[2], F. Buttari[2], S. Bernardini[1], M. Dessì[1]

[1]Department of Experimental Medicine and Surgery, University of Rome "Tor Vergata", Rome
[2]MS Research Center, Tor Vergata University, Rome & Unit of Neurology and Neurorehabilitation, Pozzilli (IS)
[3]Laboratory, I.R.C.C.S. Neuromed, Pozzilli (IS)

Trial design: The diagnosis of Multiple Sclerosis (MS) is based on focal lesions dissemination in time and space. After 2010 lack of evidence for dissemination in space (DIS) could be substituted by laboratory test as oligoclonal bands (OCBs) in cerebrospinal fluid (CSF). OCBs detection in CSF by isoelectrocfocusing and immunodetection is an important support to determine the inflammatory demyelinating nature. Determination of free light chains (FLCs) in the CSF, for their diagnostic accuracy, could become a promising biomarker to represent intrathecal synthesis in MS. Our study's objective is assessing the diagnostic accuracy of a new highly sensitive latex-enhanced nephelometric assay for free light chains determination in CSF/serum as an alternative to traditional tests and its clinical application. In this study we will perform kFLC Index in an increased cohort of patients in order to validate the cut off previously described by us and to improve the sensitivity and specificity of this method.
Methods: kFLCs were measured in CSF/serum pairs from 180 patients from Tor Vergata University Hospital and from I.R.C.C.S. Neuromed, by the use of a nephelometric automated immunoassay using monoclonal antibodies for determination. All the samples were divided into three groups according to the neurological diagnosis.
Results: Compared to previous published results, we obtained a K Index sensitivity and specificity of 94% and 99% (vs 96% and 91%) respectively; recalculating K Index the new cut off was 12.8 slightly higher than the previous (cut-off=12).
Conclusions: The K Index is more effective to diagnose MS in comparison to the reference method (OCBs) and could be useful for evaluating treatment response. Finally, it has been proposed that the presence of FLCs in CSF is associated with recent demyelination activity in MS and might be used as a prognosis marker.
Duranti F, Pieri M, Centonze D, et al. Determination of kFLC and K Index in cerebrospinal fluid: a valid alternative to assess intrathecal immunoglobulin synthesis. J Neuroimmunol 2013;263:116-20.

P184
CSF FLC AND IL-10 CONCENTRATIONS IN MULTIPLE SCLEROSIS

P. Cardelli[1], G. Ferranti[1], M.R. Favaroni [2], M.T. Corsetti[1], G. Salerno[1], M.L. Troccoli[1]

[1]Dipartimento di Medicina Clinica e Molecolare, Sapienza Università di Roma
[2]A.O.U. Policlinico S. Andrea, Roma

Background: Multiple Sclerosis (MS)is an autoimmune disease, an inflammatory process involving B cells and consequential intrathecal immunoglobulin synthesis, leading to demyelination. MS work-up thus requires cumbersome diagnostic procedures. To date, the only biochemical analysis employed for the diagnostic support is serum and cerebrospinal fluid (CSF) oligoclonal band investigation as "gold standard". Nonetheless, this investigationis qualitative, requiring extensive manual expertise and experience for its interpretation. Along with IgG, also free light chains are secreted. The recently introduced commercial assayfor FLC quantification has opened up to new scenarios, enabling the assessment of this novel parameter in a quantitative manner, displaying similar sensitivity to OCB detection. Other parameters, including IL-10, an anti-inflammatory cytokine have parallel been tested. Indeed, IL-10 plays an extensive role in immunoregulation and inflammation, contributing to down-regulation of other pro-inflammatory factors, possibly leading to limitation of disease progression.The aim of our study was to assess the utility of FLC analysis, along with IL-10 CSF concentrations.
Methods: A total of 44 patients referring to the Neurology unit of the S. Adnrea Hospital of Rome, undergoing lumbar puncture for OCB analysis were recruited (following written informed consent). CSF and serum samples were collected and stocked. IgG and albumin were routinely assessed (Dade Behring, Siemens BN II) along with FLCs (FreeliteTM, The Binding Site) and IL-10, according to the manufacturer's instructions. Patients were then stratified according to diagnosis: 18 Multiple Sclerosis (Grp1), 8 Transverse Myelitis (Grp2), 11 Polyneuropathy (Grp 3) and Controls-other non-inflammatory neurological diseases (Grp4).

Results: κFLC along with IL-10 specifically discriminate MS patients from other neurological diseases. SM patients have statistically significant elevated κFLC ($P < 0.001$) and low IL-10 ($P = 0.034$) as opposed to all other groups, with a negative correlation ($r2 = -0.70$, $P < 0.05$).

Conclusion: κFLC and IL-10 concentrations were both indicative of MS. Thus, these may in the future represent two parameters to be used as further tool in order to specifically identify MS patients upon dubious diagnostic results.

P185
THIOLS METABOLISM IN MITOCHONDRIAL DISEASES

J. Gervasoni[1], S. Servidei[2], G. Primiano[2], A. Schiattarella[1], A. Primiano[1], C. Zuppi[1], A. Urbani[1], S. Persichilli[1]

[1]Laboratorio Analisi I, Università Cattolica del Sacro Cuore, Fondazione Policlinico A. Gemelli, Roma
[2]Istituto di Neurologia, Università Cattolica del Sacro Cuore, Fondazione Policlinico A. Gemelli, Roma

Introduction: Mitochondrial diseases (MDs) are heterogeneous genetic disorders caused by impairment of the oxidative phosphorylation (OXPHOS) system, which affects tissues that are heavily energy dependent, including the brain and skeletal muscle. Mitochondria, the main source of cellular adenosine triphosphate (ATP) and, at the same time, the primary source of reactive oxygen species (ROS) are ubiquitous. Therefore MDs may present with a multitude of clinical features in different combinations. While in normal conditions the cell neutralizes ROS, in case of mitochondrial DNA (mtDNA) mutation and/or insufficiency, the oxidative stress may exceed the capacity of cellular antioxidant mechanisms, leading to cellular damage and apoptosis. For this reason, oxidative stress is plausible pathogenic mechanism leading to cellular dysfunction and phenotypic expression. The thiols, reduced and in their oxidized forms, are an important component of intra and extra-cellular districts, especially for their involvement in detoxification processes toward oxidative injury.
This study is aimed to investigate the role of thiols metabolism as a marker of oxidative stress in a cohort of adult patients with genetically confirmed mitochondrial disease.
Material and method: We use high performance liquid chromatography (HPLC) with fluorimetric detection to analyze serum glutathione, cysteine and homcysteine in twenty adult patients with defined mitochondrial disease on the basis of molecular genetic, histochemical and biochemical analysis from the Neuromuscular Center at Gemelli Hospital (7 MERRF, 5 MELAS, 6 PEO, 2 other mitochondrial myopathies).
Results and discussion. GSH concentration was significantly lower ($p=0.008$) in MDs compared to controls, in particular in samples from MERRF syndrome ($p=0.0006$), and serum homocysteine was significantly higher in the patients than in healthy controls ($p <0.001$). Although our findings are preliminary, serum GSH and homocysteine are promising biomarkers for detecting underlying oxidative stress in these pathological conditions and, in particular, GSH have a potential role as prognostic biomarker for monitoring disease progression and to evaluate the efficacy of new therapeutic approaches.

P186
POTENTIAL DIAGNOSTIC APPLICATION OF SIDE CHAIN OXYSTEROLS ANALYSIS IN PLASMA AND CEREBROSPINAL FLUID

V. Leoni[1], C. Caccia[2]

[1]Laboratorio Analisi Chimico-cliniche, Ospedale di Circolo di Varese, ASST-Settelaghi, Varese
[2]Laboratorio di Patologia Clinica e Genetica Medica, IRCCS Carlo Besta, Milano

The neurospecific cholesterol 24-hydroxylase converts the excess of brain cholesterol into 24S-hydroxycholesterol (24OHC) which, via LXR, can increase the expression and synthesis of ApoE by astrocytes. 24OHC effluxes directly from brain into plasma where it is considered an indicator of brain cholesterol turnover and it was found reduced in neurodegenerative diseases proportionally to the severity of disease and the degree of brain atrophy. In early phases of an active disease, a higher rate of the turnover may result into transitory increased plasma 24OHC (Leoni et al, 2011).
Less than 1% of the total excretion of 24OHC occurs via cerebrospinal fluid (CSF) where almost all of 27-hydroxycholesterol (27OHC) depends on the function of the blood cerebrospinal fluid barrier: increased oxysterols were found in CSF from patients with neurodegenerative and neuroinflammatory diseases in presence of barrier dysfunction.
Along neurodegeneration, apoptosis and autophagy concur to increase the amount of free cholesterol released from dying cells engulfing neurons. Cholesterol also increases Amyloid β deposition and tau pathology. ApoE, 24OHC, Tau and soluble APP were found correlated in Alzheimer disease (AD) samples: the excess of cholesterol converted into 24OHC may up-regulates the synthesis of ApoE which acts as a scavenger for Aβ and Tau. In case of AD this protective mechanism seems to be inefficient, probably affected by presence of high concentration of 27OHC, microvascular dysfunction and lower efficiency of ApoE4 as lipid transporter and Aβ scavenger. 24OHC was found cytotoxic itself.
The analysis of side chain oxysterols by isotope dilution mass spectrometry in CSF is likely to offer information about cholesterol metabolism and ApoE function involved in the pathogenesis of AD.

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METABOLOMIC EVIDENCE OF THE MITOCHONDRIAL DYSFUNCTIONS IN 7-KETOCHOLESTEROL-TREATED 158N OLIGODENDROCYTES

V. Leoni[1], C. Caccia[2], T. Nury[3], A. Vejux[3], A. Zarrouk[4], G. Lizard[3]

[1]Laboratory of Clinical Chemistry, Hospital of Varese, ASST-Settelaghi, Varese

[2]Laboratory of Clinical Pathology, Foundation IRCCS Istituto Neurologico Carlo Besta, Milan

[3]Biochemistry of the Peroxisome, Inflammation and Lipid Metabolism' EA 7270 / Univ. Bourgogne Franche Comté / INSERM, Dijon, France

[4]Univ. Monastir, Faculty of Medicine, LR12ES05, Lab-NAFS 'Nutrition - Functional Food & Vascular Health', Monastir, & Univ. Sousse, Faculty of Medicine, Sousse, Tunisia

Background: In multiple sclerosis (MS), the white matter degradation leads to demyelination. Oxidative stress, inflammation, apoptosis, necrosis and/or autophagy result together into the progressive loss of oligodendrocytes. 7-ketocholesterol (7KC), product of cholesterol autoxidation, is increased in the cerebrospinal fluid of MS patients and it triggers a rupture of RedOx homeostasis associated with mitochondrial dysfunctions, aptoptosis and autophagy (oxiapoptophagy) in cultured murine oligodendrocytes (158N).
Methods: Oligodendrocytes (158N) were cultured in standard conditions. Overproduction of reactive oxygen species was quantified by staining with hydroethidine and mitochondrial changes by staining with DiOC6(3), nonyl acridine orange (NAO) and MitoSox Red. Sterols, oxysterols, fatty acids and metabolic organic acids were measured by isotope dilution gas chromatography-mass spectrometry (GC/MS) after ethanolic saponification, liquid to liquid and cartridge separation and TMS-derivatisation.
Results: In presence of 7KC, the amount of adherent 158N cells was decreased, oxidative stress enhanced with evidences of apoptosis (increased caspase-3 and PARP degradation) and autophagy (increased LC3-II/ LC3-I ratio). The mitochondrial membrane potential ($\Delta\psi m$) was reduced together with OXPHOS (reduced NAD + and ATP). The cellular lactate was higher while pyruvate, citrate, fumarate, succinate (tricarboxylic acid (TCA) cycle intermediates) were significantly reduced, suggesting that an impairment of mitochondrial respiratory functions could lead to a reduced amount of ATP and acetyl-CoA available for the anabolic pathways. The concentration of lathosterol, lanosterol and desmosterol were significantly reduced together with satured and unsatured long chain fatty acids (C16:0 - C18:0, structural elements of membrane phospholipids).
Discussion: The cell death induced by 7KC is associated with mitochondrial dysfunctions, including alterations of OXPHOS, resulting into lipid anabolism dysfunctions. MS based metabolomics offers a better knowledge of mitochondrial associated dysfunctions triggered by 7KC will and contribute to bring new information on the demyelination processes in MS.

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IMPROVING THERAPEUTIC APPROPRIATENESS OF MULTIPLE SCLEROSIS TREATMENTS USING BIOLOGICAL APPROACHES TO PERSONALIZE THERAPY AND SAVE PHARMACEUTICAL SPENDING: 20 YEARS' EXPERIENCE

M. Caldano, A. Sala, F. Marnetto, A. Bertolotto

Neurologia 2 – Centro Riferimento Regionale Sclerosi Multipla (CReSM) & Neuroscience Institute Cavalieri Ottolenghi, Osp. San Luigi, Orbassano, Torino

Multiple Sclerosis (MS) is an autoimmune, progressive inflammatory disease of the Central Nervous System that affects almost 70000 people in Italy. All drugs for MS treatment are very expensive: as over 30000 patients (pts) are under treatment in Italy, the National Health Service (NHS) total expense is more than 400 million €/ year. The goal of therapy is to reduce the disease activity that contributes to long-term disability. Regardless of the availability of different treatment options, the response of each patient to these treatments remains unpredictable. The selection of the best treatment for each patient as soon as possible upon clinical manifestation of MS is needed to avoid exacerbation. Aim of this study is the evaluation of biological parameters such as Anti-Drug Antibodies (ADA), serum drug level or biological activity to early identify Non-Responders pts to treatment, to improve appropriateness and to save or better allocate a huge amount of NHS money.
Since 1996, the Clinical Neurobiology Laboratory of S. Luigi Hospital has been involved in standardization of assays to evaluate the development of ADA, becoming the unique Italian referral center for the assessment of immunogenicity in MS pts treated with Interferon-beta (IFNß), and later in pts treated with Natalizumab (NAT) and Rituximab (RTX). Moreover, the evaluation of biological activity of IFNß and RTX, using the Mixovirus Resistance Protein A (MxA) and CD19 gene expression respectively, and the quantification of RTX serum level has been standardized and currently used in clinical practice.
The evaluation of biological parameters allowed neurologists to monitor the effectiveness of the drug in more than 1800 pts followed at CReSM (Centro Riferimento Regionale Sclerosi Multipla) and to establish European Guidelines on the use of anti-IFNß antibodies in the management of MS pts (1). This approach, applied to other specialties used for MS, allowed us to better allocate more than 1,2 million € of the pharmaceutical expense.
The biological parameters monitoring in MS treated pts improves the appropriateness and allows to better allocate an enormous amount of NHS money. A specialized laboratory in drug monitoring is needed to reach a personalized treatment in a very expensive disease such as MS.
1. Polman CH, Bertolotto A, Deisenhammer F, et al. Lancet Neurol 2010;9:740-50.

P189
DETECTION OF ANTI MOG ANTIBODIES IN DEMYELINATING DISORDERS OF THE CENTRAL NERVOUS SYSTEM (CNS)

A. Sala, M. Spadaro, M. Capobianco, A. Bertolotto

Neurologia 2-Centro Riferimento regionale Sclerosi Multipla (CReSM) & Neuroscience Institute Cavalieri Ottolenghi, Osp. S. Luigi Orbassano (TO)

Myelin-oligodendrocyte glycoprotein (MOG) is a CNS-specific antigen expressed on the surface of myelin sheaths. Anti MOG antibodies (Abs) have been recently described as diagnostic marker of acquired demyelinating diseases of the CNS different from Multiple Sclerosis (MS) and in particular in seronegative Aquaporin-4 Neuromyelitis Optica spectrum disorders, pediatric ADEM or very early onset pediatric MS. At the moment there is not a standardized protocol to detect MOG-Abs but it is known that the most specific method is an cell based assay (CBA). Here we present our work to set up the FACS procedure to detect the MOG-Abs in routine.
We incubated a Glial LN18 cell line untransfected and stably transfected with full-length MOG with serum patient diluted 1:200,then cells were incubated with goat anti-human IgG conjugated secondary antibody Alexa Fluor 488 diluted 1:100 and analyzed immediately by FACS. In each assay, we tested one negative commercial control serum, one positive commercial control and one human positive patient serum. We expressed the levels of Abs titers as the difference in median fluorescence intensity (#MFI) between the MOG-transfected and untransfected LN18. The assay cut-off was based on the average #MFI plus six times the SD of healthy control (HC).
The medium values of positive controls are 2028 and 242. The low and high limit detection of CBA were verified using serial dilution up to 1:2560 of both positive controls (R2= 0.99 for both regression curve). All 61 healthy controls were MOG-Abs negative (mean 2.16, SD 3.5), like the 13 MS sera patients (mean 1.5, SD 2). No statistical differences between HC and MS groups; $p<0.0001$ between HC and both positive controls.
Our CBA test is reliable and reproducible without false positive samples. We are the only one Italian laboratory using the CBA and starting from June 2015 we tested for routine 171 samples coming from different Italian neurology department with 6 MOG-Abs + patients (3.5%), consistent with literature data. Our main purpose, like all researchers in this field, is to identify a clinical phenotype associated to anti-MOG Abs, because this golden standard is lacking.
Ketelslegers IA, et al- Anti-MOG antibodies plead against MS diagnosis in an Acquired Demyelinating Syndromes cohort. Mult Scler 2015;21:1513-20.

P190
VALUTAZIONE DELLA 1.25-DIIDROSSI VITAMINA D CON DOSAGGIO IN CHEMILUMINESCENZA NELLA STADIAZIONE DELLA MALATTIA RENALE CRONICA

B.C. Creanza[1], E. Fasianos[2], M. Guida[1], G. Dirienzo[1]

[1]U.O.S.D Lab. Analisi Patologia Clinica, Ospedale della Murgia "F. Perinei", Asl Bari - Altamura
[2]U.O.S.D Nefrologia e Dialisi, Ospedale della Murgia "F. Perinei", Asl Bari - Altamura

Introduzione: La determinazione della 1α,25diidrossivitamina D, sta rapidamente diventando uno strumento efficiente nell'individuazione di malattie e patologie che colpiscono il normale metabolismo del fosforo e del calcio. Lo scopo di questo lavoro è stato quello di dosare la 1α,25diidrossivitamina D in pazienti afferenti all'Ambulatorio di Nefrologia nella stadiazione della malattia renale cronica e nel monitoraggio della terapia sostitutiva.
Materiali e metodi: La determinazione dell'analita è stata effettuata su campioni di plasma (provette BD Vacutainer PSTII) in 60 pazienti provenienti dall'ambulatorio di MRC dell'Ospedale della Murgia "F. Perinei" di Altamura. Il test adoperato, LIAISON® XL 1,25 Dihydroxyvitamin D (DiaSorin), è un dosaggio modificato in 3 fasi di tipo sandwich che utilizza una proteina ricombinante di fusione per catturare la molecola di 1,25(OH)2D e un anticorpo monoclonale murino che riconosce specificamente il complesso formato dalla proteina ricombinante di fusione e dalla molecola di 1,25(OH)2D.
Risultati: Il 36.7 % del campione preso in esame presentava dei valori inferiori al range di normalità con simile distribuzione nei due sessi (il 36.4% nei maschi ed il 37.0% nelle femmine); tuttavia all'avanzare della malattia renale cronica si è osservata una progressiva riduzione della percentuale dei pazienti "carenti" di 1,25D (il 40.0% nei pazienti al II° stadio e il 26.6% in quelli al V° stadio) con simile andamento in entrambi i sessi (42.9% "carenti" maschi nello stadio II vs 30.1% nello stadio V; 37.5% "carenti" femmine nello stadio II vs 23.4% nello stadio V), evidente segno di una supplementazione farmacologica che ha come obiettivo il ripristino dei normali livelli plasmatici della vitamina D.
Conclusioni: Il dosaggio della 1,25 diidrossivitamina D può rappresentare un aggiunto strumento diagnostico nella valutazione del metabolismo minerale nei pazienti affetti da MRC e può essere un buon indicatore nella scelta dell'appropriata terapia di supplementazione. Per questo motivo un dosaggio in chemiluminescenza rapido, efficace e meno complesso, rispetto ai precedenti dosaggi utilizzati, può essere di ausilio nella pratica clinica.
Santoro D, Gitto L, Ferraro A, et al. Vitamin D status and mortality risk in patients with chronic kidney disease. Ren Fail 2011;33:184-91.

P191
VARIAZIONE DEI LIVELLI DI CHINURENINA E TRIPTOFANO IN SEGUITO A TRATTAMENTO FARMACOLOGICO IPOLIPEMIZZANTE IN PAZIENTI CON INSUFFICIENZA RENALE CRONICA

S. Ena[1], D. Arru[1], A. Baralla[1], V. Bulla[1], L. Urru[2], A.E. Satta[2], A. Zinellu[1], C. Carru[1]

[1]*Dipartimento di Scienze Biomediche*
[2]*Dipartimento di Scienze Chirurgiche, Microchirurgiche e Mediche*

La degradazione del Triptofano (Trp), attraverso la via indolamina2,3-diossigenasi (IDO), con conseguente aumento della concentrazione di chinurenina (Kyn), viene considerato un importante indice di stress infiammatorio. Come ben noto, l'infiammazione è un condizione presente costantemente in pazienti con insufficienza renala cronica (IRC) [1] e, sebbene i meccanismi responsabili dell'infiammazione in tali soggetti non siano ancora chiari, lo stress ossidativo potrebbe contribuire ad attivare lo stato proinfiammatorio. Lo scopo dello studio è stato quello di osservare l'effetto della terapia ipolipemizzante sui livelli di Kyn e Trp plasmatici in pazienti IRC.
Per lo studio sono stati reclutati 30 pazienti IRC suddivisi in tre gruppi in base alla terapia farmacologica in atto: 40 mg/die di simvastatina per il gruppo 1, 10/20 mg/die di ezetimibe/simvastatina per il gruppo 2 e 10/40 mg/die di ezetimibe/simvastatina per il gruppo 3, per una durata totale di 12 mesi di trattamento.
I livelli di Kyn, Trp, malondialdeide (MDA) ed il rapporto allontoina/acido urico (All/UA) sono stati determinati tramite elettroforesi capillare UV detection. I dati ottenuti mostrano che i livelli basali di Kyn e il rapporto Kyn/ Trp sono più alti nei pazienti IRC rispetto ai controlli (rispettivamente 1.67±0.62 μmol/L vs 1.25±0.40 μmol/ L, p <0.01 e 0.036±0.016 vs 0.023±0.010, p <0.001). Inoltre i due parametri sopracitati diminuiscono dopo un anno di trattamento ipolipemizzante (rispettivamente 1.67±0.62 μmol/L vs 1.31±0.51 μmol/L, p <0.0001 and 0.036±0.016 vs 0.028±0.012 p <0.0001), fino ad arrivare a valori sovrapponibili a quelli dei controlli sani. L'effetto più intenso nel ridurre i valori di Kyn era osservabile nel gruppo 3. Parallelamente è stata osservata una riduzione nei livelli di MDA (218±143 nmol/L vs 176±123 nmol/L, p <0.01) e All/UA (1.47±0.72 vs 1.19±0.51, p <0.01).
Il trattamento farmacologico ha portato ad una significativa riduzione dei valori di Kyn e del rapporto Kyn/ Trp. Tale riduzione era associata al decremento dei livelli di stress ossidativo.

1. Nakanishi I, Moutabarrik A, Okada N, et al. Interleukin-8 in chronic renal failure and dialysis patients. Nephrol Dial Transplant 1994;9:1435-42.

P192
WE KNOW ALL THE COMPONENTS OF URINARY STONES?

A. Primiano[1], P.M. Ferraro[2], S. Persichilli[1], G. Gambaro[2], A. Schiattarella[1], C. Zuppi[1], A. Urbani[1], J. Gervasoni[1]

[1]*Laboratorio Analisi I, Fondazione Policlinico A. Gemelli, Roma*
[2]*Unità Operativa Complessa di Nefrologia, Complesso Integrato Columbus, Roma*

Introduction: nephrolithiasis continues to be a major cause of morbidity and health care costs. A recent survey in Italy has shown a prevalence of 7.5% in an urban population mainly affecting adults aged 20–60 years. The most common urinary stones types are calcium oxalate monohydrate and dehydrate, uric acid and its salts, carbonate apatite, struvite (magnesium ammonium phosphate) brushite, and rarely cysteine stones. But we must also consider spurious or factitious stones presented to a clinical laboratory for analysis.
Different methodologies exist for the analysis of renal stones, these include wet chemistry tests and X-ray crystallography, infrared spectroscopy (FT-IR), stereomicroscopic study. Guidelines on Urolithiasis of European Association of Urology 2015 underlines the obsolescence of chemical analysis and recommends the use of FT-IR for urinary stone analysis, this method methods can be combined together with morphological analysis (stereo microscope).
In our laboratory, from 2013 the chemical method has been replaced with the FT-IR and we are currently improving approach by inserting morphological analysis with the stereomicroscope in order to obtain a more complete morphocostitutional analysis. In this way it was possible to find substances considered rare (for example drugs), particular calculi, crystals of unexpected material and identify false stones.
Material and method: we studied 500 urinary stone with FT-IR and same of this also with morphological analysis.
Results and discussion: among 500 calculi, 491 (98.2%) had a known composition (calcium oxalate, uric acid, carbonate-apatite, struvite, brushite and cystine); 2 (0.4%) corresponded to protein; 3 (0.6%) were false calculi (e.g polyglactine, albite, wax-paraffin); 3 (0.6%) contained drugs or metabolites (Atazanivir and Silicate), 1 (0.2%) had an unusual composition (Aragonite).
Due to its intrinsic characteristics, recognition of substances by FT-IR, on the basis of characteristic IR spectra, allows to identify, theoretically, any substance, including drug-containing calculi or calculi with unusual composition. To confirm drug-containing calculi can facilitate modifications in drug therapy.

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CLINICAL AND ANALYTICAL EVALUATION OF FTIR SPECTROSCOPY FOR KIDNEY STONES IN A ROUTINE LABORATORY PRACTICE

G. Musso[1], M.G. Epifani[1], N. Gallo[1], M. Plebani[2]

[1]Department of Laboratory Medicine, University-Hospital of Padova, Padova
[2]Department of Medicine-DIMED, University of Padova, Padova

The prevalence rates for urolithiasis vary from 1% to 20% and is noteably high in countries with a high standard of life (>10%). Stone incidence depends on geographical, climatic, ethnic, dietary and genetic factors. The recurrence risk is determined by the disease or disorder causing the stone formation. The risk status of stone formers and stone composition is of particular interest because it defines the probability of recurrence or regrowth, and is imperative for pharmacological treatment.
The European Association of Urology (EAU) Urolithiasis Guidelines Panel (Skolarikos A et al., Eur Urol 2015) recommends stone analysis should be performed in all first-time stone former, with repeat stone analysis needed in case of recurrence under particular circumstances. The preferred analytical procedures are infrared spectroscopy (IR) or X-ray diffraction (XR). Chemical analysis (wet chemistry) is deemed to be obsolete.
Last year we upgraded from a chemical method (DiaSys Diagnostic System) to a FTIR spectrometer (Nicolet iS5 Thermo Fisher) with Kidney Stone Basic and Daudon as spectral reference libraries. Our laboratory receives stone samples of either inpatients or outpatients both from our hospital and from hospitals of a wide area of the Veneto Region. For this retrospective study we collected data of stone analysis from January to May 2016 and compared to a similar period of 2015. Out of 690 results with chemical analysis we found 54% calcium oxalate, 13% uric acid, 5% cystine and 28% of mixed inflammatory calculi. Out of 484 results with IR we had 68% whewellite or wheddelite, 11% uric acid monohydrate or dihydrate, 14% dahllite, carbonite apatite or struvite, 2% cystine and 4% not calculus. Data show agreement with the known prevalence of chemical compounds. Moreover IR recognizes crystalline form and unusual compositions and can better discriminate small percentages of compounds in mixed inflammatory calculi, which is critical for medical follow up. Overall IR spectroscopy speeds up kidney stone analysis and guarantees a better standardization of results. In spite of this, it requires well trained technical operators and qualified clinical pathologists for spectra interpretation and clinical evaluation for a complete patient's workup.

P194
cFGF-23 MEASUREMENT IN PERITONEAL DIALYSIS PATIENTS

C. Cosma[1], M.T. Vilei[2], M. Zaninotto[1], S. Giannini[2], M. Plebani[1]

[1]U.O.C. Medicina di Laboratorio, Azienda Ospedaliera-Università degli Studi di Padova
[2]Dip. di Medicina, Clinica Medica 1, Azienda Ospedaliera-Università degli Studi di Padova

Introduction and aim: Fibroblast Growth Factor 23 (FGF-23) is a bone-derived hormone involved in the regulation of phosphate homeostasis. It is a protein secreted by osteoblasts and osteocytes. Two native, biologically active forms (intact — iFGF23) and inactive c-terminal fragments (cFGF23) were found in circulation. Aim : to examine the usefulness of FGF23 measurement in peritoneal dialysis (PD) and in the chronic kidney disease (CKD) patienΩts in comparison to a bone diseases (BD) and a control group (CG) subjects.

Methods: Creatinine (Crea, μmol/L), Ca(mmol/L) and P(mmol/L) were measured using Cobas 8000 platform (Roche Diagnostics) and c-FGF23(pmol/L) using ELISA method (Biomedical, Pantec). The results were expressed as median (minimum -maximum) using Mann-Whitney (p <0. 05 as significant).
Results: 10 patients with PD, 10 with CKD, 43 with bone diseases and 20 as control group were recruited. Crea μmol/L: PD= 891 (460-1086), CKD= 136 (72-168), BD=59 (10-131), CG=77 (52-100); the differences in creatinine values were statistically significant between all groups. Ca mmol/L: PD=2.325 (1.98-2.77), CKD=2.47 (2.35-2.73), BD=2.34 (2.04-2.57) and CG=2.375 (2.19-2.64) (PD vs CKD p=0.0079, CKD vs BD p=0.0004 and CKD vs CG p=0.0019); P mmol/L: PD=1.735 (1.05-5.00), CKD:1.09 (0.64-1.51), BD=0.9 (0.55-1.4), CG=1. (0.7-1.9) (PD vs CKD p=0.0004, PD vs BD p <0.0001, PD vs CG p=0.0452, CKD vs CG p=0.0047, BD vs CG p <0.0001); c-FGF23 pmol/L:PD=46.244 (4.224-220); CKD=1.0101 (0.638-5.071); BD=1.18 (0.084-22.913) and CG=0.69 (0.107-1.476) (PD vs CKD p <0.0001, PD vs BD p <0.0001, PD vs CG p <0.0001 CKD vs CG p=0.0154).
Conclusions: CKD patients are characterized by increased FGF23 levels, being the concentrations progressively increased as renal function declines. FGF-23 might be a sensitive biomarker of phosphorus metabolism disorders in patients suffering from CKD with normal phosphate level, because increase earlier than either phosphate or PTH concentrations. Present data furthermore provide additional informations for a better understanding of the association between P homeostasis and FGF23 in patients on PD.

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DATI PRELIMINARI SULL'USO DI NEPHROCHECK, NUOVO MARCATORE DI RISCHIO DI DANNO RENALE ACUTO

M. Carta, S. Indico, R. Tomasiello, A. Tomasini, D. Giavarina

Lab. Analisi, Osp. S. Bortolo, Vicenza

Nephrocheck è un nuovo test per la valutazione precoce del rischio di danno renale acuto (AKI). Comprende la valutazione dell'inibitore tissutale della metalloproteinasi 2 (TIMP-2) e la proteina 7 legante il fattore di crescita simil-insulinico (GFBP7). Nell'ambito di una valutazione di efficienza clinica ed economica, si sono valutati i primi risultati dopo 21 giorni dall'introduzione del test. Il test è stato eseguito sul primo campione di urine proveniente da pazienti ricoverati in Terapia Intensiva (ICU) o dopo intervento cardiochirurgico (CC). Le urine di 11 volontari sani sono state analizzate come gruppo di controllo. L'esame è stato eseguito con immunodosaggio a fluorescenza Nephrocheck®, su apparecchio di misura Astute 140TM. Il risultato è espresso come AKIRisk = ([TIMP-2] * [IGFBP-7] / 1000 (unità = $(ng/ml)^2$/1000). Da indicazioni del produttore, sono stati considerati negativi i campioni <0.3, positivi tra 0.3 e 2.0, fortemente positivi > 2.0 ng/ml2/1000. Nella maggior parte dei campioni (66 su 85) e sui controlli è stata misurata anche la creatinina urinaria. In 21 giorni sono stati valutati 85 pazienti, 46 dalla ICU e 39 dalla CC. Complessivamente, i risultati sono stati tra <0.02 e 36.47 $(ng/ml)^2$/1000, con una mediana di 0.17. Sono risultati negativi, positivi e fortemente positivi, rispettivamente, per i pazienti in ICU 25 (54%), 12 (36%) e 9 (20%) campioni; in CC 30 (77%), 9 (23%) e 0 (0%) campioni. Il gruppo di controllo ha avuto risultati tra 0.06 e 1.08 $(ng/ml)^2$/1000, con una mediana di 0.52 e la percentuale dei positivi del 72%. Valutando il livello di AKIRisk e la creatinina urinaria, questo è risultato inversamente proporzionale nel gruppo di controllo (r= 0.85, p=0.001), mentre non correla nel gruppo campione (r= -0.16 (95%CI -0.25 to 0.22), P= NS) né analizzato in toto, né suddiviso nei due diversi reparti di provenienza. Conclusioni. Il 46% dei soggetti in ICU e il 23% dei soggetti in post cardiochirurgia risultano positivi allo screening. La percentuale di forte positività è prossima alla prevalenza stimata di AKI per i pazienti in terapia intensiva. I pazienti in terapia intensiva presentano caratteristiche particolari di idratazione indotta tali da spiegare come i livelli decisionali debbano essere riferiti esclusivamente a questa particolare popolazione di pazienti.

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ARE NORMALITY RANGES BASED ON A NORMAL HEALTHY POPULATION TRANSFERABLE TO PROFESSIONAL ATHLETES? FIRST CONSIDERATIONS ABOUT THE STEROID PROFILE IN ELITE FEMALE VOLLEYBALL PLAYERS

L. Roli[1], G. Savino[2], T. Trenti[3]

[1]SSD Laboratorio di Endocrinologia, Azienda USL Modena

[2]UOC Tutela della Salute nelle Attività Sportive, Medicina dello Sport, Azienda USL Modena

[3]Dipartimento Interaziendale ad Attività Integrata Medicina di Laboratorio ed Anatomia Patologica, Modena

Background: Testosterone (T) and Cortisol (C) ratio, representing the anabolic/catabolic balance, is useful in the early detection of overtraining syndrome in almost all type of sports. Increasing T and C levels have been described in volleyball male and female players suggesting exercise-related anabolic adaptations to intense workout and individual response to emotional stress due to competition and role in the team. Accordingly to these evidences, may be necessary to develop different reference ranges to assess what is "normality" in professional athletes.

Aim of the study: to assess the basal levels of serum T and C in female elite volleyball players and their changes during 4 main phases of a regular season, athletic Preparation (P), Beginning (B), Middle (M) and End (E) of the championship and to evaluate the transferability of reference ranges of serum T and C used in our laboratory.

Methods. The longitudinal, retrospective study included 47 female elite volleyball players belonging to the same team, whose state of health was evaluated 3/4 times per sportive season, from 2013 to 2016. A blood sample was collected during each physical examination after an overnight fast for routine hematological and hormonal laboratory test, including T and C.

Laboratory tests: T and C levels were measured with CMIA assays Architect 2ng Generation Testosterone (Abbott GmbH & Co, Germany) and Access Cortisol (Beckman Coulter Inc, USA) respectively.

Results: Mean age 23,57 + 5.86 years, median of age 22.94 years. Mean+SD of steroids serum levels: T: 0.35ng/ml+0.14. C: 18.22 μg/dl+4.20. T/C ratio + SD during different championship phases: P: 0.138+0.049; B: 0.022+0.007; M: 0.019+0.009; E: 0.020+0.011. Number (%) of samples with steroids levels out of the laboratory normality range: T (range 0.6 to 6 ng/ml): P: 0; B: 1(2.86); M: 2 (4.65); E: 1 (3.23). C (range 6.7 to 22.6 μg/dl): P: 2(14.29); B: 6(17.14); M: 6(13.95); E: 4(12.90).

Conclusions. The T/C ratio decreases by 80% from P to the E of championship suggesting an intense strain during the regular season; the establishment of reference ranges for hematological parameters which are known to increase accordingly to physical and psychological stress should be considered in the perspective of the individual Athlete Biological Passport.

Meeusen R, Duclos M, Foster C, et al. Med Sci Sports Exerc 2013;45:186-205.

P197
EFFETTO STABILIZZANTE DELL'ALBUMINA SIERICA UMANA SULLE CATECHINE DEL TE VERDE

S. Assaretti, D. Arru, S. Ena, E. Sotgiu, R. Giordo, G. Pintus, C. Carru, A. Zinellu

Dipartimento di Scienze Biomediche, Università di Sassari

L'epicatechina (EC), l'epigallocatechina (EGC), l'epicatechingallato (ECG) e l'epigallocatechinagallato (EGCG) sono composti polifenolici antiossidanti presenti nel tè verde, una bevanda dai riconosciuti benefici. Altamente instabili in soluzione, possono essere degradate attraverso processi ossidativi e di epimerizzazione in funzione di fattori quali pH e temperatura. Diversi studi indicano come il legame con proteine plasmatiche possa modularne la concentrazione plasmatica, il trasporto nei tessuti e l'attività biologica [1]. Lo scopo del lavoro è stato quello di indagare come il legame delle catechine all'albumina sierica umana (HSA) possa influenzare la stabilità delle stesse in condizioni fisiologiche, confrontandole con le loro forme libere. La valutazione della stabilità delle catechine è stata eseguita mediante elettroforesi capillare UV detection dopo precipitazione dell'albumina ed estrazione delle catechine con acetonitrile. L'analisi è stata eseguita in un tampone sodio borato 200 mmol/L (pH 8.4) a 37°C, in capillare di silice fusa (40cm x 75µm ID), valutando l'assorbanza a 214 nm. EC, EGC, ECG e EGCG (0.5mmol/L) sono state incubate in PBS, sia in presenza che in assenza di HSA (0.75mmol/L). Aliquote da 200µL sono state analizzate in diversi momenti durante un periodo di 48 h.

In generale l'albumina ha mostrato un effetto stabilizzante su tutte le catechine. In particolare dopo 48h di incubazione è stato possibile recuperare il 29% di EGCG, l'85% di EC, il 70% di ECG ed il 50% di EGC. In assenza di HSA, EGC e EGCG sono scomparse in meno di 24h, mentre solo il 5% di ECG ed il 50% di EC sono state rilevate al termine dell'incubazione. I dati ottenuti indicano che l'albumina è in grado di stabilizzare le catechine in soluzione acquosa riducendone la velocità di degradazione. Ciò potrebbe avere una certa rilevanza sulla regolazione della biodisponibilità e dell'attività biologica delle catechine in vivo.

1. Bae MJ, IshiiT, Minoda K, et al. Albumin stabilizes (-) -epigallocatechin gallate in human serum: Binding capacity and antioxidant property. Mol Nutr Food Res 2009;53:709–15.

P198
N- E S- OMOCISTEINILAZIONE RIDUCONO L'INTERAZIONE TRA L' ALBUMINA UMANA E LE CATECHINE DEL TÈ VERDE

D. Arru, S. Assaretti, G. Pira, A. Baralla, A.M. Posadino, G. Pintus, C. Carru, A. Zinellu

Dipartimento di Scienze Biomediche, Università di Sassari

Le catechine sono composti polifenolici presenti ad alte concentrazioni in diverse piante (es. tè verde) che pare possano svolgere un ruolo importante nella prevenzione o nel trattamento di patologie quali cancro, diabete, malattie cardiovascolari e neurodegenerative. Le principali catechine del tè verde sono l'epicatechina (EC), l'epicatechina gallato (ECG), l'epigallocatechina (EGC) e l'epigallocatechina gallato (EGCG). Tali catechine interagiscono nel circolo ematico con l'albumina sierica (HSA) che contribuisce al trasporto verso i tessuti modulandone biodisponibilità ed efficacia[1].

In condizioni di iperomocisteinemia la HSA può subire due modificazioni post-traduzionali, S-omocisteinilazione della cisteina 34, da parte dell'omocisteina (Hcy) con formazione di S-Hcy HSA, o N-omocisteinilazione della lisina 525, da parte dell'omocisteina tiolattone (HcyT) con formazione di N-Hcy HSA.

Scopo del lavoro è stato quello di determinare se tali modificazioni possano alterare i livelli di interazione tra HSA e catechine valutando le costanti di legame (K_b) mediante elettroforesi capillare di affinità (ACE).

Per i nostri studi S-Hcy HSA è stata preparata incubando HSA con Hcy 50 e 500µM per 16h; N-Hcy HSA è stata preparata incubando HSA con HcyT 2.5 e 5mM per 4h e la mercaptoalbumina è stata ottenuta riducendo HSA mediante trattamento con ditiotreitolo (DTT) 2.5mM per 35'.

I nostri risultati mostrano che S-Hcy HSA presenta valori di K_b inferiori rispetto ad HSA con un decremento compreso tra il 14 e il 18% per EC ed EGC e tra il 24 e 30% per ECG ed EGCG. Un andamento simile è stato osservato per N-Hcy HSA, con una riduzione dei valori di K_b tra il 17 e 22% per EC ed EGC e tra il 23 ed il 32% per ECG ed EGCG. Per contro la riduzione dell'albumina a mercaptoalbumina non ha mostrato avere effetti sull'interazione con le catechine.

In conclusione possiamo affermare che le modificazioni post-traduzionali dell'HSA tipiche della condizione di iperomocisteinemia riducono la sua affinità nei confronti delle catechine, con probabili conseguenze a carico della loro farmacocinetica e bio-disponibilità.

1. Zinellu A, et al. Evaluation of non-covalent interactions between serum albumin and green tea catechins by affinity capillary electrophoresis. J Cromatogr A 2014;1367:167-71.

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TROPONIN-I, GALECTIN-3 AND NTPROBNP LEVELS IN TRAINED SUBJECTS PARTICIPATING IN AN HALF-MARATHON RUN

C. Vassalle[1], G. Basta[1], C. Prontera[1], P. Di Cecco[1], S. Masotti[2], S. Del Turco[1], L. Sabatino[1], A. Pingitore[1]

[1]Fondazione CNR-Regione Toscana G Monasterio & Istituto di Fisiologia Clinica, CNR, Pisa
[2]Scuola superiore di studi universitari e di perfezionamento Sant'Anna, Pisa

Introduction: Troponin-I (TnI), NTproBNP and galectin-3 (GAL) can mirror cardiomyocyte injury and stretch, and cardiac fibrosis, which represent the most important and prevalent injuries to myocardial tissue. However, the significance of these biomarkers in paraphysiologic states, as endurance exercise, is less known (Salvagno GL, Schena F, Gelati M, Danese E, Cervellin G, Guidi GC, Lippi G. The concentration of high-sensitivity troponin I, galectin-3 and NT-proBNP substantially increase after a 60-km ultramarathon. Clin Chem Lab Med 2014;52:267-72).
Aim: to evaluate the trend of cTnI, NTproBNP and GAL in trained runners before an half-marathon run and a 48-h recovery period.
Methods: Trained runners (N = 18, 15 males, 46±6 years) participated to the half-marathon run with serial evaluation of cTnI (DxI 800, Beckman Coulter), NTproBNP (Cobas e411, Roche) and GAL (Boster Biological Technology Co., Ltd. Pleasanton, CA) at rest, immediately post-run and at 24 and 48 h post-exercise.
Results: At baseline, all runners had NTproBNP values within the reference range. Concentration of NTproBNP and GAL transiently increased after acute exercise (from 31± 32 to 62± 50, 50± 43, 30± 27 ng/L, p <0.001; and from 4869± 4200 to 5443±4576, 4942± 4699, 4853± 4348 pg/mL, p <0.001, respectively) although TnI did not significantly vary (from 42± 13 to 34±1, 43± 12, 32± 1 ng/L). No significant correlation was found among the three biomarkers.
Conclusion: No significant increase of cTnI was found, although this result will be further confirmed by using another cTnI assay (Architect, Abbott; "guideline acceptable" method). The transient increase in NTproBNP and GAL levels may suggest a temporary stress on the myocyte, and being the increase in these biomarkers moderate and reversible, although more slowly for NTproBNP, it may represent a physiological response to acute exercise.

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INFLUENCE OF POLYMORPHISM OF MCT1 GENE AND LACTATE CONCENTRATION IN ELITE FOOTBALL TEAM

G. Canu, M. De Bonis, A. Minucci, E. Capoluongo

Department of Diagnostic and Laboratory Medicine, CriBeNS: Centro di Ricerca in Biochimica e Nutrizione dello Sport, Catholic University, "A. Gemelli" Hospital, Rome

Background: Polymorphisms of proton linked monocarboxylate transporter (MCT1) gene (c.1470A>T) could influence the incidence of muscle injuries and fatigue in elite football players. A recent study has found a higher venous blood lactate levels in AA genotype carriers compared to carriers of the AT and TT genotypes. Lactate transporter defects in skeletal muscle might provide an explanation for some cases of easy fatigue and muscle cramping upon exercise due to delayed removal of the protons accumulated during anaerobic work. Muscle fatigue has been shown to predispose to injury. Lactate is highly accumulated during high-intensity exercise and transport across the plasma membrane is mainly mediated by MCT1. The aim of this study was to compare genotype of MCT1 gene and lactate concentration in 21 players from "Serie A" national football team.
Methods: Genomic DNA were obtained to perform MCT1 polymorphism from whole blood EDTA samples. Lactate test were performed on plasma samples obtained before training T_0, immediately after training T_1 and at two hours from the end of training T_2. Genotyping assays were performed by PCR and direct sequencing. The primers used were homemade by us.
Results: The 21 players showed the follow DNA profiling: 12 (57%) are AA (reduced lactate clearance), 8 (38%) are AT and 1 (5%) is TT. The lactate concentrations from all athletes resulted at T_0, T_1 and T_2 1.39, 2.7 and 1.33 mmol/L, respectively. After the first training, lactate concentration increased significantly (p <0.0001), decreased at T_2 (p <0.005) and returned baseline when compared with T0. Athletes with AA genotype have 1.42, 2.99, 1.21 mmol/L lactate concentration at T_0, T_1 and T_2, respectively. Athletes with AT and TT genotypes have 1.34, 1.62, 0.66 mmol/L lactate concentrations at T_0, T_1 and T_2, respectively.
Discussion: This study highlighted that athletes with AA genotype had reduced lactate clearence with higher venous lactate concentrations at different times compared with AT and TT genotype. To avoid sport performance and decrease muscle fatigue and injuries related to lactate clearance, are need know DNA profiling. This study showed the importance of genetics on athletes in comparison with chemical tests to avoid individualized nutritional and training programs.
Massidda M, et al. Sports Med Open 2015;1:33.

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SPORT PERFORMANCE: COMPARISON BETWEEN MCT1 POLYMORPHISM/LACTATE LEVELS AND PERSONALIZED NUTRITIONAL PROGRAM IN ELITE FOOTBALL TEAM

G. Canu[1], M. De Bonis[1], A. Vitaterna[2], M. Pirelli[2], E. Capoluongo[1]

[1]*Department of Diagnostic and Laboratory Medicine, CriBeNS: Centro di Ricerca in Biochimica e Nutrizione dello Sport, Catholic University, "A. Gemelli" Hospital, Rome*
[2]*Staff Sanitario Frosinone Calcio, Stagione 2015/2016" Serie A Tim"*

Background: Lactate is highly accumulated during high-intensity exercise. The transport across the plasma membrane is mainly mediated by proton-linked monocarboxylate transporters (MCT1) that is particularly important in skeletal muscle and it is transported out of the cell through this transporter. MCT1 gene polymorphism (c.1470A>T) could influence the incidence of muscle injuries and fatigue in elite football players. Lactate transporter defects and accumulation in muscle might cause easy fatigue and cramping upon exercise due to delayed removal of the protons accumulated during anaerobic work. Muscle fatigue has been shown to predispose to injury. The aim of this study was compared genotype of MCT1 gene and lactate levels with personalized nutritional program in 21 players from "Serie A" national football team.
Methods: Genomic DNA and plasma samples were obtained to perform MCT1 polymorphism and lactate chemical test before training T_0, immediately after training T_1 and at 2 hours from the end of training T_2 in 2 different days of championship, at beginning and after personalized alkaline nutritional program.
Results: The 21 players showed the follow DNA profiling: 12 (57%) are AA (reduced lactate clearance), 9 are AT/ TT (43%). The lactate concentrations from all athletes at beginning of championship was 0.64, 8.31, 0.92 mmol/ L at T_0, T_1 and T_2, higher than concentrations measured after nutritional modifications (1.39, 2.7 and 1.33 mmol/L), respectively. At beginning and after nutritional program, the athletes with AA genotype have 0.59, 9.42, 0.99 and 1.42, 2.99, 1.21 mmol/L lactate levels at T_0, T_1 and T_2, respectively; the athletes with AT and TT genotypes have 0.71, 6.66, 0.81 and 1.34, 1.62, 0.66 mmol/L lactate concentrations at T_0, T_1 and T_2, respectively.
Discussion: Athletes with AA genotype showed a reduced lactate clearence with higher venous lactate concentrations compared with AT and TT genotypes. To avoid sport performance and decrease muscle fatigue and injuries related to lactate clearance, is need know DNA profiling. With important nutritional intervention, lactate levels and accumulation in muscle are decrease, avoiding athletic performance e reducing injuries. This study highlighted importance of nutritional intervention for decrease lactate levels and accumulation.
Massidda M, et al. Sports Med Open 2015;1:33.

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CASI DI MALARIA A CARPI: MIGLIORE ACCURATEZZA O MAGGIORE INCIDENZA?

M.F. Borghi[1], V. Favale[1], D. Bisi[1], P. Coppolecchia[1], T. Trenti[2]

[1]*Lab. Patologia Clinica, Osp. B. Ramazzini, Carpi (MO)*
[2]*Dip. Patologia Clinica SC Patologia Clinica Tossicologia Diagnostica Avanzata, Nuovo Osp. S. Agostino Estense, Modena*

Scopo del lavoro: analizzare criticamente i dati raccolti riguardo alla ricerca del Plasmodio della Malaria (P.m.) nell'anno 2015 in cui sono arrivate al Lab. Pat. Clin dell'H. Carpi 35 richieste consecutive di ricerca del P.m. (22 M/ 13 F) età 4-41 anni, il 66% ≤12 anni. Il 77% erano pazienti stranieri. Il percorso è stato: 1) allestire e colorare (Giemsa) più vetrini di cui 2 venivano mandati a NOCSAE per conferma di positività; 2) ricerca di Ag malarici nel sangue mediante test immunocromatografico (IC) su membrana che utilizza due anticorpi monoclonali (MAB). Il primo MAB riconosce una proteina ricca di istidina (HRP-2) caratteristica del P.falciparum, l'altro riconosce un Ag comune alle 4 specie di malaria (PMA); 3)la provetta di sangue viene mandata a NOCSAE per effettuare PCR. HPR-2 è prodotta negli stadi asessuati di P.f. Lo svantaggio dell'utilizzo della HPR-2 come RDT è quando la parassitemia è inferiore a 100/µl e che la proteina è assente nei gametociti. 20/35 campioni sono risultati negativi sia al microscopio che con IC. 15 campioni provenienti da 11 paz.sono positivi al microscopio. Sono casi tutti di importazione. Di questi 11 pazienti 6 provenivano dal Pakistan. La sensibilità diagnostica dell'IC rispetto al microscopio 80% e specificità 87% CASO n°1 M/40 anni provenienza Ghana prelievo H8 Test IC neg microscopico pos per P.f.rari trofozoiti Prelievo H14 Test IC pos per P.f. Test microscopico pos per P.f. CASO n°2 F/4 anni proveniente Togo prelievo 18/5 Test IC pos per P.f.o mistoTest microscopico: numerosi trofozoiti di P.f. Dopo una settimana dalla fine della terapia antimalarica c'era completa risoluzione. Prelievo del 21/5Test IC pos per P.f. Test microscopico pos per P.f. Prelievo del 25/5Test IC pos per P.f. Test microscopico pos per P.f.Ci potevamo aspettare (caso n° 1) che una bassa parassitemia potesse causare un risultato FN in quanto una limitazione all'uso dell'IC è proprio questa. Nel caso n° 2 invece abbiamo un FP in una paziente trattata per P.f. La proteina HRP-2 persiste in circolo anche per 28 giorni. RDT è capace di una diagnosi rapida di infezione di P.f.o infezione mista, comunque la rilevazione di FN può portare al rischio di una diagnosi sbagliata. Per questa ragione è raccomandata la conferma mediante visione microscopica.

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PRE-ANALYTICAL PHASE IN FLOW CYTOMETRY: THE KEY ROLE OF SAMPLE PREPARATION

V.E. Muccio, E. Saraci, M. Gilestro, M. Ruggeri, D. Oddolo, S. Caltagirone, E. Marzanati, P. Omedè

Laboratorio di Citofluorimetria, Dipartimento Universitario di Ematologia, A.O.U. Città della Salute e della Scienza di Torino, Torino

Introduction: Flow cytometry is a useful tool to diagnose hematological diseases and to evaluate Minimal Residual Disease (MRD). The standard sample preparation is based on Stain-Lyse-Wash method (SLW) but washing the samples before staining can prevent unspecific antibody bindings. In our study we performed a total of 468 test with the two methods: 104 surface antibodies were tested in a total of 5 PB and 5 BM, and 13 antibodies, used to identify lymphocyte subpopulations, were tested in 10 samples.

Methods: LWS procedure: 100μL of peripheral blood or 1x106 white blood cells were incubated at RT for 10 min with 3mL of NH4Cl lysing solution, centrifuged at 4°C for 5 min at 1500 RPM, washed in 3 mL of PBS + BSA 0.5%, stained in darkness at RT, with the appropriate amount of antibodies, for 15min and washed in PBS. In SLW procedure the steps are shifted. MFI and percentage values were obtained by positive populations among all white blood cells gated by physical cytometric parameters. 100.000 total events were acquired with Navios flow cytometer and analyzed by Kaluza software.

Results: MFI and percentages obtained by the two methods were not different (p=0,224 and p=0,186, respectively) and highly correlated (r^2=0,835 and r^2=0,991 respectively, p <0,001). The Bland Altman analysis of differences between coupled percentages has shown -0,324 as a mean and above all differences did not exceed 5% except for 3 cases where debris in SLW samples cannot be excluded by cytometric physical parameters. The amount of debris was higher in SLW than LWS samples (mean=31,8% vs 19,3%, p <0,001). The analysis of 13 selected antibodies in 10 patients has shown a significant difference between percentages of positive cells for CD45 and surface kappa and lambda chains.

Conclusions: The percentages of positive cells obtained by LWS or SLW are comparable. The high amount of debris in SLW samples explain the high number of positive cells for CD45 in LWS samples. The higher percentage of positive cells in LWS samples for surface kappa and lambda chains are justified by their increased brightness after washing samples. We suggest to use the LWS preparation to reduce debris, to prevent artifacts, and to better compare data obtained at diagnosis and at MRD investigation with bulk lysis method.

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ALTA AUTOMAZIONE, EMOGASANALIZZATORE E GLUCOMETRO: LA GLICEMIA DAL LABORATORIO AL SELF-TESTING

B. Bernardi, A. Panetto, G. Bonfant, M. Di Benedetto

S.C. Analisi Cliniche, Osp. U. Parini, Aosta

La misura della glicemia è una delle principali applicazioni nella diagnostica POCT, in quanto l'immediata disponibilità del valore permette monitoraggio e terapia tempestivi, migliorando la qualità dell'assistenza e semplificando la complessità organizzativa del territorio.
Metodi: è stato effettuato un confronto tra una catena automatizzata, metodo di riferimento, e dispositivi portatili, valutandone caratteristiche e prestazioni. 40 pazienti esterni sono stati prelevati e, entro 14 minuti, sono stati eseguiti i dosaggi su Cobas8000 (Roche), ABL825flex (ABL, De Mori), Epoc Reader e StatStrip (ER e SS, Menarini).
Dopo aver escluso l'effetto della glicolisi, è stata valutata la concordanza tra i valori di ABL, ER e SS con il metodo di riferimento, ai fini dell'inquadramento diagnostico mediante analisi statistica (Bland e Altman, griglie di Clarke e di Shermok).
Risultati: n=40, ABL glicemia media 117,5, max 252, min 80 mg/dL, differenza media (ABL-Cobas) 2,28, Limits of Agreement (LOA) 7,4 e -2,8 mg/dL; ER glicemia media 111, max 255, min 67 mg/dL, differenza media (ER-Cobas) -4,2, LOA 4,5 e -12,9 mg/dL; SS glicemia media 106,5, max 225, min 68 mg/dL, differenza media (SS-Cobas) -8,7, LOA 4,1 e -21,6 mg/dL.
Griglia di Clarke: i dati di tutti gli strumenti si situano nelle zone A e B.
Griglia di Shermok: la % di osservazioni che porterebbe a decisione clinica discordante rispetto al Cobas è simile per ABL e ER (7,5 e 12,5%), mentre per SS è il 20%.
Conclusioni: la differenza media tra ER e ABL ed il Cobas è simile, i limiti di agreement di SS sono più ampi rispetto ad ABL e ER. Tutti gli strumenti nella Griglia di Clarke si situano nelle zone A e B di decisione terapeutica concordante.
I criteri di accettazione ISO 15197 sono soddisfatti da ABL e ER, confermandone la possibilità di utilizzo ospedaliero. I risultati di SS pur essendo accettabili solo per concentrazioni di glucosio pari o superiori a 100 mg/dL, grazie alla connettività e al facile utilizzo dello strumento, ne permettono l'impiego al letto del paziente.
Luukkonen AA, Lehto TM, Hedberg PS, et al. Evaluation of a hand-held blood gas analyzer for rapid determination of blood gases, electrolytes and metabolites in intensive care setting. Clin Chem Lab Med 2016;54:585–94.

P205
EVALUATION OF KFLC AND K INDEX IN CEREBROSPINAL FLUID: OUR EXPERIENCE

C. Ferraris Fusarini[1], S.M.G. Cioffi[2], A. Maregnani[1], I. Silvani[1], D. Galimberti[2], R. Maiavacca[1]

[1]Lab. of Clinical Chemistry and Microbiology, Fondazione IRCCS Ca' Granda Osp Maggiore Policlinico, Milan
[2]Neurology Unit, Dep of Pathophysiology and Transplantation, University of Milan, Fondazione IRCCS Ca' Granda Osp Policlinico, Milan

Background: Multiple sclerosis (MS) is an inflammatory autoimmune disease that causes demyelination of central nervous system. In MS intrathecal immunoglobulin (Ig) synthesis is commonly observed: it can be evaluated through the detection of IgG oligoclonal bands in cerebrospinal fluid (CSF) with isoelettrofocusing and immunoblotting, operator depending methods. The measurement of free light chains (FLC) can represent a valid alternative.
Methods: We have performed a turbidimetric assay of λ and k FLC (Freelite™, Binding Site, Birmingham), demonstrating that only kFLC is useful for diagnosis of MS. We collected serum and CSF of 172 patients with different neurological disorders. On the basis of consensus definition and application guidelines for CSF studies in MS, patients were divided into four groups: MS (65), other neurological disorders (OND, 66), other inflammatory neurological disorders (OIND, 10) and control (CTRL, 31).
Results: About serum, we reported for kFLC significantly more elevated values in OND (26.40±16.54 mg/l), OIND (20.63±11.68 mg/l) and CTRL (33.38±23.36 mg/l) than MS (14.42±3.74 mg/l); the same result was found for λFLC (OND = 18.65±10.13 mg/l; OIND = 18.84±9.48 mg/l; CTRL = 19.07±7.04 mg/l, MS = 10.95±3.54 mg/l) ($p < 0.001$). On the contrary, kFLC in CSF are significantly higher in MS (4.34±5.10 mg/l) than in OND (0.93±1.46 mg/l), OIND (0.72±0.63 mg/l) and CTRL (0.77±0.68 mg/l) ($p < 0.001$); λFLC are not statistically significant ($p=0.02$) so we did not considered them. We then evaluated the k index as CSF/serum quotient: it was higher in patients with MS (0.35±0.39) compared to other groups (OND = 0.04±0.09; OIND = 0.06±0.07; CTRL = 0.03±0.03). ROC curves were performed for kFLC and k index: areas under curves (AUC) were 0.74 and 0.87 (95% confidence interval), respectively.
Discussion: As other authors reported, we found values of kFLC and k index more elevated in patients with MS compared to other groups (OND, OIND, CTRL), but k index seems to be a more useful diagnostic tool, with an AUC of 0.87. Additional data is needed to confirm the results obtained and to evaluate the correlation between kFLC and k index and progression and monitoring of MS.
Teunissen C, Menge T, Altintas A, et al. Mult Scler 2013;19:1802-9.

P206
VALIDATION STUDY OF THERMO SCIENTIFIC CEDIA CYCLOSPORINE/MYCOPHENOLIC ACID AND QMS TACROLIMUS/EVEROLIMUS IMMUNOASSAYS ON THE BECKMAN COULTER AU680 ANALYZER IN A CLINICAL SETTING.

A. Veronesi, C. Carone, E. Cariani, C. Rota, N. Castellana, D. Gallesi, T. Trenti

Department of Laboratory Medicine & Pathology, Nuovo Ospedale S. Agostino-Estense, Modena

Immunosuppressive drugs (ISD) are used for the control of tissue rejection following transplantation [1]. Although liquid chromatography coupled to mass spectrometry (LC-MS) represents the gold standard for ISD quantification, immunoassays might prove faster, easier and prone to automation for clinical ISD monitoring. The study evaluates, in a clinical setting, the suitability of Thermo Fisher Scientific (TFS) immunoassays for the determination of cyclosporine both in Low and High range (LR and HR CsA), mycophenolic acid (MPA), tacrolimus (TAC) and everolimus (EVER) on the AU680 analyzer (Beckman Coulter). CsA/MPA assays used a CEDIA while TAC/EVER assays used QMS technology. Blood samples from patients receiving CsA (n=169 kidney, n=58 bone and n=11 unknown transplants), MPA (n=134 kidney transplants), TAC (n=93 kidney, n=83 liver and n=15 unknown transplants) or EVER(n=66 kidney, n=39 liver and n=23 unknown transplants) were collected and tested by both LC-MS/MS (Chromsystems reagents) and immunoassays.
All reagents, calibrators, controls were supplied by TFS. Precision, sensitivity, linearity and accuracy were evaluated according to the CLSI guidelines. Comparison method was carried out by Spearman's correlation and Passing-Bablok regression.
The coefficient of variation for all immunoassays was lower than 6% (within day) and 9% (between days); linearity and sensitivity were consistent with manufacturer's specifications.
Correlation analysis revealed a good agreement ($r>0.87$, $p < 0.0001$) between the immunometric and LC-MS/MS results for all the ISD patient samples. Nevertheless, immunoassay data showed a positive average bias respect to LC-MS/MS measures for all the ISDs: 14.9% for LR CsA, 19.1% for HR CsA, 28.6% for MPA, 47.9% for TAC and 28.6% for EVER. Moreover, after stratification groups for transplant type a significantly different bias was detected after bone respect to kidney transplants (23.8% vs 13.7%) in CsA samples, while no significant difference was observed for the other ISD. Data demonstrated acceptable performance of AU680 analyzer in terms of precision and sensitivity. The bias between LC-MS/MS and immunoassay results is consistent with previous data and suggests appropriate therapeutic reference ranges based on the detection method used.
1. Morris RG. Ann Pharmacother 2005;39:119-27.

P207
STUDIO COMPARATIVO PER L'INTRODUZIONE DI UN NUOVO METODO DI DOSAGGIO DELLA PCR MEDIANTE POCT

E. Perri, A. Periotto

Lab. analisi chimico-cliniche, Società Ricerche Biomediche Valle d'Aosta, Aosta

Ormai è noto che la proteina C reattiva (PCR) è presente nel sangue e fa parte della famiglia delle proteine di fase acuta che vengono sintetizzate durante uno stato infiammatorio. La misura della VES come marker infiammatorio, pur avendo minor specificità e sensibilità rispetto alla PCR, è ancora largamente richiesta. Lo studio si è basato su una comparazione tra metodi di dosaggio della la PCR per valutare se il nuovo metodo immunochimico in fase solida mediante POCT possa eventualmente sostituire quello turbidimetrico attualmente in uso. In aggiunta, è stato fatto anche un confronto dei risultati di PCR con quelli della VES. Nel periodo di osservazione (gennaio-maggio 2016) sono state eseguite 146 PCR e 172 VES (15% di differenza) ed è stata fatta un'osservazione sul 64% di PCR rispetto al totale dei dosaggi eseguiti. Sono stati analizzati 94 risultati di cui il 47% ha richiesto contemporaneamente il dosaggio di VES e PCR. Sulla base di quest'ultima percentuale di risultati della PCR, è stata calcolata la differenza media ottenuta tra i due metodi (circa 20%) ed è stato elaborato l'intervallo di confidenza che comprende il 95% delle differenze tra i due metodi (±1,96×DS). Dall'elaborazione del grafico di Bland-Altman, si è evinto che il 98% dei dati rientra in tale intervallo ($-1,6 \leq x \leq 2,41$) e che i punti si addensano casualmente intorno alla linea media delle differenze (0,41). Dall'analisi del confronto eseguito tra i risultati di PCR e VES, il 76% dei risultati correla per entrambi gli analiti (PCR <0,5 mg/dL - VES <15) mentre il restante 24% ottiene risultati discordanti.
In conclusione, nonostante non sia stata individuata alcuna dipendenza delle differenze dalla concentrazione, il laboratorio si propone comunque di proseguire l'osservazione effettuando un confronto su range di valori di PCR più elevati poiché il 70% dei valori raccolti in questo studio sono risultati essere inferiori a 0,5 mg/dL. Ciononostante, la comparazione effettuata dei due metodi può non precludere l'introduzione del nuovo metodo poiché non sussiste compromissione del significato e dell'impatto clinico.
Vidali M, Tronchin M, Dittadi R. Protocollo per la comparazione di due metodi analitici di laboratorio. Biochim Clin 2016;40:129-42.

P208
VALIDATION OF HEMOGLOBIN AND HEMATOCRIT MEASUREMENTS ON GEM PREMIER 4000 BLOOD GAS ANALYZER

E. Aloisio, A. Panzeri, S. Birindelli, A. Dolci, M. Panteghini

Clinical Pathology Unit, 'Luigi Sacco' University Hospital, and Department of Biomedical and Clinical Sciences, University of Milan

Background: With the introduction of a new blood gas analyzer (GEM Premier 4000, Instrumentation Laboratory) in our laboratory, we preliminarily checked in a correlation study the interchangeability of hemoglobin (Hb) and hematocrit (Hct) results with those obtained with the automated blood cell counter used in our core-lab.
Methods: Hb and Hct were assayed in duplicate on 46 fresh EDTA whole blood samples by both the GEM analyser and the Sysmex XN-9000 hematology system. Hct was also estimated (eHct) on GEM from Hb concentrations with the formula: eHct=0,03*(Hb/10). Correlations were performed using linear regression analysis and biases evaluated using difference plots.
Results: Hb and Hct concentrations at XN-9000 ranged from 56.0 to 175.5 g/L and from 0.16 to 0.53 L/L, respectively. Regression analyses gave the following equations: for Hb, GEM=1.00 (95%CI: 0.97-1.03)*XN + 3.9 g/L (95%CI: 0.3-7.5), r=0.996; for Hct, GEM=1.14 (95%CI: 1.06-1.22)*XN – 0.02 L/L (95%CI: –0.05-0.01), r=0.973; for eHct, GEM=1.07 (95%CI: 1.01-1.13)*XN – 0.007 L/L (95%CI: –0.03-0.02), r=0.983. When compared with minimum goals for bias derived from biological variability (≤2.8% for Hb and ≤2.6% for Hct, respectively), GEM Hb displayed a slightly elevated bias (+3.4%), whereas Hct (both measured and estimated) showed a markedly positive bias (+8.7% and +4.7%, respectively).
Conclusions: GEM Hb showed a constant positive bias of ~4 g/L, which appears tolerable if the use of these results is limited to screening purposes. On the other hand, GEM Hct displayed a suboptimal accuracy, making this measurement probably unsuitable for clinical use.
Budd JR, Durham AP, Gwise TE, et al. Measurement procedure comparison and bias estimation using patient samples; Approved Guideline - Third edition. The Clinical and Laboratory Standards Institute 2013, document EP09-A3.

P209
LETTURA DEI FLUIDI BIOLOGICI: DUE METODI A CONFRONTO

M. Ammirabile, C. Ferraris Fusarini, A. Maregnani, M. Speroni, M. Cambisano, R. Maiavacca

Lab centrale di analisi chimico-cliniche e microbiologia, Fondazione IRCCS Ca' Granda Osp Maggiore Policlinico, Milano

Introduzione: La conta dei leucociti nei liquidi biologici (BF) è un elemento utile per valutare eventuali stati infiammatori o infettivi. Nel nostro laboratorio analizziamo più comunemente i liquidi cerebrospinale (CSF), pleurico (PLE) e ascitico (ASC). Ad oggi si esegue una conta microscopica in camera di Burker/ Nageotte con opportuna diluizione (ingrandimento 250x). Questo metodo richiede personale formato, è operatore-dipendente e presenta una certa imprecisione. Da qualche anno sono stati sviluppati analizzatori ematologici automatizzati applicabili ai BF.
Metodi: È stato effettuato un confronto tra i risultati ottenuti con il microscopio e lo strumento XN-1000 (Sysmex Corporation, Kobe, Japan). Sono stati valutati 113 BF (50 CSF, 47 ASC e 16 PLE).
Risultati: Valutando la correlazione tra i metodi abbiamo ottenuto i seguenti coefficienti R^2: 0.98 per CSF, 0.97 per ASC e 0,94 per PLE. La differenza tra le due misurazioni effettuate è statisticamente non significativa (test t di Student, $p > 0.05$). Per discriminare tra condizione fisiologica e patologica, è stato usato uno specifico valore di cut-off (cell/mmc) per ogni BF: >5 per CSF, >250 per ASC e >1000 per PLE. Utilizzando questi cut-off abbiamo ottenuto risultati diagnostici statisticamente dissimili: 10%, 2.2% e 12.5% rispettivamente per CSF, ASC e PLE (test di Fischer, $p < 0.05$). Mediante le curve ROC abbiamo un diverso cut-off per ogni BF: >7 cell/mmc per CSF con sensibilità e specificità pari a 93.7% e 91.2% (AUC=0.976, intervallo di confidenza (IC) 95%=0.887-0.997, $p < 0.05$); >228 cell/mmc per ASC con sensibilità e specificità di 88.2% e 97.2% (AUC=0.899, IC 95%=0.784-0.964, $p < 0.05$); >1438 cell/mmc per PLE con sensibilità e specificità di 71.4% e 100% (AUC=0.781, IC 95%=0.555-0.926, $p < 0.05$).
Conclusioni: Lo strumento XN-1000 potrebbe essere introdotto nella pratica di laboratorio, considerando la buona correlazione con l'analisi al microscopio. Tuttavia abbiamo rilevato alcuni falsi positivi e falsi negativi (in totale, rispettivamente 6 e 2) che potremmo ridurre eseguendo una conta microscopica in caso di risultati compresi tra 5 e 7 cell/mmc (CSF), 228 e 250 (ASC), 1000-1438 (PLE). Ciò permetterebbe di ottimizzare l'uso delle risorse disponibili.
Fleming C, Brouwer R, Lindemans J, et al. Clin Chem Lab Med 2012;50:1791-8.

P210
96-WELL MICROELUTION PLATES USED FOR SIMULTANEOUS QUANTIFICATION OF EIGHT ANTIBIOTICS IN HUMAN PLASMA: AN UHPLC-PDA METHOD VALIDATION

F. Favata, G. Fatiguso, M.T. Terzulli, L. Baietto, S. Allegra, G. Di Perri, A. D'Avolio

Department of Medical Sciences, Unit of Infectious Diseases, University of Turin, Amedeo di Savoia Hospital, Turin

Background: Therapeutic Drug Monitoring(TDM) is an emerging tool used to minimize toxicity in drugs with narrow therapeutic window or complex pharmacokinetics. Its application is important, expecially in patients with serious infections and sepsis but TDM is limited by technical problems and cost restrictions. 96-well SPE plates could be a good strategy to increase throughput and to reduce solvent and material consumption, a single rapid method could optimize laboratory routine. We aimed to develop and validate a new chromatographic method for simultaneous plasma quantification of: ceftriaxone, ceftaroline, ciprofloxacin, daptomycin, ertapenem, levofloxacin, linezolid and moxifloxacin.
Method: plate wells were activated with methanol, then conditioned with KH2PO4 pH 1,9 (phosphoric acid). 125 μL of sample, 125μL of KH2PO4 pH 1,9 and 50 μL of internal standard working solution were loaded in plate wells; two different consecutive washes and elution phases were made. Finally, samples were diluted with 200μL of HPLC grade water and 10 μL were injected in the UHPLC-PDA system. Chromatographic separation was performed at 45 °C using a column ACQUITY UHPLCTM HSS
T3, 1.8 m, 2.1 × 150 mm, at 0.4 mL/min using solution A, potassium dihydrogen phosphate buffer 10 mM (pH 5.5 with sodium hydroxide, NaOH) and solution B, acetonitrile.
Results: the method was validated according to Food and Drug Administration and European Medicine Agency guidelines. Each antibiotic has a different retention times and well-separated peak, with no interference to endogenous compounds and other drugs. Following parameters were evaluated: calibration curve (r^2 >0.999), limit of quantification, limit of detection, intra (4-7%) and interday (9-15%) imprecision, intra (±15%) and interday (8-15%) accuracy, mean recovery (44-83%) and stability. Samples have been analyzed both with validated methods (1) and new method without any significant difference.
Conclusions: this method could be useful to facilitate antibiotics plasma exposure, allowing TDM employment in laboratory routine: a single method with 8 antibiotics can change radically sample management, time and resources.
1. Baietto L, D'Avolio A, De Rosa FG, et al. Ther Drug Monit 2009;31:104-9.

P211
DETERMINAZIONE DEI LIVELLI EMATICI DI EVEROLIMUS: CONFRONTO TRA DUE METODI ANALITICI

A. Del Viscovo, E. Assentato, M. Caputo, G. Longo, W. Utech

U.O.S.D. Biologia Molecolare di Virologia, Centro NAT e Immunodiagnostica dei Trapianti. Dipartimento dei Servizi A.O. "A. Cardarelli", Napoli

Scopo dello studio è di confrontare i risultati ottenuti con il dosaggio QMS (Quantitative Microsphere System), in uso presso il nostro Centro per il monitoraggio della terapia con Everolimus, con quelli ottenuti con il metodo ECLIA (ElectroChemiLuminescence).
Materiali e metodi: Campioni di sangue intero di 62 pazienti trattati con Everolimus (Evr) sono stati contestualmente processati con l'analizzatore CDX90 TEMA (QMS, ThermoScientific) e con l'analizzatore COBASe601 (ECLIA, RocheDiagnostics). Sono stati testati, con entrambi i metodi, campioni di VEQ (Bioanalytics and Toxicology,London). Per lo studio sono stati eseguiti test statistici di base, studi di correlazione e comparazione.
Risultati: I risultati ottenuti con i due metodi hanno mostrato un'apparente sovrastima del metodo ECLIA. L'analisi di Bland-Altman mostra un bias medio di -2,10 ng/mL e r^2=0,43. L'intervallo di confidenza della media delle differenze al 95% varia tra + 0,2 e -4,4. I risultati della VEQ mostrano che con la metodica ECLIA essi sono in linea con quelli attesi e i risultati ottenuti con il test QMS sono sottostimati per il fattore di correzione introdotto dalla Ditta al fine di allineare i risultati del test QMS con la tecnica gold standard che in routine risulta complessa e laboriosa (Thermo Scientific QMS Everolimus Assay, Product Design and Background Information,2010). L'analizzatore COBASe601 ha rilasciato i risultati dei test in tempi inferiori rispetto all'analizzatore CDX90 per le caratteristiche strumentali.
Conclusioni: La metodologia ECLIA offre la possibilità di effettuare su un'unica piattaforma il dosaggio di più farmaci immunosoppressori che sono in uso presso la nostra A.O. e di velocizzare la fase pre-analitica con un unico pretrattamento del campione nei pazienti in terapia con combinazione di farmaci. I risultati dei due metodi sono sovrapponibili solo dopo aver introdotto un fattore di correzione. Nel fornire il dato analitico al clinico deve essere segnalato con quale delle due metodiche è stato ottenuto per evitare un sovradosaggio con conseguente tossicità, o un sottodosaggio con possibilità di rigetto. Tale accorgimento consentirà il migliore adeguamento terapeutico anche ai pazienti che dovessero rivolgersi a Laboratori diversi nel corso del monitoraggio.

P212
EVALUATION OF THE CORRECTIVE PERFORMANCE OF NON-DEUTERATED INTERNAL STANDARD ON ANALYTICAL RESULTS: THE INTERNAL-STANDARD-NORMALIZED MATRIX EFFECT

A. De Nicolò[1], D. Pensi[1], M. Pinon[2], C. Pisciotta[1], P.L. Calvo[2], A. Nonnato[4], R. Romagnoli[3], F. Tandoi[3], G. Di Perri[1], A. D'Avolio[1]

[1]Unit of Infectious Diseases, Univ. of Turin, Dept. Medical Sciences, Amedeo di Savoia Hosp., Turin
[2]Unit of Pediatric Gastroenterology & Hepatology, Univ. of Turin, "Città della Salute e della Scienza" Hospital, Turin
[3]Liver Transplantation Center & General Surgery 2U, "Città della Salute e della Scienza" Hosp., Univ. of Turin
[4]Clin. Biochem. Unit, Dept. Diagnostic Lab., A.O.U. "Città della Salute e della Scienza" Hosp., Turin

Aim: Although tandem mass spectrometry (MS/MS) is now widely used, the impact of matrix effect (ME) is an important problem and its evaluation is mandatory. In order to balance ME deuterated internal standards (IS) are used, but these are sometimes not available or too expensive. In this work we propose a formula to describe the IS-normalized-ME (IS-nME), estimating the impact of ME on analytical results when non-deuterated ISs are used. We applied IS-nME on a method for the intracellular quantification of tacrolimus (TAC) and everolimus (EVE) in PBMCs. Experimental: PBMC samples were isolated through CPT vacutainers and counted through an automated cell coulter. After lysis and addition of IS (ascomycin), samples were analysed through UPLC-MS/MS (ESI+) coupled with on-line SPE (OSM®, Waters). Method validation was conducted in accordance with FDA and EMA guidelines. Matrix effect was evaluated at different cell concentrations (3x106 to 24x106 cell/mL). Besides the usual evaluation of ME, we evaluated the IS-nME through the formula: IS-nEM ={[(PAAna-matrix/PAIS-matrix)/(PAAna-neat/PAIS-neat)-1]*100}, where PA is peak area of the analyte and of the IS (in matrix or neat samples). The RSD was calculated on "(PAAna-matrix/PAIS-matrix)/(PAAna-neat/PAIS-neat)". Results: Linearity, accuracy, precisions and recovery fitted the limits of acceptance indicated by FDA and EMA guidelines. Since the RSD of ME was too wide for EVE (13.4%) in samples with more than 12x106 cell/mL, the validation was performed for samples with a lower cell number. Mean ME for TAC, EVE and IS were -29.48% (RSD 3.35%), +17.45% (RSD 1.67%) and -31.71% (RSD 8.00%), respectively. Mean IS-nME was low for TAC (+3.90%, RSD 9.50%) and high, but still reproducible, for EVE (mean +72.81%, RSD 8.39%). Conclusion: The method was successfully validated according to current guidelines. The proposed formula confirmed ascomycin as optimal IS for TAC, while highlighted a high IS-nME for EVE. However, the mean IS-nME was successfully corrected by the adoption of a calibration curve in PBMC matrix: in this context, the real amount of error is due to the variability of IS-nME between different matrix lots, which resulted lower than 10% for both drugs, thus avoiding any significant impact on analytical results.

P213
BASIC VALIDATION OF A NEW METHOD FOR ACE DETERMINATION

S. Brambilla[1], V. Bellomo[2], L.M. Motta[1], S. Valaperta[2]

[1]*Clinical Lab., Humanitas Research Center, IRCCS, Rozzano, Milan*
[2]*Struttura Complessa Clinical Lab., Fond. Policlinico San Matteo, Pavia*

Before introducing a new analytical method replacing the previous one a laboratory should investigate, as required by IVD 98/79 and UNI ISO 15189 Standard, if it can really replace the former in terms of practicability and costs and also of analytical performance. We compared the two methods for Angiotensin Converting Enzyme (ACE) and we studied the features of the new one. The new method (ACE Liquid, Sentinel CH, kinetic, the first automated assay on chemistry analyzers - Architect Ci16000Abbott) has been compared to the former (ACEcolor, Fujirebio Inc., manual colorimetric end-point on Beckman Du640 Spectrophotometer) measuring 41 routine serum samples in duplicate in order to cover a wide range of concentrations (5.3–35.3 U/L and <6.0–118.0 U/L for ACEcolor, Fujirebio and ACE Liquid, Sentinel CH, respectively). Considering the respective cut-offs (21.4 U/L for the former method and 63.0 U/L for the new one) 93.2% (41/44) of patients showed classification agreement. Passing-Bablock regression gives an intercept of -6.6698 and a slope of 3.236 (y=3.326x-6.6698). Slope >1 suggests the presence of a proportional difference. A negative sample, distilled water and saline solution (measured concentration <6.0 U/L) gave respectively average concentration values (DS) 3.0 U/L (1.4), -4.2 U/L (20.2) and 1.1 U/L (1.4). Saline solution therefore reveals to be the best diluent in case of samples needing prediluition. Within-run precision using two levels control materials had average values (CV%) 43.5 U/L (0.75) and 85.0 U/L (0.65), between-run precision 45.0 U/L (2.82) and 85.0 U/L (3.03). The new method has a good linearity all along the investigated range of concentration (0.0-117.0 U/L): r=0.998. Plotting precision in terms of CV% vs concentration we obtained good precision even at elevated concentrations: at 0.0-40.0 U/L CVs were 1.7-4.7%, at 40.0-75.0 U/L 1.1-1.2% and at 75.0-117.0 U/L 0.4-1.0%. We plotted precision expressed as SD and we extrapolated the regression curve at concentration 0 U/L getting SD 0.58 U/L. This means that the limit of quantitation could be fixed at 1.16 U/L (2SD). The new method has sure practical advantages being totally automated. Furthermore it is a good method in terms of sensibility, linearity and precision even at high concentrations.

P214
ISO15189 ACCREDITATION: THE MANAGEMENT OF LABORATORY EQUIPMENT

L. Baccarin, F. Gentile, M. La Malfa, L. Sciacovelli, M. Plebani

Dept. of Laboratory Medicine, University-Hospital, Padova

Introduction: Laboratory equipment management is an important issue of the technical requirements of the International Standard ISO 15189. In particular, the laboratory shall have a documented procedure for the calibration of equipment, together to records that include the identity of the equipment, unique identification, location and the acceptability for use.
Aim of this work is to propose a software to manage all the laboratory equipment, including the calibration metrological traceability.
Methods: After the analysis of the available scientific documents, the following steps have been considered for the laboratory equipment management: a) the typology of equipment; b) characteristics to record; c) the calibration procedure with the criterion for the acceptability; e) the feasibility; f) the procedure sharing to all laboratory personnel.
Results: A label was create to identify the equipment with a barcode constituted by a first code to identify the typology, a second code to detect the location, a third code for the progressive number of the same equipment typology. The equipment records were managed with“home-brew” software that includes the identity of the equipment the barcode, the calibration traceability, the acceptability for use, the maintenance carried out and the schedule for preventive maintenance. To guarantee a complete application of this procedure, an educational training to the laboratory personnel was carried out.
Conclusions: Laboratory equipment management was achieved with a “home-brew” software that guarantees a complete traceability according to the ISO 15189 requirements. In this way, all the laboratory personnel can monitor their own equipment. Moreover the quality assurance manager can supervised the conceived and developed system for the equipment management.
ISO 15189:2012 Medical laboratories- requirements for quality and competence. Geneva, Switzerland: ISO, 2012.
Evaluation of measurement data — Guide to the expression of uncertainty in measurement. JCGM 100:2008, 2008.

P215
EVALUATION OF A NEW LABORATORY MEDICINE MODEL: HTA FOR THE GOVERNANCE OF PDTA

M.R. Cavallo, C. Vella, V. Granero, D. Fortunato, S. Crema, M.G. Boglione, C. Priotto, E. Richetta

S.C. Lab. Analisi, O.R. Pinerolo ASL TO3

Introduction: The changes of the NHS have made the necessary strategic choices of reorganization of services, hospital and territorial networks. Models Hub-Spoke outlined by regulations want to respond to the clinical needs of the stakeholders and health, rationalizing resources, and implementing processes of prevention and guarantee quality of clinical data. While there are different national realities considered benchmark, there is no experience to the definition of a Guidelines for the governance of the activities of Laboratory Medicine (Mlab) aimed at improving the appropriateness prescriptive and pre/post-analytical processes.
Our team, through a structured path, was confronted with the professionals of the area to gain awareness of the role that the Mlab has in the prevention route, screening, care and patient assistance.
The aim of our work, on the basis of regulatory and scientific guidelines, was defined a shared model of governance to answer the question: "What is a spoke laboratory and how it enhance its professionalism?".
Materials and methods: Training Field: laboratory professionals, physicians of the hospital departments, family doctors for the territory and delegates of scientific society of Mlab. Consensus conference: May-11-2016 for evaluation of the proposed model.
Methodology: Working Group, Plenary, professional and multi-disciplinary multi comparison, round table.
Conclusions: Our Laboratory Spoke model offers a highly integrated laboratory in the territory which works in synergy with stakeholders.
The skills of the laboratory professionals represent a fundamental step for structuring of the prevention paths, appropriate care and support so that they are effective and efficient.
Both Regional and University representatives have shown that this model well adheres to the initiated reorganization, focusing on the phases of the process which, if not governed, would lead to an increase in spending and a low benefit to the patient.

P216
VERIFICATION OF ANALYTICAL AND CLINICAL PERFORMANCE OF THE FUJIREBIO 25-OH VITAMIN D ASSAY AND COMPARISON WITH THE REFERENCE METHOD

S. Giuliani[1], M. Lucchiari[1], M. Giuliani[1], D. Corvetta[2], M. Herrmann[1]

[1]Dept. of Clinical Pathologhy, District Hospital, Bolzano
[2]Dept. of Haematology, District Hospital, Bolzano

Measurement of 25(OH)vitD by automated immunoassays is challenging. Many of the recently introduced automated assays have sown variable performance. Therefore, liquid chromatography tandem mass spectrometry (LC-MS/MS) remains the gold standard. Recently, Fujirebio has launched a two-step non-competitive sandwich immunoassay that uses a novel steroid compound to extract 25(OH)vitD from VDBP. The Lumipulse G®25-OH vitaminD method represents a new approach that may overcome the inherent difficulties of previous immunoassay.
The aim of this study was to evaluate the analytical performance of the Lumipulse G®25-OH vitaminD assay on a Lumipulse G600 System (Fujirebio). In addition, we compared this method with a validated LC-MS/MS assay in different populations.
To evaluate the analytical performance of the Lumipulse G®25-OH vitaminD assay we studied the following aspects: LoB, LoD, LoQ, intra- and inter-assay precision, linearity and recovery. In addition, bias was assessed by comparison of the Fujirebio assay with a validated commercial LC-MS/MS method (25-OH vit D2/D3 kit, ClinMass®Recipe) using 450 randomly selected serum samples that were send to our laboratory for 25-(OH) vitD measurement, 60 hemodialyzed patients, 60 patients with eGFR <60 mL/min/1.73m^2 and 60 pregnant women. The agreement of both methods was compared by Passing-Bablock regression and Bland-Altman difference plots.
The assay was linear into the range proposed by the manifacture. The performance indices of the Fujirebio assay were as follows: LOB = 1.09 ng/mL, LOD = 1.6 ng/mL, LOQ = 4.2 ng/mL, intra-assay precision: 3.0% (at 19.0 ng/mL), 5.6% (at 38.2 ng/mL) 12.1% (at 90.2 ng/mL), inter-assay precision: 10.3–13.3 %, recovery (at expected concentrations of 25.3, 42.3, 59.3 ng/mL): 112,116, 101%. Comparing the Fujirebio method with LC-MS/MS by Passing Bablock regression revealed the following equation: Fujirebio=1,535+0,89LC-MS/MS. In samples from nephropathic and pregnant patients the agreement was inferior.
The new 25(OH)D immunoassay from Fujirebio has acceptable sensitivity and precision. In unselected patients the assay shows a significant 11% negative proportional bias when compared to a validated LC-MS/MS method. In nephropathic patients the Fujirebio method showed no significant bias.

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VALIDATION OF ALDOSTERONE MEASUREMENT WITH CHS™ MSMS STEROIDS KIT ON LC-MS/MS SHIMADZU 8050 PLATFORM

S. Giuliani[1], M. Lucchiari[1], M. Giuliani[1], I. Gentilini[2], G. Rademaker[1], C. Crivellaro[3], M. Herrmann[1]

[1]Department of Clinical Pathology, District Hospital, Bolzano
[2]Transfusional Department, District Hospital Bolzano,
[3]Endocrinology Department, District Hospital, Bolzano

Primary aldosteronism (PA) is a potentially curable disease that cause hypertension (HT). The first step in diagnosing PA consists in the determination of the aldosterone-renin-ratio (ARR). Recently, new methods with superior analytical performance for the measurement of both, aldosterone and renin, have been introduced in clinical practice. While aldosterone can be measured by liquid chromatography tandem mass spectrometry (LC-MS/MS) or automated immunoassays, renin is predominantly measured by direct immunoassays. ARR is strongly method dependent. Insufficient data exists on ARR in healthy subjects and PA patients when aldosterone and renin are measured with one of these new methods.
The present study evaluated the analytical performance of a commercial LC-MS/MS method from Perkin Elmer (CHS™ MSMS Steroids) for the measurement of aldosterone. In addition, we established a reference range for ARR when aldosterone is measured with the before mentioned LC-MS/MS method and renin is measured by a direct immunoassay from DiaSorin on a Liaison XL auto-analyser.
The Perkin Elmer CHS ™ MSMS Steroids method is a multiplex assays. Analyses were performed on a Shimadzu 8050 LC-MS/MS. The analytical performance of aldosterone measurement was evaluated determining the following performance indices: LoD, LoQ, recovery, carryover, linearity and precision. For the establishment of the ARR reference range serum aldosterone and plasma renin were measured in samples from 128 normotensive blood donors.
LOD and LOQ were respectively 17 pg/mL and 26 pg/mL. Intraassay precision at different concentrations was 7% (at 79.9 pg/mL.), 7.6% (at 161.8 pg/mL) and 1.5% (at 3506.08 pg/mL). Interassay precision was 8.5%, 9.6% e 5.1% respectively. Recovery ranged between 80.5 and 104.4%. Between 27.3 and 3580 pg/mL the method was linear. ARR in healthy blood donors varied between 0.6-62.4 pg/µIU with a median of 5.7 pg/µIU. The 2.5th and 97.5th percentiles were 1.06 and 39.5 pg/µIU respectively. The performance characteristics of the here tested LC-MS/MS method for the measurement of aldosterone are satisfactory and justify the use of this test in clinical practice. When combined with the direct renin assay from DiaSorin the ARR reference range is between 1.2 and 62.4 pg/µIU.

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SCREENING, IDENTIFICAZIONE E QUANTIFICAZIONE DELLE COMPONENTI MONOCLONALI SIERICHE: CONFRONTO DI DUE PERCORSI ANALITICI

D. Giavarina[1], C. Bonamigo[2], G. Marchesini[1], G. Marzotto[1], L. Bedin[1], A. Paternoster[1]

[1]Lab. Analisi, Osp. S. Bortolo, Vicenza
[2]Laureanda TSLB, Univ. Padova, Sede Vicenza

Scopo di questo lavoro è stato valutare, in un ampio campione di studio, il miglior percorso tra differenti tecnologie per raggiungere le migliori performance nella valutazione delle componenti monoclonali, da quattro punti di vista: sensibilità (SE) e specificità (SP) nella identificazione e tipizzazione delle componenti monoclonali; tempi; carico di lavoro; costi.
Materiali, soggetti e metodi: Sono stati studiati 990 campioni di siero (resi anonimi) ottenuti in un periodo di 5 settimane, da soggetti afferenti al laboratorio dell'ospedale, esterni ed interni. Tutti i campioni sono stati analizzati sia con elettroforesi capillare (CE) sia su gel di agarosio (S-PAGE). Due patologi esperti (GM e AP) hanno esaminato tutti i gel SPAGE, così come i tracciati elettroforetici in capillare. Nessuna informazione clinica era disponibile agli esaminatori. Ogni minima banda o picco rilevato da almeno uno dei due esaminatori su almeno une dei sue sistemi ha determinato l'esecuzione della seconda fase, con una immunosottrazione in CE (IS-CE) e una immunofissazione (IFE). Campioni senza picchi o bande, pur in presenza di ipogammaglobulinemia, sono stati esclusi dalla seconda fase. IFE è stato considerato sistema di riferimento. Sono stati utilizzati gli strumenti Capillarys™2, Hydrasys™2, dispensatore Assist™ con reagenti dedicati (Sebia Italia S.r.l.).
Risultati: Gli screening con S-PAGE e CE hanno identificato rispettivamente 120 e 159 campioni positivi. 10 casi sono stati classificati come inconclusivi. Complessivamente, 183 campioni sono stati sottoposti al secondo livello con IS-CE e IFE. Considerando il gruppo di 980 campioni classificati allo screening, la SE e SP per S-PAGE è stata rispettivamente di 0.77 e 0.97, per CE 0.98 e 0.96. Per una routine di 180 campioni/die, il percorso S-PAGE-IFE richiede 7:13 ore di tempo macchina, CE-IS-CE 5:36 ore. Il tempo-operatore è risultato 100% del tempo macchina per S-PAGE/IFE, 50% per CE/IS-CE. I costi a mercato per strumenti e reagenti, forniti dall'azienda fornitrice, sono equivalenti.
Conclusioni: Il percorso CE/IS-CE si dimostra più sensibile (97 vs 77%) e solo lievemente meno specifico (96 vs 97%) del percorso S-PAGE/IFE. Esso è inoltre più economico in termini di tempo macchina e carico di lavoro. Non ha sostanziali differenze in termini di costi di acquisto.

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CORRELATION OF LEUKOCYTE COUNTS BETWEEN SYSMEX XN 1000, SIEMENS ADVIA 2120i AND TRUCOUNTTM BD OF PERIPHERAL BLOOD SAMPLES AND BAGS OF APHERESIS

F. Dima[1], D. De Sabata[2], E. Barison[2], M. Montagnana[1], F. Benedetti[2], G. Lippi[1]

[1]*Sez. di Biochimica Clinica, Università di Verona, Verona*

[2]*Centro Trapianti Midollo Osseo, AOUI, Verona*

Background: A growing number of studies has been published on mobilization process and collection of hematopoietic stem cells in peripheral blood. The aim of this work is to compare the WBC counts of two automated cytometers and TrucountTM BD system in flow cytometry. Materials and methods: The white cell count was performed on peripheral blood samples and bags apheresis. The analysis was performed on Sysmex XN-1000 (Sysmex Corporation, Kobe, Japan), Advia 2120th (Siemens Healthcare Erlangen, Germany) and FACScalibur flow cytometer (BD) with Trucount™ tubes (BD) ISHAGE protocol. For the statistical analysis the software Analyse-it (Analyse-it Software Ltd, Leeds, UK) was used. Results: On peripheral samples the Passing-Bablok regression displayed correlation (r) of 0.99, slope of 0.822 (95% CI: 0.807 to -0.838) and intercept of 47.4 (95% CI: -74.1 to 276.2) with a bias (Bland-Altman) of -4710 x 10^9/L (95% CI: -5448 to -3973 x 10^9/L) for Advia versus Trucount. For Sysmex versus Trucount the correlation was 0.99, the slope 0.878 (95% CI: 0.860 to 0.893), the intercept 106.3 (95% CI: 57.7 to 279.6) and the bias -3261 x 10^9/L (95% CI: -3847 to -2675 x 10^9/L). The analysis of samples from apheresis bags with >200 x 10^9/L yielded a correlation of 0.93, slope of 0.734 (95% CI: 0.617 to 0.882), intercept of 16817 (95% CI: -19128 to 46288) and a bias of -54365 x 10^9/L (95% CI: -63827 to -44904 x 10^9/L) for Advia versus Trucount. The comparison of Sysmex versus Trucount yielded a correlation of 0.91, slope of 0.819 (95 %CI: 0.668 to 0.985), intercept of 24640 (95% CI: -13083 to 60953) and mean bias of -21112 x 10^9/L (95% CI: -29246 to -12979 x 10^9/L). Conclusions: These results suggest that the count of both analyzers are significantly underestimating the data compared to the Trucount system. In particular, the underestimation with Advia increases with increasing cell values in the bags, whereas the underestimation is more constant and independent of the amount of cells present when analyzing peripheral blood. In the latter case, the differences between XN and Trucount are smaller than with Advia but still significant.

Sutherland DR, et al. The ISHAGE guidelines for CD34+ cell determination by flow cytometry. International Society of Hematotherapy and Graft Engineering. J Hematother 1996;5:213-26.

P220

DETERMINAZIONE DELLA FORMA ATTIVA DELLA VITAMINA B12 E UTILITÀ NELL'IDENTIFICAZIONE DEGLI STATI DI CARENZA

A. Fortunato, A. Tomasini, D. Giavarina

Laboratorio di Chimica Clinica ed Ematologia, Ospedale "San Bortolo", Vicenza

Introduzione: l'insufficienza di vitamina B12 (B12) è un problema che si presenta con elevata prevalenza soprattutto nella popolazione anziana. Questa insufficienza, come per la carenza delle altre vitamine del gruppo B, risulta strettamente correlata con il riscontro di elevate concentrazioni di omocisteina (HCY) in circolo che, a sua volta, rappresenta un importante fattore di rischio cardiovascolare in quanto è altamente lesiva per l'endotelio dei vasi. La determinazione della forma attiva della VitB12 (olotranscobalamina, aB12), che rappresenta circa il 20% della quantità totale circolante, rispetto alla forma non biodisponibile (legata alla aptocorrina) risulta importante per la corretta identificazione dei soggetti che non hanno un sufficiente apporto di vitamina.

Materiali e metodi: in un gruppo di 151 soggetti afferenti al nostro laboratorio per la valutazione della concentrazione della B12 (79 donne e 72 uomini, età media 68,1 anni) sono state determinate le concentrazioni di B12 e aB12 (ADVIA Centaur, Siemens) e di HCY (Dimension VISTA, Siemens).

Risultati: la concentrazione media misurata di B12 è risultata di 254,9 pmol/L (52,0–1154,0) nelle donne e di 316,4 pmol/L (86,0-1250,0) negli uomini (p <0,05). Analogamente per la aB12 le concentrazioni misurate sono 53,6 pmol/L (6,0-144,0) e 60,1 pmol/L (9,0-143,0) con differenza non significativa tra i due gruppi (p=0,272). Per l'HCY sono state trovate le seguenti concentrazioni medie: 22,2 μmol/L (5,4-94,9) nelle donne e 28,8 μmol/L (9,0-92,6) negli uomini con p <0,05. Il rapporto medio tra le concentrazioni di B12 e aB12 è risultato 22,9 (6,2-65,3) nelle donne e 23,8 (4,3-59,3) negli uomini (p=0,612).

Discussione: la valutazione dei risultati ottenuti dimostra che, se si considerano tutti i campioni, la classificazione dei soggetti con non sufficiente apporto vitaminico si ha nel 4,0% dei casi (6 su 151) in base alla concentrazione di B12, mentre nel 19,2% dei casi (29 su 151) se si osserva la concentrazione di aB12. La differenza di sensibilità tra i due parametri è amplificata se si considerano i soggetti con B12 inferiore al primo quartile della distribuzione dei valori di riferimento impiegati (B12 <200pmol/L), infatti in questo gruppo il carente apporto di B12 è attribuito al 12,5% dei soggetti (6 su 48) e al 37,5% (18 su 48) se rispettivamente si valuta la B12 o la aB12.

P221
VALUTAZIONE PRELIMINARE DELL'ANALIZZATORE AUTOMATICO PER IMMUNOCHIMICA ROCHE COBAS e801

M. Pontillo, L. Fasoli, V. Gioia, M. Locatelli, F. Ceriotti

Servizio di Medicina di Laboratorio, Ospedale San Raffaele, Milano

Introduzione: La richiesta di analisi immunochimiche costituisce una frazione sempre maggiore della routine quotidiana, anche come analisi urgenti; inoltre nei laboratori di dimensioni medio—grandi sono spesso integrate in sistemi di automazione globale o dell'area siero. In questo contesto è fondamentale disporre di analizzatori in grado di fornire analisi accurate, ma anche rapidi e soprattutto che possano operare a ciclo continuo. Il nuovo analizzatore Roche Cobas e801, mantenendo il principio analitico del predecessore (Cobas e602, elettrochemiluminescenza) permette ora il caricamento in continuo dei reattivi ed offre una cadenza analitica teorica di 300 risultati/ora.
Materiali e metodi: Sono stati valutati i seguenti esami: FT3, FT4, TSH, CEA, Testosterone, FSH, LH e Ferritina. Per ciascuno di essi si è effettuata una valutazione della precisione (analisi in triplo per 5 giorni) su 5 materiali di controllo (Roche Precicontrol Universal, 2 livelli e BioRad Lyphocheck Immunoassay, 3 livelli) ed un confronto su 50 - 70 campioni di pazienti con Roche Cobas e602 e con Tosoh AIA 2000 (tiroide e CEA) e con Siemens Centaur (FSH, LH, Testosterone).
Risultati: La precisione è risultata ottima, con i seguenti CV% complessivi (minimo e massimo per i 5 materiali): FT3 da 1.98 a 4.35, FT4 da 0.74 a 2.22, TSH da 0.82 a 1.63, Testosterone da 1.73 a 2.86, FSH da 1.44 a 2.83, LH da 0.96 a 3.25, CEA da 1.58 a 2.16, ferritina da 2.39 a 6.94. La correlazione con Cobas e602 è risultata eccellente per tutti i metodi con intercette non significativamente diverse da zero, pendenze che si scostavano da 1.0 per FT4 (0.90) e LH (1.06) con r^2 tra 0.9966 (FT4) e 0.9993 (Testosterone). Il confronto con gli altri sistemi analitici ha dato i seguenti risultati: FT3 e801=1.24 AIA +0.09 pg/mL; FT4 e801=1.05 AIA +0.03 ng/dL; TSH e801=1.07AIA + 0.28 mU/L; CEA e801=0.798AIA – 0.01 ng/mL; FSH e801=0.94Centaur + 1.2 U/L; LH e801=1.05Centaur + 1.0 U/L; Testosterone e801=1.13Centaur – 0.03 ng/mL.
Conclusioni: Il sistema ha fornito prestazioni eccellenti in termini di precisione per tutte le analisi eseguite, i risultati sono molto ben correlati con quelli ottenuti con il sistema Roche oggi in uso mentre rispetto ad altri sistemi analitici si osservano in alcuni casi significative differenze.

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PROTEINURIA DI BENCE JONES: IMMUNOFISSAZIONE ED IMMUNOSOTTRAZIONE A CONFRONTO

F. Spolaore , S. Altinier, M. Zaninotto, M. Plebani

UOC Medicina di Laboratorio, Azienda Ospedaliera-Università degli Studi, Padova

La proteinuria di Bence Jones (BJ) è fondamentale nel monitoraggio delle patologie plasma-proliferative. In questo studio sono state confrontate le prestazioni del metodo in elettroforesi capillare ed immunosottrazione con il metodo di immunofissazione su gel di agarosio attualmente in uso.
Materiali e metodi: Sono state analizzate le urine (raccolta 24h) di 56 pazienti (14 femmine, 42 maschi età 30-90) monitorati per patologia plasma-proliferativa (tra cui 20 mieloma multiplo, 6 MGUS, 2 amiloidosi e 1 Waldenstrom). La proteinuria totale (uPT, metodo colorimetrico, Dimension Vista-Simens) risultava compresa tra 0,005-0,86 g/L (0,008-1,6 g/24h). In base alla concentrazione di uPT i campioni sono stati suddivisi in 3 gruppi <0,1 g/L (n=18); tra 0,1 e 0,3 g/L (n=27); >0,3 g/L (n=11) e sono stati analizzati mediante immunofissazione su gel di agarosio (Hydragel 9 IF-Sebia) con antisieri Anti-catene kappa e Lambda libere. 49 campioni sono risultati positivi (27 kappa e 22 Lambda) e 7 negativi. Gli stessi campioni sono stati dializzati come previsto dal produttore, sottoposti ad elettroforesi urinaria (uETF) (Capillary/minicap-urine, Sebia) e successivamente ad immunosottrazione con antisieri anti IgG, IgA, IgM, anti-kappa e anti-lambda.
Risultati: In 41/49 campioni BJ positivi (83,6%) uETF risultava indicativa di picco monoclonale; l'immunosottrazione confermava in 28 la positività per catene leggere libere kappa o lambda; in 13 forniva risultati negativi o dubbi (88% uPT <0,1 g/L). In 8/56 uETF non è risultata sufficientemente sensibile nonostante la positività all'agarosio (100% uPT <0,2 g/L). I 7 negativi presentavano totale concordanza tra metodi.
Il numero maggiore di risultati positivi e la più elevata percentuale di concordanza tra metodi (53%) era osservabile in campioni con valori di uPT >0,13 g/L.
Conclusioni: Il metodo valutato ha prestazioni e concordanza con il metodo di riferimento soddisfacenti solo per valori di proteinuria elevati: l'applicabilità alla routine di laboratorio per ricerca, identificazione e quantificazione delle BJ è strettamente correlata alle caratteristiche della popolazione assistita che, nella nostra routine, presenta in una percentuale significativa (32%) valori inferiori a 0,1 g/L.

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STUDIO MULTICENTRICO PER LA VALUTAZIONE DEI PARAMETRI ANALITICI E RISULTATI CLINICI DEL NUOVO METODO DI DOSAGGIO ACCESS TSH (3° IS) PER L'ORMONE TIREOSTIMOLANTE

S. Masotti[1], C. Prontera[2], M. Migliardi[3], A. Fortunato[4], A. Alfano[5], C. Carrozza[6], M. Correale[7], R. Dittadi[8], G. Gessoni[9], S. Rizzardi[10], R. Ndreu[11], G. Zucchelli[11], A. Clerico[2]

[1]*Scuola Superiore Sant'Anna, Pisa*
[2]*Fondaz. CNR Regione Toscana G. Monasterio, Pisa*
[3]*Ospedale Umberto I dell'Ordine Mauriziano di Torino*
[4]*Ospedale San Bortolo di Vicenza*
[5]*Ospedale Civile di Ciriè ASLTO4*
[6]*Dip. Med. Lab., Univ. Cattolica del Sacro Cuore, Roma*
[7]*IRCCS Gastroenterologico S. de Bellis, Castellana Grotte (BA)*
[8]*Ospedale dell'Angelo di Mestre (VE)*
[9]*Ospedale di Chioggia*
[10]*Istituti Ospitalieri, Cremona*
[11]*QualiMedLab, Ist. di Fisiologia Clinica del CNR, Pisa*

Scopo dello studio è valutare le caratteristiche analitiche del nuovo metodo per la misura del TSH che utilizza la piattaforma UniCel DxI800 [Access TSH (3°IS), Beckman Coulter] e confrontare i risultati con quelli ottenuti con il metodo precedente Access HYPERsensitive hTSH. La valutazione dei parametri analitici è stata effettuata presso il laboratorio di riferimento dello studio (FTGM, Pisa). Il bianco del metodo (LoB) e il limite di rivelabilità (LoD), determinati seguendo il protocollo CLSI EP17-A, sono risultati 0.00043 e 0.00485mIU/L, rispettivamente; risultando migliori rispetto a quelli riportati dall'azienda per il vecchio metodo (LoB: 0.003mIU/L). Per il confronto tra "vecchio" e "nuovo" metodo, sono stati raccolti 593 campioni di siero con concentrazioni di TSH che variavano da 0.002 a 100mIU/L, includendo sia soggetti normali che pazienti con malattie tiroidee. I campioni sono stati inviati al laboratorio di riferimento e misurati con entrambi i metodi. I due metodi presentano una stretta correlazione (nuovoTSH=0.220+0.875vecchioTSH; R= 0.980), anche se è stata riscontrata una differenza significativa tra i valori medi di TSH misurati [vecchio metodo: mediana (25°-75° percentile) 2.73 (1.04-6.59)mIU/L; nuovo metodo: mediana (25°-75°percentile) di 2.34 (0.96-6.08)mIU/L]. Sono stati dosati 15 campioni di controllo distribuiti nei cicli di VEQ Immunocheck 2010-2015 (range di TSH: 0.120-17.635 mIU/L) e confrontati i valori di TSH misurati con il nuovo metodo nel laboratorio di riferimento e i valori trovati come media di consenso dai laboratori partecipanti, utilizzatori del vecchio metodo Beckman Coulter. È stata trovata una lieve ma significativa differenza tra i valori medi [vecchio metodo: mediana (25°-75° percentile) 2.89(0.47-4.30)mIU/L; nuovo metodo: mediana (25°-75° percentile) di 2.42(0.45-4.27)mIU/L; p=0.02]. In conclusione, il nuovo metodo Acces TSH (3°IS) presenta parametri di sensibilità analitica migliori rispetto al metodo Access precedente, con concentrazioni di TSH perfettamente allineate per tutto il range di misura.

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EVALUATION OF THE ANALYTICAL PERFORMANCE OF 5 IMMUNOTURBIDIMETRIC ASSAYS FOR SPECIFIC SERUM PROTEINS COMPARISON WITH IMMUNONEPHELOMETRY ACCORDING TO THE SIBioC GUIDELINES

C. Canali, L. Giampaolo, S. Tagliavini, P. Natali, D. Carra, G. Patelli, M. Varani, T. Trenti

CoreLab NOCSAE, Department Of Laboratory Medicine, Modena

Background: Despite improved standardization due to the availability of a reference material, inter-assay comparability for specific serum proteins is still problematic. Moreover, for some of these analytes no reference material is actually available, making inter-method agreement difficult to reach. We evaluated the analytical performance of turbidimetric assays for the measurement of β-2 microglobulin (B2M), haptoglobin (HP), α-1 acid glycoprotein (AAG), α-1 antitrypsin (AAT) and lipoprotein(a) (LPA), in comparison to the old nephelometric tests employed in our laboratory, following the new guidelines issued by SIBioC.

Methods: Internal quality control materials (two levels) were used to calculate method imprecision with a 3x5 protocol. Turbidimetric and nephelometric assays for the quantitation of B2M, HP, AAG, AAT and LPA were performed on consecutive serum samples submitted to our laboratory for routine testing on the Beckman Coulter AU680 and IMMAGE, respectively.

Passing-Bablok (PB) regression analysis, Bland-Altman (BA) plots, imprecision and acceptability of the new methods have been calculated with SIBioC MetComp ver 1.0.

Results: Total imprecision calculated in our laboratory was consistent with that declared by the manifacturer for the 5 immunoturbidimetric methods. 45, 47, 111, 63 and 32 serum samples were included for comparison of AAG, AAT, B2M, HP and LPA, respectively. PB regression analysis revealed a significant bias (constant and/or proportional) between turbidimetry and nephelometry for every assay except for AAT. Accordingly, BA plots showed a systematic difference for AAG, B2M, HPT and LPA. Nonetheless, taking total allowable errors based on biological variability (9.2% AAT, 27.3% HP, 9% B2M, 24.1% LPA, 16.2% AAG), the acceptability of the new methods were judged good for all the assays.

Conclusions: Using SIBioC guidelines for method comparison, the new assays were found acceptable for replacement of the old ones, according to quality specifications based on biological variability.

This allowed us to consolidate specific protein analyses on an integrated clinical chemistry/immunoassay system, resulting in improved efficiency, more rapid analysis, random access, high volume testing and cost reduction.

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P225
VALUTAZIONE DI eGFR MEDIANTE CKD-EPI E BIS1 IN UNA POPOLAZIONE DI SOGGETTI ANZIANI

S. Da Molin[1], F. Cappellini[1], R. Falbo[1], S. Signorini[1], P. Brambilla[2]

[1]S.C. Analisi Chimico Cliniche, ASST Monza, P.O. Desio, MB
[2]Dipartimento di Scienze della Salute, Università degli Studi di Milano Bicocca, Monza, MB

Introduzione: La malattia renale cronica (CKD) è definita dalla presenza di danno renale o ridotta funzionalità d'organo presente da più di tre mesi. Le linee guida KDIGO 2012 (Kidney Disease Improving Global Outcomes) stabiliscono che la valutazione e la stadiazione della ridotta funzionalità renale avvengano tramite stima del GFR (eGFR) mediante equazione CKD-EPI basata sulla creatinina plasmatica (IDMS). Poiché l'equazione CKD-EPI è stata sviluppata considerando una popolazione con ridotta percentuale di soggetti sopra i 70 anni, l'eGFR potrebbe risultare in questi pazienti sovrastimato. Alcuni studi hanno perciò proposto equazioni alternative specificatamente elaborate per la popolazione in età avanzata. Scopo del presente lavoro è stato comparare l'equazione CKD-EPI$_{crea}$ con la BIS1$_{crea}$ (Berlin Initiatives Study) (1) nella valutazione dell'eGFR in una popolazione di individui con età ≥70 anni.
Materiali e metodi: Lo studio include un totale di 465 soggetti afferenti al Pronto Soccorso del P.O. di Desio senza successivo ricovero. I soggetti sono stati suddivisi in tre gruppi in base all'età (A: 70-74 anni, n=150; B: 75-79 anni, n=150; C: ≥80 anni, n=165) per ognuno dei quali è stato calcolato l'eGFR mediante CKD-EPI$_{crea}$ e BIS1$_{crea}$. Per ogni gruppo, i pazienti sono stati classificati nei diversi stadi di funzionalità glomerulare definiti nelle linee guida KDIGO (G1, G2, G3a, G3b, G4, G5) secondo CKD-EPI e successivamente riclassificati sulla base di BIS1. Il confronto tra le proporzioni di soggetti appartenenti alle diverse classi con le due equazioni è stato effettuato utilizzando il test di McNemar.
Risultati e conclusioni: Nel gruppo A il 13% dei soggetti, classificati come G1 o G2 dall'equazione CKD-EPI, viene riclassificato come G3 o G4 o G5 secondo la BIS1. Nel gruppo B questa percentuale sale al 24% e nel gruppo C viene riclassificato il 28% dei pazienti. In tutti i casi le differenze di classificazione erano statisticamente significative con p <0.001. I dati ottenuti, indicano che nei soggetti con età ≥70 anni l'equazione BIS1 classifica una percentuale maggiore di casi con ridotta funzionalità glomerulare rispetto all'equazione CKD-EPI. Questa differenza di classificazione cresce con l'aumentare dell'età.
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VALUTAZIONE DI UNA METODICA AUTOMATIZZATA PER LA DETERMINAZIONE DELLA PRA

G. Priolo, G. Martinasso , D.M.L. Sapienza , A. Ianniello , S. Limoncelli , G. Mengozzi

A.O.U. Città della Salute e della Scienza di Torino, Presidio Molinette, Lab. Biochimica Clinica

Per identificare un iperaldosteronismo primitivo (IP) è utile il rapporto Aldosterone/PRA. Dosare l'attività reninica plasmatica (PRA) a valori molto bassi (<1 ng/ml/h) tipici dell'IP richiede tecniche complesse, completamente manuali, con lunghi tempi di esecuzione e l'utilizzo di radiomarcato. Pertanto alla PRA viene spesso preferito il dosaggio della renina attiva (DRA). Purtroppo la correlazione tra DRA e PRA è poco soddisfacente proprio per i valori bassi che sono tipici nell'IP. Per questo alcuni centri specialistici ricorrono ancora al dosaggio diretto, che comporta però l'adozione di particolari misure di radioprotezione.
Recentemente la ditta Medical System ha immesso sul mercato una metodica, applicata sul sistema Maglumi SNIBE, che ricalca la metodica RIA e permette di dosare la PRA con una procedura semi automatizzata ma, soprattutto, senza ricorso a radiomarcato. Scopo del nostro lavoro è stato ricavarne la correlazione con il RIA tradizionale.
Sono stati testati in parallelo 70 campioni di pazienti sospettati di IP. La correlazione globale risulta discreta (R=0.803), mentre l'analisi della regressione (Passing e Bablok) mette in evidenza un maggiore divario che si presenta soprattutto per i valori elevati, meno determinanti nella diagnosi di IP: Valori PRA MAGLUMI = - 0.478 + 3.836 Valore RIA
I dati clinici preliminari sembrano confermare la validità del metodo che, pertanto potrebbe rappresentare una buona alternativa alla procedura con radiomarcato.
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DIESSE CHORUS 25 OH VITAMIN D TOTAL AND SIEMENS ADVIA CENTAUR VITAMIN D TOTAL ASSAY METHOD COMPARED

M. Lorubbio[1], F. Petrini[2], P. Buccato[2], C. Rocci[2], C. Paoli[2], M. Tognini[2], D. Soldateschi[2], A. Ognibene[1]

[1]*Laboratorio Generale, AOU Careggi, Firenze*
[2]*DIESSE Diagnostica Senese S.p.A., Monteriggioni (SI)*

Aim of the study: Measurement of serum 25-hydroxyvitamin D [25(OH)D] is generally considered to be an indicator for bone homeostasis. Deficient or insufficient 25(OH)D values, supported by clinical evidence, are linked to bone diseases as osteoporosis, rickets, osteomalacia and malabsorption syndromes, so a reliable measurement is required.
In this study, we evaluate the performance of the Chorus 25OH Vitamin D Total kit Assay method compared to the Siemens ADVIA Centaur Vitamin D Total Assay, used as reference method that has joined to "The Vitamin D Standardization Program" (VDSP) of the NIH Office of Dietary Supplements (NIH ODS) and the National Institute of Standards and Technology (NIST).
Materials and methods: A total of 217 random samples of serum were evaluated. All samples were examined by Siemens ADVIA Centaur Vitamin D Total Assay as daily routine clinical analysis at General Laboratory in Careggi Hospital, Florence. Analyzed samples were kept in storage at 4 °C (according to Quality Manual of Laboratory) and later they were recalled for be analyzed by Chorus 25 OH Vitamin D Total kit Assay during about 10 days. The results were evaluated by Bland and Altman and, Passing-Bablok statistical analysis. The statistical analyses were performed with SPSS version 11.0.
Results: The results at the Passing-Bablok analysis showed r=0.95; Intercept = 1.4875 (95% CI=0.2822-2.3985), while at the Bland and Altman analysis the mean differences was -0.61 (+1.96 SD=+8.68 and -1.96 SD=-9.89). Chorus 25OH Vitamin D Total Assay method showed an Intra-assay imprecision CV%=4.54, and an Inter-assay imprecision CV%=6.87. Moreover the Chorus 25OH Vitamin D Total Assay showed the LOB 0.9 ng/mL and the LOD 1.6 ng/ml.
Conclusions: The Chorus 25 OH Vitamin D Total Assay is comparable to Siemens ADVIA Centaur Vitamin D Assay. The comparison between the two methods in terms of imprecision (Intra-assay and Inter-assay), LOB and LOD shows acceptable differences.

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DEVELOPMENT OF A RAPID AND RELIABLE METHOD FOR DIRECT IDENTIFICATION WITH HIGH PERFORMANCES OF FASTIDIOUS MICROORGANISMS AND AEROBIC BACTERIA GROWN IN POSITIVE BLOOD CULTURES USING VITEK® MS SYSTEM AND SARAMIS™ DATABASE

J. Intra, M.R. Sala, R. Falbo, F. Cappellini, P. Brambilla

Department of Laboratory Medicine, University Milano Bicocca, Desio Hospital, Desio (MB)

Rapid and early identification of microorganisms in blood has a key role in diagnosis of a febrile patient, in particular to guide the clinician in the definition of a correct antibiotic therapy. This study presents a simple and very fast method with high performances for identifying bacteria by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), using PolyViteX chocolate agar plates inoculated with 5 drops of blood-broth medium, and a direct transfer procedure without additional modification. Ninety-nine percent of aerobic bacteria were correctly identified from 600 positive blood cultures after a 4-hour incubation on the agars. The performances are optimal for Enterobacteriaceae, Staphylococci, Listeria monocytogenes, and fastidious species Haemophilus influenzae, Streptococcus pneumoniae, and Neisseria meningitidis, that need complex nutritional and environmental requirements in order to grow, and results for Gram-positive bacteria, such as Enterococci and Streptococci, and Pseudomonas are positive. Our results are in agreement with data obtained in a recent retrospective analysis performed by Gonzalez et al. (2016). In fact, they illustrated that early growth on solid media is accurate for Enterobacteriaceae identification, and they demonstrated that implementation of a similar method statistically significant decreases the turn-around time of identification for these Gram-negative bacteria in routine practice, allowing an improvement in patient outcome and optimal antimicrobial therapy. The reliability of results, rapid performance and applicability of this protocol have contributed to the reduction of time to optimal antimicrobial treatment. In conclusion, our method, not requiring additional cost and particularly skilled personnel, is a very straightforward procedure and, compared to the different extraction protocols and standard procedures, is very easy to be performed into routine clinical use.
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DETERMINAZIONE DELLE COMPONENTI DI UN CALCOLO URINARIO: NUOVI ACCORGIMENTI METOLOGICI ED INFORMATICI

G. Cangiano[1], E. Di Maina[1], G. Buccino[1], A. Errico[1], P. Improta[1], C. Taranto[1], M.M. D'Ambrosio[1], M. Iappelli[1], P. Raffio[2], M. Terribile[3], M. D'Amora[4], A. Sarappa[1]

[1]Lab. di Patologia Clinica, Osp. dei Pellegrini ASL NA1 Centro, Napoli
[2]Lab. di Patologia Clinica, P.S.I. NA Est ASL NA1 Centro, Napoli
[3]Nefrologia e Dialisi, Osp. dei Pellegrini ASL NA1 Centro, Napoli
[4]Lab. di Patologia Clinica, Osp. S.maria del Popolo degli Incurabili, ASL NA1 Centro, Napoli

Premessa e scopo dello studio: La determinazione da noi proposta prevede il dosaggio di ossalato (ditta LTA) e cistina (metodo al fosfotungstato) su Viva E Siemens e di ammonio, acido urico, magnesio, fosfato e calcio su Cobas 6000 Roche. I dati ottenuti, opportunamente elaborati in Excel, sono trasformati nelle possibili componenti presenti nel calcolo urinario.
Materiali e metodi: Circa 30 mg di calcolo frantumato si solubilizzano con 3 gocce di acido solforico concentrato. Successivamente si aggiungono 10 mL di acqua distillata e si centrifuga. La soluzione è pronta per i dosaggi sopra citati, tranne che per l'ossalato che necessita di una diluizione 1:10 con tampone EDTA pH 7,0.
Excel converte le concentrazioni degli analiti sopra citati in mg/dL ed in μmoli/dL e riesce in tal modo a quantificare la presenza di calcio ossalato, struvite, acido urico e suoi sali, cistina e infine di calcio fosfato.
Risultati: Lo stesso numero di micromoli di calcio ed ossalato portano alla formazione di whewellite; identiche quote di fosfato, magnesio ed ammonio mettono in evidenza la struvite. La presenza di acido urico e l'eccesso di ammonio identificano urato di ammonio. L'urato in eccesso rappresenta invece il contenuto di acido urico. Anche il calcio in eccesso può evidenziare presenza di urato di calcio. L'uso del rapporto Ca/P, riesce ad individuare le diverse forme di calcio fosfato.
I diversi sali ottenuti, espressi in #μmoli/dL, sono trasformati in mg/dL e percentualizzati. Le concentrazioni degli analiti non utilizzate rappresentano il residuo inorganico.
Conclusioni: Rispetto al lavoro proposto nel 2013 si propone una più idonea diluizione del calcolo urinario e l'uso del tampone EDTA per la correzione del pH nel dosaggio Trinder degli ossalati. Il rapporto Ca/P, elaborato in Excel, consente di refertare, in modo ottimale, il risultato di calcio fosfato. Per evidenziarne lo specifico tipo (brushite, fosfato octacalcico, whitlockite, idrossiapatite ed apatite) è indispensabile usare la metodologia all'infrarosso (FTIR).
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P230
L'ESAME DI BASE DEL LIQUIDO SEMINALE: QUESTO SCONOSCIUTO

G. Previtali, F. Baderna

Laboratorio Analysis, Busseto, Parma

Dal 2010 sono entrate in vigore le nuove linee guida per l'esame del liquido seminale. Le linee guida del WHO da un lato descrivono con chiarezza quali siano gli esami di base e le metodiche per eseguire correttamente l'analisi del liquido seminale e dall'altra costituiscono un tentativo di standardizzare questo esame rendendo comprensibile e il più possibile omogeneo fra i vari laboratori. Riportiamo il caso di un paziente che ha eseguito quattro spermiogrammi negli ultimi tre anni in quattro laboratori diversi della provincia di Parma. Tutti i referti non sono risultati compatibili con le indicazione del WHO. Il dato più rilevante è quello riferibile al numero di spermatozoi che varia da 0 a 83 milioni/ml. Le indicazione del manuale del WHO prevedono in caso di assenza di spermatozoi una centrifugazione del liquido seminale e successiva ispezione al microscopio ottico. Questo fatto avrebbe evitato un errore grossolano che ha comportato una pesante ricaduta, in termini psicologici, al paziente. In conclusione seguendo le linee guida del WHO per l'esame del liquido seminale è possibile analizzare quelli che sono considerati i parametri base per una valutazione clinica corretta, rendere omogenei i dati fra i vari laboratori ed evitare errori grossolani. Dove non è possibile seguire le metodiche WHO anche per l'introduzione di strumenti automatici dovrebbe sempre essere indicata sul referto la metodica eseguita per poterne valutare i limiti ad esempio è fondamentale la differenza se la morfologia viene valutata " in vivo" o da vetrino colorato. La centralità del paziente è fondamentale in questo tipo di analisi che in questi ultimi anni, grazie alle tecniche di fecondazione assistita , è diventata una vera e propria disciplina.

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ANALISI PROTEOMICA IN CAMPIONI DI PLASMA DI SOGGETTI AFFETTI DA BPCO LIEVE E MODERATA

A. Baralla[1], E. Sotgiu [1], G. Pira [1], E. Zinellu[2], V. Marras[2], A.G. Fois[2], P. Pirina [2], C. Carru[1], A. Zinellu [1]

[1]Dip. di Scienze Biomediche

[2]Dip. di Scienze Medicina Clinica e Sperimentale

La broncopneumopatia cronica ostruttiva (BPCO) è attualmente una delle principali cause di morbilità e mortalità in tutto il mondo, caratterizzata da ostruzione delle piccole vie aeree, sviluppo di enfisema, allargamento degli spazi aerei e distruzione del parenchima polmonare. Attualmente la diagnosi della malattia viene effettuata considerando il rapporto tra il volume espiratorio forzato in un secondo, e la capacità vitale forzata (FEV1/FVC), in accordo con le linee guida del "Global initiative for Obstructive Lung Disease(GOLD)" che suddividono i pazienti sulla base della severità della malattia in lieve, moderata e grave [1]. Il presente lavoro aveva lo scopo di individuare potenziali biomarcatori nel plasma di soggetti affetti da BPCO lieve e moderata in grado di fornire indicazioni sull'avanzamento della malattia. Sono stati reclutati 43 pazienti con BPCO (età media 74.8±5.9 anni), divisi in due gruppi con una forma lieve (N=29) e moderata (N=14) della malattia, e un gruppo di controlli sani (N=43). Le proteine del plasma sono state purificate utilizzando un kit di deplezione di albumina e IgG e successivamente visualizzate mediante 2D elettroforesi. Gli spot che variavano in maniera significativa rispetto a un gruppo di controllo (sex and age matched), sono stati identificati mediante spettrometro di massa MALDI-TOF. Tale analisi ha rivelato la presenza di 28 spot corrispondenti a 16 proteine differenti, che variavano in maniera significativa tra BPCO lieve e moderata rispetto al gruppo di controllo. Le proteine individuate appartengono ai seguenti gruppi: protein binding, hemoglobin binding e antioxidant activity secondo la classificazione GeneOntology (GO) di funzione molecolare. Tra le proteine sovraespresse nei pazienti con BPCO rispetto ai controlli sono state individuate alpha-1-antitrypsin, aptoglobina, vitamin D binding protein, mentre tra quelle sottoespresse la catena gamma del fibrinogeno, l'emopexina e il plasminogeno. Questi risultati rivelano come le proteine coinvolte nell'infiammazione sistemica potrebbero rivelarsi utili nel monitorare l'avanzamento della patologia.

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DIAGNOSTIC PERFORMANCE OF URINE AND SERUM OSMOLALITY DURING THE WATER DEPRIVATION/DDAVP TEST

D. De Falco[1], D. Arpaia[2], R. Faraonio[1]

[1]Dip. di Medicina Molecolare e Biotecnologie Mediche, Università Federico II, Naples

[2]Dip. di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Università Federico II, Naples

Introduction: Polyuria-Polydipsia refers to a pathological condition in which the diuresis exceeds 3 liters/day. Generally, Polyuria-Polydipsia is caused by different conditions including Diabetes Mellitus, Diabetes Insipidus (DI) and Primary Polydipsia (PP, also referred as Psychogenic Polydipsia). The gold-standard test to distinguish Central or Nephrogenic DI from PP is the water deprivation test that usually is associated with desmopressin (DDAVP) test. In order to characterize the patient's responses to the mentioned tests, urine and serum osmolality are assessed (de Fost M & al. Endocr Connect. 2015, 4: 86-91).

Aim: Our study was focused on the determination of the diagnostic performance of urine and serum osmolality measures during the water deprivation/DDAVP test in a group of patients with Polyuria-Polydipsia syndrome (age 21-64 years), collected between 2014-2016.

Method: A total of 15 patients were enrolled for water deprivation/DDAVP tests using serum and urine osmolality as reference standard. The OSMO-STATION OM6060 Menarini Arkray instrument was used to assess osmolality. For the measures, samples were collected every hour during the tests.

Results: The study was performed on a total of 15 patients who underwent first to the water deprivation test. Among them, 10 patients were also subjected to DDAVP test. By using only the urine/serum osmolality measurements obtained during the water deprivation test, the 15 patients were classified in 3 groups: group I (7 patients) with diagnosis of DI (47%); group II (5 patients) with diagnosis of PP (33%); group III (3 patients) with uncertain diagnosis (20%). Then, the DDAVP test was performed on group I and group III patients (n=10) and the urine/serum osmolality assessed during the test. For all these patients, the final clinical diagnosis was no Nephrogenic DI. Among the patients included in the group I, 2 patients have Central Total DI and 5 patients have Central Partial DI. In the group III the final clinical diagnoses after DDAVP test were: PP in 1 patient, Polyuria syndrome in 1 patient and no diagnosis for 1 patient.

Conclusion: The urine and serum osmolality measures during the water deprivation test and the DDAVP test allowed the final clinical diagnosis for 93% of patients.

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LA CONCENTRAZIONE DEI GRUPPI SULFIDRILICI DELLE PROTEINE (PSH) PLASMATICHE COME MARCATORE PRECOCE DI STRESS OSSIDATIVO IN PAZIENTI AFFETTI DA BPCO E ASMA

E. Sotgiu[1], S. Ena[1], S. Assaretti[1], E. Zinellu[2], A. Arcadu[2], A.G. Fois[2], P. Pirina[2], A. Zinellu[1], C. Carru[1]

[1]*Dipartimento di Scienze Biomediche*
[2]*Dipartimento di Medicina Clinica e Sperimentale*

Tra le patologie croniche ostruttive polmonari presentano una significativa rilevanza la broncopneumopatia cronica ostruttiva (BPCO) e l'asma, le quali sono caratterizzate da una consistente infiammazione cronica delle vie aeree e da stress ossidativo. Diversi studi sottolineano l'importanza dello stress ossidativo in queste malattie, in quanto esso gioca un ruolo chiave nella patogenesi, andando ad aumentare la severità della patologia e riducendo la funzionalità polmonare (1). Non è ancora chiaro se esso sia presente già negli stadi precoci della malattia e su quale sia il modo migliore per stimare meglio la sua presenza.

Lo scopo di questo studio è stato quello di valutare diversi indici di stress ossidativo in pazienti affetti da BPCO e asma al fine di individuare il biomarcatore più precoce. Sono stati quindi selezionati un gruppo di pazienti affetti da BPCO lieve (n=29) e un gruppo di soggetti con asma lieve (n=24) e sono stati confrontati con due differenti gruppi di controllo, sovrapponibili in termini di genere, età e stato di fumatore. Tutti i soggetti in studio sono stati sottoposti a misurazione dei biomarcatori plasmatici di stress ossidativo, come PSH, taurina, TBARS, GSH, paraoxonasi 1 e ergotioneina, e ai test di funzionalità polmonare (FEV_1, FVC e FEV_1/FVC). L'analisi di regressione univariata indica che nei pazienti affetti da BPCO la FEV_1 è correlata con l'età (rho=-0.49; P=0.007) e con i livelli di PSH (rho=0.49; P=0.007), mentre nei soggetti asmatici la FEV_1 è correlata solo con l'età (r=-0.60; P=0.003). Le analisi di regressione logistica multipla, ottenute includendo come covariate età, genere, BMI, fumo di sigaretta, ergotioneina, GSH, TBARS, PSH, paraoxonasi 1 e taurina, mostrano come solo la concentrazione di PSH sia indipendentemente associata alla forma lieve di BPCO (OR=0.50; 95% CI 0.26-0.95; P=0.03) e alla forma lieve di asma (OR=0.32; 95% CI 0.13-0.78; P=0.01).

Questi risultati suggeriscono che la valutazione dei livelli dei gruppi sulfidrilici delle proteine plasmatiche può rappresentare un indicatore di stress ossidativo sensibile e precoce nei pazienti affetti da BPCO e da asma.

1. Chung KF, Marwick JA. Molecular mechanisms of oxidative stress in airways and lungs with reference to asthma and chronic obstructive pulmonary disease. Ann N Y Acad Sci 2010;1203:85-91.

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DISSIPATIVE FORCE MICROSCOPY: AN EFFECTIVE TOOL TO DISTINGUISH BETWEEN HEALTHY AND PATHOLOGICAL RED BLOOD CELLS

C. Rossi[1], E. Minelli[2], G. Ciasca[2], M. Papi[2], M. De Spirito[2]

[1]*Dipartimento di Medicina di Laboratorio, Fondazione Policlinico Gemelli, UCSC, Roma*
[2]*Istituto di Fisica, UCSC, Roma*

We describe a scanning probe methodology that permits to evaluate quantitatively the role of dissipative forces in the biomechanical response of cells and tissues. To this purpose we measured the local distribution of the percentage of energy dissipated during the AFM indentation process, or hysteresis (H). Although this quantity is rarely considered in AFM experiments, nevertheless it can provide useful information, given its unique capability to distinguish between structures with a nearly elastic behaviour from those whose mechanical response is ruled by viscous and dissipative forces. Red blood cells (RBC) extracted from healthy donors and patients with iron overload and hyperferritinemia were used as a model system. In physiological conditions, RBCs are characterized by extreme deformability properties [1]. A wide range of pathological conditions, including iron overload and hyperferritinemia, have a major effect on such an extreme deformability. Our nanoscale mapping unveil the local changes in the biomechanical response of healthy RBC, pointing out that the cell centre and the cell periphery have a markedly different biomechanical response. The cell centre has a nearly elastic behaviour, being characterized by small values of H. Conversely the biomechanical response of the cell periphery is more dominated by dissipative forces, showing high H values. In pathological conditions such behaviour appears to be dramatically changed. RBCs indeed show an increased average H value, hinting at global impairment of the elastic properties of the cell. Our high resolution mapping highlights that the cell centre undergoes the most relevant changes, showing an increase of H from approximately 0.1 to approximately 0.4. These results open up wide applications in diagnostics, having the potential to promote the development of novel mechanical biomarkers of pathologies.

1. Ciasca G, et al. Mapping viscoelastic properties of healthy and pathological red blood cells at the nanoscale level. Nanoscale 2015;7:17030-7.

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DOSAGGIO E SIGNIFICATO DELLA VITAMINA D NELLE PAZIENTI AFFETTE DA ENDOMETRIOSI

M.C. Filippone[1], B. Colaprisca[1], M.G. Porpora[2], E. Fuggetta[2], A. Angeloni[1], E. Anastasi[1]

[1]Dipartimento Medicina Molecolare
[2]Dipartimento Scienze Ginecologiche-Ostetriche e Scienze urologiche

Nel sistema circolatorio umano, le vitamine D2 e D3 biologicamente inerti sono legate alla proteina legante la vitamina D e trasportate al fegato per essere sottoposte ad idrossilazione, generando la 25-OH vitamina D. La 25-OH vitamina D ha riconosciute attività anti-proliferative, anti-infiammatorie e immuno-modulatorie mediate dal suo recettore VDR che è ampiamente espresso nei tessuti. L'endometriosi è una patologia ginecologica benigna estrogeno-dipendente, caratterizzata dalla proliferazione di tessuto endometriale in sedi ectopiche con un aumento dei livelli sierici del biomarcatore CA125. Lo scopo di questo studio è quello di dimostrare una correlazione tra i livelli ematici di CA125 e 25-OH vitamina D nell'endometriosi, nonché valutare se i valori di 25-OH vitamina D siano associati al dolore, principale sintomo di questa patologia. A tale proposito sono state reclutate 104 pazienti (età media 35 aa) afferenti alla Clinica Ginecologica del Policlinico Umberto I di Roma e sono stati determinati i livelli sierici di CA125 e 25-OH vitamina D. I dosaggi di questi analiti sono stati eseguiti con un sistema automatizzato in chemiluminescenza (CLEIA, LUMIPULSE® G 1200). Abbiamo osservato che 83/104 (82%) delle donne affette da endometriosi aveva un deficit di 25-OH vitamina D (v.n $\geq$20 ng/mL). La correlazione con il CA125, ha messo in evidenza che 42/83 (51%) delle pazienti con deficit di 25-OH vitamina D aveva elevati livelli di CA125 (v.n. <35 IU/ mL) ($p < 0.02$). Delle 83 pazienti con deficit di 25-OH vitamina D 79 (95%) riferivano dolore, questo dato ha messo in evidenza l' esistenza di una correlazione statisticamente significativa ($p < 0.01$). Questo studio dimostra la presenza di deficit di 25-OH vitamina D nelle donne affette da endometriosi e la sua correlazione con il dolore. Futuri studi prospettici saranno volti a verificare se il ripristino dei valori normali di 25-OH vitamin D con supplementazione esogena in queste pazienti, possa avere un effetto benefico sulla sintomatologia.

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DEFICIT DI 25-OH VITAMIN D E ALGORITMO ROMA NELLE DONNE OBESE

S. Tartaglione[1], V. Viggiani[1], T. Granato[2], C. Del Monte[1], F. Leonetti[3], A. Angeloni[1], E. Anastasi[1]

[1]Dipartimento Medicina Molecolare
[2]IBPM-ROMA
[3]Dipartimento Medicina Sperimentale

Recentemente è stato riscontrato che bassi livelli sierici di 25-OH Vitamina D sono correlati con un'aumentata incidenza di neoplasia. Numerosi studi hanno dimostrato come l'obesità rappresenti un fattore di rischio per diversi tipi di cancro. Il carcinoma ovarico epiteliale (EOC) è la neoplasia a mortalità più elevata tra quelle ginecologiche. Per riuscire a determinare il rischio di insorgenza di EOC è stato recentemente sviluppato un algoritmo, il "risk of malignancy algorithm" (ROMA). Questo algoritmo diagnostico è calcolato a partire da un indice predittivo (IP) che deriva dalla determinazione di due biomarcatori sierici: CA125 e Human Epididymis protein 4 (HE4). Obiettivo di questo studio è stato valutare se il deficit di Vitamina D rappresentasse un fattore di rischio aggiuntivo per l'insorgenza di EOC e potesse aumentare il valore predittivo dell'indice ROMA in una popolazione di donne obese. Presso il centro dell'Obesità del Policlinico Umberto I (Sapienza-Roma, sono selezionate con età e stile di vita omogeneo 118 pazienti aventi un indice di massa corporea (IMC) >30 kg/m2 (Gruppo 1) e 80 donne con un IMC <25 kg/m² (Gruppo 2). Il dosaggio di CA125, HE4 e 25-OH vitamin D è stato effettuato tramite il LUMIPULSE® G 1200. E' stato scelto come valore di riferimento, identificato dall'analisi con curva ROC, il valore di 20.2 ng/mL (sensitivity 73.3%, specificity 84%) in accordo con il limite stabilito dall'Organizzazione Mondiale della Sanità (OMS). Sono stati osservati bassi livelli di 25-OH Vitamina D nel 64% delle donne del Gruppo 1 e nell'11% delle donne del Gruppo 2 ($p < 0{,}001$). Un indice ROMA >13% (considerato predittivo di rischio) è stato riscontrato solo nelle pazienti obese (19%). Inoltre è stata osservata un'associazione tra bassi livelli di 25-OH Vitamina D e indice ROMA >13%, infatti il 64% di donne obese con un indice ROMA >13% aveva contemporaneamente anche insufficienti livelli di 25-OH Vitamin D, mentre solo il 36% delle donne obese con ROMA score <13% aveva livelli sufficienti di 25-OH Vitamina D ($p < 0.0001$). Questo studio suggerisce che, nelle donne obese, la valutazione del deficit di 25-OH Vitamina D in combinazione alla valutazione dell' indice ROMA potrebbe migliorare il potere predittivo per l'insorgenza del cancro ovarico.

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GASTROPANEL: LA BIOPSIA SIEROLOGIA NEL PAZIENTE DISPEPTICO

E. De Santis[1], G. Grande[2], A. Melegari[1], E. Baraldi[1], R. Conigliaro[2], T. Trenti[1], A.T. Scacchetti[1]

[1]Laboratorio di Tossicologia e Diagnostica Avanzata, Nuovo Ospedale Civile S. Agostino Estense, Modena
[2]Dipartimento di Endoscopia Digestiva, Nuovo Ospedale Civile S. Agostino Estense, Modena

Il cancro gastrico rappresenta la seconda causa di morte tumore-correlata al mondo che si sviluppa a partire da lesioni pre-cancerose; la provincia di Modena può essere considerata a rischio intermedio per l'incidenza di questo tipo di tumore.
Sebbene il gold-standard sia a tutt'oggi l'Esofagogastroduodenoscopia, numerosi studiosi hanno inserito l'utilizzo dei marcatori sierici di funzionalità gastrica, tra gli esami appropriati per la diagnosi ed il follow-up di pazienti portatori di lesioni pre-cancerose gastriche (Gastropanel: pepsinogeni, gastrina-17, anticorpi IgG anti-Helicobacter Pylori).
Abbiamo messo a punto uno studio prospettico ed osservazionale condotto con il Dipartimento di Endoscopia Digestiva (NOCSAE) mirato ad ottimizzare l'approccio al paziente dispeptico mediante l'utilizzo del test sierologico Gastropanel.
Sono stati selezioni 80 pazienti con disturbi dispeptici a cui, dopo prelievo di sangue, è stato eseguito il Gastropanel per studiarne la funzionalità gastrica e la ricerca di anticorpi anti-cellule parietali gastriche e anti-fattore intrinseco (per discriminare le gastriti di origine autoimmune). I nostri risultati indicano la presenza di Helicobacter Pylori nel 25% dei casi a cui si associano alterazioni di gastrina-17 e dei pepsinogeni sierici. Questi risultati suggeriscono la presenza di gastrite di diversa natura e severità che correlano con gli esami bioptici eseguiti. Gastropanel si conferma essere un test di screening rapido, affidabile, non invasivo ed utile per la diagnosi ed il monitoraggio dei pazienti dispeptici. Dopo aver ampliato la casistica e valutato questo test in termini di efficienza-efficacia, l'obiettivo futuro è quello di mettere a punto un algoritmo diagnostico che eviti l'approccio diretto all'esame bioptico e che segua i pazienti con sintomatologia dispeptica nel tempo.
Di Mario F, Cavallaro LG. Non invasive tests in gastric disease. Dig Liver Dis 2008;40:523-30.

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DETERMINAZIONI DELL'ALCOLEMIA (EX ART. 186 CODICE DELLA STRADA) NEL TRIENNIO 2013/15

P. De Luca, S. Messina, A. Previti, F. Picciolo

U. O. C. Patologia Clinica, P.O. "Fogliani" Milazzo - ASP Messina

Introduzione: L'applicazione dell'art.186 del codice della strada ha coinvolto la nostra UOC per le determinazioni del test ALCOLEMIA in modalità screening di 1° livello richieste dalle autorità giudiziarie e pervenute tramite il nostro P.S.; in tal senso è stata implementata ed adottata insieme alla Direzione Sanitaria di Presidio una specifica procedura in cui vengono definite modalità di prelievo, trasporto, esecuzione del test ed eventuale custodia del materiale biologico.
Scopo dello studio: Verificare nel corso del triennio 2013-15, la popolazione afferente per questa tipologia di esame, identificandone le principali caratteristiche statistico-epidemiologiche.
Materiali e metodi: L'esame alcolemia viene eseguito con strumentazione Architect Plus C4000 Abbott utilizzando il reagente Ethanol; la matrice biologica utilizzata è plasma da provetta LH 68 IU.
Risultati: L'accesso al DB del nostro LIS ci ha consentito di identificare le caratteristiche dei 379 soggetti esaminati nel triennio, di cui 281 uomini (74%) e 98 donne (26%); i casi riscontrati sono stati: nel 2013(137), nel 2014(142), nel 2015(100).
La suddivisione è stata fatta in funzione delle sanzioni amministrative previste dal vigente codice della strada: i casi negativi sono stati 274(72.3%) e 105 i casi positivi (27.7%); di questi, 9 erano compresi in un range alcolemico tra 0,50÷0.79; 33 tra 0,80÷1,49; 63 ≥1,50
La distribuzione dei valori di alcolemia in relazione alle età suggerisce un più forte coinvolgimento della fascia compresa tra 26 e 45 anni, con 156 casi di cui 51 positivi (di questi ben 35 risultavano essere superiori a 1,50 gr/L). Gli over 66, come era logico attendersi, sono significativamente meno rappresentati.
Conclusioni: La valutazione retrospettiva della nostra casistica ci ha permesso di quantificare la rilevanza del problema della guida sotto la eventuale influenza dell'alcool nel nostro territorio, la rispondenza della procedura implementata in relazione alle varie esigenze man mano evidenziatesi ed anche un riscontro epidemiologico locale sulla diffusione del fenomeno abuso di alcool in relazione alle varie fasce di età ed alla stagionalità.

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LIQUIDO SEMINALE: CASISTICA NEL TRIENNIO 2013/2015

P. De Luca, S. Messina, M. Cirilli, F. Picciolo, A. Previti

U. O. C. Patologia Clinica, P.O. "Fogliani" Milazzo, ASP Messina

Introduzione: L'OMS definisce sterilità la situazione in cui uno o entrambi i membri di una coppia sono affetti da una condizione fisica permanente che non rende possibile il concepimento. L'esame del Liquido Seminale, soprattutto eseguito secondo le indicazioni delle L.G. più recenti, consente una valutazione affidabile della componente maschile.
Scopo dello studio: valutare il liquido seminale di 205 utenti pervenuti nel triennio 2013/15 con richiesta di Spermiogramma associata o meno a Spermiocoltura, identificando le principali caratteristiche epidemiologiche dei soggetti esaminati.
Materiali: Il liquido seminale è stato esaminato secondo le indicazioni previste dalle relative Linee Guida WHO del 2010. Sono stati attenzionati, tra i numerosi parametri, VOL_EIAC (volume eiaculato); NDN (num nemaspermi x ml); NDN_EIAC (num nemaspermi x eiaculato).
Risultati: Nel nostro Laboratorio sono stati valutati 205 soggetti (60 nel 2013; 83 nel 2014; 62 nel 2015) di età compresa tra i 14 ed i 66 anni (Media 32.7). Ai fini dello studio è stata articolata una suddivisione in 5 fasce di età: ≤25 anni (Media 21,5 ds 2.59); 26÷35 a.(Media 30.3 ds 3.09); 36÷45 a. (Media 40.0 ds 3.04); 46÷55 a.(Media 47.6 ds 1.65); ≥56 a. (Media 60.7 ds 3.30).
Il parametro VOL_EIAC ha un valore medio di 2.74 ml, ds 1.40, valore minimo 0.2, valore massimo 8.0; il parametro NDN ha un valore medio di 17.7 10^6x ml, ds 20.4, valore minimo 0, valore massimo 95.2; il parametro NDN_EIAC ha un valore medio di 50.7 10^6, ds 74.3, valore minimo 0, valore massimo 524.
Secondo i parametri della WHO sono stati riscontrati 20 (10%) azoospermici; 109 oligozoospermici (53%). Nella casistica a nostra disposizione si rileva che 109 soggetti (58%) hanno in concomitanza effettuato il test spermiocoltura che è risultata essere positiva in 48 casi (23% complessivo).
Conclusioni: In questi ultimi decenni, specialmente nei paesi occidentali, si è assistito ad una documentata diminuzione della fertilità della coppia e nello specifico dell'uomo. I tentativi di standardizzazione della valutazione del Liquido Seminale possono fornire un validissimo aiuto al Laboratorista per mettere a disposizione dati sempre più attendibili e soprattutto comparabili agli specialisti del settore.

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CLINICAL RISK MANAGEMENT AND CHROMATOGRAPHY TECHNOLOGY: ROOM FOR IMPROVEMENT

P. Pezzati, A. Bandinelli, A. Casalucci, L. Centola, N. Cini, A. De Stefano, V. Fulgido, O. Gioia, F. Luceri

Lab. Generale, Dip. dei Servizi, AOU Careggi, Firenze

It has been shown and its is generally accepted, that errors in Laboratory Medicine occurs mainly in the pre-analytical phase (1). Although this may be the case in fully automated analytical activities, methods involving manual samples and reagents preparation, must be regarded as potentially prone to analytical and even post analytical errors. Liquid Chromatography technology, coupled either with Mass Spectrometry (LC-MS) or with common detectors (UV, fluorescence, electrochemical) represents nowadays a widespread reality in routine laboratory, but the potential risks of manual work involved, must be acknowledged.
The Laboratorio Generale AOU Careggi (Florence, Italy) performs routinely immunosuppressant therapeutic drug monitoring (TDM) in LC-MS and measures drugs, biogenic amines and vitamins in HPLC. All methods are time consuming and involve manual steps. Seven full time, trained technicians perform the bench work and three biochemists (2 PhD, 1 MD) supervise all activities. Results are released only after evaluation from both technician and supervisor.
We conducted a review (December 2015- May 2016) of HPLC and LC-MS errors occurred from December 2015 to May 2016. All events were recorded according to local Quality System.
In a 6 month period 29 events occurred; 25 errors become apparent before data release and were classified as "near misses"; the remaining 4 events originated wrong information actually passed on to the clinicians, i.e. were "adverse events" and, although no health damage was done to the patients, they have to be considered as potentially disruptive.
A further sub-analysis showed that 41 % of errors was due to incorrect chromatogram peak interpretation; 32 % to lack of compliance with procedures or reagents handling instructions; 18 % to erroneous use of units of measure and formulas and 9 % to patients mismatch.
The majority of the errors reviewed appears related either to poor manual activities performances or to insufficient knowledge, however the root cause analysis unlighted cofactors such as workload, schedule constrain and relational climate. Continuous education is an unquestionable priority, however adequate working conditions may contribute to support patient safety culture.
1. Lippi G, Guidi GC. Risk management in the preanalytical phase of laboratory testing. Clin Chem Lab Med 2007;45:720–7.

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CLINICAL RISK MANAGEMENT AND PATIENT MISIDENTIFICATION: A CASE REPORT AND THE SUBSEQUENT CLINICAL AUDIT AND IMPROVEMENT PLAN

F. Balboni[1], A. Tomei[2]

[1]*Laboratorio Analisi Istituto Fiorentino Cura e Assistenza IFCA, Firenze*
[2]*CRM Istituto Fiorentino Cura e Assistenza IFCA, Firenze*

Patient misidentification is a fearsome adverse event whose effect can be disruptive. Tuscany Clinical Risk Centre produced a Patient Safety Practice (PSP) that is mandatory for all healthcare structure in Tuscany. The PSP requires active identification of patient. IFCA adopted, disseminate and trained all personnel on the PSP. Despite this, every year, in IFCA several misidentification events take place. All adverse events are analysed through the human factor method FMEA and audits are performed, followed by improvement plans. The following case report shows in detail the methodology implemented.
In the outpatient ward the nurse fails the patient identification. She relies on her own memory, instead of perform an active identification. Moreover, the informatic procedure used to register patient is set on a inappropriate screenshot showing the name of a previous patient. The nurse labels the tubes mismatching the patient. The laboratory receives two different sets of samples few minutes apart entitled to the same person and they are analysed. A deltacheck failure is noted and the laboratory personnel phones to the ward. All the patients demographic data are checked and laboratory data of a patient are missing, although the phlebotomy has been performed. The patient is a 15 years old girl, very anxious who fainted when phlebotomy was performed. She is asked to repeat the phlebotomy, together with all the other patients admitted in that day in order to exclude any other adverse event.
An audit was performed and an improvement plan was defined introducing a double check performed by two different operators for outpatients. The adverse event was classified as “with harm”.
Phlebotomy is the most common invasive procedure performed in hospital and without a patient identification and tube labelling procedure, the risk of misidentification is very high (1).
Several studies confirm that healthcare personnel is not enough compliant with best practice procedures , possibly because the procedure is perceived as minimal invasive and, not to be excluded, because of the burden of activities in the ward.
1. van Dongen-Lases EC, Cornes MP, Grankvist K, et al. Patient identification and tube labelling – a call for harmonisation. Clin Chem Lab Med 2016;54:1141–5.

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WITHIN AND BETWEEN-SUBJECT BIOLOGICAL VARIATION DATA OBTAINED FROM 91 HEALTHY SUBJECTS FOR SERUM CREATININE USING ENZYMATIC AND JAFFE METHODS

I. Marino[1], A. Carobene[1], E. Guerra[1], N. Jonker[2], G. Barla[2], W.A. Bartlett[3], S. Sandberg[4], M. Sverresdotter Sylte[4], T. Røraas[5], U. Ørvim Sølvik[6], P. Fernandez-Calle[7], J. Díaz-Garzón[7], F. Tosato[8], M. Plebani[8], A. Co#kun[9], M. Serteser[9], I. Unsal[9], F. Ceriotti[1]

[1]*Serv. Med. Lab., Osp. San Raffaele, Milan, Italy*
[2]*Certe, Wilhelmina Ziekenhuis Assen, the Netherlands*
[3]*Blood Sciences, Ninewells Hosp., Scotland*
[4]*Lab. of Clin. Biochem., Haukeland Univ. Hosp., Bergen, Norway*
[5]*Norwegian Quality Improvement of Primary Health Care Laboratories (Noklus), Haraldsplass, Hosp., Bergen*
[6]*Dept. Global public health & primary care, Univ. Bergen*
[7]*Hosp. Univ. La Paz, Madrid, Spain, and Quality Analytical Commission of Spanish Society of Clinical Chemistry (SEQC)*
[8]*Dept. of Lab. Med, University Hospital, Padua, Italy*
[9]*Acibadem Univ., School of Med., Atasehir, Istanbul, Turkey*

An EFLM project was established to deliver new biological variation (BV) data for serum creatinine obtained using enzymatic and Jaffe methods. A cohort of 91 healthy subjects (38 male and 53 female, 21-69 years old) were bled for 10 consecutive weeks at one of six European laboratories. An equivalent and stringent pre-analytical protocol was followed at each center to deliver the blood samples. Separated sera were stored at -80°C prior to analysis in duplicate within a single run on ADVIA 2400 (Siemens Healthcare). Biorad control materials at two different concentration 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 (CI). CVA (1.1%) calculated by ANOVA on sample’s replicates for enzymatic method, was below desirable analytical performance specifications for imprecision based on current BV data (2.98%). On the contrary CVA for Jaffe method (4.7%) was higher than quality specification. Similar overall CVs were obtained on QC materials. For the two methods there were no statistical differences between genders in within-subject BV estimates (CVI(95%CI)): 4.5% (4.3-4.7), Enzymatic method [Crea=70.7 #mol/L]; 4.7% (4.4-4.9) Jaffe method [Crea=65.6 #mol/L]. Both CVIs were significantly lower than the one reported in Westgard database (CVI=5.95%). Statistical differences between genders were found in between-subject BV (CVG) estimates. Enzymatic: CVG male 14.2% (11.4-18.2), CVG female 12.9% (10.7-15.9). Jaffe: CVG male 17.2% (13.8-22.1), CVG female 13.9% (11.5-17.1). CVG obtained are closed to BV data currently used (14.7%). CVI found are statistically significantly lower than existing published data whereas CVG are similar. The new BV data deliver lower analytical goals for imprecision for serum creatinine.

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WITHIN AND BETWEEN-SUBJECT BIOLOGICAL VARIATION DATA OBTAINED FROM 91 HEALTHY SUBJECTS FOR TOTAL CHOLESTEROL, HDL, LDL-CHOLESTEROL AND TRIGLYCERIDES IN SERUM SAMPLES

I. Marino[1], A. Carobene[1], E. Guerra[1], N. Jonker [2], G. Barla[2], W.A. Bartlett[3], S. Sandberg[4], M. Sverresdotter Sylte[4], T. Røraas[5], U. Ørvim Sølvik[6], P. Fernandez-Calle[7], J. Díaz-Garzón[7], F. Tosato[8], M. Plebani[8], A. Co#kun[9], M. Serteser[9], I. Unsal[9], F. Ceriotti[1]

[1]Servizio Medicina di Laboratorio, Osp. San Raffaele, Milan, Italy

[2]Certe, Wilhelmina Ziekenhuis Assen, Europaweg-Zuid 1, 9401 RK Assen, the Netherlands

[3]Blood Sciences, Ninewells Hospitall, Scotland

[4]Lab.of Clin. Biochem., Haukeland Univ. Hosp., Bergen, Norway

[5]Norwegian Quality Improvement of Primary Health Care Laboratories (Noklus), Haraldsplass, Hospital, Bergen

[6]Dept. of Global Public Health & Primary Care, Univ. Bergen

[7]Hosp. Universitario La Paz, Madrid, Spain, and Quality Analytical Commission of Spanish Society of Clinical Chemistry (SEQC).

[8]Dept. of Lab.Med. Univ. Hosp., Padua, Italy

[9]Acibadem Univ., School of Medicine, Atasehir, Istanbul, Turkey

Here we focused on total cholesterol (TC), LDL-cholesterol (LDL), HDL-cholesterol (HDL) and triglycerides (TG). Separated serum collected from 91 healthy subjects were analyzed in duplicate within a single run on ADVIA 2400 Siemens, using Siemens reagent. LDL results were calculated using Friedewald formula. Two BioRad controls were used. Moreover frozen sera at two levels for TC and HDL with reference values assigned by a reference laboratory of CRMLN network, were analyzed to guarantee traceability. CVA calculated on sample replicates were always lower than desirable analytical goals for imprecision based on current BV data (in parenthesis): TC: 0.97% (2.98%); TG: 1.5% (9.95%); HDL 0.57% (3.65%) and LDL 1.4% (3.9%). Also CVAs obtained, on Biorad and on reference materials, were lower than desirable analytical goal. Bias% from target values calculated on reference materials were lower than CDC limits: low TC: 2.9%, high TC: -0.35%; low HDL: 2.8%, high HDL: 1.4%. With the exception of TG, within and between-subject BV (CVI and CVG) found were significantly lower for women in menopause compared to those of the whole population. On the contrary, statistical difference between genders was not found. Excluding women in menopause the CVI of whole population, compared to those currently used, were: TC: 5.57% (5.3–5.9) vs 5.95%, HDL 5.9% (5.6–6.2) vs 7.3%, TG 22.3% (21.3–23.5) vs 19.9%, LDL 8.0% (7.6–8.5) vs 7.8%. CVG estimates, were: TC: 16.1% (14.0–19.2) vs 15.3; HDL: 23.9% (20.5–28.1) vs 21.2%; TG: 41.6% (35.9–48.8) vs 19.9; LDL 27.1% (23.4–32.0) vs 20.4. CVI obtained were slightly lower for TC and HDL and slightly higher for TG than those currently used. Conversely for all lipids CVG found were significantly higher than current CVG.

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CATENE LEGGERE LIBERE (FLC) E PROTEINA DI BENCE JONES (BJP) A CONFRONTO NELLA GESTIONE DEL MIELOMA MULTIPLO A CATENE LEGGERE

M. Berardi[1], M. Staderini[2], C. Nozzoli[2], T. Biagioli[1], M. Brogi[1], A. Terreni[1], L. Torreni[1], A. Gironi[1], F. Cassani[1], A. Bosi[2], A. Caldini[2]

[1]Laboratorio Generale, Azienda Ospedaliero Universitaria Careggi, Firenze

[2]Ematologia, Azienda Ospedaliero Universitaria Careggi, Firenze

L'utilizzo delle FLC nella valutazione della risposta alla terapia o della progressione di malattia in pazienti con mieloma multiplo (MM), viene raccomandato dall'IMWG solo nel caso in cui la CM sierica e urinaria non siano misurabili e per la definizione di risposta completa stringente (sCR). La possibilità di sostituire la talvolta problematica misura della BJP con quella delle FLC è oggetto di acceso dibattito.

Scopo di questo studio retrospettivo è stato quello di confrontare l'uso delle FLC e della BJP nella gestione di pazienti con MM a catene leggere. A questo scopo sono state analizzate 696 determinazioni di 43 pazienti. Come già riportato in altri studi, non è stata trovata alcuna correlazione quantitativa tra la misura delle FLC e della BJP, ma è stata osservata una concordanza del 79% tra l'alterazione o meno dell'FLC ratio (FLCr) e della BJP. Nel 20% dei casi, è stata osservata una alterazione di FLCr con BJP negativa mentre solo nell'1% dei casi la BJP è risultata positiva pur in presenza di FLCr normale.

Per ciascuno dei 43 pazienti è stata confrontata la capacità delle FLC e della BJP nella valutazione della risposta terapeutica e dell'eventuale progressione (PD), classificate in maniera indipendente l'uno dall'altra secondo i criteri dell'IMWG [1]. La concordanza tra le "best response" definite dai due parametri è stata del 78%. In 13 pazienti entrambi i parametri hanno rilevato la CR. In 7 pazienti su 13, la CR è stata raggiunta più tardivamente dalle FLC mentre il contrario è avvenuto solo in un caso. In 21 pazienti su 43, è stata osservata una PD. In 16 pazienti sia FLC che BJP hanno rilevato la PD. In 7 pazienti su 16 le FLC l'hanno rilevata più precocemente (dai 5 ai 14 mesi prima), mentre in 4 casi la PD è stata rilevata più precocemente dalla BJP (dai 2 agli 8 mesi prima). In 4 pazienti su 21 la progressione è stata rilevata solo dalle FLC, mentre in 1 caso su 21, dalla sola BJP.

I dati ottenuti quindi, pur mostrando una buona concordanza dei due parametri, fanno ipotizzare una maggiore sensibilità delle FLC nella rilevazione della malattia residua e della progressione/ricaduta di malattia.

1. Durie BG, Harousseau JL, Miguel JS, et al. International uniform response criteria for multiple myeloma. Leukemia 2006;20:1467-73.

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STANDARDIZATION AND IMPROVEMENT OF THE BLOOD SAFETY FROM DONORS WITH OCCULT HEPATITIS B VIRUS INFECTION IN LOMBARDIA

M. Spreafico[1], L. Raffaele[1], B. Foglieni[1], I. Guarnori[1], D. Girgenti[1], P. Lanzini[4], L. La Rosa[3], F. Rossi[2], D. Prati[1]

[1]Dip. Transfusion Medicine and Haematology, ASST-Lecco, Lecco
[2]Dip. Transfusion Medicine and Haematology, ASST-Monza, Monza
[3]Servizio Immuno-trasfusionale, ASST-Vimercate, Vimercate
[4]Dip. Transfusion Medicine and Haematology, ASST-Valtellina, Sondrio

Background: In countries with a medium prevalence of anti-HBc, as Italy, the decision not to use anti-HBc screening and to accept HBV DNA results to screen blood donations was considered safe, as there was no evidence that HBV infection can be transmitted in the absence of detectable HBV-DNA. However we identified 2 cases of HBV transfusion-transmission from donors with occult hepatitis B virus infection (OBI: HBsAg negative, low/undetectable circulating HBV DNA), undetected by minipool-based NAT screening. Both were anti-HBc positive [Spreafico M et al; J Hepatol 2015]. These results let us to finalize an algorithm to improve blood safety. With the re-organization of the blood transfusion network in Lombardia, we became one of the 9 regional CLQV, centralizing processing, qualification and validation activities of blood donations coming from the areas of Lecco, Monza, Sondrio, Vimercate-Desio-Carate. The aim of the study was to apply the newly adopted algorithm for the NAT screening to all processed donations of our macroarea to evaluate the impact on the number of newly diagnosed OBI carriers and on the safety of the blood supply.
Methods: The algorithm includes: adoption of an ID-NAT screening method, analysis of anti-HBc and anti-HBs in NAT reactive samples and application of confirmatory tests (NAT assay on enriched plasma samples, real-time and/or nested PCR and HBV DNA sequencing). A working group among the 9 CLQV of Lombardia discussed this algorithm to define a regional standard for the blood investigation.
Results: From January 2014 to April 2016, the algorithm application allowed to confirm the OBI carrier status in 29 ID-NAT positive over 144.039 blood donations (20,1:100.000 donations). In all of them the two mandatory test replicates on initially reactive sample were negative. 83% OBI carriers were anti-HBc positive with a mean number of donation of 31 (5-64). The regional working group of Lombardia approved the algorithm with minor modification.
Conclusions: A standardized protocol for the molecular investigation of blood units in Regione Lombardia, including the re-introduction of anti-HBc screening of donors with a NAT positive result, will allow to improve safety of our blood supply, particularly from donors with OBI.

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GONADOTROPINA CORIONICA GLICOSILATA POSSIBILE MARCATORE PREDITTIVO DI GRAVIDANZA IN PMA

R. Lanzano, A. Lanzano, S. Abbadessa , G. Lanzano, G. Abbadessa

Azienda Ospedaliera Universitaria, Policlinico SUN

La possibilità di diagnosticare lo stato di gravidanza in fase pre-clinica nelle Tecniche di Procreazione Medicalmente Assistita (PMA), rappresenta una possibilità' molto vantaggiosa ai fini, sia prognostici che terapeutici per le persone che si sottopongono a tali tecniche. La blastocisti è un embrione a uno stato di sviluppo avanzato corrispondente a 5 giorni successivi alla fecondazione. L'embrione in IV giornata va incontro ad un processo detto di "compattazione" in seguito al quale subisce delle modifiche morfologiche che lo rendono simile ad una modula.

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THE ROLE OF ANTI-MITOCHONDRIAL M2 ANTIBODY IN PRIMARY BILIARY CIRRHOSIS: ACCURACY OF TWO TESTS NOT ONLY IN THE DIAGNOSIS BUT ALSO IN THE MONITORING OF DISEASE ACTIVITY

A.M. Alfano[1], A. Romito[2], C. Marchese[3], T. Manetta[4], M. Battistini[1], A. Ferrini[1], C. Mancinetti[1], G. Mengozzi[4], P. Merlach[1], M. Migliardi[3], M.T. Tambuzzo[1]

[1]Hospital ASL TO4, Clinical Pathology, Ciriè (TO), G.A.L. Piemonte

[2]Hospital Maria Vittoria ASL TO2, Analysis Laboratory, Torino, G.A.L. Piemonte

[3]A.O. Ordine Mauriziano, Analysis Laboratory, Torino, G.A.L. Piemonte

[4]A.O.U. Città della Salute e della Scienza, Biochimical Laboratory, Torino, G.A.L. Piemonte

AMA and the specific M2 antibody represent one of the three diagnostic criteria for the primary biliary cirrhosis (PBC) (1).
Objective: To evaluate the analytical agreement between results obtained from the indirect immunofluorescence methods and from the multiplexed line-blot assay and EliA M2, to analyze the diagnostic accuracy in a cohort of PBC patients and in control patients of 2 different types of tests for anti-M2 and assess whether, with the advent of a quantitative test, the possibility exists to correlate disease activity with the value of AMA.
Methods: Serum analysis of 67 patients with fluorescence patterns detected on Hep-2 cells suggestive of PBC-related antibodies and three groups of patients (15 PBC, 16 PBC suspect and 48 disease controls) were carried out. All samples were tested by both a qualitative test multiplexed line-blot ALD Profile Euroline and by a quantitative test EliA M2 IgG. In order to evaluate a possible correlation between the quantitative M2 and disease activity, we divided patients mixed in a further three groups based on the value EliA-M2. For each of these groups was calculated the average values of the main indices of cholestasis.
Results: A perfect agreement was shown between the EliA M2 and the multiplexed line-blot method for AMA detection. All sera of patients with PBC were positive with both tests, with a 100% sensitivity. 47 of the 48 sera of the control group were negative for both tests with a 100% next specificity. We had also observed in the other three groups of patients that the average of the values of γ-GT and ALP increases with the increase of the value EliA-M2. The difference between the mean values of the most significant parameter which the alkaline phosphatase of the three groups is significant, mostly between the first and the third group (p-Value 0.023).
Conclusions: Both the qualitative method Profile Euroline that the quantitative EliA-M2 have a high diagnostic accuracy for PBC, with a specificity higher than the immunofluorescence method. These preliminary data might suggest the possibility of using the dosage EliA-M2 not only in the diagnosis phase but also in the monitoring of disease activity.
1. Selmi C, Bowlus CL, Gershwin ME, et al. Primary biliary cirrhosis. Lancet 2011;377:1600–9.

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AGING AND MULTIMORBIDITY: ASSOCIATIONS WITH INFLAMMATORY BIOMARKERS

E. Garrafa[1], N. Casnici[2], D. Bianchini[3], F. Squazzoni[4], A. Marengoni[4]

[1]Dip. Medicina Molecolare e Traslazionale, Università degli Studi di Brescia

[2]Dip. di Economia e Managment, Università degli Studi di Brescia

[3]Dip. di Ingegneria dell'Informazione, Università degli Studi di Brescia

[4]Dip. Scienze Cliniche e Sperimentali, Università degli Studi di Brescia

Background: Even if is well-established that multimorbidity, the co-existence of two or more chronic diseases within the same individual, increases with age and, independently of age, it is strongly associated with frailty, disability, hospitalization, and mortality, little is known about factors associated with multimorbidity beyond age.
A role for inflammation in the process of multimorbidity has been suggested , therefore we investigated the relationship of different biomarkers with multimorbidity in the partecipants of the Anziani In Rete (AIR) study.
Methods: We built a randomized sample of the older population of Brescia, Italy, in three city districts;each participant was evaluated with a comprehensive geriatric assessment. Multimorbidity was defined as number of chronic diseases. Blood was collected and different biomarkers analyzed.
Results: The sample included 118 women and 82 men. The mean age of the participants was 77.7 years. The mean number of chronic diseases was 3.5. Between the different markers analyzed CRP, Lp(a) and Cystatin C strongly correlated with multimorbidity. Hight levels of these biomarkers were associated with a higher number of diseases independently of age and gender.
Conclusion: CRP, Lp(a) and cystatin C are correlated with multimorbidity in the old age. Further prospective studies are needed to confirm the causal pathway.

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Indice degli Autori