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BC biochimica clinica

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Legenda:

SP	Sessione Plenaria
SS	Sessione Parallela
CO	Comunicazione Orale

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

SS01-CO01

UNCONFORMITIES IN DIRECT ORAL ANTICOAGULANTS REQUESTS

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Introduction: Classic dicumarolics have been replaced by direct oral anticoagulants (DOACs), a new generation of drugs. The Clinical Pathology Laboratory of Siena's University Hospital introduced a specific assay for DOACs since 2016. In order to orient clinicians towards an appropriate use of requests, an operative protocol has been drawn up, in collaboration with the Thrombosis Centre of the hospital, providing indication about timing and request procedures.
Objective: To assess the protocol effectiveness one year after, analyzing the number and type of unconformities in DOACs requests.
Methods: Thrombin Time Diluted assay for dabigatran and chromogenic anti-Xa activity assay for rivaroxaban and apixaban on BCS XP (Siemens) instrumentation.
Results: To this day we received 15 requests for rivaroxaban, 13 for apixaban and 18 for dabigatran. The overall unconformities were 12: 2 erroneous requests (the patient was not taking the drug); 1 sample not received; 3 samples collected in wrong container; 4 haemolysed or inappropriate sample-anticoagulant volume ratio; 1 double request for the same patient; 1 failure to comply with the operative protocol. In the latter case, despite of the rivaroxaban interruption for three days, its plasmatic levels were still inexplicably high (30 ng/ml) because of the undeclared administration of heparin. It is well known that heparin causes positive interference in rivaroxaban assay, a detail missed by the clinician, although specified in the protocol.
Conclusions: The few number of DOACs requests collected in one year demonstrates both that the patient in DOACs therapy management is considerably simplified and the improvement in request's appropriateness. The few pre-analytical unconformities are operator dependent, related to difficult blood sampling or erroneous data entry. Our work focuses on the failure to comply with the operative protocol, even though happened only once, with the aim to spread the protocol to the clinicians concerned through personalized audit.

SS02-05

MOLECULAR ADAPTATION INDUCED BY FOOTBALL TRAINING IN SKELETAL MUSCLE: INFLUENCE ON HUMAN HEALTH AND LONGEVITY

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Aim: Football training improves cardiorespiratory (VO_2max) and body composition in players (1). Recently, we demonstrated that lifelong football training improves the expression of molecular markers related to oxidative metabolism and senescence suppression in muscle of veteran players (2). Here we performed a comparative analysis of messenger expression profiles in muscle biopsies from veteran soccer players (VSP) versus age-matched healthy untrained (CG; 65-75y) males.
Methods: Total RNA was obtained from 6 VSP and 6 CG muscle biopsies, provided by Copenhagen group; RNA integrity number (RIN) was determined on all samples. Single strand biotinylated cDNA was generated and used to hybridize the Human Genechip HTA 2.0 Array (Affymetrix). Results were filtered for fold change >1.5; statistical analysis was performed by ANOVA test. Validation of differently expressed messengers was performed by RTqPCR on total RNA from 12 VSP and 12 CG muscle biopsies.
Results: 430 messengers (including small non coding RNAs, miRNAs) differentially expressed between groups were identified (p-value <0.05). Among them, we confirmed an increased expression of RAD23A, HSPB6, HSPB1 (HSP25), TRAP1, Sirt2, RAB1B messengers, belonging to auto-lysosomal and proteasome degradation pathways and involved in protein integrity process and of RPL1, RPL4, RPL36, MRLP37, ribosomal messengers, in muscle from VPG compared to CG subjects.
Conclusions: Our preliminary results indicates that Lifelong football training positively affects the regulation of messengers involved in the integrity and function of nervous and muscular tissues and in cellular growth and differentiation pathways, respectively, in VPG. Our results also suggests that football training would be used as a tool to prevent and delay age-related decline.

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SS02-06

EFFICACY OF AN INTENSIVE PHYSICAL RETRAINING PROGRAM IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

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Chronic obstructive pulmonary disease (COPD) is the most common chronic lung disease, and a major cause of lung-related death and disability. This pathology is characterized by chronic airflow limitation associated with an abnormal inflammatory reaction (1). It is also characterized by very disabling and features extra-pulmonary manifestations. An important tool to manage this pathology is the exercise retraining, that is the cornerstone of pulmonary rehabilitation programs because offers many benefits, all well documented in patients with COPD. Physical training improving exercise capacity, muscle strength, body composition, cognitive capacity and quality life, but still there is no consensus about the optimal training strategy, same problem persists to define the ideal working intensity. Some studies report the efficacy of combined training: aerobic exercise associated with strength training, performed at high intensity, for the additional benefits that occurs (2). Aerobic workout provides benefits for exercise tolerance and cognitive capacity while strength training improving peripheral muscle function, body composition and same cognitive domains (3). The high intensity exercise in COPD, offers greater benefits in less time, compared with low intensity (4).

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SS02-07

EFFECTS OF CONCURRENT AEROBIC AND STRENGHT TRAINING ON BREAST CANCER SURVIVORS

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Breast cancer is one of the most commonly diagnosed types of cancer in women. Its pathogenesis involves genetic, hormonal, and environmental factors. Large evidences indicate that physical activity has positive effects on every aspect of breast cancer evolution, including prevention, medical treatment, and aftercare clinical settings. Thus, different types of exercise can influence the prevention and progression of the disease. Literature from the past focused major attention on the relationship between malignant disease and aerobic training; fewer studies were conducted on the effects of resistance training on the physical work capacity of cancer patients or survivors. However, resistance exercise should be an integral component of any exercise training program particularly because of its positive effects on muscle atrophy induced by the treatments and the sedentary habits in breast cancer survivors. The aim of our study was to evaluate the effects of a combined aerobic and strength program on physiological and psychological parameters in female breast cancer survivors.
20 patients (age: 45.6±2.7 yrs) surgically treated for breast cancer that had completed all cancer therapies at least 6 months before and with no contraindications to physical activity, were recruited and randomly assigned to an intervention group (n ¼ 10) and a control group (n ¼ 10). Intervention group patients attended a 24-week combined aerobic and strength training program. Physiological (i.e. VO2max, bioelectrical impedance test, maximal strength of principal muscular groups) and psychological (i.e. functional assessment of chronic illness therapy e fatigue: FACIT-F) parameters were assessed at baseline and after 24 weeks. After 24 weeks the intervention group showed significant improvement in VO2max (38.8%), strength of upper and lower limbs (ranging from 13 to 60%) and decrease in fat mass percentage (6.3%). The FACIT-F showed significant increase in all of the three scores that can be derived (FACIT-F Trial outcome: 13%; FACT-G total score: 18%; FACIT-F total score: 15%) showing patient's quality of life (QOL) improvement. No significant changes in all the parameters were found for the control group. These results show the positive effects of a combined aerobic and strength training program on breast cancer survivors and underline the importance of the early inclusion of structured physical activity in the rehabilitation protocol.
Future investigation will explore the possible relationship between this and other different protocols of physical activity and disease, on the methylation level and the expression of specific genes involved in the pathophysiology of breast cancer. We strongly believe that the results obtained through this research project will provide real guidelines for a well-structured exercise protocol designed to improve both the survival and quality of life of people affected by breast cancer. Indeed we believe that physical activity could be an important tool to be used alone or in combination with traditional therapies (i.e. medicine, drugs), to improve the efficacy of strategies for prevention and treatment of different chronic diseases.

SS02-CO02

A SMALL SIDED GAME SESSION AFFECTS SALIVARY METABOLITE LEVELS IN YOUNG SOCCER PLAYERS

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High-strength endurance sports such as soccer are known to generate many metabolic changes in athletes. The majority of studies concerning the impact of physical exercise, investigated a limited number of analytes with the aim of discovering biomarkers able to correlate with the actual performance in competition and predict the progress in the improvement. The use of saliva for monitoring metabolic variations in physical exercise and in different sports gained ground in recent years. Several studies showed that saliva reflects biochemical changes useful for analytical purposes in clinical investigations and in physiological research.The aim of this study was to explore the profile of salivary metabolite changes due to a session of small sided games (SSG) in elite soccer players, searching for a correlation between metabolic changes and athlete performance as GPS measured distances covered in the match. Ten under-20 elite soccer players participated to the study. The SSG were conducted with two goalkeepers and a maximum of two ball touches in a 30×40 m pitch. SSG had an overall duration of 24 min and it consisted of 4 bouts of 6 minutes duration with 2 minutes passive recovery between exercise bouts. Saliva samples were collected before and after the game and physiological parameters evaluated, namely the distances covered by players and blood lactate. Samples were analyzed by Nuclear Magnetic Resonance spectroscopy. Multivariate data analysis showed that SSG session affected salivary metabolite levels in players. We observed no relationship between concentrations of hematic and salivary lactate, nor found any changes in the metabolic profiles that correlate with the blood lactate values. Among the identified metabolites, taurine was instead found to correlate with distances covered by players during the game. Taurine level in saliva is a potentially significant marker which needs further investigation to be correlated with physical effort. Altogether, these results point to a potential use of saliva to follow metabolic changes during an athletic competition, and opens the possibility of using this non-invasive bio-fluid for the study of athlete training state and performance.

SS03-10

IPERTENSIONE SECONDARIA: PREVALENZA E SCREENING GENETICO E BIOCHIMICO

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Per la sua alta prevalenza (40% circa) della popolazione adulta e per l'alto numero di complicanze, (infarti e ictus, che sono la prima e terza causa di morte e invalidità prematura) ad essa imputabili, l'ipertensione arteriosa (IA) è il principale fattore di rischio cardiovascolare. Le forme secondarie di IA, cioè quelle imputabili a una causa eliminabile, un tempo considerate rare, oggi costituiscono circa il 25% delle ipertensioni arteriose nei pazienti che afferiscono ai Centri specialistici. Tra queste forme, la più frequente è l'iperaldosteronismo primario (PA) che in Italia colpisce circa l'11%-14% dei pazienti riferiti a tali centri (1). Tuttavia, dati recenti indicano che persino tra gli ipertesi visti dai medici di famiglia, circa il 6% ha un PA (2). Nonostante ciò, uno studio condotto recentemente tra i medici di famiglia italiani e quelli tedeschi ha mostrato che solo il 2% e 3%, rispettivamente, degli ipertesi in Italia e in Germania, vengono sottoposti a un qualsivoglia test di screening per questa malattia, che è potenzialmente guaribile (3). Ciò pare dipendere dal fatto che la stragrande maggioranza di tali medici o ignora l'esistenza di PA o non ne ha mai diagnosticato un caso e quindi lo ritiene essenzialmente raro. A ciò s'aggiunga il fatto che molti medici ritengono la diagnosi di PA particolarmente complessa e quindi evitano di intraprendere un iter diagnostico, che pone lor difficoltà gestionali ed esegetiche insuperabili. In antitesi con queste vedute un ampio studio italiano appena pubblicato ha invece dimostrato che è possibile semplificare notevolmente lo screening PA, evitando nella maggioranza dei casi il ricorso ai cosiddetti test di conferma. Infatti un valore assi elevato del rapporto aldosterone renina (ARR) può essere già ritenuto diagnostico di PA, senza bisogno di ulteriori conferme (4). Infine, negli ultimi anni vi sono stati formidabili progressi nella diagnosi e nel subtyping genetico-molecolare non solo del PA, ma anche di molte altre forme di IA secondaria, quali feocromocitoma/paraganglioma, che ha portato a una nuova classificazione delle forme familiari (5). L'esistenza di un gap profondo tra i dati ottenuti dalla ricerca e nella diagnosi a livello di grandi centri accademici, e lo stato di applicazione delle nuove conoscenze generate nella pratica clinica corrente, è, tuttavia, evidente. La diagnosi precoce delle forme secondarie di IA si traduce non solo in enormi vantaggi per il paziente, che può guarire del tutto dall'IA evitando una terapia farmacologica *ad vitam*

e le complicanze, ma anche per il SSN, che risparmia in termini di spesa farmaceutica e di gestione delle complicanze. Pertanto, è evidente che adeguati investimenti ai fini di colmare questo gap attraverso l'educazione continua del medico sono ormai indilazionabili.

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SS03-11

IL RUOLO DEL LABORATORIO NELLA DIAGNOSI DELL'IPERTENSIONE SECONDARIA

A. Fortunato

Ascoli Piceno

Per Ipertensione Arteriosa Secondaria si intende uno stato ipertensivo di cui è nota la causa patogenetica che, nella maggior parte dei casi, risiede in una alterata funzione del sistema endocrino: aumentata secrezione delle catecolamine adrenalina e noradrenalina (feocromocitoma), di cortisolo (sindrome di Cushing) e di aldosterone (iperaldosteronismo primario: IA). L'IA è identificato come una situazione in cui la produzione di aldosterone risulta elevata in maniera incongrua e relativamente autonoma dal sistema renina-angiotensina-aldosterone (Renin-Angiotensin-Aldosterone System) e non sopprimibile con un carico di sodio. Mentre tecniche di laboratorio, con prestazioni adeguate, per la determinazione di cortisolo e catecolamine sono disponibili da tempo, la più recente introduzione dei metodi per la determinazione diretta della renina (come proteina e non più come misura indiretta della sua attività enzimatica) e il contestuale miglioramento delle caratteristiche analitiche dei metodi per la misura dell'aldosterone hanno amplificato il ruolo del laboratorio nel contribuire alla diagnosi dell'IA, in particolare valutando il rapporto aldosterone-renina (ARR). Nella determinazione dell'aldosterone si sommano i problemi che si riscontrano nella misura degli ormoni steroidei con quelli per la rilevazione di concentrazioni molto limitate di analita, in particolare rispetto a quelle delle potenziali molecole interferenti; di conseguenza le caratteristiche di sensibilità e specificità dei reagenti assumono una grande rilevanza. Per quanto riguarda la misura della renina diretta, oltre al vantaggio della quantificazione della proteina, si deve tenere presente la possibile armonizzazione dei risultati ottenuti dai vari laboratori con il riferimento allo Standard Internazionale WHO IRP NIBSC 68/356. L'IA era considerato una condizione rara, tranne nei pazienti con ipopotassiemia, mentre attualmente, grazie alla migliore capacità diagnostica raggiunta, è ritenuto la più comune delle forme di ipertensione (dal 5 al 10 % dei pazienti ipertesi) di cui si identifica la causa e per la quale può essere adottata una terapia adeguata. L'ARR, derivando dalla combinazione matematica di due parametri, è evidentemente sensibile in maniera amplificata e spesso imprevedibile alle problematiche analitiche (e, nel caso specifico della renina, pre-analitiche) che possono influenzare i due ormoni coinvolti. Ne deriva la necessità, soprattutto da parte del laboratorio, di una attenta standardizzazione delle procedure di campionatura, di un efficace controllo delle procedure analitiche e di una corretta definizione, riferita strettamente ai metodi impiegati e non esclusivamente mutuata dalla letteratura o dalle indicazioni dei produttori, degli intervalli di riferimento da utilizzare.

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SS03-CO03

APPLICATION OF THERAPEUTIC DRUG MONITORING TO ANTIHYPERTENSIVE THERAPY FOR SCREENING OF TREATMENT ADHERENCE IN PATIENTS WITH RESISTANT HYPERTENSION: DEVELOPMENT AND VALIDATION OF A UHPLC-MS/MS METHOD FOR URINE SAMPLES

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The real prevalence of the apparent treatment-resistant hypertension (TRH) is difficult to be measured since very

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

SP05-12

ACCREDITATION OF CLINICAL LABORATORIES IN EUROPE - THE EFLM PERSPECTIVE

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Professional associations in laboratory medicine (e.g. European Communities Confederation of Clinical Chemistry, EC4) in Europe have had an important role already in the early days of the introduction of quality management systems and accreditation in Europe (1). Furthermore, quality management, accreditation of medical laboratories in Europe are among the core values of EFLM (2). Despite certain variations in the approach to accreditation of medical laboratories in Europe, ISO 15189 standard has been widely accepted and is being increasingly implemented throughout Europe. Still, there is a great heterogeneity in the number of laboratories being accredited in different countries in Europe. In some countries, almost all laboratories are accredited (e.g. Finland, Ireland), whereas in others there is only a minority of laboratories which are accredited. Accreditation is still not mandatory in most of Europe. According to EFLM data, in 2016, there were only 3 countries in which accreditation is mandatory for at least some disciplines of laboratory medicine, but not all (e.g. in Belgium, Ireland and Lithuania), whereas only in France and Hungary accreditation is mandatory for all fields of laboratory medicine (in Lithuania it will be mandatory in 2020). In countries where National Accreditation body does not offer accreditation for medical laboratories, there is commonly some other entity providing a formal recognition of competence and quality (e.g. in Slovenia, Ministry of Health provides a licence to medical laboratories). Within EFLM, since 2007, there is a Quality and Regulations Committee whose general goal is to promote and improve the quality and safety of patient care through establishing the highest standards of laboratory medicine. Their specific objectives are to develop initiatives to improve the quality of clinical chemistry and laboratory medicine in Europe, to harmonise standards of practice in quality management through production of guidance documents defining best practice in areas of clinical chemistry and laboratory medicine and to supporting the establishment of effective accreditation schemes in all European countries. One of their aims is also to represent EFLM, liaise and cooperate on accreditation matters with International Organization for Standardization (ISO) and European Accreditation and national accreditation bodies, European co-operation for Accreditation (EA) and European Committee for Standardization (CEN). This Committee and its working groups have so far delivered several documents, recommendations, original articles with results from European surveys (3, 4). The aim of this lecture is to provide an overview of the current achievements and future plans of EFLM in the accreditation of clinical laboratories in Europe.

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SP05-13

ACCREDITATION OF MEDICAL LABORATORIES. DUTIES AND OPPORTUNITIES

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The accreditation of medical laboratories according to the International Standard ISO 15189 aims at demonstrating to its patients and users the reliability of their performances through the implementation of a quality management system but, above all, qualified and technically competent personnel in performing specific examinations. The compliance with the ISO 15189 requirements, therefore, allows going beyond quality as expressed in ISO 9001, which only certifies the compliance with management system requirements. The accreditation ISO 15189, recognizing the technical and organizational competence of the laboratory staff in carrying out specific and well defined performances included in the scope of accreditation, is the tool that increases the confidence of stakeholders about the ability of the laboratory to satisfy its users with a high level of reliability. It assures laboratory compliance in minimizing errors by planning, preventing, implementing, evaluating, and improving its processes. A key element in the accreditation process is the way of assessment, during accreditation visit, that is based on the concepts of independence and peer-review and is representative of all stakeholders. The standard takes into account all the needs of medical laboratories, namely all steps of the entire testing process, starting from the appropriate test request to the right notification of laboratory reports and the role of further clinical advice provided by laboratory professionals. It focuses the attention on both the items of the intra-analytical phase (e.g. verification and validation of examination procedures, measurement uncertainty, metrological aspects, etc.), and to the pre- and post-analytical phases (peculiar features of the medical laboratories in comparison to testing laboratories). The scientific community promotes accreditation with flexible scope for the complete service, based on description of coherent groups of tests (same medical field, same test typology, same analytical principle, same sample type and same medical area). However, the spread of accreditation ISO 15189 has highlighted the need of harmonization of the list of groups in which tests have to be included. The use of the same list assures the clear understanding of the tests under accreditation for the scientific community from different countries and for patients, and allows distinguishing between laboratories with different quality level of service. Similarly, concerning the check-lists used by assessors during the audits, they should be standardized in order to guarantee a congruent evaluation among different countries. Moreover, in order to comply with ISO 15189 requirements the use of approved guidelines and recommendations often do not take into account the costs and the workload needed for their implementation and, therefore, they may represent a type of procedures affected by a high level of complexity and not easy to be performed. A pragmatic approach is, therefore, needed in order to assure the reliability of results and promote the introduction of accreditation process in the Medical Laboratories, balancing technological possibilities, risk and personnel and time constraints.

SP05-15

L'ACCREDITAMENTO DELLE STRUTTURE PRIVATE

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Affrontare il tema dell'accreditamento delle strutture private esige di fugare ogni potenziale equivoco: non esiste l'accreditamento per il pubblico e quello per il privato. La norma è unica. Scopo di questa presentazione è di mostrare la dimensione del "problema" e soffermarsi brevemente su alcuni passaggi dell'ISO 15189 che possano renderne incerto il recepimento in quell'ambito. Fonte il Ministero della Salute, è noto che in Italia, nel 2015, sono stati eseguiti 562.351.260 esami di Laboratorio, erogati dal SSN dal pubblico e dal privato (373.052.750 dal primo e 189.298.510 dal secondo). Il privato, dunque, concorre per 1/3 alla soddisfazione della domanda di Medicina di Laboratorio. Valgano qui alcune considerazioni dell'Autore solo su due specifici punti della norma. Al punto 4.1.1.3 tra l'altro si legge: "Management and personnel are free from undue commercial, financial, or other pressures and influence that may adversely affect the quality of their work". Valga un commento. Il limite tra ciò che è accettabile e ciò che non lo è, da parte della componente finanziaria e commerciale di qualunque organizzazione produttiva, non è sempre nettamente demarcata. Al contrario, la qualità della produzione analitica e il valore degli accorgimenti pre e post analitici sono ben delineabili. Indubbiamente l'aderenza alla medicina basata sulle evidenze (in primo luogo ogni utile linea guida) è la migliore garanzia d'indipendenza della professione. La cultura specifica, e il coraggio di difendere i propri spazi, sono quindi indispensabili per far valere le proprie posizioni professionali. Al punto 4.1.1.4 della norma, riguardante le prerogative del direttore del Laboratorio, tra l'altro è previsto che egli "provide effective leadership of the medical laboratory service, including budget planning and financial management, in accordance with institutional assignment of such responsibilities". Non v'è dubbio che la pianificazione del budget e la gestione finanziaria debbano correre

parallelamente, essendo nelle mani di un unico autorevole regista. Nelle grandi organizzazione private non funziona proprio così. La pianificazione del budget e la gestione finanziaria ricadono su figure diverse, se non vanificando il dettato della norma, certo rendendolo di più difficile attuazione. Si sente spesso classificare prevalentemente i grandi Laboratori, forse non solo privati, come "esamifici". Non è la dimensione che dovrebbe generare un simile negativo giudizio. Lo meritano, in realtà, tutti quei laboratori (qualunque sia la dimensione) nei quali non si affianchino, a una sufficiente e diffusa accuratezza analitica, un severo controllo della fase pre-analitica e un giudizioso governo di quella post-analitica. Per attuare tutto questo, gli strumenti culturali ci sono tutti, unitamente a quelli per evitare l'autoreferenzialità. L'unica certezza di unificare verso l'alto l'attività di ogni Laboratorio risiede nell'accreditamento a mente dell'ISO 15189. Questo processo sarà realmente diffuso solo se lo Stato lo renderà obbligatorio, il vantaggio competitivo difficilmente sarà sufficiente.

SP05-CO04

RISK MANAGEMENT: A MODEL TO BE IMPLEMENTED IN MEDICAL LABORATORIES FOR ISO 15189 ACCREDITATION

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Introduction: Assuring patient safety before an injury occurs is the main goal of medical laboratory. The Risk Management, understood as a systematic process of identifying and treating the present/potential risks for patient safety, becomes then an integral part of Quality Management Systems so that it is also required by ISO 15189.
Aim of the work is to propose the workflow planned by the Laboratory Medicine of Padua to implement a risk management process and meet the ISO 15189:2012 requirement (4.14.6).
Method: Available standards/guidelines and literature have been analysed to select technique and scales to apply. Then, was organized a training course consisting of: an in-class stage on risk management principles and an on-the job training to conduct risk identification, analysis, estimation and control. The risk management process was applied to the most errorsprone phase of the process, according to literature data. The preanalytical process, was retrospectively studied through Failure Reporting and Corrective Action System (FRACAS) by a multidisciplinary team using the self-risk assessment approach. Quality indicators (QI) data from IFCC-MQI model were used to identify the critical stages and ISO 14971:2009 scales to estimate the risk.
Results: The results demonstrated that the use of FRACAS improved the processes studied. Concerning the sample acceptance steps, for example, the overall risk index (IR) was 748. Failure in emptying transportation containers, collecting/storage samples, detecting suitability and priority of samples, showed the highest risk priority number (18<RPN>32). After the implementation of two corrective actions (distribution of detailed operating instructions and introduction of a new label to distinguish stat from routine samples) the IR decreased to 685, demonstrating the effectiveness of the implemented actions and the suitability of the proposed model.
Conclusion: To minimize risks, the risk management process has to begin with the staff awareness of safety issues. The self-risk assessment approach allowed, not only to identify the failure modes but also to make the staff more aware and responsible in risk managing. Moreover, the availability of QI data, has allowed to promptly focusing on the more critical stages of the processes.

SS06-16

GESTIONE CLINICA DEL MODY E DELLE ALTRE FORME DI DIABETE MONOGENICO

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Roma

Monogenic diabetes is more prevalent than previuosly thought, accounting for over 6% of cases in the pediatric diabetes clinic (Delvecchio M et al., JCEM, 2017) (1). In particular, M(aturity) O(nset) D(iabetes) of the Y(oung) (MODY) alone represents 5.5% of a group of 3781 patients referred to Italian pediatric diabetes clinics during years 2007-2012 and confirmed by molecular genetic analysis (1). Monogenic diabetes awareness combined with availability of genetic testing/diagnosis, translated into profound changes of clinical management, at least in the pediatric setting. Heterozygous, loss-of-function mutations of glucokinase (GCK) are the commonest form of MODY in Italy (1) and are easily identified in children (or even neonates; Prisco F et al., Diabetologia, 2000) (2) with mild, not progressive fasting hyperglycemia in the range of impaired fasting glucose or diabetes (between 100 and 140 mg/dl). Individuls carrying these mutations do not require any form of pharmacological therapy, or diet, and are not prone to any diabetic complication. Instead, HNF1A/MODY (previously known as MODY3) often presents with a severe clinical phenotype, resembling type 1 diabetes. Interestingly, HNF1A/MODY patients can respond to oral hypoglycemic agents of the class of sulfonylureas (SU) with optimal metabolic control ((1) and may be switched from insulin to SU upon genetic diagnosis (1). The same applies to patients with

neonatal diabetes (diabetes onset before 6 months of age) due to mutations of genes (KCNJ11, ABCC8) encoding for the subunits of ATP-sensitive potassium channel (KATP; subunits:KIR6.2 and SUR1). Sulfonylureas are adiministered in liquid form along with mother's milk or formula since genetic diagnosis (even at 1 week of age). The metabolic control is incredibly good and HbA1c close or equal to normal values. A 10-y follow-up of a large international cohort of patients with neonatal diabetes due to KCNJ11 mutations shows that this therapy is well tolerated and efficacious on the long term (submitted to NEJM). SU are also useful in patients with mutations of KCNJ11 or ABCC8 with diabetes onset in childhood/adolescence/adulthood (KCNJ11/MODY, or MODY 13 and ABCC8/MODY or MODY 12) and long-standing insulin therapy (Iafusco D et al., Acta Diabetol, 2012, and Barbetti F unpublished observations). In summary, the discovery of more than 25 genes responsible of various forms of monogenic diabetes either in isolation or syndromic has revolutionized the therapeutic approach(es) and genetic counseling of this subtypes of diabetes.

SS06-18

GLYCATED ALBUMIN: RESULTS FROM THE FIRST ITALIAN CLINICAL STUDY

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Glycosylated hemoglobin (HbA1c) has been established as the gold standard index for long-term glycemic control and, in year 2009, an International Expert Committee reported values of HbA1c ≥48 mmol/mol as the cut-off point for diagnosing diabetes (1). The United Kingdom Department of Health recommends the use of algorithms for diabetes screening in high-risk individuals that include traditional glucose diagnostic criteria or, alternatively, HbA1c measurements combined or not with glucose measurements. In non-symptomatic patients, a confirmed HbA1c ≥48 mmol/mol is enough to diagnose Type 2 diabetes (2). However, this guideline also recommends that patients with HbA1c levels ≥42 mmol/mol and <48 mmol/mol should undergo an oral glucose tolerance test to establish a diabetes diagnosis. Serum albumin glycation (GA) has been suggested as an additional or alternative clinical parameter to circumvent some of the limitation of HbA1c. Levels of GA are unaffected by conditions that alter erythrocytes lifespan or by genetic variation in hemoglobolin. In contrast to haemoglobin glycation, which resides inside erythrocytes, albumin glycation occurs in the extracellular volume, particular in plasma, but also to a high degree in other fluids such as CSF, interstitial fluid, and amniotic fluid during pregnancy. The much shorter half-lives of albumin compared to haemoglobin makes it more responsive to changes in glycemic status. Based on the half-lives of albumin, GA levels reflect the mean blood glucose levels in the previous 2 week. This makes GA a much more dynamic marker for glycemic control that can be used to assess the efficacy of diabetes therapy. Moreover, GA shows a stronger correlation with continuous glucose measurement over 1 to 2 days than HbA1c, so it may more accurately reflect glycemic variability and glucose excursions. The majority of data regarding clinical use of GA has been obtained in Asiatic populations, especially in Japan where GA has been proposed for diabetes screening in blood donors. Results arising from the ARIC study demonstrated the association between GA and incident diabetes over two decades of follow-up (3), highlighting its potential role in predicting diabetes. At present, it is not clear which relationships exists between GA, the other markers of glucose homeostasis and insulin resistance in subjects at risk of developing diabetes or subjects with diabetes. Moreover, no specific cut off has been established for diabetes diagnosis, nor reference intervals, in European studies. This multi-centric study for the evaluation of the clinical performances of GA is the first large clinical study conducted in Italy aims to 1) define the upper reference limit of GA in blood donors (4); 2) define the contribute of GA to traditional markers (namely HbA1c, fasting plasma glucose and 2h-plasma glucose after OGTT) for diabetes diagnosis; 3) define the role of GA in short term monitoring of diabetics patients. Secondary aim of the study is to explore the association between GA and the metabolic impairment of T2DM evaluated by overweight or obesity and insulin resistance.

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SS06-19

GLYCATION GAP: A NEW LABORATORY PARAMETER USEFUL FOR THE EVALUATION OF GLYCOMETABOLIC CONTROL

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The determination of glycated albumin (GA) has been suggested as an additional parameter having an independent added value respect to that of HbA_{1c}. The determination of glycation gap (*gg*), calculated from the deviation between the measured HbA_{1c} respect to that calculated from other indexes of glycemic control, such as fructosamine (FA) or GA, has been investigated in some recent studies, and proven to be reliable in diabetic patients with stable glycemic control, significantly associated with retinopathy, nephropathy and mortality in diabetes, although not associated with chronic kidney disease in individuals without diabetes. However, few studies have addressed its potential impact in the routine evaluation of glycometabolic control. In our experience, we have studied a total of 157 subjects presenting normal whole blood cell count, no hemoglobin variants, normal creatinine levels and serum protein electrophoresis patterns in order to estimate a reference range for the *gg*. In a second phase, a total of 205 subjects with no restrictions as those of the first phase study, were analyzed. HbA_{1c} was measured by capillary electrophoresis, glycated albumin by an enzymatic method and their *gg* were then calculated. The correlation between HbA_{1c} and GA for the subjects of phase 1 was strong (r=0.8927) and significant correlation between *gg* and age was remarked (r=0.4486). No significant differences between genders were evidenciated. We found 17.1% of phase 2 subjects with *gg* falling outside the 95% prediction intervals. Various clinical conditions seemed to affect these subjects, in our experience mostly often related to impaired renal function, with typical negative *gg* values. In conclusion, we believe that the glycation gap may be useful to alert clinicians about patients under unstable glycemic control or when various pre-analytical conditions my affect the reliability of the measurement of GA or HbA_{1c}.

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SS06-CO05

MOLECULAR DIAGNOSIS OF NON-AUTOIMMUNE DIABETES MELLITUS (DM) IN PEDIATRIC AGE: AN ATYPICAL CASE OF THE NOVO DELETION ON CHROMOSOME 12q24.31 IN A PATIENT WITH MATURITY ONSET DIABETES OF THE YOUNG (MODY)

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MODY is an autosomal dominant disease that includes clinically heterogeneous forms of not autoimmune diabetes. MODY2 and MODY3, due to GCK and HNF1α mutations respectively, are the most frequent forms.We describe a 15-years-old patient with not-autoimmune DM diagnosed at 11 years with ketoacidosis. The molecular analysis for MODY revealed a whole deletion of HNF1α gene in agreement with a diagnosis of MODY3. The clinical history of the patient also highlighted neurological and cardiac disorders, such as interatrial defect treated surgically at 2 years, a slight mental retardation, epilepsy and Hashimoto thyroiditis. Therefore to clarify this complex phenotype and to define the extension of the deletion, we performed array Comparative Genomic Hybridization analysis that revealed a deletion of 1.04 Mb on chromosome 12q24.31, that encompasses 23 genes including HNF1α. This deletion was not found in parents and paternity and maternity were confirmed. Few 12q24.31 deletions have been reported in the literature, all with a greater extension and different phenotypes compared to that observed in our patient. In fact, in our case, the phenotype is overall milder probably because the number of genes involved in the deletion is lower. Differently, comparing diabetic symptoms of our patient respect to other MODY patients with HNF1α mutation, different from a deletion, these were more severe and the diagnosis of diabetes was earlier. Despite this, the patient could replace insulin therapy with oral drugs, as in other cases of MODY3. In our proband, the deletion of ACADS gene could be the cause of epilepsy and of mild mental retardation, as well as of cardiac anomalies, while it could be hypothesized that autoimmune thyroiditis that is difficult to associate with a not autoimmune form of diabetes may be due to the deletion of the ORAI1 gene that could have caused a functional abnormality of T lymphocytes resulting in the autoimmune thyroid process. In conclusion our case shows that syndromic symptoms in notautoimmune diabetes should lead to look for deletions involving neighboring genes to better understand the aetiology and pathogenesis of the different defects, to prevent the misclassification of any clinic feature and also to offer appropriate genetic counselling.

SS07-20
DIAGNOSIS OF NEUROMUSCULAR DISORDERS

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Neuromuscular Disorders comprise all those diseases that affect spinal motor neurons, peripheral nerves, neuromuscular junctions and muscle fibers. These entities may be genetically determined or acquired, may develop at any age and may present with acute, subacute or chronic course. The diagnosis of neuromuscular disorders usually relies on neurological and muscular examination, on electrophysiological studies, and on biochemical, histopathological and molecular genetic workup. Clinical chemistry and laboratory medicine techniques may be essential or provide additional information when diagnosing a neuromuscular disorder. Among biochemical markers, creatine-kinase (CK), and its muscular isoform (CK-MM) are by far the most sensitive serum enzymatic correlates of primary muscle fiber damage, indicating cell necrosis or plasma membrane leakage. Very high levels of CK are usually found in Duchenne muscular dystrophy, rhabdomyolysis and myositis. The detection of increased serum lactate levels at baseline or a failure to rise after a forearm ischemic exercise test can reveal defects of mitochondrial functioning or anaerobic glycolysis within muscle fibers, respectively. Moreover, fine tuning in diagnosis of metabolic myopathies may be performed by measuring the activity of single enzymes with different techniques in serum or muscle tissue obtained by biopsy. The search for circulating autoantibodies is now a consolidated aspect of the diagnostic process of autoimmune disorders affecting peripheral nerves and muscles. Indeed, the detection of anti-acetylcholine receptor or anti-muscle specific kinase, two components of the neuromuscular junction, is essential for the diagnosis and classification of autoimmune myasthenia gravis. Moreover, immunoblotting or ELISA techniques may reveal the presence of autoantibodies reacting against glycolipids belonging to the ganglioside family or against sulphatides or the myelin-associated glycoprotein, therefore contributing to classification of autoimmune peripheral neuropathies. Similarly, several autoantibodies defined as myositis-specific or myositis-associated, help differentiating among subtypes of idiopathic inflammatory myopathies, thus facilitating the search for co-morbidities, a better prognostic definition and a correct therapeutic approach. Finally, autoantibodies against the so-called onco-neural antigens may be detected in patients presenting a neurological paraneoplastic syndrome associated with specific malignancies. These antibodies are markers of a molecular mimicry between cell surface antigens shared by neurons subpopulations and some kind of tumours. Although a pathogenic role of these antibodies is still debated, their presence may anticipate the finding of malignancy, therefore prompting an early diagnosis and, possibly, a better outcome.
Progress in the laboratory diagnosis of neuromuscular disorders proceeds at a fast pace: this requires a continuous refresh by the specialists in order to transfer up to date concepts into clinical practice.

SS07-23
LABORATORY DIAGNOSIS OF ACUTE NEUROLOGICAL DAMAGE: GUIDELINES AND NEW PERSPECTIVES

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Acute neurological damage is mostly due to traumatic brain injury, with millions of people suffering each year worldwide and is recognized as the leading cause of mortality and morbidity in young adults. Despite this, a PubMed search on guidelines for laboratory diagnosis of acute neurological damage identify in the last ten years only one article, published in 2012 by Eastern Association for the Surgery of Trauma (1). Authors suggest that routine use of laboratory biochemical markers for the clinical management of traumatic brain injury is not supported at the present time. Lots of guidelines have been published for the second cause of acute neurological damage: central nervous system infections. A very exhaustive one is the diagnostic pathway proposed by Italian Association of Clinical Microbiologists (AMCLI). Also the European Federation of Neurological Societies (EFNS) published guidelines on diagnosis and management of acute and chronic neurological diseases; they are particularly useful since Clinical Features, Investigations and Therapy are reported. In 2009 EFNS published guidelines on disease-specific CSF investigations, including also diseases with acute neurological damage (2). Italian Association of Neuro Immunology (AINI) published various papers on laboratory diagnosis of neuroimmunological pathologies, reporting preanalytical and analytical procedures, quality control and sample storage. An update will be published in 2017. Revised national (UK) guidelines on Cerebrospinal fluid (CSF) analysis for suspected subarachnoid haemorrhage were published in 2008. The document reaffirmed that for bilirubin detection, due to conversion in vivo of liberated oxyhaemoglobin in a time-dependent manner, spectrophotometry should be used instead of visual inspection. Nine CSF and serum biomarkers were studied in a systematic review published in 2013 on cerebro spinal fluid and serum biomarkers for the clinical differential diagnosis in traumatic brain injury. Authors concluded that at present there are insufficient literature data to support a role for diagnostic biomarkers in distinguishing focal and diffuse injury and

in evaluating raised intracranial pressure (3). In the last ten years new perspectives come from studies on neurofilament light (NF-L) protein, first in CSF then also in serum. NF-L are neuron-specific major structural proteins, abundantly expressed in the long myelinated white matter axons. In 2015 Kuhle and coworkers, for the first time, provided evidence that serum NF-L chain is a good biomarker and has prognostic value in Spinal Cord Injury patients (4). In 2016 Shahmin and coworkers demonstrated that measurement of serum NF-L may be useful to assess the severity and outcome of neuronal damage following traumatic brain injury (5). Moreover, NF-L has also very good diagnostic performance as a marker of brain atrophy in Multiple sclerosis and neuronal damage in AIDS patients. We believe that this molecule is a promising biomarker of acute neurological damage in the field of Laboratory diagnosis.

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SS07-CO06

CEREBROSPINAL FLUID TAU PROTEIN DISCRIMINATES JAKOB-CREUTZFELDT DISEASE FROM NON-PRION FORMS OF ACUTE AND SUBACUTE RAPIDLY PROGRESSIVE DEMENTIAS

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Rapidly progressive dementias (RPDs) are neurological conditions with different etiologies, which typically manifest subacute or acute symptoms over months, weeks or days. The Jakob-Creutzfeldt disease (CJD) is a common form of RPD and is invariably fatal, while some cases are due to other non-prion conditions, many of which are treatable. Moreover, Alzheimer's disease (AD) is rarely rapid, but unusual presentations can be misdiagnosed as CJD. So, an early diagnosis is essential to identify RPDs that are treatable and potentially reversible. The cerebrospinal fluid (CSF) proteins 14-3-3 and total tau reflect neuronal damage and in several studies have been suggested in the differential diagnosis of CJD. We assessed the potential of Tau as early biomarker to discriminate in a common clinical setting CJD from other non-prion forms of RPDs with acute onset. We evaluate the level of Tau in 26 cases with acute/subacute dementia and 13 non-demented patients, admitted to the Tor Vergata General Hospital during the 2015-2016 and followed prospectively. After one-year of follow up, 11 were diagnosed as CJD, 5 suffered of AD and 10 suffered of other conditions including acute psychotic, dysmetabolic or inflammatory disorders (OD). The presence of 14-3-3 protein in CSF was also investigated. We found that all CJD patients showed level of tau higher than 1300 pg/ml. Oppositely, tau in patients with non-prion conditions were in the pathological range but significantly lower than CJD (AD 517±176; OD 432±321 pg/ml; normal value <350 pg/ml). Level of tau in control patients was in the normal range. Of interest, in one case with clinical suspect of CJD, which was negative for 14-3-3 and had uncommon higher level of tau but lower than 1300 pg/ml (1018 pg/ml), the CSF analysis was repeated after two months to definitely exclude the diagnosis of CJD (tau 341 pg/ml). Our results demonstrate that tau could discriminate CJD from other non-prion forms of acute and subacute dementia. In cases of rapidly presentation of dementia, measurement of tau could be considered as a preliminary step to identify potentially treatable and reversible conditions. The analysis of CSF Tau could be a reliable tool to be included in the diagnostic procedures for the diagnosis of nonprion forms of RPDs.

SS08-27

THE ROLE OF SIBIOC IN OPTIMIZING TEST USE AND DIAGNOSTIC INFORMATION

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Laboratory medicine plays a crucial role in the clinical decision making throughout a broad spectrum of human diseases. Although approximately 60-70% of the most important clinical decisions about admission, discharge or therapeutic management entail laboratory tests (1), over- or under-utilization of laboratory diagnostics (i.e., inappropriateness) may impact the quality and the value of laboratory testing. The leading consequences of inappropriate use of laboratory resources include unjustified incremental costs, derangement of laboratory

efficiency and patient safety issues related to the possible generation of false positive or false negative results (2).
In a meta-analysis including studies published between 1997–2012, Zhi et al. reported a mean rate of laboratory tests overutilization of 20.6% (95% CI 16.2–24.9%) (3). In Italy the estimated inappropriateness of laboratory tests ordering was also found to be considerably high, in particular in the field of cancer biomarkers (4). Improving appropriateness of laboratory diagnostics is a challenging issue. In the last decade, the Italian Society of Clinical Biochemistry and Clinical Molecular Biology (SIBioC) has published several documents, guidelines and recommendations, and has also organized meetings and seminars for improving and promoting the appropriateness of laboratory tests ordering and interpretation, both for common diseases (i.e. diabetes mellitus, myocardial infarction, cancer) and for less frequent conditions. This important target has been achieved by establishing a large number of valuable collaborations with the other important national and international clinical societies. Among the various examples attesting the important activity of our Society, a close collaboration has recently been established between SIBioC and the Academy of Emergency Medicine and Care (AcEMC) for reaching a tentative consensus about the most informative diagnostic tests in emergency settings (5), along with the publication of ANMCO/ISS/AMD/ANCE/ARCA/FADOI/GICR-IACPR/SICI-GISE/SIBioC/SIC/SICOA/SID/SIF/SIMEU/SIMG/SIMI/SISA consensus guidelines about the laboratory diagnosis of dyslipidemia. Last but not least, SIBioC has edited the Italian translation of the Website LAB tests Online (6), developed by the American Association for Clinical Chemistry (AACC) and designed to help patients to better understanding the clinical lab tests that are part of routine care as well as diagnosis and treatment of a broad range of conditions.

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SS08-CO07

A MOBILE APP TO IMPROVE THE APPROPRIATENESS OF LABORATORY TEST REQUEST BY GENERAL PRACTITIONERS

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Background: The prevalence of consultations of general practitioners (GP) is huge, and for an unknown number of patients, a consistent number of diagnostic laboratory tests will be requested. A scope of GP is define patients that need or not a specific test, improving the patients outcomes given the available invested resources, and considering as well to reduce the risk of overdiagnosis and to avoid overtreatments. However, GP choices depend on knowledge and time to search for valid (updated, correct and synthetic) information about how a given test can contribute to the diagnosis, prognosis, therapy and prevention of a disease.
Methods: We developed a dedicated software application (APP) for mobile devices to support GPs in their daily practice with valid information, improving the patients referral and satisfaction. The APP contents was developed in collaboration with GP, students of a GP training course and medicine laboratory specialists. We identified the laboratory tests useful for management of the most frequent diseases observed in GPs' ambulatory, starting from information provided by SIBioC FAD ("Appropriatezza prescrittiva nell'ambulatorio del Medico di Medicina Generale") and supported by the best available evidence.
Results: In the preliminary phase of the APP development, we included knowledge about hypertension, thyroid and anaemia diseases, completed with data from systematic reviews, guidelines and health technology assessment reports. For each pathology we reported clinical information and laboratory test descriptions to lead GP in appropriate diagnostic test request.
Conclusions: This promising tool could help GP to prescribe suitable laboratory tests in different clinical scenario (diagnosis, therapy, monitoring) and to promote the implementation of best laboratory medicine daily practices, reducing inappropriate tests and accurately identifying patients who need specialist referral.

SS09-32
GENETIC PROFILE AND AGGRESSIVE BEHAVIOR

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Recent developments in molecular biology are shedding new light on the neurobiological underpinnings of human social behavior, including moral choices (1) and antisocial behavior (2, 3). Specific genetic variants may increase individual vulnerability to develop impulsive and aggressive behavior by modulating the impact of the environment on behavioral traits. These genetic variants play a role in regulating the cognitive and emotional processes underlying antisocial behavior, mostly in response to markedly negative stimuli and events. More specifically, some allelic variants, reported in literature as being associated with alterations of personality traits, may increase the risk of antisocial behavior in individuals who have been maltreated and abused as children (4). Within a larger collaborative research project aimed at investigating the neurobiological bases of antisocial behavior, here we report preliminary data obtained in a pilot sample by comparing the genetic profiles of 37 criminal males with those of 30 matched controls with no history of aggressive behavior. Six polymorphisms were genotyped: MAOA (Monoamine oxidase A) uVNTR, 5HTTLPR (serotonin-transporter-linked polymorphic region), STin2 VNTR in the SLC6A4 (solute carrier family 6 (neurotransmitter transporter), member 4) gene, COMT (Catechol-O-methyltransferase) Val158Met and DRD4 (dopamine D4 receptor) exon3 VNTR. The combination of three or more “risk” allelic variants had a significantly higher frequency in criminals than in control subjects. Particularly, the concomitant presence of five or six risk variants was observed only in criminals. Thus, we hypothesize that a “risk genetic profile”, instead of single alleles, may predispose to antisocial behavior. Interestingly, according to a recent hypothesis (5), these genetic variants would influence the individual susceptibility to both negative and positive environments, thus being “plasticity genes” rather than “vulnerability genes”. In this light, our findings provide a novel strategy to understand aspects of human behavior that have been a main focus of interest since the very early days of humanity.

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SP10-33
DEVELOPING MEDICAL TESTS FOR IMPROVING PATIENT OUTCOMES

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Like all other interventions in health care, medical tests should be thoroughly evaluated before they are introduced into daily practice. In this era of evidence-based medicine, there should be sound evidence to support the use of laboratory and other tests, to include such tests in practice guidelines, or to provide coverage through reimbursement decisions. For a very long time, such evidence came from evaluations of the analytical performance of medical tests. To be ready for clinical practice, tests should have acceptable precision, trueness, linearity, and limits of detection. Typically, goals for desirable precision and trueness were based on biological variation. Increasingly, however, there is shift towards patient outcomes in the appraisal of health care interventions. Practice guidelines are increasingly based on the effect of clinical actions on patient outcomes, not just on technology or clinicians’ preferences. In more and more countries reimbursement decisions are guided by considerations about effectiveness and cost-effectiveness. Laboratory medicine cannot, must not, and will not escape from this transition towards patient outcomes. In this presentation, we will highlight the evolution from results to consequences in laboratory medicine. We will illustrate how the effectiveness of medical testing – its effect on patient-important outcomes – can be evaluated. We will also discuss how this transition feeds in to evaluations of analytical and clinical performance. To remain effective, laboratory professionals should be aware of these patient-oriented comparative evaluations of outcome, and engage in them whenever possible and needed.

SP10-35
POTENTIAL USE OF BIOMARKERS IN CLINICAL PRACTICE: ALPHA DEFENSIN AND SYNOVIAL FLUID

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Alpha defensin in synovial regards potential biomarker (1,2) for identification of periprosthetic joints infections

(PJI). The only commercially available test is an in situ immunocromatographic device. Orthopedics use the device under their direct responsibility, either as part of pre surgery patient assessment or, intra operatively, to make treatment decisions according to the likelihood of infection. In this diagnostic path flow, the availability of a method not prone to interferences, with high sensitivity and specificity, can be of great help to clinicians. LC-MS methods are considered to meet needs in terms of analytical performance and are widely used to measure new biomarkers. The aim of our study was to implement a methods to accurately measure Alpha defensin in synovial fluids. Several issues had to be addressed: the synovial fluid matrix viscosity, the need of a standard Alpha defensin peptide and a valid Internal Standard (IS), the definition of analytical protocol and, finally, the method validation. As preliminary step the uniqueness of peptides derived from trypsin digestion of Alpha defensin was checked by liquid chromatography - time of flight mass spectrometry (LC-QTOF - Agilent Technologies). Data were analyzed by a proteomic software (Spectrum Mill - Agilent Technologies). Synovial fluids samples from primary knee arthroplasty were used as negative matrix; a synthetic matrix (simulant) was produced and measures of spiked samples were run in parallel. The quantitative analysis was performed with two different instruments (liquid chromatography with time of flight or triple quadrupole mass spectrometry, LC-QTOF or LC-MS/MS - Agilent Technologies) and two different methods (negative matrix spike and simulant synovial fluid spike) by spike of different concentrations of synthetic marker peptide (LWAFCC, DBA Italia). The quantitative method was developed with dynamic range from 0.1 to 100 μg/ml. LOD (μg/ml) and LOQ (μg/ml) were respectively 0,14 and 0,47 μg/ml. CQ samples were prepared using both simulant and negative synovial fluid spiked at 5 μg/ml and 50 μg/ml and were run in multiple replicate to determine the intra-day and inter- day accuracy. The mean percent inaccuracy and imprecision at CQs levels were always lower than 10%.

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SP10-CO08

VALIDATION OF AN IMMUNOTURBIDIMETRIC ASSAY FOR MEASURING C REACTIVE PROTEIN IN SYNOVIAL FLUID

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Background: Periprosthetic joint infection (PJI), a rare and severe complication which may occur after total joint arthroplasty, has high morbidity and mortality rates, along with considerable economic implications. Although C-reactive protein (CRP) is a common and inexpensive test for screening PJI, its serum concentration is poorly specific for diagnosing localized infection, since serum CRP can also be increased in several non-infectious inflammatory conditions. A recent study (1) showed that PCR in synovial fluid (SF) may have high sensitivity (0.92) and specificity (0.90) for diagnosing PJI. These promising data are however biased by the fact that the method has not been validated in SF. According to the ISO 15189 standard, method performance should be always validated before changing either the sample matrix or the test purpose. Therefore, we assessed the analytical performances of the Beckman Coulter CRP immunoturbidimetric assay OSR6147 using AU480 Olympus in SF.
Methods: SF samples were collected in serum tubes (Vacuette Grainer Bio-One GmbH Austria) and sent to the local laboratory within 6 hours from collection, at room temperature. The samples were separated and stored at -30°C until further analysis. Hyaluronidase was added the SF immediately before analysis (final concentration, 0.5 mg/mL) and samples were then centrifuged at 3000 rpm for 15 min. Imprecision, linearity, limit of detection (LoD), limit of blank (LoB), limit of quantitation (LoQ) and carry-over were assessed according to CLSI protocols.
Results: The LoD, LoB, LoQ were 1.95, 0.3 and 2.92 mg/l, respectively. Imprecision was comprised between 1.36-3.48 %, linearity was excellent (r=0.99) and carry-over was negligible, dynamic range was between 5 and 250 mg/L in a six point calibration.
Conclusions: We suggest that this immunturbidimetric assay may be suitable for measuring CRP in SF. However, additional research is needed to confirm the clinical significance of measuring CRP in SF to rule out PJI.

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SS13-43

BLOOD GASES ANALYSIS IN CRITICAL CARE SETTINGS: NEW SOLUTIONS FOR OLD ISSUES

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Blood gases, pH, glucose, hemoglobin and electrolytes beside to coagulation parameters represent the most important analyses in emergency and intensive care medicine. In particular, arterial and venous blood gases tests provide fundamental insight into a patient's ability to oxygenate, ventilate and into assess the acid-base status. Fluids, electrolytes and acid-base disorders represent a very common condition in patients presenting to the emergency department as well as in patients admitted to the intensive care units. The risk of death is high because of failure to rapidly identify a life-threatening situation and initiate the appropriate treatment. As a matter of fact, the first step in the acid-base disorders is to evaluate the type and cause of the disorder as well as the degree of compensation, so that the correct treatment could be administered. The need for a rapid blood gas analysis has strongly increased in the past decades. A fast turnaround time (TAT) for laboratory tests has been shown to improve clinical outcomes and decrease time for decision making as well as the medical costs. Point of care testing (POCT) for blood gases and electrolytes reduces the TAT and potentially the pre-analytical errors, shortening the time between blood collection and measurements, in comparison to the testing performed in the central laboratory. However, as for other in vitro diagnostic tests, there are several possible errors that can commonly occur with blood gases analysis, especially in the pre-analytical phase. In fact, identify potentially unsuitable specimens for blood gas analysis is a fundamental activity of the healthcare professionals for preventing inaccurate results from being released. Noteworthy, the crucial challenge in moving critical care testing from the central laboratory to the POCT network is the mandatory assurance of high quality standards for the analytical results across all the POCT sites. Based on a patient centered approach, there is the need to evaluate all steps of the total testing process, irrespective of whether they are related to procedures and personnel outside the laboratory wall. We will present the data obtained in the evaluation of the performaces of the new GEM PREMIER 5000 instrumentation by using an health technology assessment study design.

SS15-47

STANDARDIZATION IN LABORATORY MEDICINE: ADVANTAGES AND CRITICISMS

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The most important goal of medical laboratories is to provide reliable and timely information to support the clinical decision-making, allowing the choice of the best available options for patients' care. Laboratory results are used not only for diagnosis, but also for monitoring patients, for administration of treatments and for risk assessment, thus underlining that the fit-for-purpose of a specific tests result depends also on the clinical needs. Requirements in standards, such as the ISO 13485, ISO 17025 and ISO 15189 have helped in clarifying these concepts, further encouraging the development of methods that follow the intended purposes, validating the appropriated methods performances, and - on the other hand - that laboratories verify the claimed specifications. ISO 15189 not only states that laboratory "shall select examination procedures which have been validated for their intended use", but also that method performances should be independently verified (if not modified with respect to the manufacturers inserts), or alternatively validated (e.g. for in-house methods). Both of these two processes require that laboratories develop and document objective evidences for verifying that methods performances are appropriated to the intended use. Notably, the sets of documents of the Clinical Laboratory Standards Institute (CLSI) are produced with the aim of providing clear and through guidance for evaluating and characterizing methods performances. The CLSI documents are derived by the consensus of experts from manufacturers, hospitals and reference laboratories and universities. For example, CLSI documents are available for evaluating methods precision (EP15) and for defining limits of detection and limits of quantitation (EP17) (1, 2). Overall, the advantages offered by the CLSI guidelines are broad and regards: a) the standardization of terminology and definitions, b) the proposal of technically and statistically sound, low-end protocols, c) the compliance with international standards and accreditation organizations. Regardless these directives, it should be taken into consideration that the quality of results should be guaranteed independently of the methods and of the instrumentations used, as inadequate laboratory performances may potentially cause patient harms. In order to assure the quality of results, medical laboratories use internal quality controls and external quality assessment schemes. These tools allowed laboratories to evaluate and monitor imprecision (random error) and trueness (systematic error) for quantitative methods, and diagnostic accuracy (in terms of sensitivity and specificity) for qualitative methods (3). Further, measurement uncertainty is also available for all the ISO 15189 accredited laboratories (4). Therefore, the

verification by clinical laboratories of imprecision/trueness or sensitivity/specificity appear feasible, even if the choice of appropriate protocols, such as those produced by CLSI, appear advisable to obtain reliable and comparable results. However, overall these processes present several difficulties for labs, as they are time-consuming and require expert technical and statistical skills (3).

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SS15-48

EVALUATING THE INNOVATION BY MEANS OF SYSTEM FAMILIARIZATION STUDIES: OUR EXPERIENCE

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We performed a familiarization study of a manufacturing prototype of the Alinity ci ® clinical chemistry (c) and immunoassay (i) instrument systems (Abbott, IL, USA). The Alinity ci-series is a fully automated analyzer allowing random and continuous access as well as priority and automated retest for both clinical chemistry and immunoassays tests. Calibration and quality controls including automated quality controls (QC) processing and QC analysis were assessed. The performance of selected assays (seven clinical chemistry parameters: ICT, calcium, glucose, creatinine and urea, and two immunoassays: TSH and hsTn-I) were evaluated through precision on QC, linearity using provided standards, and limits of blank (LoB), detection (LoD) and quantitation (LoQ), based on CLSI guidelines. Method comparison was performed using the ARCHITECT instrument system C8000 and i2000 system. Throughput and internal controls stability on board were also evaluated. Performance analyses were completed using quality control materials from the manufacturer and biological samples from our institution. Statistical analyses were performed using PC SAS 9.3. The CVs for within-run precision for clinical chemistry tests ranged from 0.5 to 2% and were of 1.77% for TSH and 3.35 % for hsTn-I. The CVs for day-to-day precision for clinical chemistry tests ranged from <1 to 2% and were of 1.2 % for TSH and 1.94 % for hsTn-I. Linearity testing verified the assay linearity claims for all parameters (r=0.999). Comparison studies showed good correlations with ARCHITECT instruments (r ranging from 0.974 to 1). All estimates of LoB, LoD and LoQ met the manufacturer's claim. Throughput study revealed 1204 tests/hour and 167 tests/hour capability for clinical chemistry assays and immunoassays, respectively. On board stability of chemistry QC was in the range announced by the manufacturer. Taken together, the familiarization study performed on a selected set of parameters tested on a manufacturing prototype of the Alinity ci-series analyzer revealed satisfactory analytical performances, allied to simplified maintenance procedures, software easy-to-use, shortened hands-on time and overall system reliability.

SS18-58

CARDIAC TROPONINS AND NATRIURETIC PEPTIDES: LAST GENERATION METHODS AND CLINICAL APPLICATIONS

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Cardiovascular diseases are the main cause of morbidity and mortality in industrialized countries; therefore, the evaluation of cardiovascular risk factors, as well as an early diagnosis of cardiac diseases, must be considered the first objective of public health in these countries. For this reason, there is an increasing interest in the development of new cardiovascular biomarkers and, consequently, a great number of laboratory tests have recently been proposed, probably more than 100 (1). However, only few of them are recommended by international guidelines, because there are no evidence-based data on their clinical usefulness in the follow-up of patients with cardiovascular disease. Among the huge number of suggested cardiovascular biomarkers, at present time, only cardiac troponin I (cTnI) and T (cTnT) and natriuretic peptides (especially BNP and NT-proBNP) have been demonstrated to be cardiac-specific biomarkers. The cardiac-specificity associated to a high analytical sensitivity are two essential pre-requisites for the diagnosis of heart failure in the first subclinical phases of disease (2), as well as for assessment of very small amounts of injured myocardial tissue (3). In the last 10 years, the analytical sensitivity of both cTn and BNP/NT-proBNP methods has been progressively improved, so that the most recent immunoassay methods are able to detect plasma biomarker concentrations of about 2-5 ng/L (4). For example, it is was calculated that the cTn concentration equal to the 99th percentile of the reference

population actually corresponds to a necrosis of about 40 mg of myocardium, which is a tissue amount too small to detect by cardiac imaging (5). The assessment of amounts of myocardial damage too small to cause specific signs and symptoms of cardiac disease is clinically relevant because only a clinical intervention during the early phases of myocardial remodeling is able to arrest or even to reverse the progression forward to symptomatic heart failure. Several studies demonstrated that progressively increased levels of cTn and natriuretic peptides even in the concentration range below the cut off level values significantly increase cardiovascular risk. Accordingly, the measurement of these biomarkers should be mandatory in al patients before the administration of cardio-toxic drugs, especially the most part of all anti-tumor agents, in order to early demonstrate the progression to heart failure, some months before the demonstration of reduction in left ventricular ejection fraction <50%. It is really important to stress the concept that an increase of cardiac biomarkers is anyway an index of cardiac functional stress or myocardial damage, even in the case of extra-cardiac diseases (including chronic inflammatory disease, end stage renal disease, or treatment with powerful cardio-toxic drugs). Therefore, the detection of increased biomarkers should stimulate a perspicacious clinician to search for a cause for this elevation in order to make an appropriate diagnosis and, when necessary, to initiate a specific treatment.

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SS20-64
PHARMACOGENETICS IN PSYCHIATRY

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In recent years, the study of genetic variability associated with the response and/or side effects of psychotropic drugs has focused on a limited number of candidate genes functional polymorphisms. More recently, the genomic approach through multicentric GWAS studies has allowed to identify new gene variants associated with the therapeutic efficacy of antidepressants, antipsychotics and mood stabilizers. These studies also allowed to identify gene variants associated with endophenotypic and/or specific side effects. These results, if confirmed in stratified populations also derived from the huge sample collected by various international consortia, could produce genomic biomarkers with levels of sensitivity and specificity useful in clinical practice. Furthermore, due to the reduction in the cost of complete genomic sequencing, the study of genetic variability will be completed by the characterization of rare variants with high functional impact. These data will enable, in the near future, the construction of a pharmacogenomic database useful in the development of precision medicine even in psychiatry.

SS20-65
CARDIOVASCULAR PHARMACOGENOMICS

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Patients' response to pharmacological therapies depends on a complex interplay among clinical, environmental and genetic factors (1). In this framework, the analysis of the genetic factors is the focus of what is usually referred to as pharmacogenomics (or pharmacogenetics), which deals with the effects of genetic variations (polymorphisms) on drug response. Indeed, polymorphisms such as single nucleotide polymorphisms (SNPs) can influence drugs' pharmacodynamics and pharmacokinetics, resulting in a range of phenotypes that, in turn, can lead to either lack of effectiveness or toxicity of a bioactive molecule (2). Main aim of pharmacogenomics is to contribute to the so-called personalized medicine, where "the right dose of the right drug" is given to "the right person at the right time".
The contribution of pharmacogenomic tests is becoming increasingly important in several medical fields including –among others- cardiology. Cardiovascular disease is undoubtedly the leading cause of mortality and hospitalization in developed countries, mainly in elderly patients, and cardiovascular medications are the most prescribed drugs worldwide (3). Since it has been proven that several pharmacogenetic biomarkers influence both the pharmacodynamics and the pharmacokinetics of cardiovascular drugs, pharmacogenomics has recently arisen great interest in this field. Several

pharmacogenetic tests are now recommended, including those predicting the response to anticoagulant, (e.g. warfarin) antiplatelet (e.g. clopidogrel) and antihypercholesterolemic drugs (e.g. statins). As an example, many studies have underlined the importance of the pharmacogenetic dosing approach to optimize the starting dosage of warfarin, thereby reducing the risk of both thromboembolic and haemorrhagic side effects. Noteworthy, a specific pharmacogenetic algorithm to evaluate the correct warfarin dosage is currently available, which stratifies patients taking into account genetic variants, demographic and clinical factors (4). Among others, the efficacy of the pharmacogenetic testing has been widely demonstrated in the case of patients affected by peripheral arterial disease or acute coronary syndrome treated with the pro-drug clopidogrel, which is activated by the enzyme CYP2C19 [5]. Despite its good efficacy and safety, there is a remarkable variability in the therapeutic response to such antiplatelet agent, partially due to three polymorphisms indicated as CYP2C19-*2; -*3 and -*17. In particular, CYP2C19-*2 and -*3, whose presence identify individuals as intermediate and poor metabolizers, reduce the conversion of clopidogrel to its active form, thereby decreasing the drug's antiplatelet activity. In this case, the pharmacogenetics analyses that we routinely perform at the University Hospital "San Giovanni di Dio e Ruggi d'Aragona" in Salerno (Italy) allow identifying patients who do not respond appropriately to clopidogrel at standard dosage (5). In conclusion, there is a strong need to promote high power studies to implement the systematic application of the pharmacogenetic analysis in the clinical decision-making process for the treatment of cardiovascular disease.

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SS20-CO09

MUTATIONAL LANDSCAPE PROFILE IN BREAST CANCER BY LIQUID BIOPSY

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Background: Cancer molecular profile evolves over time in response to a wide variety of endogenous and exogenous selective pressures. The identification of tumor specific molecular landscapes is a crucial aspect of the success of targeted therapy. Tumor dynamic plasticity could not be captured in its complexity by the single molecular source offered by a one-time tissue biopsy. Straightforwardly, diagnostics approaches for cancers clearly benefit from more snapshots at the same time. This cutting-edge clinical applications led to the development of several monitoring tools to study circulating tumor-derived components, including circulating cell-free DNA (cfDNA) and Circulating Tumor Cells (CTCs), widely known as "liquid biopsy". In this study, we performed liquid biopsy strategy to define the mutational landscape of five breast cancer metastatic untreated women focusing on low frequency pathogenic alleles (³1%) and also on tumor clonality status.Methods. Single EDTA blood tube was collected for each subject (n=5) and prepared for targeted NGS analysis following Liquid Biopsy Platform workflow by Thermo Fisher. CTCs, cfDNA, and gDNA (WBC) were processed in a single run (2chip/run) on Ion S5 System. In addition to the variant analysis using the Ion AmpliSeq Cancer Hotspot Panel v2 and Ion Reporter v5.2 Software, an in house UNIX script was designed to gather additional variant annotations from reference diagnostics databases (ClinVar, dSNP, COSMIC) and, to retrieve variant statistics for data reporting.Result. Custom Ion Reported filter chains coupled with ad hoc UNIX script allowed: a) to filter out ~95% of variants from raw dataset. b) to collect/compare pathogenic variants (~20/sample) in each blood component. c) to highlight unbalanced allelic distributions (up to ~20fold) reflecting potential cancer clonality.Conclusion. Liquid biopsy approaches could potentially lead to define genomic profile of patients with cancer, to quantify minimal residual disease and to monitor treatment responses. In the era of high-throughput NGS tools, the possibility to better understand the nature of tumor clonality has emerged matching the need of a new diagnostic strategy chasing tumor profile's remodeling.

Strauss WM, Carter C, Simmons J, et al. Analysis of tumor template from multiple compartments in a blood sample provides complementary access to peripheral tumor biomarkers. Oncotarget 2016;7:26724-38.

SS21-68

HEMOCYTOMETRIC VIEW OF MIELODISPLASY-INSTRUMENTAL AND MICROSCOPIC MORPHOLOGY

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Salerno

Mielodysplastic syndromes (MDS) are a heterogeneous group of clonal hematogical diseases of hematopoietic stem cell characterized by peripheral blood cytopenia(s), ineffective hematopoiesis and increased risk of development of acute myeloid leukemia (1). In the early phase of the disease, the diagnosis of MDS, often occasional, is based essentially on haemochromocytometric examination that evidences cytopenia(s) and on the morphological assessment of dysplasia with standardized methods (2). Morphological alterations of the dysplastic cells, regardless of the thecnology employed, give different instrumental signals than those generated by normal cells. Morphological abnormalities of red blood cells are: anisocytosis, poikilocytosis, anisochromasia or polychromasia, basophilic stippling, Howell-Jolly bodies, Pappenheimer bodies. Erythroid dimorphism may be present with a normal population and a dysplastic microcytic or macrocytic population. Macrocytosis, often associated with erythroid dysplasia, is well expressed by hematology analyzers by volume measurements (MCV) and the degree of anisocytosis is quantized by RDW (Red Cell Distribution Width). Some analyzers are also able to provide an index of red blood cells anisochromasia (HDW) (3). Morphological alterations of neutrophils may involve the nucleus (abnormal nuclear segmentation, abnormal clumping of the chromatin, presence of nuclear projections) and/or the cytoplasm (reduction of the content of granules) (4). Depending on the specific analytical techniques in use (optical, fluorescence, cytochemical, electrical conductivity) many of these alterations generate numerical and graphical informations that are indicative of these anomalies. Platelet dysplasia morphologically appears with variations in size and density of the granules. MPV parameter measures the size of the platelets while the degree of platelet size variability is expressed by PDW (Platelet Distribution Width) parameter. Some specific-instrument parameters may provide an estimate of the mean platelet component (MPC) that appears to be reduced in myelodysplasia because of platelet granule deficiency or a measure of the fractional platelet immature (IPF) that is increased in MDS and can be a marker for karyotypic abnormalities with a poor prognosis, including chromosome 7 abnormalities. The presence of blasts in the peripheral blood is a decisive factor for WHO classification and prognosis. Definition of a precise and accurate morphological count of blasts in peripheral blood is crucial for values of 1%, <2%, and between 2% and 5% (5). The presence of blasts is well underlined with different accuracy in relation to morphological and structural anomalies and to the thecnology employed but remains a data to be defined exclusively with microscopic observation. The latest generation hematology analyzers are for rapidity, cost and multiplicity of information provided, a powerful screening tool of the population to be subjected to diagnostic deepening. The most important limit of abnormalities found in automation is the technology-dependence of the alarm signals, with difficulty to translate instrumental finds in diagnostic informations understandable to the clinician.

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SS21-CO10

SATELLITISM OF PLATELETS TO NEUTROPHILS, MONOCYTES, LYMPHOCYTES AND LARGE GRANULAR LYMPHOCYTES ASSOCIATED WITH PLATELET PHAGOCYTOSIS: AN UNUSUAL MULTI-CELLULAR INVOLVEMENT IN A RARE PHENOMENON

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Platelets satellitism (PS) is a rare phenomenon (1 of 12,000 blood count. Bizzaro, 1995) consisting in an adhesion of platelets PLTs) to surface of other cells. This fact occurs in vitro and in EDTA anticoagulated samples only. Therefore, a variable number of circulating PLTs are subtracted to automated blood cell count, leading to a pseudo-thrombocytopenia. The recognition of PS in light microscopy is easy because the images of platelets surrounding the contour of leukocytes are unmistakable and evocative. After the first descriptions of PLTs rosetting around neutrophils (NE) (Reisman, 1974; Kjeldsberg, 1974; Mende, 1975), the involvement of other cellular types it was described: basophils (BA) (Liso, 1981); eosinophils (EO)(Fábryová, 1991); monocytes (MO) (Djaldetti, 1978); lymphocytes (LY) (Tun, 2016); LY from lymphomas; (Cesca, 2001); large granular cells (LGC) (Espagnol, 2000). In addition, it was

reported PS satellitism around different cell types in the same sample: NE and MO (Greip, 1976); NE, EO and MO (Lazo-Langner, 2002). Rarely, PLT phagocytosis by NE was observed in association whit satellitism (Yoo, 1982; Preethi, 2012) or isolated (Criswell, 2001). In both cases, the phenomenon appeared to be EDTA-dependent by inducing the hypothesis of a common pathophysiology. We report a case of PS and phagocytosis in a 42- year-old- asymptomatic woman presented to our Laboratory for mild thrombocytopenia and neutropenia (50x10^9/L and 0.90x10^9/L respectively), absolute monocytosis (1.21x10^9/L) and relative lymphocytosis (57.3%; 2.58x10^9/L). PLT count was confirmed but microscopic blood smear examination revealed PLT rosetting NE (100% of these), MO (27.1%), LY (2.7%) and LGC (5.6%). In some case in the NE and in the MO engulfed PLTs were present (20% and 5.9% respectively). Some aspects of "pre-phagocytosis" (platelet that were housed in low lying areas of the nuclear contour) as wells as "post-phagocytic" vacuoles were even observed. BA and EO were not involved. PLT count in sodium citrate was equal to 147x10^9/L and a finger-pick blood smear demonstrated the demise of PS as well as phagocytosis. Based on this observation we posed the diagnosis of "pseudo thrombocytopenia due to platelet satellitism EDTA dependent".

SS22-70
TRE NEONATI CON SINDROME DI DOWN E ALTERAZIONI EMATOLOGICHE

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Riportiamo il caso di tre neonati affetti da Sindrome di Down ricoverati presso il nostro Ospedale. Nel primo caso l'emocromo evidenzia una leucocitosi (30 x10^9/L globuli bianchi), emoglobina nella norma e piastrinopenia. Alla revisione microscopica dello striscio di sangue periferico, si evidenzia la presenza di rari blasti, con caratteristiche morfologiche riconducibili ai blasti megacariocitari, in numero progressivamente crescente nei giorni successivi. Nel secondo caso la paziente viene ricoverata ad un mese di vita, per grave anemia. L'emocromo mostra emoglobina 48 g/L, con leucopenia e in particolare neutropenia (0.09x10^9/L neutrofili). L'osservazione del vetrino di sangue periferico conferma la presenza di anemia ed evidenzia la presenza di rari blasti indifferenziati. Il terzo caso riguarda invece un neonato prematuro, nato alla 32 settimana di gestazione, con idrope fetale e cardiopatia congenita. L'emocromo mostra un conteggio di 172x10^9/L globuli bianchi ed anemia. Lo striscio di sangue periferico mostra caratteristiche peculiari: eritroblasti e piastrine displastiche, alcune piastrine giganti, prevalenza di blasti con cromatina sottile con uno o più nucleoli, citoplasma basofilo e granulazioni di tipo piastrinico, alcuni blasti con blebs citoplasmatici e micromegacariociti. In tutti e tre i casi è stata posta diagnosi di Transient Abnormal Myelopoiesis (TAM), confermata dall'esame citofluorimetrico, una sindrome transitoria da alterata ematopoiesi caratterizzata dalla presenza in circolo di blasti di aspetto megacarioblastico e mutazione del gene GATA 1. Il 20-30% dei casi di TAM evolve a leucemia mieloide acuta. Il ruolo della lettura dello striscio di sangue periferico, spesso non effettuato negli emocromi della popolazione in età neonatale, può indirizzare il clinico, soprattutto nei casi in cui il dato quantitativo non risulti particolarmente indicativo, alla diagnosi corretta e all'impostazione dell'adeguato trattamento.

SS22-71
UN CASO DI LINFOADENOPATIA, EPATOSPLENOMEGALIA E TRIPTASI ELEVATA

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La mastocitosi è una malattia clonale rara dovuta alla proliferazione neoplastica di mast cellule all'interno di uno o più organi, frequentemente cute e midollo osseo. Alla diagnosi può essere presente splenomegalia e più raramente linfoadenopatia ed epatomegalia. La diagnosi e la classificazione della mastocitosi è basata sull'identificazione di mast cellule neoplastiche dal punto di vista morfologico, immunofenotipico e/o molecolare secondo i criteri stabiliti dal documento WHO 2008. Utile è il dosaggio della triptasi, enzima prodotto quasi esclusivamente dai mastociti, i cui livelli sierici correlano con la quantità e l'attività cellulare. Dal punto di vista morfologico sono descritti 4 tipi di mast cellule: (i) matura tipica (rotonda, ben granulata, con nucleo rotondo centrale); (ii) atipica tipo I con estroflessioni citoplasmatiche, nuclei ovali in posizione eccentrica, citoplasma ipogranulato con accumulo focale di granuli; (iii) atipica tipo II con nuclei bi o multilobati e (iv) simil-blastica con granuli metacromatici. Le mast cellule sono CD45++, esprimono elevati livelli di CD117 e sono negative per l'antigene CD34. Le mast cellule neoplastiche coesprimono CD25 e/o CD2. Un uomo di 60 anni giunge alla nostra attenzione per epatosplenomegalia e presenza di una modesta componente monoclonale al quadro proteico. L'esame ecografico evidenzia iperplasia linfonodale reattiva in regione laterocervicale e sottomandibolare. Per l'insorgere di una moderata anemia (Hb: 129 gr/L) lievemente macrocitica (MCV: 99 fl) il paziente pratica un agoaspirato midollare. L'osservazione morfologica del sangue midollare mostra aree di infiltrazione di mast cellule che appaiono

atipiche di tipo II e raramente di tipo I. L'esame citofluorimetrico evidenzia immunofenotipo patologico: CD45++CD117++CD2+CD25+. Per confermare il sospetto diagnostico viene richiesto il dosaggio della triptasi che risulta significativamente aumentata (551 ng/L; V.R <12 ng/L). Essendo soddisfatti tre criteri minori secondo quanto previsto dalla organizzazione mondiale della sanità del 2008 viene posta diagnosi di Mastocitosi sistemica. La mastocitosi è una malattia rara la cui diagnosi è di fondamentale importanza essendo spesso richiesta una immunoterapia cronica per evitare rischi di reazioni allergiche o complicanze ossee di rilievo.

SS22-72

EVALUATION OF RESPONSE TO TREATMENT IN A PATIENT AFFECTED BY AL AMYLOIDOSIS WITH LOW FREE LIGHT BURDEN

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In patients affected by light chain (AL) amyloidosis, the reduction of the concentration of the amyloidogenic circulating light chains translates in improvement of organ dysfunction and is associated with an increase in overall survival. This allowed the definition and validation of the criteria for hematologic response to therapy that are based on FLC quantification (Palladini et al. JCO 2012). A 69 years old man with nephrotic syndrome (proteinuria 5 g/24h), was evaluated in another center in December 2015. A renal biopsy revealed amyloid deposits. In January 2016 at our center, no monoclonal components were detected at serum and urine immunofixation, proteinuria was 6 g/24h with creatinine 0.99 mg/dL (u.r.l 1.17 mg/dL). The λ-FLC concentration was 54 mg/L (κ/λ ratio 0.25, dFLC 41 mg/L) and a bone marrow aspiration revealed 11% of monoclonal plasma cells. Echocardiography did not reveal signs of cardiac amyloidosis. The abdominal fat aspirate for amyloid typing by immune-electron microscopy showed λ-LC deposits. The diagnosis of AL (λ) amyloidosis with renal involvement was made and the patient was started on treatment with cyclophosphamide, bortezomib and dexamethasone. After 2 courses, at our center, the λ-FLC concentration was 59 mg/L (κ/λ ratio 0.24, dFLC 45 mg/L) and an increase of proteinuria (9 g/24h) and creatinine (1.25 mg/dL) was documented. Treatment strategy was changed because of the lack of hematologic response and the progression of renal damage. The patient was stated on therapy with melphalan and dexamethasone. After 3 courses a reduction of the λ-FLC concentration was noticed (k/λ ratio 1.36, dFLC 0 mg/L). A significant reduction of proteinuria was revealed (5.5 g/24h) with improved serum creatinine (1.0 mg/dL). After 3 other cycles, no monoclonal proteins were detected at serum and urine immunofixation, dFLC was 2 mg/L (k/λ ratio 1.42) and a further decrease in proteinuria was documented (3.2 g/24h). In this case, as reported in two recent manuscripts (Milani et al. and Dittrich et al. Blood 2017) a reduction of dFLC <10 mg/L after therapy was associated with an increase of organ dysfunction. These data confirm the central role of the FLC assessment in the monitoring also of patients with a low-dFLC burden affected by AL amyloidosis.

SS22-73

INCIDENTAL DETECTION OF A NEW HAEMOGLOBIN BETA VARIANT DURING HbA_{1c} MEASUREMENT

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Introduction: a number of hemoglobin b chain variants has been described. Their presence can be suspected on the basis of typical changes in hemochromocytometric analysis or incidentally detected during chromatographic analysis of glycated hemoglobin (HbA_{1c}). The availability of capillary zone electrophoresis (CZE) for HbA_{1c} measurement could improve their detection.
Methods: a non anemic 58-year-old female of sardinian origin was investigated for quantification of HbA_{1c}. The blood was collected in EDTA-containing tube. CZE was performed with Sebia Tera 3 Capillarys system (Sebia Italia Srl), using programs and reagent for HbA_{1c}. Standard high performance liquid chromatography (HPLC) was performed with VARIANT II™ Analyzer (Biorad).
Results: the quantification of HbA_{1c} was invalidated by the presence of a double peak in the HbA_0 zone. HPLC analysis did not allow to separate the variant from Hb A_0. Despite this Hb variant was clinically silent, DNA analysis and b-globin gene sequencing was performed and showed a heterozygous variation of nucleotide sequence HBB:c.376C>A; beta 125 (H3) Pro>Thr. The new variant was denominated Hb Novara. The same variant was found in the daughter of the proband during HbA_{1c} analysis, together with a marked decrease of HbA_2 (1.4 %). It was associated with a deletion in the a-chain gene. The daughter showed a moderate anemia (Hb 9.0 g/dL, MCV 61.3 fL, MCH 17 pg). The hemoglobin stability tests were normal in both patients. Oxygen

affinity was different (P_{50}: 22.68 and 29.38 mmHg respectively for the mother and the daughter).
Conclusion: the discovery of the Hb Novara has been possible by the use of a procedure not originally developed for variant detection. It clearly suggests that sometimes a combination of different technologies (such as HPLC and CZE) can be extremely useful in detection of an increasing number of hemoglobin variants (even those of apparent little clinical interest), in order to better understand the pathophysiology of hemoglobin. Although the carrier of Hb Novara seems to be essentially asymptomatic, is not possible to exclude that this mild defect could produce more relevant hematological phenotypes when associated with other α or β chain defects.

SS22-74
COMBINED PLATELET FUNCTION DISORDER AND FACTOR XI DEFICIENCY

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We present a case concerning a 17 year old boy with positive bleeding history and neck lymphadenopathy scheduled for fine needle aspiration biopsy (FNAB). The patient referred for evaluation of haemostasis status in preparation for biopsy. His medical history included bleeding after appendectomy when he was 6 year old. The family history was significant for a father with bleeding after dental extraction. Laboratory coagulation and platelets profile performed when he was a child showed slightly prolonged PT and APTT ratio (PT ratio = 1.25 and APTT ratio = 1.22) with normal values of all coagulation factors, normal values of Platelet function analyzer (PFA-100) and abnormal platelet aggregation to ADP. Coagulation laboratory investigation performed in our laboratory showed, normal PT and slightly prolonged APTT ratio (PT ratio = 1.08 and APTT ratio = 1.22) and reduced factor XI levels (49%, normal values >55%). Platelets investigation showed, normal platelets count (248.000) and morphology, normal PFA-100 closure times to Collagen/ADP and Collagen/Epinephrine and decreased secondary platelets aggregation to ADP and epinephrine on Light Trasmittance Aggregometry. Laboratory findings were consistent with an inherited platelets function defect (storage pool disease or dense granule defect) and hereditary factor XI mild deficiency. Management of this patient included careful monitoring for needle aspiration biopsy bleeding complications, prophylactic tranexamic acid, as well as having platelets readly available during FNAB. In conclusion detailed medical and family history is a key to determining the cause of bleeding, specialized tests including old-fashioned platelet function assays performed as recommended by laboratory guidelines (recommended agonists concentrations) and experienced individuals interpreting tracing and results can provide diagnosis in these patients.

SS22-75
ISCHEMIC STROKE SECONDARY TO SEVERE REACTIVE THROMBOCYTOSIS IN A PATIENT WITH CELIAC DISEASE AND LONG-TERM IRON DEFICIENCY ANEMIA

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We report a case of a 51-year-old woman who was admitted to emergency room due to an ischemic stroke. She underwent to several laboratory and neuroradiological examinations. Computed tomographic angiography showed a thrombus in the right internal carotid artery. Laboratory tests showed an extreme thrombocytosis (PLT: 1963*10^9/L, RI 130-400*10^9/L) and a severe microcytic anemia (Hb: 4 g/dL, RI 12-16 g/dL; MCV: 61 fL, RI 78-99 fL). Serum iron (8 μg/dL; RI 37-145 μg/dL) and ferritin levels (1 μg/L, RI 15-150 μg/L) were very low; lactate dehydrogenase was slightly elevated (276 U/L, RI 135-214 U/L); coagulation profile was found in the normal range and antiphospholipid antibodies were absent. The marked thrombocytosis has led to evaluate the presence of myeloproliferative disorders, particularly essential thrombocytemia. Research of JAK-2 gene mutation resulted negative. Bone marrow exam (biopsy and aspiration) and cytofluorimetric analysis allowed to rule out hematological malignancies. Anamnestic data revealed that anemia was present since adolescence and was refractory to oral iron treatment. All these evidences led to hypothesize the celiac disease (CD) as possible cause of anemia due to iron malabsorption. No gastrointestinal symptoms have been referred. CD is an autoimmune disorder triggered by gluten ingestion and targeting small intestine. Among extra-intestinal manifestations of CD iron-deficiency anemia is not so rare and sometimes associated to reactive thrombocytosis. According to guideline, total serum IgA levels were measured, ruling out a deficit of this immunoglobulin isotype; IgA anti-tissue transglutaminase (tTG) and IgA endomysial antibody (EMA) were performed and both resulted strong positive (tTG: 275 U/mL, cut-off <7 U/mL; EMA: 1/80). Endoscopy investigation to confirm the serological diagnosis of CD is in progress.The patient was treated with packed erythrocyte transfusion, intravenous iron and antiplatelet drug with a progressive improvement of neurological signs along with an increase of hemoglobin levels (Hb: 8.0 g/dL) and a reduction of platelet count (PLT: 627*10^9/L). These results suggest that the heavy

thrombocytosis is likely secondary to iron deficiency anemia due to an underlying CD and could represent a risk for thrombotic complications.

SS22-76

A CLINICAL AND SEROLOGICAL STUDY OF IgA DERMATOSIS WITHOUT IMMUNOGLOBULIN DEPOSITION OTHER THAN IgA IN DIRECT IMMUNOFLUORESCENCE: A CASE REPORT

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Intercellular IgA dermatosis (IAD) is a subset of autoimmune blistering disorder, characterized by immunoglobulin (Ig) A anti keratinocyte cell-surface antibodies. We would like to report our experience with a 54-year-old Caucasian woman who presented with a 2 years history of a persistent and steroid-resistant dermatosis. Clinical examination revealed multiple coalescent large pustules, containing clear to slightly cloudy fluid, some of which displayed hypopyon formation. Mucous membranes were uninvolved. Electrophoresis of serum immunoglobulins did not reveal a monoclonal gammopathy. Bacterial cultures were negative. Histopathologic examination of skin biopsy showed a subcorneal cleft with acantholysis. Direct immunofluorescence of the perilesional skin biopsy showed exclusively IgA deposition on keratinocytes cell surfaces. Staining was evident in particular in the superficial layers of the epidermis. Indirect immunofluorescence microscopy performed on monkey esophagus did not detect circulating IgA or IgG anti-cell surface autoantibodies. Enzyme-linked immunosorbent assay (ELISA) for IgG autoantibodies against desmogleins (DsG1, DsG3), performed using commercially available kits (MBL, Nagoya, Japan) showed no reactivity. Commercial IgA ELISAs are not available and so to detect IgA reactivity against desmogleins, ELISA kits were modified applying peroxidase conjugated anti-human IgA. At cut-off value of optical density (OD) more than 0.150, as described previously by Hashimoto et al., IgA reactivity (OD) to DsG1 was 0.206 and to DsG3 was 0.165. Moreover, commercial kits for autoantibodies against desmocollins (Dscs) were not available. In conclusion, basing on clinical appearance and immunopathology results, was made diagnosis of unclassified intercellular IgA dermatosis. Lacking circulating IgA specific autoantibodies results, we are not able to confirm this subtype accordingly to Hashimoto's classification.
Recently, a novel IgA ELISAs was developed to detect circulating IgA antibodies anti-Dsc. We hope these IgA ELISAs will introduce in routine practice to facilitate the diagnosis and understanding of pathophysiology in IAD in the future.

SS22-77

STIFF-PERSON SYNDROME CASE REPORT: FROM LABORATORY DIAGNOSIS TO TREATMENT

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Background: Stiff-person syndrome, also called "rigid man syndrome" or "stiff man-syndrome" (SMS), is a rare neurological disease characterized by fluttering chest and limb stiffness, painful muscle spasms, phobia related to certain tasks, abnormal tendency and anchilous deformities. The onset occurs around the age of 45 and the symptoms develop over the course of months or years. The pathophysiology of the disease is autoimmune, this disease seems to be associated with the presence of antibodies to the glutamic acid (GAD) decarboxylase, usually directed against peripheral nervous system but sometimes also against central nervous system parts.
Case report: Woman, age 65, hospitalised in January 2016 at U.O. of Neurology due to a two-to three-month, short-to-left motor lumbar spine, characterized by floating stiffness that appears and accentuates in orthostatism with a tendency to fall, does not exhibit axial rigidity. The patient has always had a mildly disturbed pathological history. Differential diagnosis was performed after hospitalization, after several instrumental assessments, it was decided to perform lumbar puncture with consequent analysis of the liquor and isoelectrophocalization showed absence of bands Intrathecal synthesis, but presence of numerous bands on both liquor and serum, oligoclonal mirror profile, type 4. It was decided to require dosage of anti-GAD antibodies on liquor and serum with "off-normal", seeking anti Amphiphysin negative antibodies.The patient was discharged with Stiff-person syndrome diagnosis and Diazepam therapy at home and Immunoglobulin e.v. with periodic monitoring.
Conclusion: the patient is in charge of the U.O. Day Service. Neurology of Vaio Hospital, Fidenza and continues the periodic cycles of Immunoglobuline e.v. and now reports clinical stability. It is therefore considered essential to carry out early diagnosis in this type of rare and ingesting pathology so as to set a correct therapy and to move as soon as possible. It is also important that there is a great collaboration between the clinic and the laboratory to reduce the waiting time and orient the clinician in the right diagnosis and in the need for the correct examinations to be performed, always considering the patient at the center.

SS22-78

ANALISI DELLE URINE DI UN BAMBINO CON OLIGURIA E ADENOPATIA CERVICALE: RUOLO DEGLI ANALIZZATORI A CATTURA D'IMMAGINE

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Premessa: L'applicazione di tecnologie a reti neurali con personalizzazione delle regole di validazione, recupera nella categoria "NSE" (non epithelial squamous cells) cellule con diametro 20-60 μm tra le quali l'operatore esperto può riconoscere decoy cells (DC), cellule epiteliali con effetti citopatici come da infezione virale in pazienti immunocompromessi o immunosoppressi (in particolare trapiantati di rene o midollo). In soggetti immunocompetenti l'infezione primaria da EBV può rappresentare uno stato di immunosoppressione transitoria con riattivazione di poliomavirus BK (BKV) latente: in letteratura sono riportati rari casi di co-infezione virale in pazienti pediatrici (Breuer C, et al. 2014).
Scopo del lavoro: presentare un caso di infezione da EBV in soggetto immunocompetente con DC nel sedimento urinario per rimarcare l'importanza del corretto riconoscimento di tali elementi non tipici riferibili a reattività.
Materiali e metodi: studio condotto su un bambino di 4 aa con adenomegalia latero cervicale e febbre con recente accesso al PS per oliguria. PCR 0,5 mg/dl (AU 680 BC, vn 0-0.7), attivazione linfocitaria. Il campione urinario processato su analizzatori iQ200 Iris BC per la microscopia automatizzata evidenziava ematuria accompagnata da numerosi elementi riconducibili a DC, confermati in microscopia a contrasto di fase. Il campione urinario è stato suddiviso in due aliquote: una inviata in citopatologia (0,5 ml di sedimento urinario + 1 ml Thinprep solution) per la conferma morfologica in citologia in fase liquida con colorazione Papanicolaou, l'altra inviata al laboratorio di Virologia per la ricerca di BKV con PCR-DNA. Eseguita sierologia per EBV IgG/M (Architect Abbott) e EBNA (Vidas Biomerieux).
Risultati: Il quadro citologico urinario è risultato suggestivo per infezione da BKV confermato con PCR-DNA (>4,3x10^7 copie virali). La sierologia per EBV ha evidenziato una infezione primaria (IgG/M positivi, EBNA negativo).
Conclusioni: Nel laboratorio ad elevata produttività la tecnologia a rete neurale, un efficiente middleware e l'esperienza dell'operatore consentono la corretta identificazione e segnalazione di elementi reattivi potenzialmente interpretabili come elementi atipici maligni cui seguirebbero approfondimenti diagnostici inutili e costosi.

SS22-79

SOFFERENZA TUBULARE IN UN PAZIENTE CON RECENTE TRAPIANTO DI RENE

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Introduzione: L'urolitiasi da DHA è una patologia rara e sotto diagnosticata causa di insufficienza renale secondaria alla deficienza di Adenina-phosphoribosyltransferase e conseguente deposito di cristalli di DHA nel lume tubulare, negli interstizi e nelle cellule epiteliali renali. La diagnosi è tardiva e spesso successiva al trapianto renale.
Metodologia: Paziente maschio, 54 anni, diagnosi di IRC ndd, sottoposto a trapianto di rene nel 2016, terapia immunosoppressiva con tacrolimus, micofenolato, corticosteroidi. Dopo un iniziale andamento post-operatorio discreto con moderata diminuzione della creatinina, a 10 gg dal trapianto si evidenzia ripresa funzionale ritardata, necrosi tubulare acuta, valori di creatinina e urato costantemente alti.
Risultati: Lo studio del sedimento urinario è stato condotto tramite Microscopia Intelligente Automatizzata, Iris Diagnostics con eventuale revisione microscopica. Si riscontra una evidente sofferenza tubulare con presenza di cellule epiteliali renali isolate o in clumps, cilindruria ed elementi cristallini suggestivi di urolitiasi da DHA. L'osservazione microscopica in contrasto di fase e polarizzatore ha confermato la presenza di cristalli all'interno di cilindri, di provenienza quindi tubulare. Gli esami metabolici di conferma sono in corso. Il paziente viene trattato con allopurinolo. Al follow-up a 10 mesi si osserva una lenta ma progressiva diminuzione dei valori di creatinina e di urato.
Conclusioni: Il caso clinico evidenzia l'utilità dello studio post-trapianto del sedimento urinario. Questo esame ha permesso di porre diagnosi di sospetta urolitiasi da DHA, patologia per la quale solo il trattamento farmacologico precoce può contrastare l'evoluzione in insufficienza renale. La frequente diagnosi post trapianto della patologia in esame è dovuta alla difficoltà di riconoscere i cristalli di DHA, spesso in forme atipiche, facilmente confondibili con altri cristalli. Lo studio automatizzato del sedimento aiuta la diagnostica nefrologica poiché, grazie alla tecnologia capillare, propone all'osservazione dell'operatore anche rari ma significativi elementi, permette di salvare le immagini per confrontarle con la microscopia e valutare nel tempo l'evoluzione della patologia.

SP23-81
CALPROTECTIN AND OTHER FECAL MARKERS OF INTESTINAL INFLAMMATION

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Fecal calprotectin (fCal) has challenged in the last decades a more effective diagnostic work-up for patients with inflammatory bowel diseases (IBDs), which comprise Crohn's disease (CD) and ulcerative colitis (UC). IBDs share intestinal inflammation, which affects the mucosa of the large intestine in UC, while in CD it may occur at any part of the gastrointestinal tract, being not limited to the mucosa, but involving the whole intestinal wall and potentially causing stenosis, perforation and fistulae. IBDs are chronic diseases characterized by alternations of flares and remissions, which onset typically occurs at a young age. To answer the question "how laboratory medicine should help clinicians facing with IBDs?", three main clinical settings should be considered: 1. Rule-in and rule-out IBDs, i.e. distinguish IBDs from irritable bowel syndrome and make a diagnosis; 2. Define type of IBDs (UC or CD); 3. Monitoring IBDs to early detect flares and predict the response to therapy. A number of blood and fecal biomarkers for IBDs are available. While blood biomarkers allow addressing the second, fecal markers allow addressing the first and the third items. Blood inflammatory biomarkers, as C-reactive protein, although useful are not specific for IBDs, while markers to define the IBDs type, as UC-associated pANCA and CD-associated ASCA, have a diagnostic accuracy which does not exceed 60-70%. The two most extensively studied fecal biomarkers are fCal, a S100A8/S100A9 calcium binding heterodimer, and lactoferrin, an iron binding protein. Both proteins have antimicrobial properties, are released by inflammatory cells - mainly polymorphonuclear cells - infiltrating the gastrointestinal mucosa, and are resistant to proteolysis, which renders their measurement in stool a reliable tracer of intestinal inflammation. However fCal was reported to be more sensitive and specific than lactoferrin. Despite the market offers qualitative fCal assays, to be clinically useful a quantitative result (μg/g) should be provided. Different thresholds allow to address different clinical questions in different settings: values below 50 μg/g in adults and 100 μg/g in children rule-out intestinal inflammation with a high negative predictive value (81% in primary and 98% in secondary care), while values above 150 μg/g in adults and 300 μg/g in children may predict IBDs with high sensitivity (>90%). In patients with an established IBDs diagnosis values above 250 μg/g in adults and 500 μg/g in children predict disease relapse with high sensitivity and specificity (>80%) and may help in predicting the response to anti-TNF treatment. From a clinical laboratory perspective both the pre-analytical (stool storage, weighting and sampling with dedicated devices) and the analytical phase (ELISA, chemiluminescence or turbidimetric assays) require to be strictly monitored through internal and external quality control programs. Since a high inter-method variability has been observed, hence harmonization projects represent a chance in the near future. In conclusion fCal, although not perfect, is actually our best choice for diagnosing and monitoring IBDs.

SP23-83
LABORATORY EVALUATION OF PANCREATITIS

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Today, laboratory tests not only play a pivotal role in the diagnosis of acute pancreatitis, but also may help to assess the disease severity and its etiology. In the diagnosis of acute pancreatitis, lipase measurement in serum is superior to (P-type) amylase in terms of cost-effectiveness and diagnostic performance. Its clinical sensitivity is 80 to 100% depending on the selected diagnostic cut-off, and the clinical specificity is 80 to 100% depending on the mix of the patient population studied. After an attack of acute pancreatitis, serum lipase activity increases within 4 to 8 h, peaks at about 24 h, and decreases within 7 to 14 days. However, the increase is not necessarily proportional to the severity of the attack. Points to remember for pancreatic enzymes in acute pancreatitis are: a) their diagnostic performance is greatly improved by restricting their use to a population with suspected disease; b) it is recommended that lipase replaces (P-type) amylase as initial test for acute pancreatitis; measuring both serum amylase and lipase is not warranted; c) the measurement of total amylase should be considered obsolete.

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SP23-84
FECAL TESTS FOR COLORECTAL CANCER SCREENING: FROM FECAL OCCULT BLOOD TEST TO DNA ANALYSIS

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Colorectal cancer (CRC) is one of the most common malignancies. Due to its biologic behavior, a screening program is easily applicable to CRC. The most used strategies are based on laboratory investigations on stool samples. Blood in the stools is the first and most used strategy. Fecal occult blood test (FOBT) and fecal immunochemical test (FIT) are the main methods. Both are economic, easy to perform with high specificity, and low sensitivity. However, such tests have a low sensitivity and the result may be affected by alimentary and drug interference (1). Based on CRC multi-step process with genetic and epigenetic alterations in large bowel cell DNA, single mutations or panels of alterations have been proposed as alternative stool test. These tests have the advantage of a marked improvement of the sensitivity when compared to fecal blood. However, high costs, poor availability, and correct choice of marker panel represent the major limits. A specific sDNA panel including aberrantly methylated BMP3 and NDRG4 promoter regions, mutant k-ras and β-actin (a reference gene for human DNA quantity), and an immunochemical assay for human hemoglobin has been recently approved by Food and Drug Administration (2). Novel promising biomarkers for CRC screening are represented by microRNAs (miRNAs), a group of 18-25 nucleotide non-coding RNA molecules that regulate gene expression. Reports on these fecal biomarkers are case-control studies, and each of them evaluates single miRNAs or multi-target panels (3). On the other hand, some fecal proteins have been studied as possible CRC screening markers, even though they demonstrated poor results (4). Finally, alterations of estrogen receptor-beta (i.e., dramatic reduction in the early stage of CRC) have been demonstrated in tissue samples (5). In conclusion, specific investigations are warranted in order to add further noninvasive markers to the panel of CRC screening tools.

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SP23-CO11

THE OROPHARYNGEAL MICROBIOME DIVERSITY IN HEALTHY INDIVIDUALS AND IN CELIAC DISEASE PATIENTS

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Celiac disease (CD) is a chronic immune-mediated enteropathy of the small intestine triggered by gluten in genetically predisposed individuals expressing the HLA-DQ2/DQ8 molecules, and likely in presence of additional factors not yet completely delucidated. Among these latter gut microbial alteration has been hypothesized to contribute to the CD pathogenesis (Cenit MA, et al. J Clin Gastroenterol 2016). In humans, the gastrointestinal tract contains approximately 10^{14} bacteria (>1000 different species, >3 million genes). The gut microbiota provides many functions that are crucial for the well-being of the human host, and gut dysbiosis has been suggested to play a pathogenic role in several diseases, including autoimmune and gastrointestinal disorders. In this context, we recently described a different duodenal microbiome composition in control subjects, active- and gluten free diet (GFD)-CD adult patients (D'Argenio V, et al. AJG 2016). In particular, a significant higher presence of Neisseria flavescens (beta-proteobacteria class), able to induce inflammation in both murine and human dendritic cells, was observed in active CD microbiome with respect to those of the other two groups. As gastrointestinal tract could be considered a single ecosystem extending from the oral cavity to the rectum, we investigated the oropharyngeal microbiome (by 16S rRNA sequencing) in 56 subjects (controls, GFD and active CD patients) and the duodenal microbiome in a small subgroup of CD patients. All collected samples were immediately cooled in dry ice and stored at -80°C until analysis. After DNA extraction, the V4-V6 regions of the 16S rRNA gene was amplified following the Illumina 16S Metagenomic Sequencing Library Preparation workflow, using a specific barcode sequence/sample. After quality and quantity assessment, all the libraries were pooled together and sequenced using the Illumina MiSeq System (PE 300x2). Analysis of the microbiome data was carried out by the QIIME v. 1.9.1. bioinformatics tool. Our findings, in confirming our previously duodenal microbiome data, highlight interesting similarities between the duodenal and oropharyngeal microbial alterations in active CD patients. The latter could have potential applications in CD diagnosis (Grant by 007_FC_2014).

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Firenze, 16-18 ottobre 2017

Riassunti Poster

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

P001
WHICH (AND HOW MUCH) LABORATORY MEDICINE IN DEVELOPING COUNTRIES: DOCEMUS EXPERIENCE ON THE PAEDIATRIC HOSPITAL (MAS CTH) OF HARGHEISA (SOMALILAND)

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Docemus Onlus "Theoretical and Practical Training School for Improving Specialty Medicine", Torrevecchia Teatina

The paediatric pathologies in the developing countries are the same than those in the western countries having better economic and social conditions. There is a different incidence of infectious diseases as well as of pathologies malnutrition-related such as the anaemias. For these reasons, there should be no differences theoretically in the type and in the quantity of diagnostic tests in respect to these needs. On the contrary, this assumption is frustrated hopelessly by economic, logistic, social and operative difficulties that are insurmountable often. So, these difficulties determine the witch and the how much for the Laboratory Medicine. In the clinical laboratory of the MAS CTH in Hargheisa the "WHICH" is: i. basic clinical chemistry (kidney and liver profiles; electrolytes; albumin; total protein); ii. blood gas; iii: Cell Blood Count (CBC); coagulative tests (aPTT and AP); iv. basic serology (hepatitis; HIV); v. urinalysis; vi. stool examination for parasites. The "HOW MUCH" depends on the mentioned difficulties: costs; sites and supply routes for reagents and their due date; other. Professional adequacy and upgrade are additional problems. However, even with these limitation, today in the MAS CTH the Laboratory Medicine is present and ensures as much as possible to improve the quality of care of young patients as well as the pre- and post- cardiac surgery support to Italian Team who periodically work in Hargheisa. An additional challenge is the abandonment of the procedural and operational improvisation: all of activity was described in a "operating instruction manual" and the traceability of events has been ensured starting from the pre-analytical phases of the request generation as well as of the identification of clinical material. Together to Lab Technicians and to Clinical Team a single model of report has been adopted. Next step will be the start of a basic microbiological activity.

P002
VALUTAZIONE ANALITICA DI UN NUOVO EMOGASANALIZZATORE: GEM 5000

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Presso ASST Bergamo Est sono presenti N. 8 emogasanalizzatori GEM 4000 e n. 6 emogasanalizzatori GEM 3000 ditta IL dislocati nei tre presidi ospedalieri (P.O.) aziendali sia nei laboratori che nelle Unità Operative (U.O.) di Terapia Intensiva, Pronto Soccorso, Patologia Neonatale , Sala Parto .
GEM Premier 5000 è un nuovo emogasanalizzatore di Instrumentation Laboratory - Werfen che consente misurazioni rapide e quantitative, attraverso l'utilizzo di una cartuccia multiuso e multitest, di pH, pCO_2, pO_2, sodio, potassio, cloruro, calcio ionizzato, glucosio, lattato, ematocrito, bilirubina totale e CO-Ossimetria (tHb, O2Hb, COHb, MetHb, HHb) e altri parametri calcolati.
Abbiamo valutato le performance analitiche di questo nuovo modello comparandole con il mod. GEM Premier 4000 attualmente in uso presso la nostra ASST, secondo le linee guida EP09-A3 del Clinical and Laboratory Standards Institute (CLSI).
Sono stati raccolti n. 274 campioni di sangue intero in siringhe eparinate per emogasanalisi , provenienti dalle diverse U.O. del P.O. di Seriate , ed eseguiti in contemporanea sui due analizzatori GEM Premier 4000 e GEM Premier 5000. I dati ottenuti sono stati elaborati statisticamente attraverso il calcolo della regressione lineare e i grafici di Bland-Altman, ottenendo come risultati uno slope compreso tra 0,945 e 1,098, un'intercetta compresa tra -1,02 e 7,75, e un indice di correlazione R^2 compreso tra 0,918 e 0,993. La distribuzione dei valori su un'ampia scala di lettura e i bias sempre inferiori ai limiti per il Total Allowable error definiti da CLIA (Clinical Laboratory Improvement Amendments) e CAP (College of American Pathologists) , permettono di affermare che le misure ottenute con GEM Premier 5000 sono perfettamente allineate con quelle del GEM Premier 4000.
Il modello GEM Premier 5000 si è contraddistinto per la semplicità e per l'intuitività d'uso , per la rapidità di analisi e l'innovativo sistema di assicurazione della qualità iQM2 in grado di identificare le possibili non conformità anche durante il processo di analisi.

P003

VALUTAZIONE DELLA QUALITA' ANALITICA NEL MONITORAGGIO DELLA GLICEMIA: CONFRONTO TRA SISTEMI POCT E ANALIZZATORE MULTIPARAMETRICO CON METODICA DI RIFERIMENTO

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Introduzione: Il monitoraggio della glicemia in ambiente ospedaliero riveste un ruolo fondamentale nelle situazioni di iperglicemia e ipoglicemia, spesso iatrogena e dovuta ai protocolli di "Tight glycemic control". Durante il ricovero il paziente può essere sottoposto a numerose misure della glicemia anche con sistemi differenti: analizzatori multiparametrici, emogasanalizzatori, glucometri. Risulta pertanto indispensabile garantire misure della glicemia che soddisfino le specifiche di qualità indicate dalle principali norme e linee guida (ISO 15197:2013, CLSI-POCT12-A3 e FDA 2014) al fine di minimizzare gli errori della terapia.
Scopo del lavoro: Valutare la qualità analitica dei sistemi di monitoraggio della glicemia in ambito ospedaliero, confrontando l'accuratezza delle misure della glicemia su campioni di sangue eseguite su sistemi POCT - glucometro Roche AccuChek Inform II (ACI II, GDH) ed emogasanalizzatore Siemens RapidPoint500 (RP500, GOD)- in relazione al metodo di riferimento (Esochinasi HK) su analizzatore multiparametrico Roche Cobas C6000.
Materiali e Metodi: Reclutati 159 campioni di sangue (left-over) raccolti in siringhe preeparinate; 52 scartati per ridotta stabilità del campione tra la prima e la seconda misura (Δ > 4%). Protocollo di studio di utilizzato: Mahoney. Il confronto fra metodi è stato valutato utilizzando il software EP Evaluator (Data Innovations).
Risultati: Imprecisione dei metodi. I CV% di ACI-II e RP500, rispettivamente pari a 1,81%-3,37% e 0,9%-3,0%, soddisfano i requisiti di precisione proposti dalle linee guida. Confronto tra metodi. GDH vs HK y=0.94x+3.98 (R 0.99) GOD vs HK y=1.05-4.17 (R 0.99). Griglia degli Errori di Clarke: GDH vs HK: 94% delle misure in zona A, 6% in zona B. GOD vs HK: 100% delle misure in zona A. Rispetto dei requisiti di accuratezza di ISO 15197:2013, CLSI POCT12 A3 e FDA (2014): percentuale di campioni che rispettano il requisito. Per ACI II, rispettivamente 91%, 89% e 81%. Per RP500, rispettivamente 100%, 99,5%, 99,1%.
Conclusioni: ACI II e RP500 presentano una buona correlazione con il metodo di riferimento, anche se ACI II non è risultato essere sufficientemente accurato rispetto ai requisiti richiesti dalle linee guida.
Mahoney J, et al. Diab Tech Ther 2007.

P004

ANALISI LEAN DEL SISTEMA POINT OF CARE TESTING (POCT) DELLE EMOGASANALISI. UN APPROCCIO PRELIMINARE.

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Introduzione: L'Unità Operativa Laboratorio Analisi Chimico Cliniche dell'Ospedale San Gerardo di Monza gestisce da più di 10 anni il POCT ospedaliero secondo il sistema di gestione della qualità ISO 9001, occupandosi in particolare di emogasanalisi e glicemie. Attualmente il laboratorio gestisce 16 emogasanalizzatori e 60 glucometri; annualmente si eseguono 135.000 emogasanalisi e 120.000 glicemie in POCT. L'attuale fornitura prevede un servizio biennale di Rapid Improvement Events (RIE) sui processi di POCT, secondo metodologia Lean come indicato nel sito del UK National Health Service.
Scopo del lavoro: Valutazione pilota dei processi POCT mediante RIE secondo metodologia LEAN per minimizzare i processi senza valore e migliorare le performance di processo.
Materiali e Metodi: Sono stati coinvolti 3 reparti con POCT, 5 tecnici di laboratorio,2 medici, 2 coordinatori infermieristici, l'attuale fornitore di emogasanalizzatori (Siemens Healthcare). L'approccio adottato si è sviluppato nei seguenti punti: conoscenza dell'organizzazione dei processi POCT, utilizzo di un assessment standard per consentire una comparazione diretta dei siti, acquisizione delle informazioni sui processi e pratiche lean in uso, evidenza delle best practices e delle aree di miglioramento, feedback dei risultati al team POCT e discussione del piano d'azione per i prossimi steps.
Risultati: Sono stati valutate 6 categorie gestionali: "Housekeeping", "Visual controls", "Maintenance", "Inventory", "Standard work", "Training", "Quality commitment", in una scala di punteggio da 0 (peggiore prestazione) a 5 (migliore prestazione). E' stato riscontrato uno score medio di 2, con alcune lacune sul rischio biologico e sull'identificazione del campione.
Conclusioni: Il RIE sui 3 reparti ha permesso di migliorare immediatamente aspetti di sicurezza dell'operatore e di sicurezza nell'identificazione del campione. La buona base di partenza ci induce ad estendere la prossima valutazione a tutti i reparti con POCT, con l'obiettivo di raggiungere uno score medio di 3 entro 1 anno. A nostra conoscenza questa è la prima esperienza italiana di un approccio Lean ai sistemi POCT.
www.institute.nhs.uk/quality_and_value/rie/rapid_improvement_events_%E2%80%93_introduction.html

P005
INTERFERENZA DELLE PROTEINE TOTALI PER LA DETERMINAZIONE DEL SODIO IN TERAPIA INTENSIVA NEONATALE

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La determinazione del sodio è di particolare rilevanza nella popolazione neonataleScopo del lavoro E' stato quello di confrontare per la determinazione della sodiemia il metodo della potenziometria (ISE) diretto con il metodo della potenziometra (ISE) indiretto. IL primo eseguito con emogasanalizzatore (GEM 4000 Werfen) in Terapia Intensiva Neonatale, e il secondo in Laboratorio su piattaforma automatizzata (Dimension Vista Siemens). E' stata anche valutata la possibile interferenza delle TP (Dimension Vista Siemens) sul dosaggio del sodio con il metodo indiretto Materiale e metodo Nel periodo Gennaio - Maggio 2017 è stata misurata la concentrazione di Na su 131 campioni di pazienti afferenti alla U.O. della TI N . Il dosaggio è stato eseguito il primo con ISE diretto, su sangue intero (siringa litio eparina o capillare), il secondo con ISE indiretto su siero. E' stata valutata anche la concentrazione delle proteine totali, e individuate 5 classi per differente concentrazione Risultati Na POCT: m = 134 mmol/L (± 3.84 DS) ; Na Lab : m = 141 mmol/L (± 3.96 DS). La concordanza tra i due metodi è stata effettuata con la retta di regressione di Passing Bablok y = -7 +1x r 0,57 (95% CI 0,450 a 0,682). La differenza delle medie dei due metodi ha evidenziato un delta pari a - 7; La media di tutte le determinazioni delle TP = 5.12 g/dL (± 0.89 DS). TP classe I (2.8g/dL - 3,9 g/dL) m = 3.61 g/dL (±0.33 DS) ; TP classe II (4,0g/dl- 4.9g/dL) m = 4.56 g/dL (± 0.08 DS); TP classe III (5.0g/dL – 5.9g/dL) m =5.41 g/dL (± 0.16 DS) ; TP classe IV (6.0 g/dL-6.9 g/dL) m = 6.37 g/dL (±0.25); TP classe V (7.0 g/dL-7.7g/dL) m = 7.28 g/dL (±0.27 DS). La differenza delle medie dei due metodi rapportata alle classi : classe I delta Na = -8,58 ; classe II delta Na = - 7,47; classe III delta Na = -6.44; classe IV delta Na = - 5.31; classe V delta Na= - 2.0 Conclusioni I dati mostrano una differenza tra i due metodi statisticamente significativa e un possibile effetto interferente positivo delle TP con incremento del Na misurato con il metodo ISE indiretto (effetto ioni esclusione). Al fine di garantire l'accuratezza del risultato del Na nei pazienti della popolazione neonatale, il metodo più appropriato appare essere ISE diretto.

P006
ANALYTICAL PERFORMANCE OF THE NEW POINT OF CARE (POC) ANALYZER GEM PREMIER 5000 IN COMPARISON TO RAPIDPOINT 405 SYSTEM

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Background: The GEM Premier 5000 is the newest, recently commercially available, critical care analyzer from Instrumentation Laboratory, providing rapid analysis of whole blood samples in a POC as well as in a central laboratory setting. Imprecision and method comparison studies were carried out for GEM Premier 5000 and for the routine POC RapidPoint 405 (Siemens Health Care Diagnostics) according respectively to the EP15-A3 and EP09-A3 CLSI guidelines. The effectiveness of the mixing procedure on sample's pre-analytical homogeneity was also studied. Methods. Evaluated parameters: pH, pCO_2, pO_2, Na^+, K^+, Cl^-, iCa^{++}, glucose, Hct, sO_2, tHb, O_2Hb, COHb, MetHb, HHb. Study period: 11/7/16 - 2/2/17. Imprecision: n=3 levels of n=2 quality control materials provided by manufacturers were used (GEM System Evaluator; RAPIDQC Complete); mean, SD and CV% were calculated. Method comparison: n=362 residual routine lithium-heparin whole blood samples were analyzed calculating Deming regression, Passing-Bablok regression and Bland-Altman plot where appropriate; criteria for refusing sample/result: >2 minutes between replicates, poor sample homogeneity [tHb and Hct bias>allowable total error (TEa); tHb or Hct bias>1.5 TEa], any result with error alarm. Acceptance criteria: total variability (SD or CV%) <1/2 TEa; 0.90<slope<1.10; r >0.95; >95% of points within the TEa in the Bland Altman plot. Results: Imprecision: no data were excluded (no pre-analytical errors occurred); results were within the acceptance criteria excluding for pCO_2 on RapidPoint 405 using level 1 (mean=79 mmHg, CV=6.07%). Method comparison: pCO_2, Na^+, COHb and MetHb did not pass the acceptance criteria for r while K^+ and Hct for slope; however, K^+, Hct, COHb and MetHb met the acceptance criteria for Bland-Altman plot while pCO_2 and Na^+ showed a significant bias, maybe attributable to inherent differences between POCs. A clear improvement of samples' pre-analytical homogeneity was observed applying a better method of sample mixing.
Conclusions: The Gem Premier 5000 system showed satisfactory imprecision performance over the reported measuring ranges for the studied parameters. Together with its high practicability, the observed results from the method comparison study confirmed its reliability for clinical use.

P007

MONITORAGGIO DELLA PERFORMANCE ANALITICA DI DUE EMOGASANALIZZATORI E VERIFICA ALLINEAMENTO RISULTATI CON IL LABORATORIO DI RIFERIMENTO SECONDO LE RACCOMANDAZIONI SIBioC

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Introduzione: La Raccomandazione Sibioc per l'implementazione e la gestione dei POCT ne stabilisce la piena responsabilità al laboratorio, al fine di proteggere il paziente dal rischio di errori, individuando come compito dei tecnici di laboratorio, affiancati dal dirigente sanitario, la verifica delle prestazioni del sistema e dell'allineamento fra risultati POCT e laboratorio di riferimento. All'Ospedale del Cuore di Massa il laboratorio ha applicato le attività descritte nel documento Sibioc alla gestione degli emogasanalizzatori presenti nei reparti.
Obiettivo: Verificare l'allineamento dei risultati forniti dagli emogasanalizzatori per emoglobina (Hb) e ematocrito (Hct) con quelli ottenuti in laboratorio.
Materiali e metodi: Hb e Hct sono stati misurati su 83 campioni (sangue intero in EDTA) su Sysmex XE2100 e su due ABL800Flex (A. De Mori) situati in due diversi reparti (A e B).
Risultati: L'Hb media misurata (g/dL) è stata 12.1 (±2.5) sui due ABL800Flex (n=83), 11.5 (±2.5) su Sysmex (n=51); l'Hct medio (%) è stato di 37(±7.7) e 34 (±6.7) rispettivamente. Non c'è differenza fra le misure di Hb (test t, p=0.209), mentre per Hct c'è una differenza lievemente significativa (test t, p=0.046). C'è una buona correlazione fra Hb e Hct misurati su Sysmex e su ABL800Flex (Hb ABL=0.001+1.05 Hb Sysmex; r^2=0.99; Hct ABL= -1.99+1.13 Hct Sysmex, r^2=0.96). Il bias medio (Sysmex-ABL) per Hb è -0,52 g/dL (±0,286) e per Hct è -2,47% (±1,70). Il bias sembra essere influenzato in modo lieve dalla concentrazione di Hb e più significativamente dal valore di Hct. Considerando i due strumenti separatamente, il bias medio per Hb (g/dL) è -0.34 (±0.225) su ABL_A e -0.72 (±0.208) su ABL_B; per Hct (%) il bias medio è -2.07(±1.71) su ABL_A e -2,89(±1.60) su ABL_B. I bias ottenuti sui due strumenti sono risultati significativamente diversi (test t, p<0.0001 per Hb ;p=0.027 per Hct).
Conclusioni: L'analisi conferma che i risultati forniti da ABL800Flex per Hb sono perfettamente correlabili con quelli forniti dal laboratorio di riferimento. Il POCT è un modello organizzativo del laboratorio clinico: il monitoraggio continuo e costante sulla qualità della performance degli strumenti da parte dei tecnici di laboratorio ha consentito di individuare un problema tecnico su uno degli strumenti, che ha richiesto un intervento specialistico da parte della ditta.

P008

VALUTAZIONE DEL SISTEMA POCT ACCUCHECK INFORM II ROCHE SECONDO IL PROTOCOLLO CLSI POCT 12-A3:2013

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Introduzione: I glucometri POCT Roche AccuCheck Inform II sono in uso di routine nei reparti dell'Ospedale del Cuore di Massa.Obiettivo. Valutare se la misura della glicemia ottenuta su POCT soddisfa le specifiche di qualità come definite dalla linea guida CLSI POCT 12-A3:2013.
Materiali e metodi: 100 campioni di sangue intero, in litio-eparina, sono stati analizzati in doppio su POCT, poi centrifugati entro 5 minuti dall'analisi. La glicemia su plasma è stata misurata in doppio su DXC600 Beckman Coulter entro 60 minuti dalla centrifugazione, accettando il risultato se la differenza fra i duplicati su DXC600 era <4 (% o mg/dL per glicemie > o <100mg/dL rispettivamente).
Risultati: La glicemia misurata è stata 120±46 mg/dL (media±st.dev, range 50-355). La misura in POCT correla con la misura su DXC600 (r^2=0.98, p<0.001). Per ciascun campione è stata calcolata la differenza fra il risultato in POCT e la media dei risultati su DXC600 (bias), in valore assoluto se glucosio <100mg/dL (n=28), in percentuale se glucosio >100mg/dL (n=72). Nel complesso, il 79% delle misure su POCT si discosta meno del ±5% rispetto al valore su DXC600 e il 100% meno del ±12.5%, dati confermati considerando solo valori di glucosio >100mg/dL; per glicemie <100mg/dL, 20/28 misure su POCT (71%) rimangono entro ±5 mg/dL rispetto alla misura su DXC600, 27/28 (96%) entro ±12 mg/dL e 28/28 (100%) entro ±15 mg/dL. E' stato poi studiato se la concentrazione di glucosio o il valore di Hct hanno influenza sulla variazione del bias.Il bias medio (glucosio POCT -glucosio DXC600) è -0,4 mg/dL; non varia significativamente con la concentrazione di glucosio, restando entro ± 1% per glicemie da 57 a 288 mg/dL; varia però significativamente in funzione dell'Hct (p< 0.0001), fino a-7.4% se Hct 50% (8.6% considerando solo glicemie<100 mg/dl).
Conclusioni: I dati ottenuti mostrano che il sistema risponde ai requisiti di accettabilità richiesti dal documento CLSI, dato che il 100% delle misure si discosta meno del ±12.5 % dalla misura fatta su analizzatore di laboratorio. Si rileva però una variazione statisticamente significativa della misura in POCT in base a variazioni dell'Hct del campione, dato che necessita di essere confermato aumentando la numerosità del campione considerato.

P009
POCT: RAPPORTO RISULTATI EMOGASANALISI

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L'emogas in POCT rappresenta un'analisi particolarmente critica per gli aspetti relativi alla produzione, trasmissione ed interpretazione del rapporto alla luce del numero di informazioni prodotte dai nuovi analizzatori e della necessità di immediatezza di risposta. Obiettivo del lavoro è stato riformulare la modalità di presentazione dei risultati dell'emogas nel rispetto delle ISO 15189:2012 (5.8-Trasmissione dei risultati e 5.10-Gestione del sistema informativo). La nostra rete POCT è costituita da 8 strumenti GEM4000 collegati al GEM WEB PLUS, Information Management System, a sua volta collegato al LIS, distribuiti in Pronto Soccorso, Rianimazione, Pneumologia, Emodialisi e Laboratorio. Presupposto per lo sviluppo della nuova modalità di presentazione dei risultati è stato il confronto con i reparti per capire quali misure o calcoli e quali informazioni fossero loro necessarie: P/F ratio e a-aDO_2 per Rianimazione e RI, O_2Cap per Pronto Soccorso. La realizzazione del nuovo report ha comportato: -configurazione dei singoli strumenti con le caratteristiche adatte al reparto; -creazione di un esame unico con più parametri in grado di gestire con opportune regole le differenti configurazioni (mascheramento di eventuali parametri non eseguiti o calcolati); -riposizionamento dei risultati e dei calcoli nel rapporto con suddivisione in base a equilibrio acido-base, idro-elettrolitico, ossimetria; -suddivisione tra risultati di Emogas e parametri di Ventilazione per valutazione stato generale e stato di ossigenazione; -creazione di commenti necessari all'interpretazione dei risultati quali modalità e tipologia di ventilazione, temperatura e posizione paziente; -creazione di regole di autoverifica e rilascio automatico per la corretta gestione dei dati: segnalazione di presenza di interferenze, campione coagulato, calibrazione fallita; In risposta alla complessità degli esami in modalità POCT è stato creato quindi un rapporto dei risultati "dinamico" in grado di adeguarsi alle esigenze dei vari reparti e di essere di facile e di veloce interpretazione. L'eliminazione di parametri ridondanti e non utili ha permesso di concentrare l'attenzione su quelli realmente necessari. Le informazioni e i commenti aggiunti hanno permesso una miglior comprensione dei valori ottenuti.

P010
CIRCULATING SERUM microRNAs AND DJ-1 IN PATIENTS AFFECTED BY ENDOMETRIAL CANCER

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Aim: Endometrial cancer (EC) is one of the most common female cancers worldwide. In the last few years several studies were carried out for identifying new tumour biomarkers. We have recently reported high serum DJ-1 values in EC patients compared to healthy controls (HC), with better diagnostic performance of DJ-1 than HE4. This follow-up study was aimed to investigate the diagnostic performance of combining DJ-1, miR-454 and miR-193a. Methods: 41 EC patients (65±12 years) and 30 HC (64±13 years) were included. Serum concentration of DJ-1 was measured with an ELISA kit (R&D, Minneapolis, USA). Total RNA was isolated from serum with mirVana PARIS Isolation Kit (Thermo Scientific, Wilmington, Delaware, USA). MiR-454 and miR-193a expression levels were detected with miRNA qRT-PCR. miR-16 was chosen as reference miRNA for normalization of expression levels. The differences between EC patients and HC were evaluated with Mann-Whitney test and correlation was assessed with Spearman's test. Diagnostic performance was defined using receiver operating characteristics (ROC) curve analysis. Results: miR-454 and miR-193a expression levels were significantly higher in patients than in HC (p=0.034 and p=0.007, respectively). No significant correlation was observed between DJ-1 values and miR-193a or miR-454 expression levels. The area under the curve (AUCs) of miR-454 and miR-193a was 0.65 and 0.69, respectively. For DJ-1 the AUC was 0.97. The multi-marker panel (miR-454, miR-193a and DJ-1) yielded an overall AUC of 0.96. Conclusion: These results suggest that use of DJ-1, miR-454 and miR-193a in combination has limited diagnostic performance for identifying EC patients compared to DJ-1 alone. Additional studies are needed to confirm the diagnostic performance of DJ-1 in this clinical setting.

Benati M, Montagnana M, Danese E, et al. The clinical significance of DJ-1 and HE4 in patients with endometrial cancer. J Clin Lab Anal 2017 doi:10.1002/jcla.22223. [Epub ahead of print]

P011

mRNA EXPRESSION LEVEL OF BORIS GENE IN OVARIAN ENDOMETRIOSIS

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Endometriosis represents a common gynecological disease wich affects more than 10% of women of reproductive age. Although it is considered a benign disease, it presents with malignant characteristics. In the last years, the contribution of epigenetic factors to endometriosis is supported by molecular studies. Epigenetic alterations encompasses several different phenomena; one of them is DNA methylation. In the present study, we investigated the mRNA expression of a panel of genes related to DNA methylation. Thirthy ovarian endometriosis specimens and 10 ovarian non-endometriosis tissues from women of reproductive age were obtained at Department of Gynecology of L'Aquila teaching Hospital. The samples tissues were freshly collected in RNA-later solution for RNA extraction. Gene expression was performed by quantitative real-time PCR using specific TaqMan probes for the following genes: DNMT1, DNMT3a, DNMT3b, TET2 and BORIS. Relative quantitative evaluation of mRNAs was performed by the comparative $\Delta\Delta$Ct method. mRNA levels were reported as relative units with respect to 18S gene used as reference gene to normalize each sample. Our results demonstrated that BORIS mRNA level was 15-fold increased in endometriosis samples compared to non-endometriosis ovarian tissues (p=0.038) and a similar but not-significant trend was observed for DNMT3b gene. Contrariwise, no significantly differences were observed for DNMT1, DMT3a, PARG and TET genes between the two groups of samples. Up to today, BORIS expression is observed in the majority of cancer tissues, probably due to global genetic or epigenetic changes typical of malignant transformation. Of note, this is the first study that describes BORIS up-regulation in a benign diseases as endometriosis.

P012

HETEROGENEITY OF BRAF AND NRAS MUTATIONAL STATUS IN MULTIPLE MELANOMAS: FINDINGS FROM MOLECULAR AND IMMUNOHISTOCHEMICAL TESTING

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Multiple primary melanomas (MPM) develop in about 5% of sporadic melanoma patients. The pathogenesis is not completely understood and data on the genetic diversity of MPM are limited. Oncogenic mutations in BRAF and NRAS genes are the most frequent alterations in melanoma, thus finding the best methods to identify mutations in these genes is mandatory for accurate patient selection for target therapy. We aimed first to assess the frequency and distribution of BRAF and NRAS mutations in subsequent melanomas of the same patient and to estimate the intrapatient inter-tumoral heterogeneity of the mutational profiles; secondly, we attempted to evaluate the combination of molecular methods and immunohistochemistry for mutational testing of challenging melanoma samples (i.e thin melanomas) in clinical practice. Ninety-seven FFPE paired samples of primary melanomas and subsequent melanomas were obtained from 44 MPM patients and were analyzed by both molecular methods (by Real-Time PCR and Sanger Sequencing) and IHC (by the VE1 and SP174 antibodies) to test the $BRAF^{V600}$ and $NRAS^{Q61}$ mutations. $BRAF^{V600}$ mutations were detected in 41.2% of melanomas and $NRAS^{Q61}$ in 2.1%. Mutational somatic profile (BRAF, NRAS) was concordant between first and subsequent primary tumors in 63.6% of patients. Concordance rate did not differ between sexes (p=0.95), synchronous or asynconous melanoma (p=0.08) and in melanomas developing at the same or different body site (p=0.31). Based on IHC, 46.4% of melanomas showed positive immunostaining with anti-$BRAF^{V600E}$ antibody, while none was positive for anti-$NRAS^{Q61R}$. The agreement among the molecular testing and IHC for $BRAF^{V600E}$ detection was very good (Cohen's kappa = 0.83, p<0.01) with 88.7% of melanomas presenting similar BRAF mutational results between molecular methods and IHC immunostaining. The $BRAF^{V600E}$ VE1 antibody sensitivity is 86.0%, the specificity is 100% while the positive predictive value is 100% and negative predictive value of IHC is 95.7%. Considering the NRAS testing, all melanomas wild-type or non-Q61R on molecular analysis were negative for the anti-$NRAS^{Q61R}$ immunostaining, showing a complete concordance among the two methods. Our results support the heterogeneity of molecular profiles in MPM of the same patient with implication in clinical practice due to the difficulties in classifying patients with discrepant primary melanomas. We demonstrated that combining molecular methods with IHC for BRAF and NRAS mutational testing was a reliable diagnostic tool to face challenging samples of melanoma.

P013
REGOLE DI APPROPRIATEZZA NEL LABORATORIO DI BIOLOGIA MOLECOLARE EMATOLOGICA

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Introduzione: il laboratorio ha impostato un documento dove vengono presi in considerazione i principali esami per i quali si registrano prescrizioni "inappropriate" indicando le relative regole di appropriatezza prescrittiva da applicare nelle prescrizioni a scopo diagnostico, basandosi su Linee Guida ed Evidenza Clinica, al fine di ottimizzare la richiesta di esami provenienti da tutti i reparti della AOUP e ASL limitrofe.
Scopo del lavoro: L'obiettivo di questo lavoro è stato quello di impostare per le neoplasie linfoidi a cellule B una flow chart e di verificare la ricaduta delle regole di appropriatezza impostate. Per queste patologie gli esami richiesti sono il riarrangiamento della catena pesante delle immunoglobuline (IgH) e il riarrangiamento delle famiglie dei segmenti genici VH (VH famiglie).
Materiali e metodi: Per gli esami oggetto dello studio, di fronte ad una richiesta contemporanea per ricerca del riarrangiamento IgH e VH famiglie, si esegue in primis l'analisi IgH (amplificando la regione CDR3); se il risultato è positivo (clonale) si ha già la risposta al quesito diagnostico, se invece abbiamo un risultato negativo (policlonale) o un caso dubbio, allora sarà necessario eseguire anche la seconda richiesta, amplificando un segmento di DNA più grande, utilizzando primers complementari alla regione FR1, posta più esternamente rispetto alla FR3.
Risultati: Nell'anno 2016 sono stati testati 577 campioni per IgH, 211 sono risultati positivi (36.5%). 10 campioni prevedevano anche la richiesta di VH famiglie che non è stata effettuata (4.7%). Dei rimanenti campioni in 32 su 366 (8.7%) è stata effettuata la determinazione delle VH famiglie, come da richiesta. L'implementazione di queste regole conferma il ruolo centrale del laboratorio nel fornire ai medici prescrittori indicazioni per la richiesta di esami "giusti" che danno un "valore aggiunto" nella condotta clinica/diagnostica/terapeutica.

P014
DISEASE BURDEN MONITORING IN ADVANCED COLORECTAL CANCER PATIENTS BY LIQUID BIOPSY

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Tumors shed fragments of nucleic acids in the bloodstream. Cancer genetic alterations and epigenetic changes represent markers of tumor origin DNA in blood cell free DNA (cfDNA). We hypothesized that changes in tumor burden could be tracked in liquid biopsy by quantitative analysis of tumor (epi)genetic alterations. We aimed to identify an optimal panel of cancer specific biomarkers for the purpose of monitoring therapeutic outcome in cfDNA in metastatic colorectal cancer (mCRC) patients. In the marker discovery step, we performed genome-wide assessment of DNA methylation in a collection of 149 CRC cell lines and compared it to normal mucosa and blood cells, to minimize false positivity in liquid biopsy tests. A novel five gene methylation signature was defined and validated in silico using methylome data from CRC specimens. In the validation step, digital PCR-based assays were designed for the five markers. Their prevalence was evaluated in tissue (N=112) and cfDNA samples from mCRC patients (N=182) and self-declared healthy donors (N=50). Digital PCR assays were run for hotspots frequently mutated in RAS/BRAF oncogenes. In patients treated with chemotherapy (N=8), methylation dynamics in cfDNA recapitulated tumor burden changes as assessed by imaging, with a decrease preceding partial response, while an increase in methylation anticipated progression. For cases with known mutations in the tumor tissue, KRAS or BRAF mutant levels in cfDNA were consistent with methylation values. In patients treated with the EGFR inhibitor panitumumab (N=5), disease progression was associated to an increase in methylation levels and the emergence of resistance causative alterations (acquired RAS mutations or MET gene amplification). Methylation values in plasma were usually much more abundant than the percentage of mutant RAS alleles, suggesting the presence of diverse drug resistant subclones. Finally, the best methylation change correlated with objective tumor response (as assessed by conventional radiological methods) and progression-free survival in 29 mCRC patients enrolled in the TEMECT clinical trial. Epigenetic and genetic biomarkers could be combined to assess clonal dynamics and allow disease burden monitoring in mCRC patients over diverse treatment courses.

P015
IL cfDNA COME BIOMARCATORE NEL FOLLOW UP DEI PAZIENTI ONCOLOGICI

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Introduzione: La biopsia liquida è un metodo a ridotta invasività per la valutazione dello stato genetico del tumore basato sull'analisi del DNA libero circolante (cfDNA) presente nella componente plasmatica del sangue. Poiché i campioni ematici sono facilmente ottenibili, la biopsia da plasma è da tempo considerata come una promettente metodica non invasiva a integrazione delle tecniche di biopsia tradizionali. Da aprile 2017 è stato implementato il laboratorio di Biologia molecolare con l'introduzione della Biopsia liquida al fine di analizzare il cfDNA ed eventuali mutazioni geniche (EGFR, N-RAS, K-RAS, B-RAF). Lo scopo di questo lavoro è stato valutare quanto il DNA libero circolante possa essere utile per monitorare il follow up della patologia.
Materiali e metodi: E' stato estratto il DNA da plasma di pazienti con neoplasia polmonare, prostatica, mammaria, gastrica e vescicale. Il DNA libero circolante è stato quantizzato con la real-time PCR mediante l'analisi del gene hTERT. I dati sono stati analizzati in relazione allo stadio della patologia ed alla eventuale terapia.
Risultati : Sono stati analizzati 66 pazienti, così divisi (media quantità di cfDNA espressa in ng/ml):
20 casi neoplasia polmonare: 1,9 in trattamento 35 non in trattamento
6 casi neoplasia mammaria: 0,9 in trattamento 42,2 non in trattamento
16 casi neoplasia gastriche: 0,7 in trattamento 2,7 non in trattamento
12 casi neoplasia prostatica: 13,5 in trattamento 17,2 non in trattamento
12 casi neoplasie della vescica: 1,9 in trattamento 2,2 non in trattamento
Nessuna mutazione genica è stata rilevata.
Discussioni e conclusioni: Dai risultati si evince che il cfDNA è un utile biomarcatore per valutare l'andamento della patologia e monitorare la terapia, infatti pazienti non in trattamento hanno mostrato avere una quantità maggiore di cfDNA rispetto ai pazienti in trattamento o con una ridotta invasività della patologia. Si evidenzia che, tale metodica può essere utile come supporto alla diagnostica classica e permette di monitorare la malattia identificando precocemente la ripresa e la presenza di metastasi tumorali durante il follow up dei pazienti, ma non è consigliabile come strumento per effettuare una diagnosi primaria.

P016
STUDIO EPIDEMIOLOGICO DELL'INFEZIONE DA VIRUS DELL'EPATITE C (HCV) IN UN CAMPIONE DI SOGGETTI DI NAPOLI E PROVINCIA

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Premessa: Il miglioramento delle condizioni socio-economiche e l'aumento dei flussi migratori sono fattori che hanno modificato il quadro epidemiologico dell'epatite C nel nostro Paese negli ultimi decenni. Scopo dello studio è stato valutare il riscontro di positività confermata per infezione da HCV in una popolazione di presunti soggetti sani (donatori di sangue di Napoli e Provincia - 2016).
Metodi: Per la rilevazione dell'HCV RNA è stato utilizzato il test Cobas TaqScreen MPX v.2 su sistema Cobas s201 (pool di 6 campioni) (Roche Diagnostics). La ricerca degli anticorpi anti-HCV è stata effettuata con metodica CMIA su piattaforma Architect (Abbott), la conferma con test Immunoblot (MikrogenDiagnostik).
Risultati: Nel 2016 sono stati individuati 36 casi HCV RNA ripetutamente reattivi e anti-HCV+ su 93503 soggetti presi in esame laddove nel 2015 i casi di positività per HCV sono risultati essere 47 su 87819. La gran parte dei soggetti HCV positivi era di sesso maschile (26 M e 10 F) e con ALT nella norma. Ben 13 casi di positività sono stati riscontrati in soggetti stranieri provenienti da Albania, Romania e Ucraina. La positività per HCV è stata rilevata nel 22% delle persone di età compresa tra 18-30 anni, nel 56% nella fascia d'età 31-50 e nel 22% nelle persone d'età compresa tra 51-65 anni.
Conclusioni: Il maggiore riscontro di positività per HCV nei soggetti di età compresa tra 31-50 anni, con una circolazione del virus limitata al di sotto dei 30 anni, attesta l'avvenuta riduzione del rischio di infezione da HCV nel tempo. Nonostante una discreta percentuale di soggetti viremici, il riscontro di positività nei soggetti più giovani risulta basso a testimonianza della limitata trasmissione di questa infezione. In Italia risiedono circa 5 milioni di stranieri (7,4% della popolazione totale) e rispetto lo scorso anno abbiamo assistito ad un aumento di tale popolazione. Rispetto al 2015 le positività per HCV si sono ridotte ma resta alta la percentuale di soggetti stranieri HCV positivi. Questo dato impone particolare attenzione e indirizza verso strategie ed azioni mirate a prevenire la diffusione dell'infezione nella popolazione generale attraverso questi soggetti.

P017
ABERRANT TELOMERE LENGTH IN SERUM CELL-FREE DNA OF ENDOMETRIAL CANCER PATIENTS

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Aim: Telomeres are highly specialized structures constitute by repetitive nucleotide sequences coupled with proteins, occurring at each end of a chromosome in eukaryotic cells. Their function is protect chromosome ends from fusion and degradation and ensure genomic stability. Telomere shortening has been demonstrated in different types of cancer and may thereby increase cancer risk. Despite telomere length (TL) is usually analyzed in leucocytes, it has been recently demonstrated that TL can be evaluated also in plasma or serum cell-free DNA (cfDNA). This study was aimed to investigate the diagnostic performance of TL in endometrial cancer (EC).
Methods: We measured TL in serum cfDNA of 40 EC patients (65±12 years) and 31 healthy controls (HC) (63±13 years).Circulating serum DNA was extracted from serum using QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. TL was measured by quantitative real time PCR as described by Cawthon. The telomere repeat copy number (T) to single-gene copy number (S) ratio was calculated using the comparative Ct method.The differences between EC patients and HC were evaluated with Mann-Whitney test and correlation was assessed with Spearman's test. Diagnostic performance was defined using receiver operating characteristics (ROC) curve analysis.
Results: cfDNA TL were found to be significantly shorter in EC patients than in HC ($p<0.0001$). cfDNA TL showed a good diagnostic accuracy, displaying an area under the ROC curve (AUC) of 0.87 (95% Confidence Interval: 0.79-0.95, $p<0.0001$). No significant correlation was observed between TL and EC stages or grades ($p=0.85$, $p=0.89$).
Conclusions: These results suggest that TL assessment may be an informative genetic biomarker for early EC detection. Further studies will be needed to confirm its diagnostic performance.
Wu X, Tanaka H. Aberrant reduction of telomere repetitive sequences in plasma cell-free DNA for early breast cancer detection. Oncotarget 2015;29795-807.

P018
DHCR7/NADSYN1 GENETIC LOCUS AND MULTIPLE SCLEROSIS RISK

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Background: Multiple Sclerosis (MS) is a neurodegenerative autoimmune disease resulting from a complex interaction of genetic and environmental factors. Considerable evidence support a role of vitamin D in MS pathogenesis. An inter-individual variability in vitamin D status has been reported and genetic factors represent the most relevant contributors accounting for 23-80% of vitamin D variation, as suggested by twin and family-based studies. In the last decade, genome wide-association study (GWAS) and an increasing number of candidate gene studies have identified genes involved in synthesis, metabolism and transport of vitamin D associated with vitamin D status. Recently, a GWAS identified single nucleotide polymorphisms (SNPs) in a novel genetic locus DHCR7/NADSYN1 associated with lower vitamin D levels. DHCR7/NADSYN1 encodes 7-dehydrocholesterol (7DHC) reductase, which catalyzes 7DHC into cholesterol, providing sufficient substrate for vitamin D synthesis. The aim of our study was to investigate the association among vitamin D, SNPs in DHCR7/NADSYN1 gene and MS risk.Methods: A total of 235 subjects from Western Sicily, 105 cases with MS and 130 healthy controls, were included. Serum 25-hydroxy-vitamin D [25(OH)D] levels were measured in both MS patients and controls by high-performance liquid chromatography (HPLC). All samples were genotyped for rs38292251, rs7944926 and rs12785878 of DHCR7/NADSYN1 gene using Real-Time allelic discrimination Taq-Man assay (Applied Biosystems, Forster City, USA).Results: 25(OH)D serum levels were significantly higher in the control group when compared to MS patients (39.1±9.3 μg/l and 21.8±7.2, respectively; $p<0.001$). The genotypic and allelic frequencies distribution of the studied polymorphisms was not found to be significantly different between MS patients and controls. Moreover, all three polymorphisms were not found to be associated with 25(OH)D serum levels. Conclusion: The findings of our study do not support a role of DHCR7/NADSYN1 locus in MS risk.
Agnello L, Scazzone C, Ragonese P, et al. Vitamin D receptor polymorphisms and 25-hydroxyvitamin D in a group of Sicilian multiple sclerosis patients. Neurol Sci 2016;37:261-7. doi: 10.1007/s10072-015-2401-0.

P019

A NEW ROLE OF CYP2R1 IN MULTIPLE SCLEROSIS SUSCEPTIBILITY

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Background: Multiple Sclerosis (MS) is a chronic neurological disease in which a complex interplay between genetic and environmental factors seems to be implicated in the susceptibility. In the last decade, hypovitaminosis D and genetic variants associated with vitamin D-metabolism gain great attention. The aim of our study was to assess SNPs in CYP2R1 genes in relation to serum 25-OH-vitamin D3 levels in MS patients and healthy controls. Methods: 25-OH-vitamin D3 levels and genotyping of CYP2R1-SNPs were analyzed both in 105 MS patients and in 130 healthy controls. In particular, we investigated rs10741657 and rs10766197 of CYP2R1 gene using Real-Time allelic discrimination Taq-Man assay (Applied Biosystems, Forster City, USA). 25-OH-vitamin D3 concentration was assessed on serum by a high-performance liquid chromatography (HPLC). Statistical analysis was performed by a SPSS software (version13.0).Results: The analysis revealed lower 25-OH-vitamin D3 concentrations in MS patients than in controls (39.1±9.3 μg/l and 21.8±7.2, respectively; p<0.001). When comparing genotype distribution and allele frequencies of the two SNPs selected between cases and controls, significant differences were observed only for rs10766197. Minor allele of rs10766197 (A) was significantly represented in MS patients (62% vs 47%, p=0.001). The frequency of GA genotype (heterozygous minor allele carriers) was 46% in MS patients vs 43% in controls (OR 2.19, 95% CI 1.09-4.39, p=0.03) and the frequency of AA genotype (homozygous minor allele carriers) was 39% in MS patients vs 25% in controls (OR 3.18, 95% CI 1.52-6.65, p=0.002), revealing a moderate association of allele A to MS. Analysis of the rs10766197 distribution in MS patients revealed that patients carrying the genotype AA had a trend of lower levels of 25-OH-vitamin D3 in comparison to those with genotype GG or GA, although not statistically significant (GG: 22.3±6.8 μg/L, GA: 22.2 ± 8 μg/L and AA: 19.2 ± 4.3 μg/L). Conclusion: The findings of our study open new perspectives for a role of CYP2R1 in MS risk.

Agnello L, Scazzone C, Ragonese P et al. Vitamin D receptor polymorphisms and 25-hydroxyvitamin D in a group of Sicilian multiple sclerosis patients. Neurol Sci. 2016 Feb;37(2):261-7. doi: 10.1007/s10072-015-2401-0

P020

UPLC-MS/MS METHOD FOR THE SIMULTANEOUS QUANTIFICATION OF SOFOSBUVIR, SOFOSBUVIR METABOLITE (GS-331007) AND DACLATASVIR IN THE PLASMA OF HCV-POSITIVE PATIENTS TREATED WITH COMBINATION THERAPY

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The emergence of direct-acting antiviral agents (DAAs) represents a major advance in hepatitis C virus (HCV) infection treatment. Therapeutic drug monitoring (TDM) is of interest to verify the adherence and to define a therapeutic range. Able to prevent therapeutic failure or adverse events. Aim of this study was to develop and validate ultra-performance liquid chromatography mass spectrometry (UPLC-MS/MS) method for the determination of sofosbuvir, sofosbuvir metabolite (GS-331007) and daclatasvir in human plasma.A simple protein precipitation extraction was applied by adding to 100 mL plasma sample 200 mL acetonitrile with internal standard 6,7-Dimethyl- 2,3-di(2-pyridyl) quinoxaline.Drug separation was performed on analytical C-18 Luna Omega column (50 mm x 2.1 mm I.D.) with particle size of 1.6 μm. The mobile phase consisting of solution A which is water containing 0.1 % formic acid and phase B consisting of acetonitrile at flow 0.4 mL/min and a gradient run time of 3.5 minutes. The injection volume was 10 mL. Anti-HCV drugs were detected in positive electrospray ionization mode. The full scan mass spectral analyses of sofosbuvir, GS-331007, daclatasvir and quinaxoline showed protonated molecule ions and transitions m/z: 530.098>243.02, 260.93>112.94, 739.4>339.27 and 313.03>77.99 respectively.The linearity of standard curves was excellent (r^2 >0.99), the absolute recovery of anti-HCV drugs ranged between 95 and 98%, the matrix effect was < 5% and both precision and accuracy were <15% according to recommendation FDA guidelines. This method was applied to 7 HCV patients treated with combination therapy. In these patients the mean value was 191.7 ± 208.21 ng/mL and 443.5 ± 290.5 ng/mL for daclatasvir and GS-331007 respectively. While sofosbuvir was undetectable in all samples.These results were according with others pharmacokinetic studies that demonstrated that GS-331007 accounted for >90% of systemic drug-related exposure after intracellular activation of sofosbuvir.The UPLC-MS/MS method here reported is sensitive, specific, robust, fast ad requiring low plasma volume allowing simultaneous determination of sofosbuvir, GS-331007 and daclatasvir in human plasma.

P021

A STANDARDIZED NOVEL MOLECULAR ALGORITHM TO PREDICT RELAPSE IN CUTANEOUS MALIGNANT MELANOMA

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Objective: The aim of this study was to evaluate if our molecular algorithm, based on tumor circulating transcripts, may predict relapse risk in cutaneous malignant melanoma (CMM).
Methods: Peripheral blood was collected from 111 CMM patients and from 87 healthy controls (HC) randomly selected. Each specimen was examined by qRT-PCR analysis for the expression of 3 tumor-related transcripts (PAX3d, MITF-m and TGF-β2) at diagnosis, and at the following 6 and 12 months during clinical monitoring.
Results: The multi-marker panel was able to differentiate patients with CMM from HC with high diagnostic sensitivity and specificity, especially for MITF-m and TGF-β2 (91-100%) whose levels decreased during follow-up of recurrence-free patients, and remained stable in the case of relapse. PAX3d higher than 2.76 copies/μL emerged as a promising biomarker [specificity=90% and negative predictive value=87%] to stratify subjects at high risk of CMM recurrence independently of age, gender and AJCC staging [OD=9.5(3.2–28.0), $p<0.001$]. The survival analysis confirmed PAX3d performance in relapse prediction with significant differences in recurrence risk 12 months after the basal time-point ($p=0.008$).
Conclusions: We demonstrated the usefulness of our molecular algorithm to indirectly detect circulating melanoma cells in blood, along with PAX3d capability to assess patients' progression and relapse prediction.

P022

CEACAM5 mRNA IN BLOOD AS PROGNOSTIC BIOMARKER OF EARLY RECURRENCE IN LIVER METASTATIC COLORECTAL CANCER PATIENTS

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Background: Several prognostic criteria have been proposed to improve the selection of the best therapeutic strategy for patients with colorectal liver metastases (CRLM), including circulating tumor cells (CTCs) detection by quantitative reverse transcription-polymerase chain reaction (qRT) of specific transcripts. Although tumor specific transcripts have been associated with outcome, new prognostic and predictive factors remains to be identified.
Methods: Peripheral blood was collected from 29 CRLM patients at the time of the liver resection vs 30 normal controls. Follow-up draws (FUDs) were also performed at 30 days, 3 months and 12 months from the surgery. On each specimen CEACAM5 (CEA), ERCC1 and GAPDH mRNAs were examined by qRT analysis. Assay detection limit was performed by mixing serial dilutions of HCT 116 with donor-derived peripheral blood. Standard curves were generated by making recombinant clones: expression of each marker was normalized for the GAPDH housekeeping gene as a ratio.
Results: CEA levels transcript was linearly correlated with numbers of spiked cells (qRT analytical limit= 5 cells). Among 13 patients (9 M/ 4 F; mean age 63 years, range 48-77; 8 with multiple CRLM, 11 with synchronous CRLM) who completed the FUD track, highly significant baseline level of CEA was detected in those with relapse (35.4%), as for the remaining check-points FUDs ($0.07 < p < 0.05$). The main differences were found by comparing the 12 months FDUs. Similar behaviors were observed when patients with relapse (29.5%) were compared with those recurrence-free. Extremely high levels of CEA at their 30 days and 90 days FUDs were also detected in 23.5% of patients who died during the follow-up. As expected, lower levels of expression of ERCC1 were detected in patients under chemotherapy regimen during the overall period of follow up.
Conclusions: Blood CEA-mRNA absolute copy number assay can represent a valid early predictor of relapse in CRLM patients. Perspective studies in the context of large clinical trials will provide further data to also qualify ERCC1 as a predictive biomarker for selection therapy.

P023

NEXT-GENERATION SEQUENCING-BASED METHODOLOGY INCREASES THE DIAGNOSTIC SENSITIVITY OF MOLECULAR DIAGNOSIS AND SPEEDS-UP DUCHENNE MUSCULAR DYSTROPHY GENE ANALYSIS

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Duchenne muscular dystrophy (DMD) is the most common inherited neuromuscular disease. It is a consequence of inherited mutations in the dystrophin gene, mostly deletions and/or duplications, more rarely, point mutations [1]. To date the molecular diagnosis of DMD is a lengthy and complex process due to the wide mutational spectrum, the large size of the gene and the multiple tests needed for complete molecular screening. In fact, the procedure entails MLPA/mPCR to detect deletions/duplications and Sanger sequencing to detect point mutations in order to identify all possible DMD mutations [2]. The introduction of next-generation sequencing (NGS) has improved molecular diagnostics and may be a suitable method for the detection of all types of mutations spanning the 79 exons of the dystrophin gene, as well as some regulatory regions. The purpose of this study was to test this hypothesis; therefore, we evaluated the efficacy of a NGS-based approach in 40 DMD patients, previously analyzed by MLPA and/or mPCR and/or Sanger sequencing. To assess the accuracy in mutation detection of the new method, patients were selected who carried different DMD mutations spanning throughout the DMD gene and analyzed in blind. Libraries were prepared using the DMD MASTR (Multiplicom) according to the manufacturer's instructions and sequenced on the Illumina MiSeq platform. The downstream data were analyzed using the Sophia DDM Software (Sophia Genetics). The NGS results were compared with those from MLPA/mPCR or Sanger sequencing. All previously identified deleted/duplicated exons and point mutations were confirmed by NGS. Furthermore, an additional point mutation was identified in a DMD patient classified "wild type" in the earlier previous MLPA and mPCR analysis. Our results show that the proposed NGS-based method is able to correctly detect all the different types of mutations affecting DMD, including underestimated mutational events not detected by currently used technologies. Thus, we propose that this NGS-based method be used to improve DMD molecular diagnosis and overcome limitations of traditionally used methods.

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P024

A NEXT-GENERATION SEQUENCING-BASED APPROACH IMPROVES AND SPEEDS-UP THE MOLECULAR NEONATAL SCREENING OF CYSTIC FIBROSIS

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The life expectancy of patients affected by cystic fibrosis (CF) largely depends on an early diagnosis (1). Consequently, newborn screening for CF (NBSCF) is now considered standard care. In this context, CFTR molecular screening usually consists in the analysis of a panel of the most common CFTR mutations, which depends on geographic prevalence. This practice could lead to false negative results, also because of frequent population migration, and to longer and more expensive molecular tests, if multiple analytic steps are required to increase the analytic and diagnostic sensitivity. Next-generation sequencing (NGS) technologies are progressively imposing new standards for molecular tests, also in the diagnostic field. Moreover, they have several advantages in the context of NBSCF (2). In this context, we validated a NGS-based procedure that detects both point mutations and copy number variations as a single-step-assay, which would increase NBSCF diagnostic sensitivity and lead to an earlier CF diagnosis. To this aim, we carried out a pilot study by analyzing, in blind, 80 dried blood spots, already processed by conventional methods and carrying different CFTR mutations. Briefly, we amplified CFTR coding regions (including their flanking sites) using the CFTR MASTR Assay kit (Multiplicom) to obtain a library/sample. The subsequent sequencing reactions were performed with the MiSeq System (Illumina) to simultaneously analyze all 80 samples. Finally, the downstream data were analyzed using the SeqNext (JSI Medical Systems) and the Sophia Genetics software. Comparative sequence analysis showed that the proposed method is reliable, since all the previously identified variants were confirmed. Additionally, 4 previously missed mutations were also identified. Therefore, our NGS-based analytic workflow improves NBSCF in terms of time and sensitivity of the molecular test, and can be easily applied in a routine lab context.

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P025

NUOVI APPROCCI DIAGNOSTICI IN ONCOLOGIA MOLECOLARE: DNA PLASMATICO E URINARIO PER IL MONITORAGGIO DELLE RESISTENZE ALLA TERAPIA IN PAZIENTI CON NON SMALL CELL LUNG CANCER (NSCLC)

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Introduzione: Nei pazienti con NSCLC, la rilevazione delle mutazioni a carico del gene EGFR (Epidermal Growth Factor Receptor) in campioni citologici o istologici è un passo fondamentale nella scelta della strategia terapeutica. Nei pazienti con materiale biologico inadeguato o non disponibile, l'analisi del DNA tumorale libero circolante nel plasma (cfDNA) rappresenta una valida alternativa. Recentemente è anche stata descritta la possibilità di utilizzo del DNA urinario (UcfDNA) [1].
Scopo: Valutare il ruolo di UcfDNA e l'utilizzo in routine del cfDNA nella diagnosi e follow-up di pazienti con NSCLC.
Materiali e metodi: Da gennaio 2017 abbiamo analizzato DNA tissutale di 134 pazienti con NSCLC; dai 24 (17%) che hanno mostrato mutazioni a carico di EGFR, abbiamo raccolto 20 campioni di sangue e 10 di urina. Entro 2 ore dal prelievo il sangue è stato sottoposto a doppia centrifugazione (4°C/2500g/10' e 4°C/3500g/10') e singola per l'urina (15°C/16000g/10'). Il plasma ottenuto (4mL) e il surnatante urinario (8mL) sono stati stoccati a -80°C fino all'analisi. cfDNA e UcfDNA sono stati estratti mediante kit "Cobas cfDNA Sample Preparation" e amplificati mediante RT-PCR su COBAS Z480 usando il kit "Cobas EGFR Mutation Test-v2" (Roche).
Risultati e conclusioni: L'analisi su cfDNA ha mostrato in 12 pazienti (60%) concordanza con i risultati ottenuti in precedenza sui campioni di DNA tissutale, inoltre ha permesso di analizzare e identificare mutazioni a carico di 4 pazienti (20%, 3 wild type e 1 delezione esone 19) che non disponevano di materiale tissutale adeguato. L'analisi molecolare su campioni di UcfDNA non permette ad oggi di trarre conclusioni significative sull'utilizzo di questo materiale biologico in quanto i nostri dati non sono confrontabili con quelli ottenuti su tessuto/plasma e con la letteratura scientifica. Tale discordanza potrebbe essere dovuta alle modalità di raccolta del campione (non standardizzata), alla bassa concentrazione di UcfDNA e anche alla minore sensibilità della RT-PCR rispetto alla Droplet Digital PCR, metodica utilizzata nei pochi lavori disponibili in letteratura [1].

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P026

DISSECTING GENETIC HETEROGENEITY IN TUMORS USING DIELECTROPHORESIS (DEP) ARRAY METHODOLOGY

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Cell-to-cell differences are always present to some degree in any population of cells, and the ensemble behaviors of a population may not represent the behaviors of any individual cell. Phenotypic and functional heterogeneity arise among cancer cells within the same tumor as a consequence of genetic change and/or environmental differences which change cellular properties. However, it remains unclear how this heterogeneity may account for therapy resistance and disease progression.The development of technologies in detection of single cancer cells within an heterogeneous sample will be sure to improve the diagnosis and treatment in cancer resulting in a positive prognosis.We used the advanced DEPArray technology platform to individually identify, manipulate and sort single cancer cells within two heterogeneous neuroblastoma cell lines (SKNBE2c, IMR32). The cells were visualized and analyzed based on GD2 antigen staining. At least 20 single cells-sorted for each cell line were recovered and subjected to whole genome amplification (WGA), followed by comparative genomic hybridization array (aCGH) and next generation sequencing (NGS) analysis. Genomic analysis of isolated single cells revealed the existence of two main subpopulations within the same cell line. Particularly, one cells population is characterized by subchromosomal deletion of chromosome 1, especially of 1q31-p21 region and subchromosomal duplication of chromosome 2, especially of 2p25-p22 region. The other cells population is characterized by subchromosomal duplication of chromosome 1, especially of 1p21-q13 region. The gain or loss chromosomal regions may contain genes or portion of genes important for therapy response. In fact, the amplified copies of oncogene N-Myc located in the 2p25-p22 region confer resistance to some treatments used in the therapy of neuroblastoma; patients with amplified N-myc have markedly poorer prognosis than those in which N-myc copy number is not elevated.Our preliminary data show that DEP-array cells isolation is a well established methodology to examine tumor genetic heterogeneity. Furthermore, deep-sequencing of the collected single cells may be useful to examine the evolutionary relationships of mutations during disease progression and therapy response.

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P027

GENETIC SCREENING IN PATIENTS SUFFERING FROM SEVERE HYPERTRIGLYCERIDEMIA

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Introduction: Severe hypertriglyceridemia is characterized by very high levels of plasma triglyceride (TG) levels, usually more than 10 mmol/L and caused by mutations in the Lipoprotein lipase (LPL), Apolipoprotein A-V (APOA5), Apolipoprotein C-II (APOC2), Glycosyl-phosphatidyl-inositol-anchored HDL-binding protein (GPIHBP1), and Lipase maturation factor-1 (LMF1) genes. Transmission is mainly autosomal recessive although some dominant cases have been described.
Methods: Twenty-one unrelated patients with severe hypertriglyceridemia were recruited based on fasting plasma TG>10 mmol/L. The presence of diabetes mellitus (when started before pancreatitis), alcoholism, renal disease, untreated hypothyroidism, estrogen use and autoimmune diseases were the exclusion criteria. The coding regions with the flanking intronic regions of LPL, APOA5, APOC2, GPIHBP1 and LMF1 genes were amplified by PCR and directly sequenced. Multiplex ligation-dependent probe amplification (MLPA) was used to search for large rearrangements in the LPL gene. For APOE gene, the presence of E2 and E4 alleles were evaluated.
Results: We found 18 different rare variants (MAF<0,01), classified based on the American College of Medical Genetics and Genomics guidelines, like pathogenic, likely pathogenic and uncertain significance variants (USV). In particular 3 patients had 2 pathogenic variants, 6 patients had 1 pathogenic variant while 12 patients had no pathogenic variant. No statistical differences of lipid levels were observed between patients carrying 2, 1 or none pathogenic variants. No statistical differences of E2 or E4 carriers frequencies have been observed between patients carrying 2, 1 or none pathogenic variants.
Conclusions: Our results indicate the high heterogeneity of genetic background of severe hypertriglyceridemia, the importance to evaluate the pathogenicity of USV by in vitro assay and the importance to identify rare variants in other gene or in other gene's regions by NGS.

P028

INTEGRITY AND QUANTITY OF TOTAL CELL-FREE DNA IN THE DIAGNOSIS OF THYROID CANCER: CORRELATION WITH CYTOLOGICAL CLASSIFICATION

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Cell-free DNA (cfDNA) quantity and quality in plasma has been investigated as a non-invasive biomarker in cancer. Previous studies have demonstrated increased cfDNA amount and length in different types of cancer with respect to healthy controls. The present study aims to test the hypothesis that the presence of longer DNA strands circulating in plasma can be considered a biomarker for tumor presence in thyroid cancer. We adopted a quantitative real-time PCR (qPCR) approach based on the quantification of two amplicons of different length (67 and 180 bp respectively) to evaluate the integrity index 180/67. Cell-free DNA quantity and integrity were higher in patients affected by nodular thyroid diseases than in healthy controls. Importantly, cfDNA integrity index was higher in patients with cytological diagnosis of thyroid carcinoma (Thy4/Thy5) than in subjects with benign nodules (Thy2). Therefore, cfDNA integrity index 180/67 is a suitable parameter for monitoring cfDNA fragmentation in thyroid cancer patients and a promising circulating biomarker in the diagnosis of thyroid nodules.

P029

MOLECULAR ANALYSIS HAS ALLOWED THE DEFINITIVE DIAGNOSIS OF MULTIPLE ACYL-COA DEHYDROGENASE DEFICIENCY (MADD)

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Multiple acyl-CoA dehydrogenation deficiency (MADD) is a rare autosomal recessive disorder due to defects in the electron transfer flavoprotein (ETF) or in the electron transfer flavoprotein dehydrogenase (ETFDH) enzymes, involved in the mitochondrial electron transport chain. Patients with MADD fall into different clinical phenotypes, ranging from a severe neonatal presentation, with metabolic acidosis, cardiomyopathy and liver disease to a mild childhood/adult disease, with episodic metabolic decompensation, muscle weakness and respiratory failure.Nowadays, the MADD diagnosis is established by the presence of dicarboxylic organic acids and acylglycine derivatives in the urine and increased levels of medium- and long-chain acylcarnitines in the blood. Mutations in ETFA, ETFB, ETFDH genes, encoding for alpha and beta subunits of ETF and for ETF-dehydrogenase are associated with MADD. We report the case of a three years old child, affected by lethargy and asthenia associated with anorexia. Biochemical analyses showed hypoketotic hypoglycemia with remarkable increments in transaminases, lactic dehydrogenase, aldolase and creatine kinase. The chromatographic layout of urinary organic acids showed a typical dicarboxylic aciduria. Thus, based on these features, MADD was suspected. Fifteen years later, at the age of 19, MADD diagnosis was confirmed by molecular analysis, showing a compound heterozygosity for the mutations c.1074G>C (p.R358S; HGMD: CM031670 in HGMD database) and c.1073G>A (p.R358K) in the ETFDH gene. The c.1073G>A (p.R358K; rs796051959) mutation is reported in ClinVar database as pathogenic allele, although lacking link to a specific clinical condition. However, familial segregation study and in silico analysis, performed by bioinformatics tools, confirmed that this substitution is likely pathogenetic. Her parents were healthy carriers of one of the two mutations. It is known that the severity of the clinical phenotype of MADD may be related to the type of mutation in the ETFA/ETFB/ETFDH genes. Particularly, missense mutations in the ETFDH gene, leaving a detectable residual enzyme activity, may account for the milder form of the disease, as is the case here. In conclusion we suggest that molecular analysis is essential to the definitive diagnosis of MADD and to direct the adequate therapeutic management. Thus, through a close nutritional follow up, a few months ago the patient gave birth to a healthy boy.

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P030

A NEW CASE OF CONGENITAL HYPERINSULINEMIC HYPOGLYCEMIA DUE TO M/SCHAD DEFICIENCY: THE CONTRIBUTION OF METABOLIC AND MOLECULAR DIAGNOSIS FOR THE MANAGEMENT

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Congenital Hyperinsulinemic Hypoglycemia (CHH) is a rare metabolic disease (prevalence <1/1.000.000) characterized by a persistent hypoglycemia and high secretion of insulin in the neonatal and infancy period. An early management of patients with CHH is mandatory to avoid brain damage. Recent advances in molecular analysis have linked CHH to mutations in nine genes: ABCC8, KCNJ11, GCK causing either diazoxide-responsive or diazoxide-unresponsive Hyperinsulinemic Hypoglycemia, and GLUD1, HADH, SLC16A1, UCP2, HNF4A and HNF1A, causing generally diazoxide-responsive CHH. However, HADH defect is the most common form in presence of consanguinity and diazoxide-responsiveness. The HADH gene codifies the M/SCHAD mitochondrial enzyme, which catalyses the penultimate reaction in the β-oxidation of medium and short-chain fatty acids, causing in some affected individuals an elevated plasmatic hydroxybutyrylcarnitine and urinary medium-chain dicarboxylic, and 3-hydroxydicarboxylic metabolites. To date about 40 cases of M/SCHAD defect have been reported in literature.We report here a new case of CHH due to M/SCHAD deficiency. The index case was a Pakistan infant, born from consanguineous parents, showing a diazoxide-responsive hyperinsulinism and organic aciduria. The M/SCHAD deficiency was confirmed by the molecular diagnosis performed by sequencing of HADH gene, which revealed the presence of the nonsense mutation c.706C>T (p.R236*) in HADH gene, at homozygous state, while both parents were heterozygous for the mutated allele. The patient started diazoxide treatment at the maximum dose of 10 mg/kg/day, which resulted in adverse drug reactions (hypertrichosis, peripheral edemas and persistent hypertension) gradually solved with antihypertensive regimen. Diazoxide was progressively titrated to 2 mg/kg/day with good results in glycemic control and no hypertensive crisis. Low organic aciduria was followed.In conclusion, when the metabolic profile suggests a CHH disorder, the molecular analysis is necessary for the precise diagnosis and the appropriate counseling to the parents, also for the possibility of a prenatal diagnosis. In this setting, the definitive diagnosis of CHH, due to M/SCHAD deficiency, may suggest also the most appropriate therapeutic intervention to avoid both risk of worsening or adverse drug effect.

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P031

TARGETED NEXT GENERATION SEQUENCING FOR THE DETECTION OF SOMATIC ALTERATIONS IN LUNG CANCER: A COMPARISON WITH ROUTINE CLINICAL METHODS

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Introduction: In recent years, the identification of molecular alterations involved in specific cancer mechanisms have provided new treatment strategies for lung cancer. Molecular tests to detect activating driver mutations in EGFR and chromosomal rearrangements of ALK gene, are prescribed for the selection of patients most likely to benefit from targeted therapy. The implementation into clinical laboratories of methods, as the next generation sequencing (NGS), able to assess multiple genes in a single analysis is advisable in view of an increasing number of predictive biomarkers.
Aims: Evaluation of an NGS panel for the analysis of hot spot regions in 22 cancer-related genes and of the main fusion transcripts of ALK, ROS1, RET e NTRK1 in NSCLC (Non Small Cells Lung Cancer) FFPE tissue samples. Comparison of the results with the methods applied in the diagnostic routine setting (Sequenom and FISH).
Materials and Methods: RNA and DNA extractions from 16 FFPE samples. NGS analysis by Oncomine Solid Tumour DNA e Fusion Transcript kit on Ion S5 platform and variant calling by Ion Reporter (Thermo Fisher). Reference methods: mutations detection in mass spectrometry by Myriapod Lung status kit (Diatech Pharmacogenetics) on MassARRAY (SEQUENOM); chromosomal rearrangements detection by Fluorescent in situ hybridization (FISH).
Results: In 8 samples a mutation in EGFR was confirmed: 6/6 in exon 19; 2/2 in exon 21; 1/1 in exon 20 for drug-resistance. One sample positive for a ROS1 fusion was correctly identified. An EML4/ALK fusion was detected in 3/5 samples, while for 2/5 RNA was inadequate. Additionally, in a sample without data from FISH analysis, another EML4/ALK was revealed. Four pathogenetic mutations were detected in TP53, reported in literature as potential prognostic factor, in addition to other sequence variants in KRAS, DDR2, FGFR3, STK11, SMAD4, ERBB4 and MET genes.
Conclusion: The application of NGS analysis may provide a complete molecular profile of NSCLCs that gives crucial information on the efficacy of targeted therapy through a single approach. The ability of NGS in the detection of several classes of somatic alterations highlights the potential strong contribution of the methodology in the routine clinical setting.

P032

PRE-ANALYTICAL VARIABLES AFFECTING FREE-CIRCULATING miRNAS MEASUREMENT: SAMPLE MATRIX, CENTRIFUGATION, AND STORAGE CONDITIONS

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Introduction: miRNAs are endogenous non-coding RNAs, post-transcriptionally regulating gene expression [1], that are released into the circulation depending on specific stimulation [2]. Although their clinical use is very close, the pre-analytical variables influencing the analytical output have not been clearly defined [3].
Aim of this study was to define the best sample matrix for free circulating miRNA, their time- and temperature-dependent stability and the assay the effect of platelet removal.
Methods: Venous blood was collected from 10 healthy young males in K2EDTA and PPT tubes (BD). Samples were centrifuged 10min at 1100g. Additional EDTA tubes were further centrifuged 15min 2500g in order to eliminate platelet residues. Plasma aliquots were stored immediately at -80°C or kept at either room temperature or 4°C for 24h before freezing.Micro RNA-enriched total RNA was extracted from plasma using 3 synthetic oligonucleotides (spike-ins: Sp2, Sp4, Sp5) as extraction controls. Reverse transcription and real-time PCR was performed using Sp6 and Cel39 as internal controls and specific primers. A panel of 179 circulating miRNAs was tested. Relative expressions were calculated by the 2^(-ΔΔCT) method using the whole mean for data normalization. Hemolysis was checked by calculating the miR-23a-to-miR-451 ΔCT ratio (positive if >7).
Results: Regardless the storage length and temperature, in pooled samples underwent to platelet depletion, 47-63% of miRNA are undetectable. In standardly centrifuged K2EDTA and PPT samples detectability was increased. Furthermore, in K2EDTA samples the frequency of detectable miRNA was increased by storage while PPT samples were not influenced by storing conditions.Those miRNAs whose fold changes exceed ±5, compared to K2EDTA immediately store at -80°C (used as reference, are currently under evaluation in single samples in order to confirm the effect of the pre-analytical variables.
Conclusions: Based on our preliminary data PPT tubes guarantee a greater stability of circulating miRNAs although the contribution of platelets-derived miRNAs should be relevant.
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P033
ANALISI QUALITATIVA E QUANTITATIVA DI SPLICING ALTERNATIVI MEDIANTE UNA SINGOLA REAZIONE DI DROPLET DIGITAL PCR

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Il gene CFTR codifica per una proteina canale per il trasporto di cloro indispensabile per la corretta idratazione di epiteli in molti organi e tessuti. Il mal funzionamento di questa proteina è causa di Fibrosi Cistica, malattia genetica con un'incidenza di circa 1 su 3000 nati. Ad oggi, sono note più di 2000 mutazioni del gene CFTR e molte di queste alterano il corretto splicing del suo mRNA.La valutazione qualitativa dell'effetto di una variante genetica sullo splicing è una procedura semplice che mediante una RT-PCR ed una separazione elettroforetica mostra eventuali prodotti di splicing alternativi. Di contro, fare una valutazione quantitativa accurata dei prodotti di splicing, al fine di valutare la percentuale di splicing corretto residuo, richiede procedure indaginose e dispendiose. In questo lavoro mostriamo come l'utilizzo della droplet digital PCR (ddPCR) fornisce in un'unica reazione un'accurata valutazione qualitativa e quantitativa dei prodotti alternativi di splicing. In particolare, abbiamo analizzato alcuni campioni di RNA estratti da cellule di epitelio nasale prelevate direttamente da paziente con il polimorfismo T5-TG12, noto alterare lo splicing. Mediante l'utilizzo della ddPCR con tecnologia EVAGreen è stato possibile stabilire che la quota di splicing corretto risulta essere di circa il 50% rispetto al controllo. Questi dati, sebbene preliminari, indicano fortemente che utilizzando una semplice coppia di primer, senza l'utilizzo di sonde particolari, è possibile quantizzare in maniera accurata la percentuale di splicing corretto residuo. Questo è di particolare interesse, soprattutto, nell'analisi di eterozigoti compositi, in cui la mutazione di splicing è associata ad un'altra mutazione severa, e si rende necessario conoscere l'esatto contributo della variante genetica in esame sul processo di splicing. Inoltre, l'analisi del processo di splicing è fondamentale quando predetto da studi bioinformatici anche per mutazioni missense o di altro tipo, ai fini di una consulenza genetica o nella scelta di una terapia.

P034
STUDIO DELLE MUTAZIONI DEL FATTORE V IN PAZIENTI CON APC RESISTENZA

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Introduzione: Il fattore V attivato è un cofattore essenziale per l'attivazione della protrombina a trombina. Il suo effetto procoagulante è inibito dalla Proteina C attivata (APC) che taglia il fattore V attivato in tre parti. La mutazione G1691A, definita variante di Leiden, ne impedisce il taglio da parte della APC con conseguente resistenza alla APC ed una maggiore attività procoagulante che predispone alla trombosi. Riguardano questo sito anche le mutazioni R306T (FV Cambridge) e R306G (FV Hong Kong). Sono state individuate altre mutazioni del Fattore V associate a trombofilia: la G4070A, che causa la sostituzione H1299A, dando vita all'aplotipo HR2 e la Y1702C.
Scopo: Scopo del presente lavoro è stato quello di evidenziare la presenza di mutazioni del Fattore V in una paziente con ricorrenti episodi di aborto spontaneo e con valori borderline della resistenza alla Proteina C attivata. Le analisi sono state poi estese ai genitori.
Materiali e metodi: Per lo studio delle mutazioni G1691A e A4070G nel gene umano del Fattore V sono stati usati il DuplicαRealTime Factor V G1691A e il DuplicαRealTime Factor V H1299R Genotyping Kit (Euroclone, Italia).
Risultato e discussioni: La paziente presentava valori borderline di APC resistenza, con 0,61 (V.N.>0,7). L'analisi genetica per la ricerca delle mutazioni del Fattore V, ha evidenziato una eterozigosi composta (G1691A/Wt-A4070G/Wt). L'analisi estesa ai genitori ha mostrato un valore di APC resistenza di 0,71 e la presenza della mutazione G1691A in eterozigosi nel padre ed un valore di APC resistenza di 1,12 e la mutazione eterozigote A4070G nella madre.
Conclusioni: È ben nota la relazione che intercorre tra la mutazione G1691A del Fattore V e la resistenza alla Proteina C attivata. Diversi studi hanno riconosciuto l'aplotipo HR2 come un fattore di rischio protrombotico, in particolare in quei soggetti che ereditavano in trans l'aplotipo HR2 e la mutazione FV Leiden. Individui doppi eterozigoti FV G1691A e H1299R presentano un rischio di trombosi venosa profonda 3-4 volte maggiore dei soli eterozigoti Leiden. Dai risultati ottenuti si evidenza l'importanza di estendere il pannello mutazionale del Fattore V ai pazienti che presentano valori borderline dell'APC resistenza.

P035

RAPID IDENTIFICATION OF THE FREQUENT BRCA1 EXON 1a-2 DELETION USING THE INNOVATIVE COMPETITIVE PCR-HIGH RESOLUTION MELTING ANALYSIS

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Background: The evaluation of Large Genomic Rearrangements (LGRs) in BRCA1/2 genes represents a crucial step of mutational analysis in hereditary breast and ovarian cancer (HBOC) syndromes. LGRs are scattered throughout the genes, with an increased frequency in BRCA1 compared to BRCA2. In particular, BRCA1 exons 1a-2 represent an important recombination hot spot due to the presence of a ΨBRCA1 pseudogene at 5' end of BRCA1. This region contains duplicated BRCA1 exon 1a-2 sequences and their surrounding introns. The BRCA1:ΨBRCA1 recombination leads to a non-functional chimeric gene contains ΨBRCA1 exons 1a-2 fused to BRCA1 exons 3-24. We apply Competitive PCR-High Resolution Melting Analysis (cPCR-HRMA) in the rapid evaluation of BRCA1 exon 1a-2 deletion as frequent Copy Number Variation (CNV) in HBOC patients.
Methods: Germline CNV status of BRCA1 exon 1a-2 was evaluated in 30 WT and in 18 deleted samples, previously analyzed by MLPA. cPCR-HRMA was performed on LighCycler 480 Real-time PCR system (Roche Diagnostics, Basel, Switzerland) until the exponential phase in a duplex reaction including BRCA1 target region and Albumin region as unchanged copy number reference. Interpretation of CNV status was based on the evaluation of melting profiles and fluorescence peaks height ratio (BRCA1/Albumin). Mean and SD of fluorescence peak height ratio of WT samples was used to set ratios for unchanged copy number (x±2SD), deletion (x<2SD) and duplication (x>2SD). Specificity of the method was assessed through the analysis of samples with point mutation and micro-rearrangements.
Results: We were able to correctly classify all BRCA1 exon 1a-2 deleted samples due to the interpretation of melting profile and fluorescence peaks height ratio. Samples with point mutations or micro-rearrangements showed an unambiguous melting profile, proving the high specificity of the method.
Conclusions: Our approach represents a robust, rapid and cost-effectiveness method for the evaluation of BRCA1 exon 1a-2 deletion occurs frequently in HBOC patients. To date, LGRs evaluation is performed mainly through MLPA and MAQ techniques and cPCR-HRMA represents a great optimization of analytical workflow, allowing a more targeted use of gold standard techniques as confirmatory and definitely tests.
Minucci A, et al. Clin Chim Acta 2017;470:83-92. doi: 10.1016/j.cca.2017.04.026. Epub 2017 Apr 30.

P036

HIGH RESOLUTION MELTING ANALYSIS FOR RAPID GENOTYPING OF A RECURRENT CYP24A1 PATHOGENIC VARIANT IN IDIOPATHIC INFANTILE HYPERCALCEMIA (IIH)

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Background: Pathogenic variants (PVs) in cytochrome P450 family 24 subfamily A member 1 (CYP24A1) gene are associated with Idiopathic Infantile Hypercalcemia (IIH), a rare genetic disease affecting infants and patients in early adulthood. The deficiency of 1,25-hydroxyvitamin D_3-24-hydroxylase leads to an impaired vitamin D catabolism pathway, resulting in increased serum levels of vitamin D_3 metabolites with persistent hypercalcemia, hypercalciuria and suppressed parathyroid hormone (PTH) concentration. Nephrocalcinosis and nephrolithiasis represent possible disease complications. Among all the reported CYP24A1 PVs, the in-frame deletion c.428_430delAAG (p.Glu143del) seems to be one of the most common and it causes the loss of enzymatic activity. In this scenario, molecular analysis of CYP24A1 gene represents an advanced step in IIH diagnostic workflow.
Methods: A rapid CYP24A1 gene testing based on High Resolution Melting Analysis (HRMA) was designed in order to detect the CYP24A1 c.428_430delAAG (p.Glu143del). The method was set up in 20 Italian subjects belonging to IIH families previously analyzed and it was validated through the examination of 80 unknown subjects, using Sanger sequencing as confirmatory test.
Results: HRMA method was able to identify all c.428_430delAAG genotypes evaluating melting curve shape and melting temperature (T_m) with a full consistency between Sanger results. In fact, heterozygous samples exhibited a unambiguous melting profile while homozygous samples were identified by a specific T_m shift of 0.3±0.1 °C compared to WT.
Conclusions: HRMA represents a powerful tool for unambiguous genotyping of CYP24A1 c.428_430delAAG allele. The identification of CYP24A1 molecular alterations is fundamental in the IIH diagnosis and, in order to fulfill the needs of rapid and cheap gene investigation, the availability of such screening tools has to be considered. Furthermore, it could be of great importance in tailored therapeutic approaches, mainly aimed to avoid renal complications. In conclusion, this molecular technology could be applied also in CYP24A1 gene scanning application. In this case, only those amplicons with an unusual melting profile should be selected for further analysis, thus limiting time-consuming and workload.
Schlingmann KP, Kaufmann M, Weber S, et al. N Engl J Med 2011;365:410-21. doi:10.1056/NEJMoa1103864.

P037
L'ESPERIENZA DEL SETTORE DI FARMACO-TOSSICOLOGIA DEL LABORATORIO UNICO DELL'AUSL ROMAGNA, IN SEGUITO ALLA LEGGE 41 DEL 23 MARZO 2016 CHE HA INTRODOTTO IL DELITTO DI OMICIDIO STRADALE E IL DELITTO DI LESIONI PERSONALI STRADALI GRAVI O GRAVISSIME

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Introduzione: Per valutare se un soggetto sia alla guida sotto l'effetto di alcol e/o sostanze d'abuso la matrice di elezione è il sangue; a tale scopo nel nostro laboratorio siamo attrezzati con un Gascromatografo Spazio di Testa Agilent, per le alcolemie, un preparatore DSX ELISA Processing System e un LC-MS triplo quadrupolo XEVO TQ-S della Waters per lo screening e le conferme delle droghe d'abuso. Le analisi delle droghe su sangue sono state implementate dal luglio 2016.In questo lavoro mostreremo l'andamento delle richieste da parte dei Pronto Soccorso, in seguito ad incidenti stradali, e le positività rilevate nel periodo luglio 2016- maggio 2017 sia per alcol sia per droghe d'abuso nel territorio dell'AUSL Romagna di 5.098 chilometri quadrati e popolazione di 1.200.000 residenti.
Risultati e metodi: Alcolemie eseguite su sangue intero senza pretrattamento sullo strumento Gascromatorafo-spazio di testa. Sostanze d'abuso dosate su sangue intero con un metodo di screening ELISA con prediluizione e confermate in LC-MS con metodo che prevede precipitazione proteica in aceto nitrile. Dal luglio 2016 fino a maggio 2017 abbiamo dosato 1342 campioni per alcolemie di cui 203 positivi (15.1%) e 713 campioni per droghe su sangue di cui 128 pazienti positivi (18%) con 253 diverse positività, (35.5%). Le positività alle droghe sono rappresentate da: THC 28.8%, Cocaina e metaboliti 18.2%, Cocaetilene 5.1%, Benzodiazepine 26,9%, Metadone 2.4%, Ecstasy 0.4%, Ketamina 10.3% e Oppiacei 7.9%.
Conclusioni: Nei mesi analizzati le richieste e le positività per alcolemia, si mostrano costanti nel tempo mentre aumentano le richieste e quindi le positività delle droghe su sangue. Nel mese di maggio le richieste per alcol e droghe sono state quasi identiche (140 e 148). Tra le positività riscontrate alcune, sono dovute alla somministrazione da parte di personale sanitario (Benzodiazepine 60%, Ketamina 100%, Oppiacei 50%) e nel 75% dei casi per i THC e Cocaina è presente solo il metabolita inattivo THC-COOH e Benzoilecgonina che non dimostrano l'attualità d'uso.

P038
LA MALARIA UN URGENZA CLINICA: RUOLO ESSENZIALE DEL LABORATORIO

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Introduzione: In Italia ogni anno si registrano migliaia di casi di importazione dovuti sia all'aumento dei flussi migratori, sia all'aumento dei viaggi turistici in aree endemiche: nel nostro Paese la malaria è una malattia per la quale è prevista la notifica obbligatoria. Scopo di questo lavoro è illustrare la gestione diagnostica di un caso clinico. Paziente nigeriana di sesso femminile, 49 anni, giunge al nostro Pronto Soccorso con febbre alta, brividi scuotenti, tosse, astenia, artralgie diffuse e mialgia. Dichiara, inizialmente, di essere rientrata già da due mesi in Italia. Trasferita in Medicina la paziente continua a presentare febbre con un andamento intermittente. Il giorno seguente al persistere dei sintomi i clinici richiedono la ricerca della malaria su sangue periferico.
Materiali e metodi: Il test rapido immunocromatografico usato: CARESTART MALARIA RAPYD TEST (APACOR) Sensibilità 98% per Falciparum, sensibilità 96% per le forme benigna, specificità 97,5% per tutte forme. Si procede con l'esecuzione di striscio e goccia spessa.
Risultati: Il test rapido immunocromatografico per la ricerca della malaria risulta positivo sia per il falciparum che per le altre 3 forme di malaria benigne. Dopo aver riferito telefonicamente della positività del test rapido, confermato al microscopio, il clinico approfondisce l'anamnesi, con un colloquio difficile, e seppur con reticenza la paziente confessa di essere rientrata dalla Nigeria solo da 8 giorni. L'esame dello striscio sottile evidenziava la presenza di numerosi trofozoiti di Plasmodio Falciparum con emazie poliparassitate. L'osservazione della goccia spessa ha confermato la presenza del Plasmodio. Durante l'attesa del trasferimento in altra struttura specializzata, la situazione clinica della paziente peggiora iniziando a presentare sintomi meningei. Attualmente la Paziente ha ripreso la sua attività lavorativa.
Conclusioni: Da questo caso si evince che: la diagnosi di infezione parte da un sospetto anamnestico-clinico, la ricerca della malaria presenta i caratteri di emergenza, striscio e goccia spessa rappresentano il Gold Standard per una corretta diagnosi clinica, lo striscio sottile consente la diagnosi di specie, la goccia spessa consente di determinare la percentuale di parassitemia.

P039
PIASTRINOSI COME CAUSA DI PSEUDOIPERKALIEMIA

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Premessa: La pseudoiperkaliemia da piastrinosi è un'evenienza di possibile riscontro qualora si esegua la determinazione della potassiemia su campioni di siero in presenza di un elevato conteggio piastrinico. Si tratta di un fenomeno in vitro che può riconoscere diverse cause e consiste in livelli di potassio nel siero falsamente aumentati in presenza di una concentrazione normale in vivo. In caso di piastrinosi la sovrastima del valore di potassio è dovuta al suo rilascio in vitro da parte delle piastrine durante la coagulazione. Per evitare questo artefatto la determinazione del potassio in tale situazione deve essere eseguita su campione di plasma.
Caso clinico: donna di 64 aa, ambulatoriale esterna, affetta da malattia mieloproliferativa. Gli esami ematochimici evidenziano un valore critico di potassiemia (potassio= 7.0 mmol/L) in presenza di normale funzione renale (creatinina=0.77 mg/dL) e una piastrinosi (piastrine=2092 10^9/L). Nel sospetto di una pseudoiperpotassiemia da piastrinosi (il dosaggio era stato eseguito su campione di siero, in assenza di emolisi) si effettua la ripetizione del prelievo e il dosaggio viene eseguito anche su un campione di plasma. Il valore di potassio riscontrato su quest'ultimo risulta 4.9 mmol/L confermando il sospetto di errore preanalitico.
Conclusioni: scopo del caso clinico presentato è segnalare la possibilità di sovrastima del potassio in condizioni cliniche di piastrinosi, che potrebbe essere causa di trattamenti inadeguati e potenzialmente pericolosi per il paziente, determinando una falsa iperpotassiemia o mascherando un'ipopotassiemia.

P040
UN FALSO NEGATIVO AL TEST DI SCREENING PER LE BENZODIAZEPINE: CASO ISOLATO O CRITICITÀ LEGATA AL TEST?

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Ad una neonata di 1 mese viene somministrato Lormetazepam, (2,5 mg pari a 0,5 mg/kg) anziché un farmaco per il trattamento sintomatico dell'aerofagia. Dopo 2 ore la madre si accorge dell'errore per sonnolenza marcata della bimba e si reca subito in PS pediatrico, ove si verifica un episodio di apnea profonda con bradicardia: la piccola viene stimolata e le viene infuso l'antidoto (Flumazenil 0,1 mg ev). A 12 ore dall'assunzione, la situazione si è normalizzata. Il PS subito dopo l'accettazione invia un campione di urine al nostro laboratorio per lo screening tossicologico delle benzodiazepine, eseguito sullo strumento Indiko con test semiquantitativo immunoenzimatico in fase omogenea DRI® test (entrambi Thermo Scientific). Esso prevede una curva di calibrazione con Oxazepam su 5 punti a concentrazione 0, 100, 200, 500 e 1000 ug/L. Si utilizzano controlli su 2 livelli a concentrazioni di Oxazepam oscillanti intorno al valore di cut-off di 300. Il Lormetazepam viene escreto nelle urine quasi totalmente come molecola immodificata in forma glucuronata, solo il 5% circa viene demetilato a Lorazepam. La ditta dichiara che la specificità anticorpale verso il farmaco consente un livello di riconoscimento dell'89% circa. Sorprendentemente, il campione di urine della bambina è risultato del tutto negativo per le benzodiazepine. Il test di secondo livello (UHPLC-MS/MS), eseguito sullo stesso campione presso il Centro Anti Doping di Orbassano, è risultato invece fortemente positivo (Lormetazepam 4.250 ug/L e Lorazepam 37 ug/l). Da questo riscontro emerge la possibilità di falsi negativi al test di screening per le benzodiazepine. Si è interpellata in merito la ditta e, nell'attesa di un riscontro esplicativo, si è ritenuto utile indagare ulteriormente per verificare un eventuale divario tra quanto dichiarato e le reali prestazioni del metodo. Con l'aiuto del SERD e della Psichiatria, sono stati raccolti campioni urinari di soggetti assumenti quantità note di diverse molecole della famiglia delle benzodiazepine, da testare con il metodo in uso e con un kit CEDIA della stessa ditta.
Kohler K, Hammer R, Riedy K, et al. Evaluation of CEDIA and DRI Drugs of abuse immunoassays for urine screening on a Thermo Indiko Plus Analyzer. J Clin Lab Anal 2017;31:e22021.

P041
FREE LIGHT CHAINS: A COMPARISON OF PERIPHERAL BLOOD AND BONE MARROW

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Plasma cell dyscrasias (PCD) are a spectrum of bone marrow disorders resulting from the clonal expansion of terminally differentiated B-cells known as plasma cells. PCD range from the pre-malignant monoclonal gammopathy of undetermined significance (MGUS) to multiple myeloma (MM). PCD are characterized by abnormal secretion, by plasma cells cloner, of a monoclonal component (CM) or else an entire immunoglobulin or part thereof. Immunoglobulins are formed by two heavy chains and two light chains called κ and λ. Plasma cells normally produce an excess amount of light chains over heavy chains, indeed in the serum of a normal person there are free circulating light chains. The measurement of free light chains (FLCs) is expected for PCD screening, in stratification of risk and in the evaluation of response to therapy. It seemed interesting to go to evaluating the values of FLCs in the bone marrow, as it is the organ involved in such pathologies, and to compare them with the tested values in peripheral blood. The following tests were performed on 94 patients (66 males and 28 females aged 40 to 80 years with MGUS to MM): FLCs in peripheral blood, FLCs in bone marrow and creatinine. Dosage of FLCs in peripheral blood and bone marrow was performed in triplicate. 70% of patients had normal creatinine and just 25% had similar FLCs and κ/λ ratio (ratio). Without considering the value of creatinine, in 34% of patients the only value of FLCs κ was similar, while in 55% of patients the only value of the FLCs λ was similar. These findings suggest that the peripheral blood FLCs do not always accurately reflect the FLCs found in the bone marrow, organ in which these pathologies develop. This may be owing, as we already know, to renal function but also to other factors that may affect FLC metabolism once the bone marrow releases them into the bloodstream.

P042
SOUTH-EAST ASIAN OVALOCYTOSIS: ERYTHROCYTE AND RETICULOCYTE CYTOGRAMS EVALUATION

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South-East Asian ovalocytosis (SAO), also known as Melanesian elliptocytosis or stomatocyticelliptocytosis, is a particular variant of endemic hereditary elliptocytosis, extremelingly frequent in South-East Asia. The Authors describe a case of SAO. A 40 years old Indonesian woman came to our observation for a routine examination. The cell blood count, performed with ADVIA 2120i (Siemens) analyzer, showed normal values of erythrocyte (RBC: 4,605x10^9/L, Hb: 140 gr/L, MCV: 88,3fl, MCH: 30,4pg, MCHC: 345g/L) and reticulocyte indexes (76,8x10^9/L, CHr: 33,7pg, CHCMr: 342g/L). However, volume/concentration cytograms of erythrocyte and reticulocyte hemoglobin showed an increase in normocytic and hyperchromic erythrocytes (MCHC>410gr/L)=10,9% (N.V.=<1%) with enlargement of the erythrocyte distribution towards macrocytosis and hypocromia. The reticulocyte distribution histogram was shifted to higher values. Morphological examination of peripheral blood showed the presence of some macro-ovalocytes with a V or Y shaped stoma or with one or two central bars and several small stomatocytes, ovalocytes and stomato-ovalocytes, characteristic of the SAO. The hemoglobin pattern, osmotic fragility test, hemolysis indexes, iron balance and lipid pattern were normal. Molecular analysis showeda heterozygous 27 bp deletion between codon 400 and codon 408 of the SLC4A1 gene encoding the band 3 protein of the erythrocyte membrane, the characteristic molecular lesion of SAO.SAO is a dominant autosomal disease, asymptomatic in heterozygous adult: however, it may present with neonatal hemolytic anemia; homozygosity leads to fetal lethality. In the last decades physicians of Western countries are more and more involved with new or completely unknown (genetic or acquired) deseases, that are instead frequent in the countries, where the immigrants come from. In SAO the observation of numerical and graphical informations provided by hematologic analyzers, of peripheral blood morphology and the knowledge of patient's country are extremely useful tools to the diagnosis.

P043

RELATIONSHIP BETWEEN 25-OH VITAMIN D AND EFAVIRENZ PLASMA EXPOSURE IN HIV-POSITIVE PATIENTS

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Vitamin D (vit.D) deficiency is common in HIV-positive patients. In vitro studies suggested the ability of vit.D to induce the expression of several cytochrome P450 (CYP) genes through competitive interaction. Indeed a role of vit.D in impacting on drug metabolism has been demonstrated for immunosoppressant drugs . The pharmacokinetic of antiretrovirals as efavirenz (EFV) is influenced by its hepatic clearance by CYP enzymes, and is characterized by substantial interpatient variability. Patients with low exposure to EFV can have an increased risk of virological failure, whereas those with higher exposure to the drug suffer from adverse reactions, mostly affecting the central nervous system. Therefore, a therapeutic range for EFV of 1000–4000 ng/mL has been recommended . Aim of the study was to evaluate a possible association between 25-OH vit.D plasma level and EFV plasma concentrations.

25-OH vit.D was quantified in serum by chemiluminescence immunoassay. Serum vit.D levels were classified on IFCC recommendation: deficiency (< 10 ng/mL), insufficiency (11 to 30ng/mL) and normal (>30ng/mL). For TDM, samples were collected at the end of a normal dosage interval (C_{trough}). The EFV C_{trough} was measured using a validated HPLC-UV method.

Seventy samples from HIV patients were included. The mean of vit.D level was 22.7±10.4ng/mL: 10.7% of cases (7/65) had normal values, 69.2% (45/65) insufficiency, 20% (13/65) deficiency. Mean EFV C_{trough} was 2440±1326ng/mL: 78.5% (55/70) were in the therapeutic range (mean+SD: 2204±849ng/mL), 8.6% (6/70) were below therapeutic range (mean+SD: 760±114ng/mL) and 12.8% (9/70) were above range (mean+SD: 5003±640ng/mL). A significant and inverse correlation was observed between serum vit.D levels and EFV C_{trough} (r =-0,4173; P=0.0005). EFV levels were significantly different among the three groups: 3753±1602ng/mL in vit.D deficiency, 2150±1069ng/mL vit.D insufficiency, and 2047±1120ng/mL in vit.D normal levels (ANOVA P=0,0079). In particular, EFV plasma concentrations were significantly higher in patients deficient for vit.D than in patients with insufficient or normal vit.D levels (P=0.0013, P=0.0197, respectively)

This study showed a strict relationship between 25-OH vit.D and EFV plasma concentrations. Deficiency of vit.D below 10ng/mL could increase EFV concentrations above the therapeutic range with consequent increased risk of neurological adverse reactions.

P044

VALORE AGGIUNTO DELLA SINERGIA TRA OCCHIO ESPERTO E ANALIZZATORE AUTOMATICO DI SEDIMENTO URINARIO

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Caso clinico: maschio di 64 anni con insufficienza renale cronica in nefropatia policistica, con recente trapianto renale in politerapia immunodepressiva (ID). Peggioramento della funzione renale con creatinina plasmatica fino a 3,7 mg/dL, in parte correlabile a tacrolemia aumentata fino a 14 ug/L a 6 settimane (wk) dal trapianto: il livello ematico del farmaco resta elevato fino a 8 wk. A 5 wk sedimento urinario (SU) spento e Poliomavirus BK DNA assente. Da 6 a 10 wk persistenza di decoy cells (DC) nel SU: dopo due riscontri di DC si ripete PBK il cui titolo positivo aumenta ai successivi controlli (9 wk). La riduzione della ID porta a scomparsa delle DC (12 wk), calo e negativizzazione della viremia (13 wk) e parziale miglioramento della funzione renale. Nel nostro laboratorio le DC sono state rilevate con l'analizzatore automatico di SU Sedimax (Menarini), grazie a filtri scelti per selezionare elementi cellulari da revisionare. Lo strumento ha classificato tali cellule come "non epithelial cell": l'osservatore ha riscontrato la presenza di nuclei con aspetto globoso e dimensioni aumentate inducendolo al sospetto di DC. L'osservazione microscopica in contrasto di fase e la colorazione di Papanicolaou ne hanno confermato la natura. Le DC sono cellule epiteliali tubulari renali o uroteliali infettate da virus BK, il quale forma una singola densa inclusione basofila intranucleare con aumento delle dimensioni e del pleomorfismo nucleare (d.d. con cellule neoplastiche). In seguito a perdita del contenuto virale, il nucleo diventa pallido e trasparente con all'interno frammenti di cromatina e cromocentri. E' risultata vincente la sinergia tra l'impostazione strumentale di filtri atti a classificare particolari categorie di campioni e l'expertise dell'osservatore nell'interpretazione visiva delle cellule sospette fotografate dal Sedimax. Nei 3-6 mesi dopo trapianto il rischio infettivo aumenta per la maggior immunodepressione farmacologica: cruciale diventa quindi il controllo routinario del SU con particolare attenzione a segni d'infezione (nel caso specifico da BKV), utili per la segnalazione precoce al clinico e la conseguente messa in atto di decisioni tempestive.

Becker GJ, Garigali G, Fogazzi GB. Advances in Urine Microscopy. Am J Kidney Dis 2016;67:954-64.

P045
TSH REFLEX MEASUREMENT: IMPROVE MANAGEMENT IN THYROID DISEASE PATIENTS IN BERGAMO HOSPITAL

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Aims: The only dose of Thyroid-stimulating hormone (TSH) is in most cases the most reliable and economical means for basic thyroid function diagnostics. The aim of this study was to evaluate how TSH Reflex determination can optimize demand management in patients with suspected Thyroid Pathologies, as indicated by the National Reference Guidelines (Istituto Superiore di Sanità, 2003) and International (American Thyroid Association, 2000). Although the normal TSH range ranges from 0.3 to 5 mcU/mL, to include all possible pathological events, the normal range for TSH Reflex in our laboratory has been reduced between 0.45 and 3.5 mcU/ mL.
Materials and Methods: The study provided for the determination of TSH Reflex in 1474 samples taken in test tubes without any additive (Becton Dickinson), centrifuged for 15 minutes at 3500 rpm. The third generation method is based on the binding of serum TSH and antibodies Labeled with FITCH, and the concentration is detected in chemiluminescence via the Advia Centaur XP (Siemens Healthcare Diagnostics) tool.
Results: In 1174 (80%) samples only TSH was determined, falling within the normal range. In 144 (9.6%), with TSH less than 0.45 mcU / mL, both FT4 and FT3 were determined; of these, only 12 in FT3 was not performed, as the FT4 value was higher than 1.80 ng / dL, confirming the suspected hyperthyroidism. In 156 (10.4%) requests, with TSH greater than 3.5 mcU/mL, FT4 was determined and in 4 of these was lower than the lower limit of 0.7 ng/ dL confirming hypothyroidism of patients with TSH above the normality limit.
Conclusions: The study has shown how TSH reflex determination is reliable for the diagnosis of thyroid disorders, while the targeted determination of FT4 and FT3 confirms the cases of patients with subclinical pathology, guaranteeing savings in the management of long term costs.

P046
ACUTE PULMONARY EMBOLISM AND SEPSIS CAUSED BY A DOUBLE GRAM-NEGATIVE INFECTION IN CHRONIC LYMPHOCYTIC LEUKEMIA: A CASE REPORT

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Introduction: Infectious complications and acute pulmonary embolism (PEA) have been known to be a major cause of morbidity and mortality in Chronic Lymphocytic Leukemia (CLL) patients who are prone to infections because of both the humoral immunodepression inherent to the hematologic disease. Early diagnosis of this hematological condition can improve the prevention of thrombotic events as acute pulmonary embolism (PEA) and infectious complications as sepsis caused by a double Gram-negative infection (E. Coli and Ps. aeruginosa).
The initial symptoms of PEA and may include dyspnea, painless and chest pain. We report a case 75-year-old man with dyspnea and lymphocytosis.
Methods: A 75-year-old man presented to an emergency department with chest pain, fever, with purulent sputum and dyspnea. On physical examination, the patient was observed to have a temperature of 40°C, tachycardia of 105 bpm, a blood pressure of 70/50 mm Hg, a respiratory rate of 30 breaths per minute and oxygen arterial saturation of 90 %, and enlarged axillary, submandibular, pectoral and supraclavicular lymph nodes and hepatosplenomegaly. Blood tests revealed a white cell count of 28.2×10 /L and a C reactive protein of 21 mg/ L. hematocrit 31 %.
An urgent CT pulmonary angiogram (CTPA) revealed a saddle embolus, with propagation of the clot from the pulmonary valve extending into both main pulmonary arteries There was occlusion of pulmonary artery outflow as well as signs of right ventricular failure, with reflux of contrast into the inferior vena cava and the hepatic veins. There were numerous cavitating lesions within the right upper lobe. These were reported as pulmonary infarcts, consistent with a large and unresolved pulmonary embolism.
Results: Bone marrow aspirate showed increased number of B lymphocytes 67%, with dysplastic features. Axillary, submandibular, pectoral and supraclavicular lymph nodes prompted the diagnosis of B cell chronic lymphocytic leukaemia. The patient was restarted on intravenous heparin until the INR reached a new target range 3–4. Empirical treatment with imipenem and gentamicin was started.
Conclusions: Encapsulated bacteria are the predominant pathogens in patients with CLL, but also various Gram-negative enteric patogens such as Ps. aeruginosa, E. coli can be responsible for bacteraemia and septicemia, especially in patients with hypogammaglobulinemia. In our opinion, a multidisciplinary collaboration between internist medical doctors, haematologist and cytologist is essential in order to obtain the diagnosis as soon as possible.

P047
CASI DI MALARIA E SCATTERGRAMS DI SYSMEX XN-MODULE

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Il numero di casi di malaria registrati nei paesi in cui la malattia non è endemica è molto basso, per cui è difficile, per lo specialista di laboratorio, mantenere l'adeguata competenza per il riconoscimento morfologico del parassita su striscio di sangue periferico, che costituisce attualmente il metodo di riferimento. Uno strumento utile per il laboratorista può essere l'analisi degli scattergrams strumentali degli analizzatori automatici, sia per i casi sospetti che, in generale, per gli emocromi non richiesti con quesito specifico. Presentiamo i casi di tre pazienti giunti all'attenzione del Pronto Soccorso dell'Ospedale Papa Giovanni XXIII di Bergamo, a cui è stato eseguito l'esame emocitometrico, i cui scattergrams ottenuti con l'analizzatore ematologico Sysmex XN 9000 sono stati un valido supporto per la diagnosi: si tratta di due casi di malaria in forma gametocitica, rispettivamente da infezione da plasmodium falciparum e da plasmodium ovale, e di un caso di malaria in forma intracellulare, da infezione da plasmodium malariae. In caso di malaria in forma gametocitica, l'osservazione degli scattergrams ha mostrato un cluster cellulare, atipico ma peculiare, apparentemente appartenente alla popolazione degli eosinofili, ma caratterizzato da una minor intensità di fluorescenza e da una minor complessità. Non è possibile confondere questo cluster cellulare anomalo con una vera eosinofilia, perché le caratteristiche citofluorimetriche della popolazione eosinofila sono molto diverse. Mentre nel caso di parassitosi intracellulare, vi è stato il riscontro di una pseudoreticolocitosi, con prevalenza di reticolociti ad alto grado di maturazione, insieme alla presenza di linfociti attivati. Questa pseudoreticolocitosi può essere spiegata dall'errata classificazione delle emazie parassitate, a causa del loro contenuto, in reticolociti. La formulazione di allarmi strumentali dedicati e la conoscenza della morfologia degli scattergrams strumentali, suggestivi di patologia malarica, deve indurre lo specialista di laboratorio alla revisione microscopica, al fine di confermare/ escludere la presenza di parassiti malarici, sia nei casi sospetti che, più in generale, negli emocromi che arrivano alla sua attenzione per i più svariati quesiti diagnostici.

P048
A NEW CLINICALLY SILENT HEMOGLOBIN VARIANT INTERFERING WITH HbA1c QUANTIFICATION

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Background: Hemoglobin (Hb) variants are well-known factors interfering with accurate HbA_{1c} testing. This report describes a novel Hb variant leading to inappropriately low quantification of HbA_{1c} by ion-exchange high performance liquid chromatography (HPLC), which is a commonly used method for this evaluating.

Aim: Here, we report a novel hemoglobin variant, named Hb Alzette, found during a routine health check Ancona Hospital. HbA_{1c} level was 9 mmol/mol and this result was not coherent with other clinical and laboratory data.

Methods and Results: Molecular characterization of β gene identified a novel sequence variation (c.314G>A, p.Arg105Lys) never reported before in Italian population. Sequencing revealed a heterozygote variation in beta gene that was responsible for the double pich in the chromatographic assay that produced the abnormally low HbA_{1c} quantification.

Conclusions: When there is no correlation between clinical, glycemic status and glycated hemoglobin of the patient, the chromatogram of HbA_{1c} should be carefully checked to detect the possible presence of variants that cause interference in their measurement. Close examination of HbA_{1c} chromatograms can reveal clinically silent Hb variat that interfere with HbA_{1c} quantification, which must be kept in mind by health providers when interpreting HbA_{1c} results.

P049
LOW PLASMA LDH AND URIC ACID DUE TO AN IGM INTERFERENCE

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Indroduction: Paraprotein analytical interference is not infrequent in a laboratory with medium-high productivity and it's difficult to predict, prevent and manage.
Aim: We studied the cause of a possible paraprotein interference following a report of LDH and uric acid concentration below the method's limit of detection in a litium-heparin sample limpid but with high lipaemia index.
Materials and methods: We present a case of a 71-year-old man who performed routine laboratory tests at our laboratory. Litium-heparin plasma was tested with Siemens Advia Chemistry XPT and showed a concentration of LDH and uric acid below the method's limit of detection, <13 UI/l e <0,5 mg/dl, respectively. Plasma was limpid but had hight lipaemia index, and normal lipid profile. We Hypotized an analytical interference and the observation of the time/absorbance curve of the analysis confirmed our hypothesis. Therefore to eliminate the interference cause by a paraprotein presence we precipitated the plasma whit PEG 6000 and retested the sample.
Results: after the precipitation LDH and acid uric concentration where 150 UI/l and 5,6 mg/dl, respectively. The patient was invited to perform supplementary analysis. We repeated the tests on serum and plasma EDTA samples; the results previously obtained with precipitated litium-heparin plasma were confirmed. Additionally serum protein electrophoresis detected a peak in the gammaglobulin zone (29,6%), and the immunoglobulin concentration were:IgG 6,65 g/L, IgA 1,52 g/L ,IgM 38,54 g/L. The monoclonal component resulted to be IgM lambda. Therefore we suppose an IgM interaction with litium-heparin.
Conclusions: the presence of abnormal or discordant value in limpid litium-heparin plasma sample with high lipaemia index can hide a paraprotein interference. Since various literature data support our hypothesis we suggest to pay particular attention to the serum index and, in case of discordant values, to confirm these index with a visual inspection of the sample. Bearing in mind both the importance to present an accurate data to the patient as well as the chance to disclose a potentially severe not yet diagnosed disease. Moreover the laboratory policy should be to adopt a warning method, in case of future analysis, to indicate a patient with a previous interference. It is always appropriate to be aware of the technical features of the equipment in use; since are reported a considerable number of different analytical interference, regarding various analytes on different analytical platform.

P050
DIAGNOSIS OF A NON-SECRETORY MULTIPLE MYELOMA IN SANTA CORONA HOSPITAL: A CASE REPORT

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Multiple myeloma (MM) is a severe malignancy previously associated with poor survival rates and represents the second most common hematologic malignancy, characterized by a typical production of large amounts of defective immunoglobulin (Ig). Diagnosing MM and monitoring treatment response, including possible relapse, is mostly based on sequential measurements of Ig by serum protein electrophoresis. A small subset of MM called non-secretory multiple myeloma (NSMM), however, is characterized by the lack of Ig production thus diagnosis is mostly based on a clinical suspect and should be managed in a strict collaboration with laboratorists with respect to detection, treatment, and monitoring.
A 50-year-old Italian male with a diagnosis of diffuse joint inflammation made by the general practitioner, was treated with non steroidal anti-inflammatory drugs without benefit. He presented himself at the first-aid unit of the Santa Corona Hospital with difficulty to deambulate, gross hematuria, anemia and weight loss. During the anamnesis, he reported a familiarity for MM and one week history of a progressively increasing bone pain. He also reported a recent rib fracture caused by a sneeze. On the basis of clinical manifestations, combined with anamnestic data, MM disease was hypothesized. Blood tests were normal except for a substantial increase of the creatinin and Ca++. Peripheral blood microscopy showed mononuclear cells with a basophilic cytoplasm. While the context was suggestive for MM, the serum protein electrophoresis was negative for monoclonal Ig. Thus we decided to perform serum and urine immunofixation assay (s-IFE, u-IFE). Both tests resulted positive for free light chain (FLC), and the result was confirmed by high κ/λ ratio. CT images revealed diffused destructive lytic lesions of the bones. The bone marrow biopsy confirmed the NSMM diagnosis according to the International Myeloma Working Group (IMWG) criteria.
As represented in the described case NSMM is challenging in term of clinical risk management because of delayed diagnosis and inappropriate therapy. Reports describing the clinical presentation and the diagnosis of cases of NSMM, as does the present one, might contribute to an increase in our knowledge of this disease and an update to its epidemiology.

P051
UN CASO DI EMOFILIA A ACQUISITA POST-PARTUM

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Introduzione: Lo sviluppo di anticorpi ad azione inibitoria contro i fattori della coagulazione si manifesta con alterazioni nei test coagulativi e/o con eventi emorragici di varia entità in soggetti senza precedenti personali o familiari di patologia coagulativa. Spesso gli anticorpi sono diretti contro il fattore VIII (emofilia A acquisita) e si sviluppano in corso di malattie autoimmuni, neoplasie, nel post-partum, in seguito a interventi chirurgici o alla somministrazione di farmaci o per causa sconosciuta (idiopatiche, 50% dei casi). Case Report: CL (F, 35 anni) accedeva all'Ambulatorio per le Patologie Coagulative dell'AOUS inviata dal medico di medicina generale per ematomi mucocutanei diffusi, associati ad un allungamento dell'aPTT (1,93 Ratio), con PT normale e emoglobina 10,1 g/dL. All'anamnesi riferiva nei due mesi precedenti, a partire da due giorni dopo il parto, la comparsa di piccoli ematomi e di dolore alla gamba destra, trattato con FANS. Si evidenziavano ematomi muscolari a coscia destra e polpaccio sinistro. Anamnesi personale e familiare negativa per sindromi emorragiche. Si sospettava pertanto una sindrome emorragica acquisita.
Risultati: Il test di miscela a t.a. (aPTTmix) correggeva l'aPTT, mentre dopo incubazione a 37°C per 2 ore non si osservava correzione, indicando la presenza di un inibitore della via intrinseca. Tra i fattori dosati su ACLTOP500 con plasmi carenti di FVIII, FXI, FXII, FIX (Werfen), solo i livelli di attività del fattore VIII risultavano estremamente ridotti (0,4%); negativa la ricerca del LAC. Il titolo dell'inibitore del FVIII (Bethesda modificato Nijmegen) risultava 20 UB (emofilia A acquisita). La negatività degli ANA ed ENA screening e della ricerca di neoplasie solide/ematologiche confermavano l'eziologia post-partum. La paziente è stata trattata con agente bypassante (concentrato protrombinico) per i sanguinamenti attivi e con corticosteroidi associati a ciclofosfamide per l'eradicazione dell'inibitore. Ad oggi, dopo 15 giorni di terapia, si è avuto accorciamento dell'aPTT (1,21 Ratio), riduzione del titolo dell'inibitore (7 UB) con aumento dell'attività del FVIII (4,2%), aumento dell'emoglobina (11,8 g/dL). All'ecografia si riscontra completa risoluzione degli ematomi muscolari precedentemente evidenziati.

P052
EARLY ISCHEMIC HEART DISEASE IN A 21-YEAR-OLD PATIENT AND THE SUSPECT OF AUTOIMMUNE DISORDERS

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Introduction: Atherosclerosis is a multifactorial pathology, which develops through a series of pathophysiological mechanisms with the formation of an atheroma plaque that suppresses the arterial vessel. Recent research has shown that besides the known risk factors known as cigarette smoking, dyslipidemia, hypertension, diabetes, the immune system and inflammatory response play a crucial role. In fact, autoimmune diseases seem to increase the early formation and progression of the atherosclerotic plaque.
Case: A 21-year-old, male, smoker, type 1 diabetic patient came to the cardiology clinic for chest pain during physical exertion. Laboratory tests showed normal lipid profile, glycemia and glycosylated hemoglobin. The ergometric test showed alterations compatible with coronary artery disease. The next coronary CT showed an incredible report. Both coronary arteries were involved in a major atherosclerotic disease with multiple non-critical stenosis. Coronary angiography confirmed the CT report and no angioplasty was required. We have prescribed a therapy for ischemic heart disease. In a non-cardiological history, the patient has reported, in recent months, arthritis to the distal interphalangeal joint of the left finger. After excluding an infectious etiology, we suspected an immunological disorder. Laboratory tests showed ANA, anti-dsDNA and LAC positivity. In the rheumatology department, a systemic lupus erythematosus was diagnosed. Actually with the cardiological and rheumatologic therapy the patient is better and the follow-up continues.
Conclusion: This case gave us the opportunity to study the literature. It has been known for some years that autoimmune diseases can accelerate the atherosclerotic process in patients with cardiovascular risk factors. However, there are no similar cases of such young people in the literature with a significant coronary heart disease. Usually, patients with autoimmune diseases periodically perform cardiological checks for the prevention of ischemic heart disease. Our patient had a diagnosis of lupus due to chest pain and coronary atherosclerosis. It is therefore important in young patients who presented early atherosclerosis, without significant risk factors, to seek autoimmune disorders that may increase the pathogenesis of atherosclerosis and the risk of myocardial infarction, which is today the first cause of death in the world.

P053
CELLULE ATIPICHE NEL LIQUIDO PLEURICO: UN CASO DI METASTASI DI MELANOMA

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Il melanoma è un tumore cutaneo molto aggressivo che può facilmente recidivare e indurre metastasi. Il trattamento terapeutico consiste nella rimozione chirurgica della lesione, seguita e/o supportata da chemioterapia, radioterapia o immunoterapia. Nell'aprile 2017 perviene al laboratorio di ematologia un liquido pleurico, di colore giallo citrino, di un uomo di 73 anni. L'analisi del liquido è eseguita con lo strumento XN-1000, modulo "body fluid" (Sysmex Corporation, Kobe, Japan). Dal grafico WDF (WBC differential fluorescence) si ipotizza la presenza di cellule grandi e caratterizzate da un'elevata fluorescenza. Inoltre, la conta dei globuli bianchi (0.232 10^9/L) è inferiore rispetto al totale delle cellule (0.307 10^9/L). L'esame microscopico (ingrandimento 600x), eseguito dopo cito-centrifugazione (Cytospin 4, Thermo Scientific) e colorazione mediante May-Grunwald Giemsa, mostra un cospicuo numero di cellule di natura non ematopoietica. Si presentano organizzate in cluster, alcune con nuclei bizzarri, altre in attiva fase replicativa, la maggior parte con dimensioni notevolmente aumentate e rapporto nucleo/citoplasma >1. La successiva valutazione anatomo-patologica conferma la presenza di cellule tumorali maligne caratterizzate da positività per il marcatore S100 e negatività per CKpool, profilo immunofenotipico indicativo di metastasi di melanoma. Il paziente aveva infatti una diagnosi di melanoma al mento, rimosso chirurgicamente, ma poi recidivato e metastatizzato a livello osseo, linfonodale e polmonare, nonostante i trattamenti radioterapico e immunologico (denosumab, 120 mg/mese). Arriva in pronto soccorso con difficoltà respiratoria e versamento pleurico. La natura definitiva delle cellule è stata chiarita solo con il referto anatomo-patologico; tuttavia l'osservazione al microscopio del liquido ha permesso di dare, in breve tempo, una indicazione ipotetica della natura neoplastica del versamento, sospetto che non era immediatamente intuibile dal colore chiaro del liquido.Nel caso in cui la strumentazione automatica di laboratorio fornisca grafici suggestivi di cellule sospette, è opportuno guardare il vetrino al microscopio ottico con la consapevolezza che si potrebbero trovare cellule di natura non ematopoietica ascrivibili a forme tumorali.

P054
UNA RECIDIVA DI LINFOMA ASSOCIATA A COMPARSA DI MALATTIA DA CATENE PESANTI

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Introduzione: Le malattie da catene pesanti (HCD) sono rari disordini linfoproliferativi in cui plasmacellule clonali producono immunoglobuline (Ig) monoclonali anomale costituite dalle sole catene pesanti (γ, α o μ), incapaci di legarsi alle catene leggere. La diagnosi di HCD richiede che l'immunofissazione (IF) delle proteine sieriche e/o urinarie evidenzi la presenza di una banda monoclonale (BM) a carico di una catena pesante, senza corrispondenza nelle catene leggere. Sono note tre forme di HCD, distinte in base alla classe di catena pesante coinvolta: la forma alfa è la più comune (circa 400 casi noti); seguono la forma gamma (circa 120 casi riportati) e quella a catene pesanti mu (meno di 40 casi segnalati in letteratura). Generalmente le HCD si riscontrano in associazione con altre malattie linfoproliferative, soprattutto come forme varianti di linfomi non-Hodgkin.
Caso clinico: Una donna di 86 anni affetta da linfoma diffuso a larghe cellule B (DLBCL) a localizzazione addominale, in remissione da due anni dopo terapia, lamenta la comparsa di adenopatie laterocervicali e cervicalgia. L'ecografia rileva diffuse linfoadenomegalie cervicali, ascellari ed addominali. Gli esami di laboratorio evidenziano una moderata anemia (emoglobina:10.4 g/dL), iperuricemia (9.5 mg/dL), un aumento degli indici di flogosi (proteina C reattiva:16.8 mg/L, β2-microglobulina sierica:7.6 mg/L) e un marcato incremento delle IgG sieriche (3443 mg/dL) accompagnato da sierologia virale negativa. L'elettroforesi delle sieroproteine mostra sia un allargamento anomalo della regione β2 sia un picco nella zona gamma anodica. Dopo aver escluso eventuali errori pre-analitici (es. presenza di fibrinogeno o emolisi), si esegue l'IF sierica, che evidenzia una BM nella sola corsia degli antisieri anti-catene pesanti gamma, senza corrispondenti bande nelle corsie degli antisieri anti-catene leggere: il laboratorio referta la presenza di una componente monoclonale a sole catene pesanti γ nelle regioni β2 e gamma. L'esame istopatologico su biopsia di un linfonodo ascellare diagnostica una recidiva di DLBCL.
Il caso riportato mostra come la comparsa di una γ-HCD possa essere contemporanea all'insorgere di una recidiva di linfoma non-Hodgkin.

P055
SEVERE HYPERTRIGLYCERIDEMIA IN A HEAVY DRINKER INDIVIDUAL: THE IMPORTANCE OF LABORATORY FOR OUTPATIENT RAPID MANAGEMENT

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Hypertriglyceridemia (HTG) is caused by a variety of genetic and acquired conditions, such as obesity, physical inactivity, smoking, excessive alcohol intake, thyroid and renal disease, and several drugs. Although doubts remain on the role of HTG as an independent cardiovascular risk factor, severe HTG (›1000 mg/dL) is certainly a major risk factor for development of acute pancreatitis. Here we report the case of a 44 years old male, known as chronic alcohol abuser, who was referred to our laboratory by his general practitioner (GP) for a periodic evaluation. As in doing measurements in our total laboratory automation the automatic detection of interference indices is preliminarily performed on all tested plasma and serum samples, an extremely high lipemic index (i.e. 2918) was immediately detected. Consequently, all requested biochemistry test results were reported as interfered by turbidity, except for triglyceride concentration that was out of the assay linearity. The sample, sequentially diluted, gave a final triglyceride result of 13,159 mg/dL. After result confirmation, a laboratory professional alerted the patient's GP, who immediately informed him about his condition and associated risk of acute pancreatitis. GP advised to call on the Emergency Department where to be treated for this massive HTG. So that, 12 hours after the blood drawing, the patient presented himself to the Emergency Room of our hospital, where an abdominal ultrasound together with a complete physical examination were performed. No signs of pancreatic inflammation were found, but only those compatible with the known alcoholic liver disease. Serum triglycerides were 11,846 mg/dL and the patient was discharged with prescription of a low-fat diet, discontinuation of alcohol intake and triglyceride lowering therapy with fibrates and omega-3 fatty acids. This case is a good example of the effectiveness of the recently proposed approach that all commonly required laboratory tests, including those of outpatients, should have short turnaround time together with mindful verification of the results (including interference indices), then offering a high-quality and timely service to all laboratory users for properly treating life-threatening conditions.

P056
COPEPTIN IN THE EARLY DIAGNOSIS OF SIADH INDUCED BY GASTRIC CANCER

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Background: Copeptin directly reflects AVP concentration and can be used as a surrogate biomarker of AVP secretion. In many studies copeptin represents AVP levels and its behaviour correlates with changes in serum osmolality and various diseases. Aim: We described an unusual association between SIADH and gastric cancer and evaluated the potential role of plasma copeptin measurement as a surrogate marker of ADH secretion for the early diagnosis of SIADH in cancer patient. Patient and Methods: We described a case of a 66-year-old woman affected by an advanced well-differentiated gastric cancer in stable disease after chemotherapy. The euvolemic patient presented to emergency room for abdominal pain, lethargy and cachexia. No intracranial metastases whereas, a disease progression in number and diameter of liver metastases a CT scan was observed. Laboratory evaluation showed a sodium level of 126 mmol/L. Liver insufficiency was excluded. Therefore, diagnostic algorithm for the etiology of hyponatremia was started. Laboratory tests: Serum and urine osmolality were 279 and 1.691 mOsm/L, respectively. Urine sodium was 16 mmol/L. TSH and cortisol levels were in the normal range. Copeptin assay: Serum copeptin levels were inappropriately high (˃7). We compared standard diagnostic criteria for SIADH to the measurement of plasma copeptin in relation to osmolality. Serum and urine sodium were examined using ISE method of Vista system (Siemens), while copeptine dosage was performed in chemiofluorescence by Kryptor (Thermo Fisher). Results: SIADH diagnosis was made and medical therapy with Tolvaptan was started. Patient's sodium levels gradually improved and became in the normal range after 10 days. A progressive improvement in neurological symptoms was observed 3 days after the onset of therapy. However, abdominal pain and cachexia persisted.

P057
FREE LIGHT CHAINS E MALATTIA DA DEPOSITO DI CATENE LEGGERE (LCDD): CASO CLINICO

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Background: La malattia da deposito di catene leggere (LCDD) è caratterizzata dalla deposizione a livello renale di catene leggere libere monoclonali, prodotte da un clone di linfociti-B o di plasmacellule. Un appropriato workup diagnostico di laboratorio ed ematologico è fondamentale per la diagnosi e la prognosi del paziente. La biopsia renale è essenziale per determinare l'esatta natura della lesione e la severità del danno renale.
Metodi: Soggetto maschio di 65aa, con storia di ipertensione dal 2008, si ricovera presso l'U.O. di Nefrologia nell' aprile 2015 per la presenza di edemi declivi. Il workup diagnostico di laboratorio all'ingresso è stato orientato sia alla valutazione del danno renale che alla ricerca della componente monoclonale, con elettroforesi sieroproteica (CZE), Immunofissazione sierica (s-IFE), Immunofissazione urinaria (u-IFE) su gel di agarosio HR (Hight Resolution) (Sebia) e dosaggio delle Free Light Chain nel siero (sFLC) (Binding Site).
Risultati: Il soggetto non presentava alla CZE alcuna alterazione morfologica, ma solo una diminuzione della zona gamma 7,9%; la s-IFE e la u-IFE erano negative, mentre le FLCk erano 217,2 mg/L, FLCλ 26,7 mg/l e la ratio 8,12. Gli esami ematochimici all'esordio erano i seguenti: eGFR 27mL/min, creatinina 2,2 mg/dl e proteinuria 6755 mg/24h. La biopsia osteomidollare evidenziava un infiltrato plasmacellulare < 10% con clonalità kappa; la biopsia renale rilevava una LCDD isotipo kappa confermata alla microscopia elettronica.
Conclusioni: L'elevata sensibilità diagnostica della ratio alterata delle sFLC rispetto ai test tradizionali (s-IFE, u-IFE), ha evidenziato la monoclonalità delle catene leggere kappa. La sola ratio k/λ di 8,12 ha permesso ai clinici di orientare le indagini verso una Gammopatia Monoclonale di Significato Renale (MGRS). La biopsia ossea e biopsia renale hanno confermato la diagnosi di LCDD. La possibilità di avere un indicatore di clonalità che permetta di individuare la malattia in fase precoce è importante, in quanto un ritardo nel trattamento può avere un impatto negativo sia sulla prognosi renale che sul paziente.
Correia SO, Santos S, Malheiro J, et al. Monoclonal gammopathy of renal significance: Diagnostic workup. Word Journal of Nephrology 2017;6:72-8.

P058
"IN TRANSAMINASES WE TRUST": CASO CLINICO DI EPATITE AUTOIMMUNE

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Introduzione-Scopo: L'epatite autoimmune (AIH) è una malattia infiammatoria cronica del fegato che, se non trattata, spesso porta a cirrosi, insufficienza epatica e morte. Le prevalenza varia da 10 a 17 per 100.000 in Europa ed il sesso femminile è colpito tre volte più frequentemente. La maggior parte dei casi non prevede fattori scatenanti e in circa il 40% dei casi AIH si presenta come Epatite Acuta (EA). Riportiamo un caso di AIH di tipo 1 tratta con successo con Prednisone (PDN) ed Azatioprina (AZA).
Descrizione caso clinico: Uomo caucasico di 38 anni accede in Pronto Soccorso per ittero e dolore epigastrico diffuso. I primi esami ematochimici mostrano segni di marcata citolisi epatica compatibili con quadro di EA. Viene ricoverato presso il reparto di Medicina Interna per procedere alla diagnosi differenziale.Dopo esclusione di patologie neoplastiche ed infettive e poiché le transaminasi (TRS) si mantengono elevate, il paziente (PZ) viene trasferito presso l'unità trapianto fegato dell'ospedale Molinette. Nel frattempo i test d'autoimmunità risultano positivi per AIH di tipo 1 e la successiva biopsia epatica ne conferma la diagnosi. Il PZ inizia terapia con PDN (75 mg/die) e AZA (50 mg/die). A distanza di tre settimane dalla dimissione le TRS tornano a valori normali, il PZ presenta condizioni buone e stabili e prosegue con terapia domiciliare a dosaggi minori. Attualmente il PZ esegue regolarmente i controlli e presenta una situazione clinica ottimale.
Risultati:
–Ematochimici:- t zero, dopo 3 settimane, dopo 2 mesi:
ALT UI/L: 2894; 252; 38
AST UI/L: 1220; 44; 16
Bilirubina Totale mg/dL:18.05; 2.86; 1.19
INR: 1.31; 1.22; 0.99
–Ricerca marcatori virali: HAV, HBV, HCV, HEV: negativa.
–Indagine per Leptospira: negativa
–Autoimmunità:
ANA: positivo 1/160 con pattern punteggiato
E.N.A.: negativo
AMA UI/L: 145.00
Ab Anti muscolo liscio: positivo
Discussione: La cooperazione tra clinica e specialisti di laboratorio risulta fondamentale per un precoce e corretto inquadramento diagnostico e trattamento terapeutico di AIH.
Gleeson D, Heneghan MA. British Society of Gastroenterology guidelines for management of autoimmune hepatitis. Gut 2011;60:1611-29.

P059

A CASE OF POLYMERIZATION IGM IN MONOCLONAL GAMMOPATHY SHOWING LABORATORY INSTABILITY AFTER 3 DAYS

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Problemi di rilevazione di alcune componenti monoclonali IgM con l'utilizzo di tecniche elettroforetiche sono già stati descritti in letteratura. L'osservazione della viscosità e/o della presenza di precipitati nei sieri conservati a 4°C, permette di adottare misure precauzionali preanalitiche: preincubare il campione a 37°C o pretrattarlo con agenti riducenti.La paziente G. E. (60 anni) è giunta alla nostra osservazione nel maggio 2017 in occasione di un controllo di laboratorio, che comprendeva l'esecuzione di un'elettroforesi siero proteica. La paziente nel settembre 2016 aveva eseguito alcune analisi biochimiche da cui era risultato un consumo accentuato del complemento (C3 minore di 1,03 g/L; C4 minore di 0,02 g/L), un elevato titolo ANA (maggiore di 1:640) e un incremento delle IgM (5,61g/L).L'elettroforesi capillare (Capillarys3Sebia) eseguita su siero fresco evidenziava una componente monoclonale in zona gamma globulinica pari a 7,4 g/L. Dopo 3 giorni di conservazione a 4°C il siero della paziente, sottoposto a tipizzazione mediante immunosottrazione (Capillarys3Sebia) e mediante immunofissazione su gel di agarosio (Hydrasis System Sebia), non ha più evidenziato alcuna componente monoclonale. Il siero conservato a 4°C non presentava un crioprecipitato né iperviscosità. L'elettroforesi ripetuta su un secondo campione di siero della paziente prelevato nella stessa giornata confermava l'assenza di componenti monoclonali.Poiché la determinazione delle catene leggere libere sieriche è un importante fattore prognostico e riveste un ruolo significativo nello screening dei disordini plasma cellulari, è stato eseguito il dosaggio turbidimetrico delle catene κ e λ free (Optilite Binding Site) e si è evidenziato uno sbilanciamento delle catene leggere libere (κ 88,8 mg/L; λ 17,6 mg/L; κ /λ 5,04).Sospettando una aggregazione delle Ig, il siero della paziente è stato trattato con un agente riducente l'acetilcisteina (Fluidil Sebia), secondo le linee guida. L'immunotipizzazione ripetuta ha evidenziato la presenza di una componente monoclonale IgM κ. Il caso descritto sottolinea come in particolari condizioni cliniche la conservazione del campione a 4°C prima dell'esecuzione dell'elettroforesi sieroproteica possa indurre la polimerizzazione delle IgM con il rischio di falsi negativi.

P060

THE DIAGNOSTIC UTILITY OF BRONCOALVEOLAR LAVAGE: CLINICAL CASES OF ALVEOLAR PROTEINOSIS AND INVASIVE PULMONARY MYCOSIS

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Broncho alveolar lavage obtained by fiber optic bronchoscopy is a low invasive and well-toleratedprocedure, which provides an important diagnostic tool in the diagnosis of variousdiffuse lung diseases. Generally, Broncho Alveolar Lavage Fluid (BALF) is analyzed in order to determine lung inflammatorycells profiles and to detect respiratory pathogens. Many cytological stains are used in orderto help the diagnosis, such as Papanicolau to identify neoplastic cells, periodic acid–Schiff (PAS) to detect yeasts, mucus or alveolar proteinosis, toluidine blue for the detection of pathogens such as Pneumocystis spp. and Perls to identify siderophagesand to establish the Golde score for alveolar siderosis.Briefly, BALF is performed injectingsterile saline solution in a sub segmental bronchus during a diagnostic bronchoscopy. Fluid is collected by gentle aspiration and sent in the laboratory at 4°C where it is immediatelyprocessed. After a gently mixing, the total number of white cells in the native, un#ltered lavage #uid is determined by a Bürker chamber. Microscope slides are prepared by centrifuging BALF at 1800g for 10 min. Cell pellet is smeared and slides are #xed in methanol for subsequent different stainings.Four hundred consecutive cells are counted under oil immersion and both the number and percent of macrophages, neutrophils, epithelial cells, lymphocytes and eosinophils are recorded. Differential cell counts in BALF (cytogram) is established on May–Grunwald–Giemsa or Papanicolaou-stained slides. The Golde score is established using Perls staining. The presence of speci#c pathogens on PAS- or toluidine blue-stained slides is also noted. We report two different clinical cases of severe respiratory deficiency with diffuse radiological lung infiltrations in which BALF analysis allowed to obtain a precise diagnosis without any other more invasive procedure. We describe BALF patterns characteristic of alveolar proteinosis, a rare disease characterized by alveolar accumulation of glicoproteins and lipids due to defective surfactant clearance by alveolar macrophages. The second case report describes an unexpected Histoplasma capsulatum lung infection in a woman with chronic lymphocytic leukemia.

P061
EARLY NORMALIZATION OF sFLC AS A PREDICTIVE FACTOR OF HIGH QUALITY RESPONSE TO DARATUMUMAB IN RELAPSED/ REFRACTORY MULTIPLE MYELOMA PATIENT

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Multiple Myeloma (MM) represents one of the most common hematological malignancies characterized by clonal proliferation of neoplastic plasma cells in the bone marrow. During the last years, the management of the disease has greatly improved both for the introduction of serum Free Light Chains (sFLC) assay and, on therapeutic grounds, for advent in the clinical practice of monoclonal antibodies like anti CD38 Daratumumab. In this issue, we report a case of a 54 years old female patient affected from 2007 by IgG/κ MM. From March 2007 to March 2016 the patient underwent several chemotherapy regimens, in sequence thalidomide-dexamethasone followed by double autologous stem cell transplantation, bortezomib- dexamethasone, lenalidomide-dexamethasone, bendamustine-thalidomide-dexamethasone, pomalidomide-dexamethasone and VD-PACE chemotherapy salvage regimen. Due to the progression of disease (serum M protein=52g/L, sFLCκ=10.500mg/L, sFLC ratio=7.200), in April 2016 she underwent to weekly Daratumumab 16mg/kg monotherapy. The first infusion, however, was interrupted due to development of fever, chills, pyrexia and discomfort. To exclude tumor lysis syndrome, biochemistry exams were performed evidencing mild hypocalcemia (1,92mmol/L) and a significant increase in lactate dehydrogenase serum levels (from 333 to 714 UL). After complete regression of the symptoms, Daratumumab therapy was continued one week after the first infusion and completed for a total amount of 4 doses weekly without side effects. A dosage of sFLCs was performed one week after the first infusion showing a significant reduction in sFLCκ levels (from 10.500 to 9.060mg/L). After the end of the first cycle, a reduction in serum M protein (24,88g/L) and a surprising normalization of sFLCκ and ratio (19.8mg/L and 3.5 respectively) with negativity of the urinary M-protein were found at biochemical revaluation. Of notice, the reduction of M-protein continued to October with a nadir of 2,44 g/L. In conclusion, in this patient the early normalization of sFLC was predictive of durable and high quality response to Daratumumab therapy, earlier than serum M protein reduction, highlighting the importance of sFLC assay in the response evaluation of MM patients.

P062
NUOVO CASO DI MGUS IgE k

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Il riscontro di una componente monoclonale (CM) IgE è un evento molto raro. Il mieloma multiplo IgE rappresenta solo lo 0.01% dei casi e poche evidenze di gammopatie monoclonali di significato indeterminato (MGUS) IgE sono riportate in letteratura. Nel marzo 2013, presso il dipartimento di dermatologia dell'AOU "Federico II" di Napoli, viene ammessa una donna di 54 anni, affetta da psoriasi acuta e diffusa, di cui soffre dall'età di 18 anni. La paziente presenta, inoltre, diabete mellito di tipo II, ipercolesterolemia e, a causa di tiroide multinodulare, assume levotiroxina sodica. L'elettroforesi capillare zonale (CZE) delle proteine sieriche evidenzia un lieve picco di probabile natura monoclonale in zona γ. Effettuata tipizzazione mediante immunosottrazione (ISE), si osserva positività per le sole catene k, quindi, eseguita immunofissazione (IFEs) con antisieri anti δ e anti ε, la CM risulta tipizzata come IgE k, 3.5 g/L. Ulteriori indagini mostrano assenza di ipercalcemia, insufficienza renale, anemia e lesioni ossee (criteri CRAB). Valori osservati: proteine totali (PT) 71 g/L (vr 64-83); creatinina (CREA) 0.7 mg/dL (vr 0.6-1.1); calcio totale (Ca tot) 9.9 mg/dL (vr 8.4-10.2); emoglobina (Hb) 14.2 g/dL (vr 12-16), diagnosi MGUS IgE k; persistono iperglicemia ed ipercolesterolemia. Ad Ottobre 2016, la paziente viene ricoverata in regime di day hospital presso il dipartimento di ortopedia per problemi all'anca sinistra ed, eseguita radiografia al bacino, si evidenzia coxartrosi bilaterale. Gli esami ematochimici effettuati confermano iperglicemia, ipercolesterolemia e PT, CREA, Ca tot, Hb nella norma. Il protidogramma in CZE risulta immodificato rispetto al precedente, con CM 3.2 g/L; il dosaggio delle catene leggere libere del siero (sFLC) mostra valori di k pari a 49.4 mg/L (vr 6.7-22.4) e di λ pari a 29.6 mg/L (vr 8.3-27) con un rapporto k/λ lievemente aumentato, 1.7 (vr 0.31-1.56). In presenza di una CM stabile in un periodo di circa 3 anni e in assenza di parametri indicativi di danni d'organo, si riconferma la diagnosi di MGUS, in ogni caso il riscontro del lieve sbilanciamento del rapporto k/λ è un dato da tenere in considerazione nel corso del follow-up.

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P063

CONFRONTO TRA DIFFERENTI PIATTAFORME ANALITICHE PER LA DETERMINAZIONE DEL FATTORE DI VON WILLEBRAND

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La malattia di Von Willebrand (VWD) può essere causata da carenze sia di natura qualitativa che quantitativa del VW Factor. I test di laboratorio disponibili per uno screening di primo livello sono la misura dell'antigene del VWF (VWF:Ag), dell'attività del cofattore ristocetinico del VWF (VWF:RCo) e la capacità di legame del VWF al collagene (VWF:CB), insieme alla determinazione del Fattore VIII (FVIII:C), come indicato nella linea guida del B.C.S.H. Scopo dello studio: Valutare e definire quali siano gli intervalli di riferimento di questi parametri. Sono stati selezionati 134 soggetti normali (82M e 52F), analizzati sia globalmente che suddivisi in base al gruppo sanguigno (Gruppo "0" = 64 e Gruppo non "0" = 70). I dosaggi di FVIII:C, VWF:Ag e VWF:RCO sono stati eseguiti in turbidimetria su ACL TOP e i dosaggi di VWF:Ag, VWF:RCO e VW:CB in chemiluminescenza su ACL Acustar con reattivi IL-Werfen. I valori al 97.5° percentile sono stati: globalmente per FVIII:C 67.4-140.7%,VWF:Ag 59.2-183.1% e VWF:RCo 48.2-126.0% su ACL TOP;VWF:Ag 51.4-175.0%,VWF:RCo 50.2-175.1% e VWF:CB 56.0-174.7% su ACL-Acustar; per il Gruppo 0 FVIII:C 66.1-134.9%,VWF:Ag 56.6-140.4% e VWF:RCo 45.0-113.2% su ACL-TOP; VWF:Ag 48.1-139.2%, VWF:RCo 47.1-138.7% e VWF:CB 50.7-137.5 su ACL Acustar; per il Gruppo NON 0 FVIII:C 73.5-141.7%,VWF:Ag 79.3-186.4 e VWF:RCo 54.2-148.4% su ACL TOP;VWF:Ag 75.3-179.2%, VWF:RCo 62.3-183.2% e VWF:CB 60.4-182.1% su ACL Acustar. Il test VWF:Ag sui dati complessivi ha una correlazione superiore tra i due sistemi rispetto al test VWF:RCo. Inoltre il confronto tra i due sistemi evidenzia: il gruppo 0 mostra una migliore correlazione per il test VWF:Ag rispetto al test VWF:RCo; il gruppo non 0 ha un bias minore per il test VWF:Ag, mentre il bias è decisamente più ampio per il test VWF:Rco. Ogni laboratorio dovrebbe individuare i propri intervalli di riferimento dei vari test rispetto alla piattaforma tecnologica utilizzata. Alla luce dei dati esposti è utile differenziarli tra gruppo 0 ed i non 0.

P064

VALUTAZIONE ANALITICA DEL DOSAGGIO DEL D-DIMERO SU DUE ANALIZZATORI AUTOMATICI CS5100 (SIEMENS) E ACL TOP500 (WERFEN)

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Elevati livelli di concentrazione di D-Dimero sono presenti in alcune condizioni cliniche, quali la trombosi venosa profonda, l'embolia polmonare, CID. Una diagnosi precoce di tali patologie garantisce un migliore management dei pazienti con riduzione anche del rischio per la stessa vita. Lo scopo del lavoro è stato quello di valutare la precisione dei due metodi per il dosaggio del D-Dimero su analizzatore CS5100 (Siemens) che utilizza il reattivo Innovance D-Dimer e su ACL TOP 500 (Werfen) che utilizza il reattivo Hemosil D-Dimer HS 500.
Materiali e metodi: Per la valutazione della precisione analitica dei due metodi sono stati utilizzati materiali di controllo (QC), a due livelli di concentrazione di D-Dimero, forniti dalle ditte produttrici, testati per venti giorni consecutivi rispettivamente sul CS5100 e ACLTOP 500. Per lo studio di comparazione e la valutazione della concordanza tra i metodi, sono stati selezionati 40 campioni di plasma con valori di D-Dimero 7023–8100 ng/ml. I dati sono stati valutati con l'analisi di Bland and Altman.
Risultati: Studio di precisione. Metodo CS5100: QC livello 1, CV% tra-serie = 2.1 (media = 265 ng/ml), CV% intra-serie =1.9 (media = 285 ng/ml); QC livello 2, CV % tra serie=3.2 (media =2677 ng/mll), CV% intra-serie = 2.6 (media = 2874 ng/ml); Metodo ACL TOP 500: QC livello 1, CV% tra-serie = 1.2 (media=560 ng/ml), CV % intra-serie = 1.39 (media = 584 ng/ml); QC livello 2, CV% tra serie=1.89 (media =1889 ng/ml), CV% intra-serie=2.89 (media = 1984 ng/ml). Per i valori patologici: bias assoluto 18,82 ng/ml (95% CI: 17,29 to 20,35); 95% limiti di agreement = 9,44 (lower), 28,2 (upper).
Conclusione: I dati ottenuti mostrano che lo strumento ACL TOP500 fornisce risultati riproducibili tra-e intra-serie, per i due livelli di controlli testati l'imprecisione totale il CV% tra 1.39 e 2.89. Lo strumento CS5100 fornisce risultati riproducibili tra-e intra-serie con l'imprecisione totale il CV% tra 1.9 e 2.6. Entrambi gli strumenti risultano idonei per un uso routinario per il dosaggio del D-Dimero. Tuttavia, la presenza di un bias statisticamente significativo, i due metodi non possono essere interscambiabili pertanto è opportuno, in caso di pazienti sottoposti a monitoraggio terapeutico utilizzare sempre lo stesso metodo analitico per ridurre errori di interpretazione post-analitici.

P065
VALUTAZIONE DELLA NUOVA TROMBOPLASTINA ESTRATTIVA STA® – NEOPTIMAL A ISI=1,0

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Il Tempo di tromboplastina valuta la via estrinseca della coagulazione. Il dosaggio si basa sull'attivazione della cascata coagulativa da parte della tromboplastina (tissue factor + fosfolipidi). Le tromboplastine presenti in commercio possono avere caratteristiche e origini diverse. Scopo dello studio: valutazione di 4 diverse tromboplastine della ditta STAGO: TriniCLOT HTF (ottenuta da colture di cellule umane con ISI pari a 1.12, STA-NEOPTIMAL estratta da cervello di coniglio con ISI = 1.01), STA-NEOPLASTINE C1 + (usata per la routine estratta da cervello di coniglio con ISI=1.26 e STA-NEOPLASTINE R (ricombinante con ISI=0.97).Materiali e metodi:I dosaggi sono stati eseguiti su STA-R MAX STAGO.In accordo con LG CLSI-H47-A2 è stato calcolato il tempo di riferimento per ogni tromboplastina su 20 campioni di donatori. Quindi in accordo con LG CLSI EP09-A3 il confronto tra tromboplastine è stato fatto valutando campioni di pazienti : 75 "normali", 100 in TAO, 5 con difetti fattoriali, 26 in terapia con DOAC e 3 con LAC.Risultati:Nei pazienti in TAO, i valori di INR hanno mostrato questi "agreement": STA-NEOPLASTINE R vs STA-NEOPLASTINE C1+ :y = 1,1268x - 0,1101 R^2 = 0,9830; bias 0.20 INR ; limiti di concordanza al 95%, da -0.9 a 1.3 INR.STA-NEOPTIMAL vs STA-NEOPLASTINE C1+ :y = 1,0774x - 0,0562 R^2 = 0,8969; bias 0.27 INR; limiti di concordanza al 95%, da -0.15 a 0.69 INR.TriniCLOT HTF vs STA-NEOPLASTINE C1+:y = 0,9433x + 0,1100 R^2 = 0,9303; bias -0.05 INR ; limiti di concordanza al 95%, da -0.55 a 0.45 INR.L'analisi complessiva delle ratio di tutti i soggetti (134) ha mostrato coefficienti di correlazione: STA-NEOPLASTINE R vs STA-NEOPLASTINE C1 + R^2 = 0.986; STA-NEOPTIMAL vs STA-NEOPLASTINE C1 + R^2 =0.923; TriniCLOT HTF vs STA-NEOPLASTINE C1+ R^2 =0.949. La tromboplastina estrattiva NEOPTIMAL (ISI = 1,01) mostra una migliore correlazione con l'attuale tromboplastina in uso, sia nel monitoraggio della TAO, sia nella valutazione complessiva di ogni condizione di riduzione dell'attività coagulante. Le performance analitiche sembrano superiori alla tromboplastina ricombinante a ISI=1,00, dello stesso produttore. Performance accettabili ha dimostrato TriniCLOT HTF. L'introduzione di una tromboplastina a ISI=1 semplifica la refertazione del PT evitando di dover distinguere tra soggetti in TAO e non. L'origine estrattiva pare fornire maggiore sensibilità al reagente in tutto il range di misura.

P066
CONFRONTO TRA STA-CLOT APCR E PEFAKIT APCR NELLA VALUTAZIONE DELLA RESISTENZA ALLA PROTEINA C ATTIVATA

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La valutazione della resistenza alla proteina C attivata (APC) è un metodo di screening utilizzato per ricercare la mutazione del fattore V Leiden.Pefakit APC-R Fcator V Leiden (Pentapharm) è un test funzionale che si basa sull'incubazione del plasma diluito in plasma carente di Fattore V (per correggere l'assenza di altri fattori) e incubato a 37° con un attivatore del fattore V (tratto da veleno di Daboia Russelli) per convertire il FV in FVa. La coagulazione viene poi iniziata grazie all'aggiunta di un attivatore della protrombina (tratto dal veleno di Notechis Scutatus). Si esegue un tempo di coagulazione aggiungendo APC (APC+) ed uno invece in assenza di APC (APC-) e si calcola il rapporto tra i 2 tempi: se tale rapporto è superiore a 2.7 (strumento dipendente) il paziente è normale, se inferiore è presumibilmente portatore di mutazione del fattore V Leiden. Basandosi sulla magnitudo del rapporto, il metodo fornisce anche la possibilità di distinguere tra portatori eterozigoti ed omozigoti del difetto ma è opportuno consigliare la conferma con la ricerca diretta della mutazione.STA-STACLOT APC-R (Stago) è un test funzionale che si basa su prolungamento del tempo di coagulazione del plasma in presenza di APC. Anche in questo test il plasma del paziente va diluito con plasma carente di fattore V e poi addizionato con un attivatore del fattore X tratto da veleno di Crotalus Viridis. Il prolungamento del tempo di coagulazione oltre 120 secondi in presenza di APC indica la capacità del plasma del paziente di inattivare il fattore Va, mentre un tempo di coagulazione più corto è compatibile con la probabile presenza di una mutazione del fattore V. Scopo dello studio è stata quindi la comparazione dei due test. Materiali e metodi: Sono stati valutati 90 soggetti, con esecuzione dei due diversi test di screening la ricerca della mutazione Leiden del fattore V con metodo real time PCR (GeneXpert, Werfen).Risultati: dei 90 soggetti studiati considerati, 15 erano portatori eterozigoti della mutazione V Leiden. Tutti i soggetti sono stati correttamente classificati da entrambi i test funzionali. Conclusioni: la resistenza alla proteina C attivata va considerata come uno screening nella ricerca della mutazione Leiden del fattore V. Entrambi i test sono stati in grado di identificare correttamente tutti i pazienti con mutazione del fattore V Leiden. Il test in fase unica ha il vantaggio di essere più rapido sia nell'esecuzione che nella preparazione dei reattivi.

P067
IMPIEGO DI RIVAROXABAN IN PAZIENTI CON TRAPIANTO D'ORGANO SOLIDO

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Introduzione: il trapianto d'organo solido da donatore e la terapia immunosoppressiva per prevenire il rigetto d'organo predispongono ad uno stato di ipercoagulabilità; la terapia anticoagulante è aggiunta nel piano terapeutico se sono presenti evidenze cliniche che aumentano il rischio tromboembolico. Lo schema standard prevede l'utilizzo del Warfarin, con monitoraggio costante di INR. È dimostrato che gli anticoagulanti orali diretti (DOAC), come Rivaroxaban, a confronto di Warfarin, hanno efficacia sovrapponibile, minore variabilità inter e intra individuale e minori interferenze farmacologiche. Lo studio ha verificato il passaggio da Warfarin a Rivaroxaban in soggetti sottoposti a trapianto d'organo.
Materiali e metodi: sono stati selezionati 3 pazienti (2 con allotrapianto di rene e 1 di fegato), con funzionalità renale ed epatica stabile, in terapia con Warfarin. E' stato effettuato il monitoraggio del passaggio a Rivaroxaban (15 mg/die) mediante due prelievi giornalieri di sangue, in corrispondenza del punto di valle (ore 8.30) e di picco (ore 12.30) del farmaco per 15 giorni. In tutti i campioni sono stati dosati il Rivaroxaban, con metodo cromogenico anti fattore Xa su analizzatore ACLTop 700 (Werfen), gli immunosoppressori Tacrolimus e Sirolimus e creatinina. Due volte alla settimana sono stati inoltre valutati emocromo, AST, ALT, INR, aPTT; il controllo è proseguito poi a cadenza mensile per 4 mesi.
Risultati: la terapia con Rivaroxaban non ha dato effetti collaterali. Il Rivaroxaban a picco è risultato 203±72 µg/L (media±DS), nel range di riferimento atteso. Il Rivaroxaban nel punto di picco dei singoli pazienti è risultato rispettivamente 99±35, 231±32 e 256±67 µg/L ($p < 0.05$). Rivaroxaban nel trapianto di fegato (99±35) è più basso che nel trapianto di rene (243±49) con $p < 0.05$. Immunosoppressori, creatinina e gli altri parametri sono risultati entro i range attesi.
Conclusioni: le scarse evidenze scientifiche sui DOAC nei trapianti non hanno favorito l'uso di tali farmaci e i dati osservazionali necessitano di ulteriori verifiche. Nei pazienti dello studio il Rivaroxaban, a funzione renale ed epatica stabile, fornisce la concentrazione attesa. È da approfondire la farmacocinetica di Rivaroxaban nei diversi tipi di organo solido trapiantato.

P068
PROTEINA C COAGULATIVA E PRESEPSPEINA NEI PAZIENTE CON SEPSI: NOSTRA ESPERIENZA

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La sepsi grave e lo shock settico rappresentano stati infiammatori che derivano da una risposta sistemica a un'infezione batterica. Durante la risposta infiammatoria (SIRS) si assiste ad un coinvolgimento di mediatori solubili sia della flogosi che della cascata coagulativa. Nei pazienti con sepsi grave e shock settico si verifica una anomala attivazione della cascata della coagulazione e della risposta infiammatoria, pertanto tali patologie necessitano di una rapida e precoce diagnosi per garantire una maggiore sopravvivenza dei pazienti. Se è noto che la emocoltura rappresenta il gold standard per la diagnostica della sepsi e la presepsina è significativamente aumentata in pazienti settici, recentemente è emerso il ruolo in tale processo di altre molecole quali la proteina C coagulativa. Questa è un inibitore fisiologico della coagulazione, in grado di regolare l'attività del fattore V e VIII, ed è in grado di inibire sia le attività infiammatorie mediate dalla trombina che l'adesione dei leucociti all'endotelio. Scopo di questo studio è stato quello di valutare se in pazienti con emocolture positive esiste una correlazione tra le concentrazioni della proteina C coagulativa e la presepsina.
Materiali e metodi: nel periodo compreso tra aprile e maggio 2017, sono stati selezionati 35 pazienti con emocolture positive (14 maschi e 21 femmine) (età mediana dei maschi 74 anni età mediana delle femmine 69 anni). Le determinazioni della presepsina (plasma litio-eparina) è stata eseguita sull'analizzatore PathFast® (Mitsubishi Gepa) con metodica immunoenzimatica in chemiluminescenza. Il dosaggio della proteina C quantitativa funzionalmente attiva è stata determinata sull'analizzatore CS5100 (Siemens). Le determinazioni sono state monitorate per tre giorni consecutivi.Risultati: retta di regressione $y = 129{,}2111 - 23{,}9553 \log(x)$ (intercetta CI 95% 94,71 to 163,70) coefficiente di regressione $R = -0.408$, CI 95% (-0,59 to -0,18) $p<0.005$ il coefficiente di correlazione mostra una correlazione inversa statisticamente significativa.
Conclusioni: I nostri dati preliminari confermano quanto riportato in Letteratura sul ruolo della proteina C coagulativa nei pazienti con sepsi. Un decremento della presepsina accompagnata ad un aumento della proteina C coagulativa soprattutto in terza giornata fornisce una indicazione sul decorso clinico della sepsi. Pertanto appare utile, in corso di sepsi, associare al dosaggio della presepsina e quello della proteina C coagulativa al fine aumentare il potere predittivo positivo del trattamento farmacologico.

P069
UPPER EXTREMITY VENOUS THROMBOSIS IN CANCER PATIENTS WITH PERIPHERALLY INSERTED CENTRAL CATHETERS: PRELIMINARY DATA FOR THE CREATION OF AN INTEGRATED CARE PATHWAY

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Introduction: Cancer patients are in need of prolonged treatment and care, so a central venous catheter (CVC) is often placed to facilitate chemotherapy. The use of peripherally inserted central catheters (PICCs) has grown rapidly, because they are inserted in the arm and avoid many complications associated with CVC insertion in the neck or chest. However, PICCs are also associated with risk of upper extremity venous thrombosis (UEVT). Objective: To evaluate the incidence of UEVT and establish the most predictive risk factors for the development of PICC-related thrombosis in cancer patients, during chemotherapic treatment, for the future design of an integrated care pathway (ICT) that could be used to prevent thrombotic events. Patients and methods: We conducted a retrospective cohort study in cancer patients who underwent PICC placement for the administration of chemotherapy between 1th August 2015 to 31th July 2016. All PICC lines were inserted in standardized fashion through a group of extensively trained nurses. Symptomatic UEVT was confirmed by ultrasound. All patients were followed for a minimum of 6 months after PICC insertion, unless they died during this period. Factors previously associated with catheter-related thrombosis, including side of catheter placement, tip location, tumor type, inherited and acquired thrombophilia and environmental factors have been evaluated. Results: Of 551 cancer patients treated with 4-Fr mono-lumen polyurethane PICCs, 19 patients (3,4%) developed UEVT. In the most common malignancies (i.e. gastrointestinal tract tumors) the percentage of UEVT was approximatively 6%. Moreover, in 3 patients with UEVT (15,8%), inherited thrombophilia (factor V Leiden and/or prothrombin mutation) was found. At the same time, coagulation status analysis revealed that d-dimer and factor VIII were the more sensitive markers during the UEVT ultrasound monitoring. On average, UEVT appeared within 60 days from PICCs implantation and approximatively the 50% of events occurred during the summer, when heat and dehydration are higher. Conclusions: In PICCs-treated patients, some tumors, as well as pro-coagulant mutations and the period of the year, appear to enhance UEVT events. Implementation of our data will help to assess the utility of an ICT to reveal patients at high risk to develop UEVT, an event that causes treatment suspension, enhancing the risk of tumor progression.

P070
DOSAGGI FUNZIONALI ED APPROPRIATEZZA DELLA RICHIESTA DEI NUOVI ANTICOAGULANTI ORALI AD AZIONE DIRETTA (DOAC)

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Introduzione: I DOAC agiscono su un singolo fattore della cascata coagulativa. Il dabigatran inibisce il fattore II attivato mentre rivaroxaban e apixaban il fattore X attivato. I dosaggi funzionali per la determinazione dei DOAC si dividono in test coagulativi tradizionali (aspecifici) e test capaci di quantificare indirettamente il farmaco (specifici).Obiettivo: Confrontare le responsività delle metodiche specifiche, test all'ecarina per dabigatran, dosaggio anti-fattore X attivato (anti-Xa) per rivaroxaban e apixaban, e aspecifiche, tempo di tromboplastina parziale attivata (aPTT) per dabigatran, tempo di protrombina (PT) per rivaroxaban e apixaban. Correlare la normalità dei test aspecifici con la concentrazione di DOAC.Materiali e Metodi: Tutti i dosaggi sono stati eseguiti con reattivi e strumentazione STA R Max (STAGO) e la responsività è stata valutata confrontando il valore del 2 clotting time (2CT), considerando più responsivo il test con il 2CT inferiore. Per ognuno dei farmaci è stata confrontata la metodica funzionale specifica con quella aspecifica utilizzando calibratori farmaco specifici. Per valutare possibili differenze di responsività delle metodiche aspecifiche utilizzando i calibratori o i plasmi dei pazienti, sono stati raccolti campioni di plasma di 40 pazienti giunti all'IRCCS Policlinico San Donato, sui quali sono stati eseguiti i relativi test specifici e aspecifici, in base al farmaco assunto.Risultati: Le metodiche specifiche mostrano maggior responsività rispetto a quelle aspecifiche per i DOAC. 2CT dabigatran: test ecarina 130 ng/mL vs aPTT 189 ng/mL; 2CT rivaroxaban: anti-Xa 162 ng/mL vs PT 352 ng/mL; 2CT apixaban: anti-Xa 166 ng/mL vs PT 1164 ng/mL. La responsività delle metodiche aspecifiche usando i calibratori risulta sovrastimata per il dabigatran: 2CT calibratori 143 ng/mL vs pazienti 228 ng/mL e per il rivaroxaban: 2CT calibratori 313 ng/mL vs pazienti 423 ng/mL al contrario sottostimata per l'apixaban: 2CT calibratori 1106 ng/mL vs pazienti 780 ng/mL. I valori di PT o aPTT nell'intervallo di normalità non correlano con l'assenza di anticoagulante in circolo.Conclusioni: Questi risultati suggeriscono che i test tradizionali non possono essere utilizzati nella valutazione preoperatoria del paziente in trattamento con DOAC.

P071

ALBUMINURIA ESPRESSA COME RAPPORTO ALBUMINA/CREATININA: RISULTATI DEL PROGRAMMA DI VALUTAZIONE ESTERNA DI QUALITA' (VEQ) DEL CENTRO DI RICERCA BIOMEDICA

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Introduzione: Sulla base delle linee guida della Kidney Disease Improving Global Outcomes (KDIGO) e di quelle del Gruppo Interdisciplinare laboratorio e clinica dell'Apparato Urinario (GIAU), l'albuminuria deve essere determinata con metodi quantitativi ed il risultato espresso in rapporto alla creatinina (ACR). Nel 2016 il Centro di Ricerca Biomedica ha implementato uno schema di VEQ specifico per l'albumina nelle urine che prevede la stima di ACR. Scopo: Valutare la "qualità" della misura in termini di variabilità interlaboratorio e di armonizzazione tra i diversi sistemi analitici.
Metodi: Sono stati analizzati i risultati di ACR di 118 laboratori relativi ai primi 12 campioni di controllo. I metodi utilizzati per la determinazione dell'albuminuria sono prevalentemente turbidimetrici (n=103), solo 12 nefelometrici (9 Siemens e 3 Beckman) e 3 chimica secca. I risultati di ACR sono elaborati per gruppi omogenei, anche se derivano da un calcolo. E' stato calcolato il CV% medio in un intervallo di concentrazione di 4,07 – 27,8 mg/mmol, il bias di ogni sistema analitico vs il valore assegnato (media delle mediane) ed il numero di prestazioni non accettabili (PNA) riscontrate per un Errore Totale ammissibile di 16,5%.
Risultati: CV% medio = 8,30±1,18. Bias% medio = Nef. Siemens: 13,7±2,9; Abbott Architect: 4,6±2,4; Beckman LX/DxC: -6,6±3,3; Beckman AU: -5,2±2,5; Roche Cobas: -0,8±2,9; Siemens Advia: -4,9±3,4; Siemens Dimension: -0,8±3,8. Il numero di PNA medio è risultato 5,0±1,2% per ACR nel range 5,7-27,8 mg/mmol; 12,5±2,3% per ACR <4,6 mg/mmol.
Discussione: Il limite dello studio è che non sono stati ancora distribuiti campioni di controllo con ACR <4 mg/mmol, quelli più clinicamente utili. Nel range di concentrazione studiato la variabilità interlaboratorio risulta contenuta. I sistemi analitici turbidimetrici presentano bias compresi tra -6,6% e +4,6% mentre i sistemi nefelometrici Siemens presentano un bias medio del 13,7% che riflette quello osservato per l'albuminuria non normalizzata in funzione della concentrazione delle urine.
Conclusioni: Per ACR >5,7 mg/mmol la qualità nella misura di ACR risulta soddisfacente ma già con ACR di 4,07 mg/mmol (46 mg/g) ben il 12% dei laboratori non raggiunge il livello di qualità minimo per l'impiego clinico della misura.

P072

HARMONIZATION E STANDARDIZATION OF CQI IN THE LABORATORIO UNICO METROPOLITANO (LUM) - BOLOGNA

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The "Laboratorio Unico Metropolitano" (LUM), inaugurated in 2016, is a complex operation unit of the USL Company of Bologna. It has been designed to provide users with qualifying, homogeneous and fast reporting performances, through innovative technological/methodological and managerial choices. The network of the USL's laboratories of Bologna is set on the Hub and Spoke model and it collects samples from the withdrawal points of the Company located in the territory of Bologna and the province. About 22 millions of analysis per year are done concerning about 1500 different types of exam. The Ospedale Maggiore's LUM is the hub of the territorial network of collection and analysis of biological samples, to which belong also 11 spoke laboratories and 65 withdrawal points. In this report we want to focus on those related to the path undertaken to evaluate the reliability of diagnostic information through the use and monitoring of the CQI Program. Choices that have been made are the result of an intense time of comparison between the professionals of all the laboratories that have shared knowledge and experiences. Having a fully coordinated CQI management for each sector of analysis means, basically, a uniformity in the choice of instrumentation, materials and custom rules for each analytic and IT tools. What has given a great added value to this path was undoubtedly the choice of exhaustive software to be used daily for technical and clinical validation of analytical sessions, enabling us to have full control over individual and cumulative performances. The features of the program have fully responded to our requests: integration into the LIS without any additional workstations, easy consultation, ability to act from all locations with centralized action, coordinated management of materials (same lot in all locations, contemporary start for each lot change), instrumental alignment control of the various locations and traffic light code for real-time alarms management with the possibility to graphically display the trend of the performances at various levels. The availability of those tools allows us to rapidly and reliable analytical evaluation and access the need for any corrective action.

P073
GESTIRE IL RISCHIO CLINICO ATTRAVERSO IL POTENZIAMENTO DELLE STRATEGIE DI CONTROLLO DI QUALITÀ': UNA "MISSION" POSSIBILE?

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Gestire il rischio clinico, cioè definire le strategie per minimizzare la possibilità di refertare risultati inaccurati che possano causare danni ai pazienti, rappresenta una sfida per ogni laboratorio. E' tuttavia possibile ridefinire i programmi di controllo di qualità con l'obiettivo di misurare la capacità di rilevare errori e progettare strategie individualizzate per la gestione del rischio. Abbiamo scelto 4 parametri: Calcio, Glucosio, Creatinina e Troponina. I controlli sono stati determinati dosando sieri forniti da Bio-Rad Laboratories su strumentazioni Cobas Roche. I risultati sono stati elaborati con il software Unity Real Time (Bio-Rad) con il cui ausilio abbiamo stabilito gli obiettivi analitici definendo l'errore totale massimo accettabile (TEa). Per calcolare il rischio clinico associato ai test in esame abbiamo utilizzato il software Mission:Control (M:C di Bio-Rad). Partendo dai valori di media, di TEa, del numero di test eseguiti giornalmente e delle frequenze di QC, M:C ha fornito per ogni test il numero di eventi di controllo necessari per evidenziare l'errore E(QCE), il numero di pazienti refertati con possibilità di errore E(NUF), e il numero di risultati errati non refertati E(NUC). Il rischio clinico è stato calcolato raggruppando i test in classi di severità del danno attribuendo al Calcio valore 1, Glucosio e Creatinina valore 2 e Troponina valore 3. Per il Glucosio modificando il numero degli eventi di QC da uno a due odierni, così come suggerito dal modulo QC-Designer del sw M:C, siamo passati da E(QCE)=4.1, E(NUF)=14.6 e E(NUC)=4.8 ad E(NUF) e E(NUC)<1 abbassando il rischio da 4.7% a <1%. Analogamente, per la Creatinina abbiamo raggiunto un E(NUF) ed un E(NUC)<1 applicando la strategia suggerita di 3 eventi di QC giornalieri e la regola di Westgard 2-2s. Per il Calcio la strategia suggerita ha portato a un dimezzamento degli E(NUF) passando da 112 a 53.4. Per la Troponina, i valori già ottimali di E(NUF)<1 ed (ENUC)<1 hanno portato ad utilizzare la regola 1-4s lasciando immutato il calcolo del rischio.L'uso del software M:C, pertanto, consente di sviluppare strategie individuali di qualità per poter rilevare errori nella prima fase del processo ed incrementare le procedure di controllo attraverso l'analisi del rischio sul paziente.

P074
SPIDIA4P: SPIDIA FOR PERSONALIZED MEDICINE - STANDARDISATION OF GENERIC PRE-ANALYTICAL PROCEDURES FOR IN-VITRO DIAGNOSTICS FOR PERSONALIZED MEDICINE

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L'uso diagnostico dei saggi molecolari "in vitro" potrebbe essere limitato dalla mancanza di linee guida per la raccolta, la manipolazione, la stabilizzazione e la conservazione dei campioni. SPIDIA,"Standardisation and improvement of generic pre-analytical tools and procedures for in-vitro diagnostics",finanziato dalla UE nell'ambito del PQ FP7, è stato un progetto sviluppato da 16 istituzioni accademiche e da organizzazioni internazionali e società scientifiche, finalizzato alla standardizzazione e al miglioramento delle procedure pre-analitiche per la diagnostica in vitro. Sulla base dei risultati derivanti da numerose Valutazioni Esterne di Qualità paneuropee (EQAs), nell'ambito del Comitato Tecnico CEN 140 per i dispositivi medici in vitro, sono stati sviluppati e di introdotti in Europa i primi nove documenti standard "evidence-based" (Tecnical Standard, CEN/TS) per i flussi di lavoro pre-analitici per saggi molecolari.SPIDIA4P è un progetto H2020 (Coordination and support action) che nasce dai risultati del precedente progetto SPIDIA. Il consorzio è composto da 19 partner altamente qualificati provenienti dall'industria privata e da istituzioni pubbliche a cui si aggiunge l'European Committee for Standardisation (CEN), una delle tre organizzazioni riconosciute ufficialmente dall'EU come responsabili per lo sviluppo di TS. Il progetto ha l'obiettivo di sviluppare e implementare un portafoglio completo di ulteriori 14 TS (CEN/TS e ISO/IS) per la standardizzazione della fase pre-analitica dei flussi di lavoro per saggi molecolari finalizzati alla medicina personalizzata.
Il laboratorio di Chimica Clinica e di Biologia Molecolare Clinica dell'Università di Firenze è stato responsabile, nell'ambito del primo progetto SPIDIA, dei WPs relativi allo sviluppo di linee guida per la fase pre-analitica dei campioni di sangue per DNA, RNA e DNA libero circolante ed è ora coinvolto, in SPIDIA4P, nello sviluppo di nuovi CEN/TS e ISO/IS sulla standardizzazione della fase pre-analitica per DNA, RNA e per l'esame citomorfologico e citoimmunochimico delle Cellule Tumorali Circolanti (CTC).
Qui presentiamo una panoramica di SPIDIA4P concentrandoci in particolare sui principali aspetti relativi agli approcci pre-analitici per l'isolamento del CTC.

P075

PROPOSTA DI UN APPROCCIO STRUTTURATO ALL'ANALISI E ALLA RISOLUZIONE DI NON CONFORMITÀ RELATIVE ALLA VEQ

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Introduzione: Quando un risultato VEQ non rispetta i criteri di prestazione stabiliti dal Provider, la norma ISO 15189 richiede che il Laboratorio esegua e documenti un'azione correttiva (punto 5.6.3.4). Condizione necessaria per soddisfare questo requisito è stabilire a priori un approccio standardizzato all'analisi delle cause che possono aver condotto all'errore. Pertanto, il gruppo di studio SIBioC "Qualità analitica" intende proporre un approccio all'analisi e alla risoluzione delle non conformità (NC) VEQ.
Metodi: L'approccio indaga "a ritroso" il processo di produzione del dato: 1) Fase post-analitica: ricerca di errori da parte del Laboratorio (di trascrizione, di unità di misura, di conversione, di decimali mal posizionati, ecc) oppure legati all'elaborazione dei dati da parte del Provider (es. assegnazione dei risultati ad un errato gruppo omogeneo); 2) Fase analitica: analisi dei dati CQI (sia "grafica" che numerica) relativi al periodo di prestazioni stabili individuabile "attorno" al giorno di esecuzione della prova, al fine di valutare la capacità del sistema analitico di rispettare i traguardi proposti dal Provider e il conseguente rischio di fornire risultati non conformi. Da ciò si può inoltre evincere se il problema sia imputabile alla presenza di bias (es. calibrazione scorretta) oppure ad un limite intrinseco della metodica (se, in assenza di bias significativo, l'imprecisione è già più ampia del range di accettabilità centrato attorno al valore target); 3) Fase pre-analitica: ripetizione del test (se campione conservato): se la NC non si conferma è probabile un errore casuale, in caso contrario si può ipotizzare un errore del Laboratorio nel trattamento del campione (se compresenza di NC a carico di altri analiti) oppure un problema a carico del campione stesso (sua inomogeneità oppure effetto matrice legato all'utilizzo di lotti di reagenti diversi all'interno del gruppo omogeneo).
Risultati: Questo metodo è stato provato nell'analisi di 35 NC e ha consentito di individuarne la causa nel 77% dei casi (post-analitica 11%, analitica 81% e pre-analitica 8%).
Conclusioni: Un approccio strutturato consente di individuare la causa della maggior parte delle NC VEQ e di impostare le appropriate azioni correttive e/o preventive.

P076

CONTROLLO DI QUALITÀ BASATO SUL RISK MANAGEMENT PER I PARAMETRI SOGGETTI A COMUNICAZIONE DEL DATO CRITICO

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Di recente la letteratura ha proposto una revisione dei CQI, attualmente basati sul modello Westgard (stabilità delle performance analitiche definendo a priori obiettivi di qualità sulla base dell'errore totale massimo accettabile/ stato dell'arte), indicando due possibili approcci: uno basato sull'incertezza di misura e l'altro sul risk management. In ospedale i risultati critici vengono tempestivamente comunicati al personale medico per una immediata rivalutazione/gestione del paziente (pts). Errori/ritardi nella comunicazione di tali dati possono avere seri esiti sul pts e conseguenze medico legali. Scopo del lavoro: sperimentare il CQI basato sul risk management (Bio-Rad Mission:Control^{TM}) per i parametri critici per misurare il rischio di refertare risultati errati e programmare piani di miglioramento. Materiali e metodi. Per i parametri critici (creatinina(CREA), sodio(Na), potassio(K), calcio(Ca), troponina(TnI) e digossina(DIG) si è effettuato un risk assessment determinando la percentuale di risultati clinici errati rispetto al numero di referti totali [ENuf%=ENuf/(EQCE*NB)*100] (ENuf=risultati clinici errati già refertati, EQCE=numero sedute CQ utili per rilevare l'errore analitico, NB=numero campioni tra le sedute CQ). In funzione degli ENuf% calcolati sono state sviluppate strategia CQI alternative volte a ridurre il rischio o mitigare gli effetti (frequenza CQI/regole Westgard). Risultati. I risultati ottenuti sono: ENuf% per K, TnI e CREA <1; per DIG=6.7 e Na=3.6. Il valore di ENuf% per i parametri K, TnI e CREA ha confermato l'adeguatezza delle strategie già in essere. Si sono invece implementate le seguenti strategie CQI di tipo individualizzato (IQCP) per DIG: da 1:3ds/1CQI die a 1:2ds/1CQI die e per Na da 1:2.5ds/1CQI die a 1:2.0ds/2CQI die. Le variazioni apportante hanno migliorato l'ENuf% di DIG e Na riducendolo, rispettivamente, a 4 e 2.6%. Discussione e conclusioni. L'analisi del rischio ha permesso di valutare oggettivamente l'adeguatezza del modello CQ in uso correlando performance analitiche, strategie CQI, carichi di lavoro e significatività clinica dei test. I metodi di mitigazione del rischio hanno ottimizzato il processo e la sicurezza del paziente. Estenderemo quindi le IQCP basate sul risk management agli ospedali periferici.

P077
INFLUENZA DELL'ALTITUDINE SULLA MISURAZIONE DELLA PO2 NEI PROGRAMMI DI CQ ALLARGATO E VEQ DEGLI EMOGASANALIZZATORI

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La gestione centralizzata degli emoganalizzatori (EGA) in regime di Point of Care Testing comporta un monitoraggio delle prestazioni analitiche tramite Controlli di Qualità (CQ) allargati, già in uso da tempo, e di Verifica Esterna di Qualità (VEQ), di più recente introduzione per tali strumenti. L'osservazione attenta dei risultati relativi alla pO_2 sui grafici dei CQ a bordo macchina (ABL800 Radiometer) non presentava criticità, mentre gli stessi dati, nell'elaborazione del CQ allargato della ditta (WDC), fornivano medie tendenzialmente al limite inferiore dell'intervallo del gruppo omogeneo e comunque sempre sotto il valore medio di quest'ultimo. Inoltre, nel 2016, il 25% (2 su 8) dei nostri risultati relativi alla pO_2 è stato classificato, nella VEQ del Centro di Ricerca Biomedica di Padova, come Non Accettabile per sottostima rispetto alla mediana dei risultati forniti da tutti i centri che utilizzano lo stesso sistema diagnostico. Escluse criticità legate alla strumentazione (membrane o elettrodi) e/o alla mancata aderenza alla procedura per la preparazione ed il campionamento, si è ipotizzato un possibile effetto dovuto alla differenza tra l'altitudine del sito di analisi (580 metri slm) e quella dei luoghi di produzione sia dei CQ sia delle VEQ: le calibrazioni degli EGA, infatti, tengono conto della pressione atmosferica attuale misurata dal barometro interno, mentre le fiale dei controlli costituiscono un sistema chiuso equilibrato con la pressione atmosferica della zona di produzione. Il documento "Blood gas measurements at high altitudes", fornito dal produttore, conferma che, mentre le fluttuazioni giornaliere di pressione sono trascurabili, la differenza dovuta all'altitudine viene compensata dal computer dello strumento. Il sistema WDC prevede la possibilità di variare l'altitudine preimpostata (0 metri slm): l'inserimento del dato reale (580 metri slm) ha migliorato la posizione dei valori di pO_2 all'interno del gruppo omogeneo. Lo stesso principio di correzione è stato applicato ai valori ottenuti sui campioni del programma VEQ (+2,3 mmHg), ottenendo un miglioramento nella classificazione dei risultati. I dati ottenuti conferiscono all'altitudine un ruolo di variabile da non trascurare nella valutazione delle prestazioni di qualità degli EGA.

P078
DEFINIZIONE E VERIFICA DELL'APPLICABILITÀ DI TRAGUARDI PER LA VALUTAZIONE DELLA QUALITÀ ANALITICA IN UN PROGRAMMA DI VEQ: RISULTATI PRELIMINARI DI UNO STUDIO COLLABORATIVO FRA ISTITUZIONE E SOCIETÀ SCIENTIFICA

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Introduzione: Da anni, il Centro di Riferimento Regionale per la Qualità dei Servizi di Medicina di Laboratorio (organo istituzionale di Regione Lombardia) promuove e verifica la qualità dei Laboratori lombardi, anche mediante l'organizzazione di programmi di VEQ. Poichè il cardine di questi programmi è l'uso di limiti di accettabilità verso cui valutare le prestazioni dei partecipanti, in questo lavoro, nato dalla collaborazione con il gruppo di studio "Qualità analitica" della SIBioC, sono stati analizzati i dati dei Laboratori partecipanti al ciclo 2016 del programma VEQ "Ormoni e marcatori tumorali", al fine di derivare possibili traguardi di massimo errore accettabile compatibili con la qualità analitica emersa dal programma.
Metodi: 32060 risultati provenienti da 158 Laboratori lombardi (24 misurandi per 12 esercizi) sono stati così elaborati: suddivisione per esercizio e per gruppi omogenei (almeno 7 utilizzatori dello stesso metodo); calcolo dello scostamento % di ogni risultato dal valore atteso (media robusta del gruppo omogeneo, dopo eliminazione dei dati aberranti secondo Huber-Hampel); calcolo del 95° percentile degli scostamenti (assunto come traguardo basato sullo stato dell'arte); individuazione dei traguardi basati sulla variabilità biologica (VB) rispettati da almeno il 90% dei risultati inviati; confronto fra le due precedenti tipologie di traguardi (sia in termini di ampiezza, che in termini di % di NC), volto a stabilire quello definitivo: se simili, scelta di quello con minor % di NC; se traguardo VB molto ampio, scelta del secondo.
Risultati: Usando come traguardo la VB, una quota di NC inferiore al 10% si è riscontrata per 15 misurandi con il livello ottimale, per 3 con il desiderabile, per 4 con il minimo; 2 non hanno traguardi di VB. I 24 traguardi analitici candidati sono stati definiti "modulando" le specifiche precedenti con lo stato dell'arte desunto dal programma VEQ.
Conclusioni: La definizione di limiti indipendenti dalle singole piattaforme analitiche per giudicare l'accettabilità dei risultati di VEQ potrebbe offrire un valore aggiunto rispetto all'uso di traguardi di tipo statistico, che, potendo variare fra i gruppi di pari (intervalli più ampi per metodi più imprecisi), disincentivano l'adozione di metodi migliori.

P079
EXTERNAL QUALITY ASSESSMENT SCHEMES (EQAS) AND ISO /IEC 15189 2012

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Background: The ISO 15189:2012 specifies requirements for quality and competence in medical laboratories. Laboratories applying for accreditation are required to participate to EQAS schemes that substantially fulfill the relevant requirements of ISO/IEC 17043 and are asked to have evidence of positive results in EQAS exercises, as stated in RT 26 Accredia rev 05. To verify to which extent the ISO 15189 requirement was met by public and private laboratories in Tuscany, the Centro Regionale di Riferimento per la Qualità in Laboratorio, reviewed data from the EQAS Clinical Chemistry. The Scheme is run according toISO /IEC 17043 (Accredia PTP 0013). Methods: A survey on Tuscany public and private Laboratories (n=96) was conducted; reports from 4 consecutive EQA samples (January-May 2017) were reviewed. Participants were considered to have obtained positive performance, when 4 consecutive results for a single analyte, were classified inside PTP limits of acceptability LA, according to ISO/ IEC 17043. Twenty parameters were reviewed.
Results: The survey showed that 18 laboratories (18.75%) fulfilled the requirement since in their reports, 20 parameters were classified inside LA in 4 consecutive exercises. Some of the participants (6,2%) did not meet the requirement because 1 result, out of 80 results, was exceeding LA; some others (8.3%) didn't because of 2 or 3 results for one or more analyte. When single analytes were examined, 80% of laboratories had evidence of positive performance, although some analyte, such as serum albumin, proved to be a challenge for most of the EQAS participants.
Conclusions: The survey shows that some laboratories have an adequate performance. However, many factors need to be taken into account when deciding whether a laboratory meet the ISO/IEC 15183 EQA performance requirements. First, PTPs should apply homogeneous evaluation criteria to enhance performance comparison, possibly based on ISO/IEC 17043. Second, synthetic performance indicators may need to be integrated with all other information present in PTP reports, to contextualize evaluations. Finally, PTPs may play a crucial role encouraging thoroughly investigation of unsatisfactory or repeatedly questionable results and supporting laboratories willing to apply for ISO/IEC 15189.

P080
STIMA INDIRETTA DEGLI INTERVALLI DI RIFERIMENTO IN AMBITO PEDIATRICO

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Introduzione: In continuità col lavoro presentato lo scorso anno (1) abbiamo utilizzato l'algoritmo in studio per la stima indiretta degli intervalli di riferimento (IR) in età pediatrica applicandolo alla Fosfatasi Alcalina (ALP).
Materiali e metodi: I dati analizzati sono stati raccolti presso gli ambulatori dell'Ospedale Pediatrico Meyer di Firenze in un periodo di circa 2 anni di attività e rispondono ad una serie di requisiti iniziali di reclutamento, in analogia a quanto adottato nel lavoro Ceriotti et al. (2).
Risultati e discussione: I risultati ottenuti dall'elaborazione dei dati provenienti dall'Ospedale Pediatrico Meyer concordano statisticamente con quelli ottenuti dallo studio multicentrico di Ceriotti et al. (2). Il prossimo obiettivo sarà applicare l'algoritmo per l'armonizzazione degli IR della ALP, nel critico ambito dei valori pediatrici, in Area Vasta Sud Est – Toscana.
1) Milletti E, Cinci F, Bellini C, et al. Algoritmo per la stima degli intervalli di riferimento; 48 Congr. Naz. SIBIOC, 18/20 ottobre 2016 – Torino.
2) Ceriotti F, Panteghini M, Guerra E, et al. Intervalli di riferimento standardizzati della Fosfatasi Alcalina serica in soggetti pediatrici. Biochim Clin 2017;41;166-74.

P081
LEAN ORGANIZATION: AUMENTO DELL'EFFICIENZA DELL'AREA CORELAB

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All'iniziale introduzione dell'innovativa Automazione Integrata Beckman Coulter, costituita da un sistema POWER EXPRESS, due analizzatori AU5800, due analizzatori DxI800 e due strumenti Automate1250, è seguito un graduale processo di ottimizzazione e miglioramento dell'efficienza del processo. Basandoci sulle metodologie della Lean Organization si è proceduto alla riqualificazione e miglioramento delle risorse presenti attraverso una continua riduzione degli sprechi e responsabilizzazione del personale.
Il gruppo del settore Chimica Clinica Automatizzata, affiancato da varie figure professionali del gruppo Beckman Coulter e dell'assistenza informatica, ha inizialmente mappato tutte le attività svolte e il flusso di informazioni esistente (Current State Map).
Ogni attività è stata quindi scomposta nelle sue componenti (Failure Mode and Effect Analysis) e per ognuna ne è stata definita la priorità di rischio (IPR).
Abbiamo pertanto individuato e distinto tra:
- attività a valore aggiunto (in grado di incrementare l'efficienza): rivalutate
- attività con nessun valore aggiunto ma necessarie: migliorate
- attività con nessun valore aggiunto: eliminate

Da questo è stato possibile definire la Future State Map cioè come dovesse essere il processo analitico per essere più efficiente e raggiungere gli obiettivi prefissati.
Sono stati sviluppati una serie di Eventi di miglioramento rapido (RIE) che hanno consentito di focalizzare l'attenzione su precise criticità e che hanno portato in tempi rapidi al raggiungimento dei vari obiettivi prefissati.
Questo ha sviluppato nel complesso un flusso continuo di miglioramento del processo che ha portato alla riduzione dei tempi di lavorazione, all'aumento della soddisfazione del personale e alla diminuzione degli errori.
L'analisi metodologica dell'intero processo analitico ci ha consentito di analizzare e ottimizzare il flusso operativo in Chimica Clinica, eliminando le azioni non produttive, concentrando l'attenzione solo sulle componenti che potevano realmente essere migliorate ed enfatizzando quelle che erano già efficienti. Inoltre l'approccio sistematico e progressivo (RIE) ha permesso di realizzare il nuovo flusso operativo in breve tempo raggiungendo tutti gli obiettivi prefissati.

P082
BIOLOGICAL VARIATION OF NEUTROPHILS CELL POPULATION DATA

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Aim of the study: Recent articles showed that neutrophils cell population data (CPD) parameters may be useful for diagnosing myelodysplastic syndromes, sepsis and acute promyelocytic leukemia. Nevertheless, no reliable information has been published on the biological variability (BV) of this parameters, which is an essential requisite for establishing analytical specifications and for accurate interpretation of tests results including assessment of significance of change in serial results (reference change values, RCV). We evaluated the BV of neutrophils CPD parameters generated by Sysmex XN-9000 according to the indications of the European Federation of Clinical Chemistry and Laboratory Medicine.
Material and methods: The study population consisted of 43 health subjects, who participated to the assessment of medium-term (21 subjects; blood sampling once a week for 5 consecutive weeks) and short-term (22 subjects; blood sampling once a day for 5 consecutive days) BV study. Samples were collected by the same phlebotomist and analyzed in duplicate within 2 hours. The analytical (CV_A), within-subject (CV_I) and between-subject (CV_G) component of variation were calculated by CV-ANOVA. Homogeneity of CV_A and CV_I were verified using Bartlett and Cochran tests, Shapiro-Wilk test and Dixon-Reed's criterion were used to verify the normality of residuals and detect outliers.
Results: In the medium-term BV arm the CV_A ranged from 0.3% (NE-SSC) to 3.2% (NE-WZ); CV_I and CV_G ranged from 0.6 (NE-SSC) to 2.6 (NE-SFL) and from 1.2 (NE-WY) to 3.6 (NE-SFL) respectively; RCV ranged from 1.8% (NE-SSC) to 9.6% (NE-WZ). In the short-term BV arm CV_A, CV_I and CV_G ranged from 0.3% (NE-SSC) to 3.3% (NE-WY); from 0.5% (NE-SSC) to 1.8% (NE-WX) and from 2.4% (NE-SSC) to 4.6% (NE-SFL) respectively; RCV ranged from 1.6% (NE-SSC) to 9.4%(NE-WX).
Conclusions: The analytic variation of Sysmex XN for neutrophils CPD parameters are very low, particularly for NE-SSC (CV_A=0.3% in both arm). We complemented this information with CV_I and CV_G data, which were not previously published . The improvement of precision of new analytical technologies entails better confidence in use and description of BV, essential prerequisites for validating the clinical application of new parameters.

P083
IMPLEMENTATION OF AN INTERNAL QUALITY CONTROL PROGRAM FOR THE PHOTOMETRIC ICTERIC INDEX DETERMINATION

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Background: In June 2016, we implemented the quantitative determination of icteric index (II) as a front-line test for screening blood samples with total bilirubin (TB) concentrations ›1.2 mg/dL. In particular, samples with II ≤0.8 are automatically reported as having TB ≤1.2 mg/dL without any bilirubin measurement. This approach, however, imposes to check the analytical performance of II measurements as inaccuracy could increase the rate of false negative results and therefore affect patients' clinical management.
Methods: Missing a dedicated manufactured control material, a complete internal quality control (IQC) program, similar to those implemented for other laboratory tests, has been designed in order to check both trueness and precision of photometric II determination on our Abbott Architect c16000 analyzers (c16-1, c16-2). Trueness is checked by measuring II on low and medium levels of Tecnopath Multichem S-Plus control material (cod. 05P78). Target values and acceptability ranges for each level were determined by calculating mean ± 2SD of 40 II preliminary measurements after the manufacturer's check of the photometer performance of the platforms. Imprecision is evaluated by analyzing daily a frozen serum pool with an II value around the cut-off. Monthly CVs for each platform are then calculated and compared with the optimal goal for imprecision derived from biological variability of TB (≤5.5%).
Results: From June 2016 to May 2017, 1962 and 1311 trueness controls were performed on c16-1 and c16-2, respectively. Mean II values were 1.28 for low level and 3.71 for medium level. Only 1.3% of controls on c16-1 and 2.3% on c16-2 fell out of the acceptability ranges. However, results returned within the range after an immediate repetition, without any further technical interventions. Monthly CV (n=12; mean II value, 1.26) was always <5.2% (mean, 2.9%) on c16-1 and <5.1% (mean, 3.3%) on c16-2, respectively, therefore fulfilling the optimal specification for the clinical use of test.
Conclusions: Although IQC programs for serum interference indices are not widely implemented, our experience with II determination shows that it is possible to organize a complete and effective IQC program for assuring accuracy of these measurements.

P084
DIFFERENT CALIBRATOR OPTIONS MAY STRONGLY INFLUENCE THE TRUENESS OF SERUM TRANSFERRIN MEASURED BY ABBOTT ARCHITECT SYSTEMS

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Background: In our Core-Lab, we measure serum transferrin (TF) by Abbott immunoturbidimetric assay on the Architect c16000 platform. Recently, we observed on EQAS results a consistent negative analytical error (-10%) in TF results, higher than the minimum quality goal derived from TF biological variability (i.e. ±5.7%). We noted that the problem had arisen with the introduction of a new lot (no. 60084) of calibrator in use [Plasmaproteins Cal (PC) (cod. 11200D), manufactured by Sentinel for Abbott Diagnostics], giving on the internal quality 3-level control (Technopath Multichem-S plus, cod. 05PT8) a systematic underestimation of ≈10%. To overcome the problem, Abbott offered to us a different calibrator [Specific Proteins Multiconstituent Calibrator (SPMC) (cod. 1E78)]. Here we present data of a correlation study performed to investigate the effect of different manufacturer's calibrators on the same measuring system for TF.
Methods: 40 leftover fresh-frozen serum samples were assayed in duplicate using Abbott TF immunoturbidimetric assay (cod. 1E04) on Architect c16000, calibrated with PC. The measurements were then immediately repeated after calibration with SPMC (lot 68002M800). Both calibrator options are CE marked for Architect and are traceable to the same higher-order reference material ERM-DA470. Declared calibrator uncertainty (expanded) is 4.17% for PC and 1.83% for SPMC, respectively. System compliance to manufacturer's declared performance was checked by measuring Multichem-S, before and after analytical runs. Correlation was assessed using Deming regression and bias evaluated using difference plots.
Results: TF concentrations ranged from 0.65 to 4.88 g/L. Regression analysis was as follows: SPMC = 1.05 (95%CI: 1.046-1.054)*PC + 0.029 g/L (95%CI: 0.019-0.039), with r^2=0.9966. In average, results obtained with SPMC were 7.6% (0.19 g/L) higher than those with PC, confirming the presumptive underestimation of PC-calibrated results.
Conclusions: Despite both calibrators used in this study are CE marked for the same measuring system and are declared traceable to the same reference material, a clinically significant bias was observed between TF results obtained with the two options. Problems in PC value-assignment protocols can be hypothesized.

P085
TRACEABILITY OF ALKALINE PHOSPHATASE (ALP) MEASUREMENT MAY VARY CONSIDERABLY ALSO USING THE SAME MEASURING SYSTEM: THE CASE OF ABBOTT ARCHITECT

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Background: Starting from 2015, Abbott correctly validates the traceability of its enzyme calibrator factors (CF) for the Architect system by comparison to results from IFCC reference procedure (RP). For ALP, they provide this experimental CF (eCF, 2290) to users as an optional alternative to the theoretical CF (tCF, 2150) derived from the p-nitrophenol molar extinction. Using this eCF, we recently observed a constant positive error on EQAS results (15.6%±3.7, n=5), higher than the desirable goal derived from ALP biological variability (±12%), when our results were compared with the median of the Architect users' group. Therefore, the trueness of ALP measurement in our laboratory was investigated.
Methods: ALP target values were assigned to 3 fresh serum pools by the IFCC RP. The pools were then assayed in triplicate using the Abbott ALP assay (cod. 7D55) calibrated with eCF, carried out on Architect c16000.
Results: The ALP target values were 55.6, 157.2, and 363.2 U/L, respectively, with corresponding expanded uncertainties of 3.1%, 2.5%, and 2.6%. The regression analysis gave the following equation: Architect = 1.058*RP – 2.8 U/L, r^2=0.9992, with the eCF Architect assay showing a negligible average bias, i.e. +1.46% vs. an optimal goal of ±2.75%. In collaboration with the EQAS provider, a survey was issued to assess among participating laboratories using Architect system which CF was used. Among 39 interviewed laboratories, the great majority (87%) used tCF, thus strongly influencing the system-specific (group) consensus value.
Conclusions: Despite observed EQAS results, the Architect ALP assay, when calibrated with eCF, shows optimal standardization. As the survey showed that most laboratories within the Architect group use tCF and not the proper eCF, we assume that this significantly lowers the EQAS median value used as reference for evaluating the performance of participants. Only true value assignment by RP to EQAS materials allows correct evaluation of the performance through a trueness-based (instead of inferior consensus-based) grading of the competency of participating laboratories. Based on our results, we expect that Abbott does indicate only one CF, i.e. that obtained by correlation results using clinical samples with RP-assigned values.

P086
PREVALENZA DI AUTOANTICORPI IN UNA POPOLAZIONE PEDIATRICA CON DIABETE DI TIPO I

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Introduzione: Il diabete di tipo I è una malattia cronica che colpisce bambini e adolescenti ed è dovuta alla distruzione, a livello del pancreas, delle β-cellule deputate alla produzione di insulina. I dosaggi degli anticorpi contro le insule pancreatiche correlati con il diabete sono utilizzati principalmente come supporto nella distinzione del diabete autoimmune di tipo I dal diabete dovuto ad altre cause. Determinare il tipo di diabete permette un trattamento precoce con la terapia più appropriata. Scopo del presente lavoro è stato quello di analizzare in maniera retrospettiva la presenza di autoanticorpi contro il pancreas, contro la tiroide e la prevalenza di autoanticorpi utilizzati nella diagnosi di celiachia in una popolazione con diabete di tipo I. Materiali e metodi: sono stati studiati retrospettivamente 4517 campioni di 542 pazienti diabetici tipo I (età media 10 anni, range 1 mese-25 anni) reclutati consecutivamente nel centro diabetologico pediatrico dell' Azienda Ospedaliera dal gennaio 2009 al dicembre 2016. Tutti sono stati sottoposti a valutazioni biochimiche e determinazioni anticorpali. Sono stati determinati i titoli anticorpali anti glutammato-decarbossilasi (GAD), anti cellule delle isole pancreatiche (ICA), anti insulina (IAA), anti tirosina-fosfatasi (IA2), gli autoanticorpi anti-tireoperossidasi (TPO), anti-tireoglobulina (TG), antitransglutaminasi e antigliadina. Risultati e conclusioni: 204 pazienti (37.6%) non presentavano autoanticorpi, 338 pazienti (62.4%) presentavano autoanticorpi contro il pancreas. 316 (93.5%) avevano positività per ICA, 283 per GAD (83.7%), 227 per IA2 (67.2%), 160 per IAA (47.3%). Di questi 54 avevano anche anticorpi anti-tiroide (16%) e 27 pazienti erano celiaci (8%). Nella nostra casistica la percentuale di diabete autoimmune è significativa. La maggior parte dei pazienti ha infatti mostrato positività per ICA e GAD confermando la diagnosi di diabete in fase iniziale. Nel diabete di tipo I i marcatori autoimmunitari sono importanti per identificare i soggetti con aumentato rischio di resistenza insulinica. Inoltre la simultanea presenza di autoanticorpi per la patologia tiroidea e celiaca suggerisce l'importanza di un monitoraggio anticorpale in questa categoria di pazienti.

P087

METABOLIC SYNDROME AND PERIODONTITIS: ASSOCIATION WITH REACTIVE OXYGEN SPECIES PRODUCTION. A PILOT STUDY

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Aim: Metabolic syndrome (MetS) is associated with an increased risk of periodontitis also if the underlying mechanisms is unknown. Since both MetS and periodontitis are characterized by an alteration of inflammation status the aim of this pilot study, was to determine if differences in ROS metabolism of phagocytes isolated from (A) patients with MetS, (B) patients with both MetS and mild periodontitis, (C) healthy subjects and (D) normal weight subjects with mild periodontitis, were present.
Methods: Polymorphonucleocytes (PMNs) and Peripheral blood monocytes (PBMs) isolationVenous blood (10 mL), obtained from each volunteers, was diluted with physiological solution (10 mL). Dextran in physiological solution (6%, 4 mL) was then added to enhance the sedimentation rate of erythrocytes at 1 × g. After 30 min, the white blood cells suspension was centrifuged on Lymphoprep (Pharmacia, Sweden).PMNs, present in the pellet, were separated from erythrocytes by hypotonic lysis. PBMs, present in the interfaces between serum and Lymphoprep, were isolated by adherenceROS metabolism of leukocytesROS metabolism was
studied by a Chemiluminescence (CL) technique: the system was made up of luminol (100 nmol/L) and cells (1 × 10^5) in the presence or absence of stimulus constituted by opsonized zymosan (0.5 mg). The final volume (1.0 mL) was obtained using modified KRP buffer. ROS production was measured at 25°C for 2 h, using a LB 953 luminometer (Berthold, EG&G Co, Germany). All the experiments were performed in triplicate. Statistical analysis: All results are mean ± standard deviation (SD). The group of means were compared by analysis of variance (ANOVA. A value of $p < 0.05$ was considered significant.
Results: Results showed that basal ROS production (both from PMNs and from PBMs) of groups A, B and D was increased respect to that obtained from group C ($p < 0.05$).
Discussion: These results are congruent with literature data, and further point to a link between oxidative stress, MetS and periodontitis. Although the actual clinical relevance of the phenomenon remains to be evaluated.

P088

DOSAGGIO DI HbA1c IN PAZIENTI CON Hb CAMPERDOWN IN ELETTROFORESI CAPILLARE

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LG è una paziente diabetica. Il suo medico chiede il dosaggio di HbA1c.Il risultato è 29 mmol/mol (4,8%), non congruo con la storia clinica della signora (glicemia a digiuno 168 mg/dL). Il dosaggio è stato eseguito in elettroforesi capillare (Capillarys 2 SEBIA).L'elettroferogramma risulta però allarmato con la dicitura "profilo atipico".Ad una valutazione più attenta si nota la presenza di un picco che precede il picco dell'emoglobina glicata e ben separato da quest'ultimo.Viene eseguito un assetto emoglobinico su MINICAP SEBIA che, pur non evidenziando la presenza di picchi anomali, mostra un profilo di HbA0 leggermente alterato.Si decide per un approfondimento con elettroforesi emoglobinica a pH alcalino e acido che mostra la presenza di una variante emoglobinica con caratteristiche di migrazione compatibili con Hb Camperdown.L'emoglobina Camperdown è una variante emoglobinica abbastanza diffusa nella nostra regione che interferisce con il dosaggio di Hba1c dosata sia con le principali tecniche HPLC che con l'elettroforesi capillare.In particolare, mentre nella maggior parte degli strumenti HPLC la variante cade sopra il picco relativo ad Hba1c che risulta quindi sovrastimata in modo molto evidente, su Capillarys 2 Sebia lo strumento fornisce un dato di Hba1c sottostimato: infatti mentre la variante e HbA0 migrano assieme, le due frazioni glicate (di HbA0 e di Hb Camperdown) migrano in zone separate, ben visibili sull'elettroferogramma.Poiché infatti il dato di HbA1c si ottiene facendo il rapporto tra HbA1c e la somma di HbA0 + HbA1c in questo caso viene a mancare l'apporto della frazione glicata della variante (Bry L, et al. Effects of hemoglobin variants and chemically modified derivatives on assays for glycohemoglobin. Clin Chem 2001;47:153-63). Tuttavia è possibile risalire al valore reale con una semplice rielaborazione matematica.E' necessario risalire alla concentrazione % del picco relativo ad HbA1c, sommarlo al picco adiacente relativo alla forma glicata di Hb Camperdown, calcolare il corrispondente valore di Hba1c facendo il rapporto tra HBA1c/HBA1c + HbA0, trasformare quindi il dato ottenuto basandosi sulla curva di calibrazione utilizzata nello strumento nel periodo di esame. In questo modo è possibile ottenere un dato corretto del valore di Hba1c (1). Nel caso di LG il valore di Hba1c corretto è risultato 57 mmol/mol (7.4%). E' necessario quindi riconoscere l'elettroferogramma con caratteristiche tipiche per presenza di Hb Camperdown, confermare il riscontro di tale emoglobina perlomeno in via presuntiva con altro test (elettroforesi emoglobinica) e procedere alla rielaborazione matematica per evitare importanti sottostime del risultato.

P089
IL DOSAGGIO DELLA GLICEMIA SU PROVETTA CON INIBITORI DELLA GLICOLISI

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Introduzione: E' risaputo che la glicolisi procede in vitro dopo il prelievo di sangue: se una provetta non viene immediatamente centrifugata si assiste ad una diminuzione della glicemia pari al 6-7% l'ora. L'utilizzo di provette contenenti sodio-fluoruro (inibitore dell'enolasi, un enzima che agisce sulla parte finale della via della glicolisi) è molto diffuso ma ha una azione tardiva, dopo 2 ore dalla raccolta del campione. E' stato quindi proposto l'utilizzo di provette contenenti tampone citrato: l'acidificazione del campione blocca istantaneamente esochinasi e fruttochinasi, due enzimi che agiscono precocemente nella via glicolitica. Le provette della ditta Terumo (Venosafe Glycemia) contenenti una miscela brevettata di fluoruro di sodio, acido citrico e NA_2 EDTA in forma liofila, hanno dimostrato buona utilità ed efficacia in numerosi studi (Gambino R, et al. Acidification of blood is superior to sodium fluoride as an inhibitor of in vitro glycolysis. Clin Chem 2009:55:1019-21), ma ora non sono più in commercio. Un'altra azienda ha proposto la combinazione di fluoruro di sodio, acido citrico e NA_2 EDTA in forma liquida (Sarsted GlucoExact) ma è necessario utilizzare un fattore di correzione per correggere l'effetto di diluizione dovuto all'anticogulante liquido. Recentemente la ditta Greiner Bio-One Italia ha introdotto sul mercato una nuova provetta (Vacuette-FC-Mix NaF-EDTA-citrato) contenente la miscela acidificata in forma liofila. Scopo dello studio è stata la valutazione di questa provetta Greiner confrontandola con la provetta Terumo già precedentemente validata.
Materiali e metodi: Sono stati arruolati nello studio 30 volontari sani afferiti ala Laboratorio Analisi dell'Ospedale di Vicenza con glicemie comprese tra 57mg/dL e 130 mg/dL. Il sangue raccolto nelle due provette Greiner e Terumo veniva mantenuto a temperatura ambiente prima di essere centrifugato dopo 2 h dal prelievo e quindi conservato a -4° fino all'analisi. La determinazione della glicemia è stata fatta su Dimension Vista in seduta unica per ogni campione e in duplicato.
Risultati: Valutata la distribuzione normale dei dati (Kolmogorov-Smirnov test) è stato eseguito il T di Student per dati appaiati che ha mostrato una differenza non significativa tra le glicemie prelevate su provette Terumo e Greiner dopo 2 h (p=0.15).
Conclusioni: Le provette della ditta Greiner contenenti la miscela acidificata in forma granulare mostrano pari efficacia rispetto alle provette della ditta Terumo e possono quindi essere utilizzate in maniera efficace garantendo il blocco della glicolisi già nelle prime 2 ore dopo il prelievo.

P090
IRON DEFICIENCY ANAEMIA INFLUENCES GLYCATED HEMOGLOBIN LEVELS

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Introduction: Several studies suggest an association between iron deficiency anaemia and higher HbA1c levels, but the results are conflicting and the matter is under debate (1, 2). We conducted a retrospective control-case study to investigate the effects of iron deficiency and erythrocyte indices on HbA1c analysis.
Methods: Starting from a large computerized database of the Italian hospital of Desio, including data from 2000 to 2016, all non-pregnant subjects aged greater than 10 years with at least one measurement of HbA1c, cell blood count, ferritin less than 200 ng/ml, and fasting blood glucose lower than 100 mg/dl in the same date were enrolled.
Results: A total of 2,831 patients met the study criteria. Eighty-six individuals were diagnosed with iron deficiency anemia, while 2,745 had normal iron state. Adjusted means HbA1c were significantly higher in anemic subjects (5.59 % [37.37 mmol/mol]), than those measured in patients without anemia (5.34 % [34.81 mmol/mol]) ($p < 0.0001$). Multiple linear regression analysis among all patients showed that hemoglobin and mean corpuscular volume values are inversely associated with HbA1c levels.
Conclusions: Iron deficiency anemia affects HbA1c measurements, as also erythrocyte abnormalities alter the test results. To the best of our knowledge, this paper is the first attempt to propose in anaemic patients a correction of HbA1c values based on haemoglobin level after an initial complete cell blood count, which may be required when diagnosing prediabetes and monitoring diabetes.
1. Son JI, Rhee SY, Woo JT, et al. Hemoglobin a1c may be an inadequate diagnostic tool for diabetes mellitus in anemic subjects. Diabetes Metab J 2013;37:343-8.
2. English E, Idris I, Smith G, et al. The effect of anaemia and abnormalities of erythrocyte indices on HbA1c analysis: a systematic review. Diabetologia 2015;58:1409-21.

P091

GLYCATED ALBUMIN IS CORRELATED TO INSULIN RESISTANCE IN SUBJECTS AT RISK OF DEVELOPING DIABETES

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Glycated Albumin (GA) has been proposed as a short-term marker of glucose homeostasis. Nevertheless, it is not clear which relationships exists between GA, the other markers of glucose homeostasis and insulin resistance in subjects at risk of developing diabetes. The aim of the study is to evaluate the relationship between GA, Fasting Plasma Glucose (FPG), 2h-PG, HbA1c and insulin resistance in a group of subjects at risk of diabetes who underwent to an Oral Glucose Tolerance Test (OGTT) basing on ADA recommendations.Two hundreds and one subjects were included in the study. At the time of the OGTT, a blood sample for HbA1c and GA, together with the complete medical history and the informed consent were collected. GA was measured on plasma-EDTA by quantILab® Glycated Albumin (Instrumentation Laboratory, A Werfen Company).When considering subjects with FPG 100-126 mg/dl; 2h-PG between 140-200 mg/dl; HbA1c: 39-47 mmol/mol, 73 subjects were classified as prediabetics. GA was slightly higher in such individuals in comparison to normal subjects (13.2 % [12.3 – 14.3] vs 13 % [11.9 – 13.8]; P=0.012). GA correlated with FPG (r=0.21; P=0.002), with HbA1c (r=0.161; P=0.024) but not to 2h-PG. Interestingly, GA was negatively correlated to insulin (r=-0.35; P=0.0007) and to HOMA-IR (r=-0.31; P=0.003), being the last still significant when considering only subjects with HOMA-IR<2.5 (r=-0.26; P=0.04). The same correlation were not detected for HbA1c. Finally, GA was negatively correlated to BMI (r=-0.20; P=0.001) while albumin didn't, suggesting that the correlation between GA and BMI is independent of the increased catabolism of albumin in overweight and obesity.GA is higher in prediabetics than in normal subjects. In our study, the association of GA with other markers of glucose homeostasis seems to be lower than expected, but it can be explained with the low grade of glucose homeostasis disturbances observed in the sample population. GA was also associated to insulin resistance and BMI, independently of albumin levels, being involved in the metabolic imbalance that can prelude diabetes onset. Nevertheless, the molecular mechanism that links low GA and high metabolic impairment requires further elucidations.

P092

DETERMINAZIONE DELL'EMOGLOBINA GLICATA: VALUTAZIONE DELLO STRUMENTO BIO-RAD D-100

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Introduzione: L'emoglobina glicata (HbA1c) è considerata il gold standard per il monitoraggio dello stato glicemico nei pazienti diabetici. Da poco è stato sviluppato un nuovo sistema diagnostico per la sua determinazione: D-100™(Bio-Rad), le cui caratteristiche tecniche sembrano garantire prestazioni, velocità analitica e semplicità di utilizzo migliori rispetto ai sistemi precedenti.
Obiettivi: Valutare le prestazioni analitiche dello strumento e confrontare i risultati ottenuti su campioni di pazienti pervenuti presso l'UOC Medicina di Laboratorio per la misura di HbA1c, con quelli ottenuti con gli strumenti Adams HA8160V e Adams HA8180V (A. Menarini Diagnostics) anche in presenza di varianti emoglobiniche.
Materiali e metodi: Precisione: valutata secondo il protocollo CLSI-EP5A2 utilizzando 4 pool di sangue EDTA a diverse concentrazioni di HbA1c; accuratezza: verificata utilizzando 11 campioni di VEQ; confronto tra i due sistemi eseguito su 1000 campioni senza varianti emoglobiniche e su 36 campioni con diversi tipi di varianti (HbS, HbD Punjab, HbHasharon, HbC, HbE, HbG, Hb Camperdown). I risultati sono stati valutati con l'analisi di Passing Bablok e Bland-Altman (Analyse-it® Software Ltd, UK).
Risultati: L'imprecisione (CV%, n=80) dei pool a 35/40/47/75 mmol/mol risulta <2.6%, il bias calcolato sui campioni di VEQ è <3.7%. Il gruppo di pazienti che non presentano varianti emoglobiniche, evidenzia un'ottima correlazione (R^2=0.99, p<0.0001) e un bias di -1.6% (-1.8- -1.4, 95%CI), statisticamente ma non clinicamente significativo. La scarsa numerosità del gruppo di pazienti con emoglobinopatie non consente di effettuare una valutazione statistica accurata, ma si può affermare che, non si sono osservate differenze >5 mmol/mol che sono considerate variazioni significative nel controllo glicemico.
Conclusioni: Le prestazioni analitiche, sia in termini di imprecisione, accuratezza e correlazione con il metodo in uso, sono risultate molto soddisfacenti. Le più comuni varianti emoglobiniche studiate (HbS, HbC, HbD, HbE) sono identificate automaticamente dal software e non hanno dimostrato interferenze analitiche con la misura di HbA1c. La facilità d'uso, la robustezza e la produttività oraria attestano la possibile applicazione nella diagnostica clinica di laboratorio.

P093
THE IMPORTANCE OF DIFFERENTIATED REFERENCE VALUES BASED ON POPULATION STUDIES

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Introduction: The flow of migrants from Africa to the European continent is gaining importance not only socially, but also on the health side. Every day there are new health issues that require the revision of diagnostic and therapeutic pathways. Recent studies on healthy subjects coming from different peoples and regions showed significant differences in laboratory parameters, determined by the combination of demographic, genetic, nutritional and lifestyle factors. Reference values used in laboratory services of the SSN are defined by studies on the indigenous population, or obtained from literature and are still referred to the European or US population (Caucasian). Recently, 26 male migrants aged 22 to 28, from the South Sahara region have been welcomed by Caritas and have been subjected to health checks. Laboratory tests include also hemocromocytometer, platelets and formula. The results of the formula examination of 26 boys showed significant neutropenia with consequent inverted leukocyte formula.
Materials and methods: Samples have been tested on 3 automated hematologic analyzers: Siemens ADVIA 2120, ADVIA 2120i and Dasit Sysmex XT1800i and reviewed in optical microscopy by May Grunwald-Giemsa.
Results: Results from the analysis of data and graphs of the leukocyte formula, using as reference values those reported in the literature for young adult Caucasian male (1.8-7.7 cells/ml), performed on the instruments, reveals neutropenia in 84.62% of the samples. If we use the reference ranges indicated in the recent scientific works, for the parameter of total neutrophils (1,1-5,1cells/ml), the number of cases of neutropenia decreases drastically to 15.38%.
Discussion: The analysis of the obtained data gives some considerations on the criteria for using the reference values for the haematologycal parameters used by the SSN laboratories. In fact, the occurrence of neutropenic and or leukopenic patients, especially if the event occurs in isolated cases, can lead to a series of diagnostic hypotheses and subsequent in-depth studies, sometimes even invasive, which are completely inappropriate. Analogous consideration may also be referred to the opposite condition where a neutrophil leukocytosis does not significantly emerge, resulting in underestimation of inflammatory and or infectious processes. Therefore it is indispensable to use reference values referring to the specific population and if this is not possible, is necessary to insert, in the report, a comment that refers to appropriate reference ranges.
Nelson Tembe. Reference Values for Clinical Laboratory Parameters in Young Adults in Maputo, Mozambique. PLOS ONE 2014;9:e97391.www.plosone.org

P094
CRIOAGGLUTININE CON BASSO RANGE TERMICO DI AZIONE: CASE REPORT

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Le anemie emolitiche autoimmuni (AEA) comprendono condizioni cliniche eterogenee caratterizzate dalla presenza di autoanticorpi che si legano ad antigeni presenti sulla superficie eritrocitaria provocandone la lisi. Le crioagglutinine sono anticorpi tipo IgM con optimun termico di azione tra 4°C e 27°C, con elevata capacità di fissare il complemento e perdita della capacità di agglutinare emazie a temperature superiori. I pazienti possono presentare acrocianosi legata all'agglutinazione delle emazie con un quadro clinico che peggiora con l'aumentare del range termico di azione. Un uomo di 70 anni giunge alla nostra attenzione per anemia. L'esame emocromocitometrico eseguito con analizzatore ADVIA 2120i Siemens evidenzia anemia (Hb:112g/L) moderatamente macrocitica (MCV:94.9fl) e reticolocitosi: $169,5x10^9$/L senza alterazioni grafiche o artefatti numerici. È presente iperbilirubinemia (BT: 2.3mg/dl) prevalentemente indiretta (BI: 1.6mg/dl), aptoglobina consumata (1mg/dl), LDH aumentato (647U/L) e test di Coombs diretto ed indiretto negativo. L'esame morfologico del sangue periferico evidenzia presenza di numerosi agglutinati eritrocitari che suggeriscono la presenza di agglutinine a freddo verosimilmente attivatesi a contatto con la superficie del vetrino. Per dimostrare tale ipotesi la provetta viene incubata in frigorifero a 4°C per 1h ed il campione è riprocessato a freddo. Ne risulta un'alterazione dei parametri eritrocitari numerici (riduzione dei globuli rossi, aumento dell'MCHC) e grafici (comparsa nel citogramma volume/concentrazione di emoglobina di una nuvola che occupa l'area di emazie normo-macrocitiche). Alla luce di tale reperto il test di Coombs viene ripetuto utilizzando sieri anti-complemento e risulta positivo contro di esso. Le proprietà termiche delle crioagglutinine vanno considerate per l'interpretazione delle indagini sierologiche perché a temperature superiori ai 4° avviene il distacco spontaneo degli anticorpi con negatività del test di Coombs diretto. Restano però adese le frazioni del complemento che possono essere svelate usando sieri contro di esse. Il tipo e le caratteristiche termiche dell'autoanticorpo determinano diverso approccio terapeutico alla AEA. Nel sospetto di crioagglutinine sarebbe indicato eseguire sempre l'emocromo a freddo.

P095

QUANDO L'OSSERVAZIONE AL MICROSCOPIO INDIRIZZA LA DIAGNOSI: UN CASO DI FAVISMO IN UN BAMBINO ALGERINO PORTATORE DI B-TALASSEMIA

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Un bimbo algerino di 2 anni e mezzo, giunto in Italia da appena 2 mesi, viene portato in Ospedale. Si presenta pallido, astenico, sub itterico, senza organomegalie. Gli esami praticati mostrano: anemia (Hb=740 g/L) e microcitosi (MCV=62fl) con indici di emolisi positivi: reticolocitosi ($123x10^9$/L), aptoglobina assente, iperbilirubinemia prevalentemente indiretta (B.T=2,47mg/dl, B.I.=1,7 mg/dl), aumento dell'LDH (842U/L), emoglobinuria e test di Coombs diretto e indiretto negativo. L'esame morfologico dello striscio periferico mostra un quadro eritrocitario caratterizzato da ipocromia e anisocitosi spiccata, cellule vescicolate, emazie contratte irregolari, numerosi ellissociti ed emazie a bersaglio. Si avviano pertanto esami per dosaggio della G6PD, elettroforesi dell'Hb e studio del fenotipo ematologico dei genitori. Per l'aggravarsi dell'anemia (Hb=59g/L) e dell'emoglobinuria il paziente viene trasfuso; le sue condizioni cliniche ed ematologiche migliorano rapidamente: si stabilizza l'Hb (Hb=110g/L), scompare l'emoglobinuria e viene dimesso. Gli esami pervenuti confermano il sospetto diagnostico: deficit di G6PD (0,39 U/L, V.N.≥ 0,85); il quadro elettroforetico dell'Hb mostra HbA2=6,4% ed HbF=0,9% come da portatore di β-talassemia. L'immigrazione che si è verificata in Italia negli ultimi decenni ha comportato, oltre ad enormi problemi economici e sociali, anche seri problemi sanitari. I medici italiani si sono trovati ad affrontare malattie del tutto nuove o poco conosciute, prevalenti invece nelle popolazioni di origine dei migranti. A ciò va aggiunta spesso la difficoltà della lingua, che impedisce o rende estremamente difficile la corretta raccolta dell'anamnesi. Nel caso illustrato l'osservazione accurata dello striscio periferico e la conoscenza del paese di origine del paziente (l'Algeria condivide con gli altri paesi costieri del Mediterraneo gli stessi difetti genetici del globulo rosso come deficit di G6PD, talassemie, emoglobinopatie, ellissocitosi ereditaria etc.) hanno indirizzato la diagnosi, permettendo di interpretare correttamente e rapidamente tutti i segni clinici e di laboratorio del paziente e di praticare la giusta terapia.

P096

IDENTIFICATION OF RARE HEMOGLOBIN VARIANT: REPORT OF FIVE YEARS OF HEMOGLOBINOPATHIES SCREENING IN A GREATER AREA LABORATORY

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Background: The Hub Laboratory of Greater Romagna Area is a "Shared Resource Laboratory" provides since 2009 diagnostic services for inhabitants of Romagna (more than one million) and consolidates the Hemoglobinopathies screening previously carried out by four local laboratories. The Guidelines for Physiological Pregnancy issued in 2011 by the Italian National Health Service recommend this screening for all the pregnant women and since then the tests requested to our laboratory continuously raised. The strong increment of migration, especially from Africa and Asia, markedly increased the incidence of rare Hemoglobin (Hb) variants previously not found in our Area.
Methods: From January 2012 to December 2016, 52210 Hemoglobinopathies screening have been carried out using the G8 system HPLC (Tosoh Corporation, Tokyo, Japan). This instrumentation can identify Hb peaks with elution time consistent with HbS, HbC, HbD and HbE. Other Hb variants were reported describing their percentage and elution time in minutes. Molecular characterization of some of them have been carried out by direct sequencing of alfa or beta globins gene (ABI PRISM 3130xl Sequencing Analyzer, AB Applied Biosystems, Foster City, CA,USA).
Results: In the last five years test ordered raised by a median of 10% per year, and in 2017 we daily carried out about 40 tests/day. The incidence of Hb variants identified as HbS (confirmed by sickling test) or presumably identified as HbC, HbD or HbE has been about 2.5% of all the tests. In the recent years the detection of rare Hb variants (e.g. Hb Leiden, Hb J Cambridge, Hb G-Accra, Hb G-Bristol, Hb Shaare Zedek) continuously increased. The recent identification of a patient with Hb alpha variant associated with Hb beta variant and alpha thalassemia is an example of the increasing diagnostic complexity.
Conclusion: The ever-increasing racial heterogeneity of the Italian society confirms the importance of the Hemoglobinopathies screening and gives reasons for the rise of ordered tests. To meet this demand, only Hub laboratories like can develop and nurture the necessary technical, professional and scientific expertise for the management of the complex cases encountered in the screening of the more and more multi-ethnic Italian population.

P097
FLOW CYTOMETRIC ANALYSIS OF THROMBOCYTOPENIA: AN AID IN THE DIFFERENTIAL DIAGNOSIS

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Identifying the cause of thrombocytopenia is crucial because the patient's life may depend on prompt treatment. Flow cytometry allows to quantify the immature platelet fraction (IPF%) and to know the proliferative state of bone marrow. High IPF values have been reported in thrombocytopenia caused by increased destruction while low IPF values are found in cases of decreased production. This study was focused on thrombocytopenia secondary to an infection. Platelets involvement in immune and inflammatory response to infectious agents is well known. Bone marrow reacts to this consumption with the release of immature platelets in the circulatory system. The aim of our study was to verify whether there was a significant difference between the proportion of circulating immature platelets in a population of infectious patients, who frequently report severe thrombocytopenia (>50.000/µL), respect a healthy control group.The IPF value of 95 infectious males and 216 healthy subjects was determined using Sysmex automatic analyzer XN-3000. This instrumentis equipped with a channel exclusively dedicatedto the count of fluorescent platelets. Mean tendencies (mean, mode, and median) were determined and the T-Student test was used to test for statistically significant differences among healthy subjects and patients. Results confirm the importance of IPF in the differential diagnosis of thrombocytopenia. In fact, the rapid evaluation ofbone marrow proliferative status may allow more correct and immediate therapeutic interventions.

P098
CORRELATION OF LEUKOCYTE COUNTS ON SYSMEX XN 1000 HEMATOLOGIC ANALYZER IN PERIPHERAL BLOOD SAMPLES

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Background-aim of the study of the Sysmex XN1000 (Sysmex Corp., Japan) is a hematologic system providing both leukocyte enumeration (WNR channel) and leukocyte differential (WDF channel). A third channel (WPC) defined "Blasts / Abn Lymph" identifies samples needing reflex testing for more specific assessment of blasts or abnormal lymphocytes (Abn Lymph). In addition to WBC (WBC-N) count, the number of leukocytes is assessed in the WDF (WBC-D) and WPC (WBC-P) channels. The purpose of this study is comparing leukocyte counts among the three XN1000 channels.
Materials and Methods: A total number of 2222 peripheral blood samples were analyzed and also tested according to WPC channel reflex test. WBC-D and WBC-P counts were compared to WBC-N by Pearson correlation and Bland-Altman plots (Analyze-it software, Leeds, UK).
Results: The WBC-N count was comprised between 0.01 -813.65x10^9/L (mean value, 22.6x10^9/L). WBC-D counts had an excellent correlation (r=1.00) and negligible mean bias (-0.09x10^9/L), with a virtually meaningless statistically significant difference (95% CI from -0.12^9 to -0.05x10^9/L). A worse correlation was found with WBC-P counts (r=0.86) along with a significant underestimation (mean bias -4.08x10^9/L; 95% CI from -5.25 to -2.92x10^9/L). In 176 samples, the bias of WBC-N channel was> 10% and 165 of these (94%) were also flagged with "Abn Lymph" (155 with WBC-N >10x10^9/L and Lymphocytes> 5x10^9/L). Excluding these samples, the WBC-P correlation become excellent (r=1.00), displaying a non-significant bias (mean 0.002x10^9/L; 95% CI from -0.057 to 0.061x10^9/L).
Conclusions: The WBC-N and WBC-D counts are highly correlated, whereas the WBC-P channel is plagued by underestimation that, in our study, was mainly due to samples with leukocytosis, lymphocytosis and probable neoplastic lymphocytes. These differences, not previously described (1), merit further scrutiny according to the clinical diagnosis, so that possible associations with lymphoproliferative diseases may be identified.
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P099
CONTRIBUTO DEL LABORATORIO NELLA DIAGNOSI E FOLLOW-UP IN UNA POPOLAZIONE DI PAZIENTI AFFETTI DA AMILOIDOSI AL

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Introduzione: L'amiloidosi da catene leggere immunoglobuliniche (AL), incidenza 10/1.000.000 è una malattia aggressiva e fatale se non diagnosticata in tempo. Il laboratorio riveste un ruolo cruciale nella diagnosi e follow-up. Scopo: Valutazione dei parametri biochimici di risposta ematologica, in pazienti affetti da AL in trattamento, secondo i criteri dell'International Amyloidosis Society [risposta completa (CR): rapporto catene leggere libere sieriche (FLC) normale (0,31-1,56) e immunofissazione di siero e urine (IFEs/u) negativa; risposta parziale molto buona (VGPR): differenza tra catena leggera libera amiloidogenica e non (dFLC) <40 mg/L; risposta parziale (PR): riduzione dFLC ≥50%; Non risposta (NR)]. Popolazione e Metodi: 25 pazienti (pz) affetti da AL, con età media di 64 anni (M/F:12/13), afferiti presso l'AOU Federico II di Napoli, sottoposti a terapia di prima linea con Bortezomib e Desametasone + Melphalan o Ciclofosfamide (massimo 8 cicli di 35 giorni). Valutazione laboratoristica a diagnosi e ogni due mesi durante il follow-up: elettroforesi capillare zonale delle proteine sieriche (CZE, Capillarys2-Sebia); IFEs, IFEu e elettroforesi delle proteine urinarie (Hydrasys2-Sebia); FLC (N-Latex, Siemens); proteinuria 24h; NT-proBNP; Troponina I e Fosfatasi Alcalina.
Risultati e Discussione: I pz in esame presentavano la seguente localizzazione d'organo: 11 pz cardiaca; 7 pz renale; 5 pz cardiaca e renale; 2 pz cardiaca, epatica, renale. Entro i primi due cicli di terapia 6 pz sono deceduti. I restanti 19 pz hanno ottenuto una risposta ematologica con una mediana di 11 mesi (2-33): la CR in 7 pz (37%), la VGPR in 7 pz (37%), la RP in 3 pz (16%) e la NR in 2 pz (10%). I 7 pz con VGPR presentavano solo la positività all'IFEs. Tra i 17 pz con CR o VGPR o RP, 6 (35%) mantenevano la risposta durante il follow-up (8-123 mesi). Inoltre, 5 dei 7 pz in CR, durante il follow-up ripresentano la CM all'IFEs (4 pz) e all' IFEu (1 pz) mantenendo un normale rapporto delle FLC. Il costante monitoraggio dei parametri biochimici è indispensabile nel follow-up dei pz con AL; nella popolazione in studio l'IFE sembra essere la tecnica più sensibile per la valutazione della risposta e più precoce in caso di recidiva ematologica.
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P100
FUNZIONI MATEMATICHE PER DISCRIMINARE LA BETA TALASSEMIA DA ALTRE ANEMIE MICROCITICHE-IPOCROMICHE

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La beta talassemia è una patologia ematologica ereditaria molto diffusa nel bacino del Mediterraneo, in Africa ed in Asia, con una incidenza globale del 5%. Il quadro clinico associato varia dalla forma grave, trasfusione dipendente, a quella del tutto asintomatica. La talassemia è una anemia microcitica ed ipocromica i cui parametri emocromocitometrici sono sovrapponibili a quelli di altre anemie con cui può essere confusa. Per una diagnosi differenziale che consenta una corretta prevenzione ed una appropriata terapia, noi proponiamo l'adozione di formule matematiche discriminanti fra talassemia e altre anemie ipocromiche-microcitiche. Ne abbiamo applicato otto diverse (England and Fraser, Mentzer, Srivastava, Sirdah, Shine and Lal, Ehsani, MDHL, MCHD) a 202 soggetti, maschi e femmine, affetti da anemia ipocromica-microcitica: 102 beta talassemici eterozigoti e 100 anemici non talassemici. Per ciascuna formula è stata calcolata la sensibilità, la specificità, il valore predittivo positivo e negativo e l'indice di Youden. La funzione più affidabile per differenziare la talassemia da altre forme di anemia microcitica-ipocromica è risultata essere quella di Mentzer.
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P101
AN UNUSUAL CASE OF PSEUDOTROMBOCYTOPENIA

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Introduction: pseudotrombocytopenia is a well-known phenomenon, frequently due to the presence of antiplatelet autoantibodies which trigger in vitro platelet aggregation in EDTA blood, so leading to underestimation of platelet counts with automated hemocytometers. We describe here the case of a patient with angioimmunoblastic T lymphoma and Epstein-Barr virus (EBV) infection, characterized by pseudotrombocytopenia in EDTA, but also in sodium citrate, lithium heparin and ACD blood samples. Case reports: A 30-year-old male with generalized lymphadenopathy associated with asthenia was hospitalized for diagnostic invetigations. A diagnosis of angioimmunoblastic T lymphoma was made with osteomidollary and lymph node biospia. Laboratory testing was normal except for the presence of mild anemia (hemoglobin, 11g/L) and an increased value of lactate dehydrogenase (LDH, 1033 U/L). In the following days, before starting the first cycle of chemotherapy, serologic testing showed an increase of anti-IgE anti-EBV antibody titer. Moreover, hematological testing performed with Advia 2120i (Siemens Healthcare Erlangen, Germany) showed decreased platelet count ($100x10^9$/L to $50x10^9$/L) in the absence of hemorrhagic diathesis. Due to the suspect that the low platelet count could be due to EDTA-induced platelet microaggregates which were not flagged by the analyzer, additional blood samples with anticoagulants other than EDTA were collected. The platelet count in sodium citrate, lithium heparin and ACD blood samples was further reduced to $10\text{-}20x10^9$/L due to the presence of visible aggregates confirmed with microscopic analysis of the blood smears. Discussion: Pseudotromocytopenia is mediated by IgG and IgM antibodies, targeting modified platelet epitopes and/or expressed in the presence of EDTA. This phenomenon does not usually occur with other anticoagulants. In our case, however, aggregation has also triggered by other anticoagulants which are typically used for overcoming EDTA-induced in vitro aggregation. Therefore, in this paradigmatic case we cannot exclude that spurious aggregation may have been caused by cross-reactivity of anti-EBV antibodies with autoimmune IgM.

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P102
PERSISTENCE OF MINIMAL RESIDUAL DISEASE BY MULTIPARAMETER FLOW CYTOMETRY CAN HINDER RECOVERY OF ORGAN DAMAGE IN PATIENTS WITH AL AMYLOIDOSIS

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Minimal Residual Disease (MRD) demonstrated by multiparameter flow cytometry (MFC) identifies subjects with significantly shorter survival among multiple myeloma patients who attain complete response (CR). The role of MRD in AL amyloidosis has been assessed only in few small series. In the present study, we assessed the MRD by MFC in patients with AL amyloidosis who attained CR. CR was defined as per current criteria (negative serum and urine immunofixation and normal free light chain ratio). For flow cytometry studies bone marrow samples were processed following the Euro Flow Protocol and stained with the EuroFlow panel and analyzed with Infinicyt software. At least $5x10^6$ events were measured using a FACSCanto II instrument. Patients were identified as having residual disease if a discreet population of clonal plasma cells comprising ≥50 events was identified (10^{-5} limit of detection). Thirty-seven patients were tested (8 were found to have relapsed) and 29 satisfied criteria for CR. Twenty-four (83%) patients had renal and 55% had cardiac involvement at diagnosis. Median time to CR was 10 months (range 3-82). Eight patients (61%) had achieved cardiac response and 13 (52%) renal response at the time of CR. The median time from CR to MRD was 30.8 months (IQR: 11-59) with no significant difference in MRD+/- patients. Flow cytometry identified MRD in 11 patients (38%). A median of 962 (range 252-2500) corresponding to 0.04% (range 0.02-0.1%) plasma cells with abnormal phenotype were detected. No differences in organ involvement, cardiac and renal stage, type of therapy, number of treatments, and organ response at the time of CR was found between the two groups. However, a further improvement of cardiac function compared to the time of CR was observed in 5/7 evaluable MRD- and in none of the 2 MRD+ (P=0.073). Compared to the time of CR, renal response was obtained in 8 MRD- (80%) and in 2 (22%) MRD+ (P=0.012). Overall, a further improvement of cardiac or renal function after CR was significantly associated with absence of MRD (P=0.018). In our study almost 40% of patients satisfying current criteria for CR have detectable MRD. MRD makes further organ improvement less likely and can explain organ progression. A validation study in a larger sample is ongoing.

P103
MIELODISPLASIA TRANSITORIA E MORBILLO

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Il morbillo è causato dal Paramyxovirus, un virus a RNA a singolo filamento negativo, del genere Morbillivirus della famiglia Paramyxoviridae. Questo virus è altamente contagioso e si diffonde attraverso la tosse e gli starnuti, con uno stretto contatto personale o il contatto diretto con le secrezioni. I fattori di rischio per l'infezione da virus del morbillo comprendono l'immunodeficienza causata dal virus dell'HIV o AIDS. immunosoppressione dopo un trapianto di organo o di un trapianto di cellule staminali ematopoietiche, terapia con corticosteroidi, viaggi in zone dove il morbillo è endemico o il contatto con i viaggiatori provenienti da quelle aree e la perdita di anticorpi, ereditati prima dell'età della vaccinazione di routine. L'immunizzazione vaccinale contro il morbillo, iniziata nel 1960, ha cambiato radicalmente l'epidemiologia della malattia.

Poiché sindromi mielodisplastiche transitorie sono possibili soprattutto in caso di infezioni da virus a RNA è stato possibile valutare alcuni aspetti clinico-laboratoristici relativi alla fase viremica dell'infezione morbillosa cosi poco frequente in passato grazie alle numerose campagne vaccinali. Donna di anni 38 con la seguente diagnosi di ingresso al pronto soccorso: sospetto morbillo, eruzione cutanea eritematosa generalizzata, paziente vigile orientata, azione cardiaca ritmica, toni puri, pause libere. Iperpiressia da 4 giorni ed esantema generalizzato da 24 ore. L'esame emocromocitrometrico rilevava leucopenia, piastrinopenia e neutropenia, LDH superiore a 1000. Nei giorni successivi in degenza presso il reparto di malattie infettive, la paziente veniva sottoposta ad approfondimenti diagnostici, per escludere la possibilità di immunosoppressione legata ad infezioni virali quali HIV, HCV, HBsAg, EBV, CMV. A tal fine, oltre a test anticorpali per la ricerca dei suddetti virus, veniva eseguita una tipizzazione linfocitaria, con esame citoflorimetrico della ditta BD e un esame morfologico su striscio di sangue periferico. I valori della tipizzazione linfocitaria risultavano patognomonici di infezione virale, con un rapporto al cut off (CD4+/CD8+ = 1,04) CD3+ = 83% - CD4+ = 42% - CD8+= 40% ; CD19 = 8%. L'esame microscopico rivelava la presenza di numerosi linfociti reattivi e spiccata displasia della serie granulocitaria. Il controllo eseguito nei giorni successivi dopo adeguata terapia dimostrava un miglioramento dello stato di salute della paziente e dei parametri ematici. Nei controlli successivi, a due mesi dalla dimissione, si notava all'esame emocromocitrometrico una completa risoluzione della leuco-piastrinopenia, l'esame microscopico su striscio di sangue periferico non evidenziava atipie morfologiche.

P104
ANALYTICAL REASSESSMENT OF THE DEHYDROEPIANDROSTERONE-SULFATE ASSAY AND COMPARISON WITH LC-MS METHOD

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Introduction: Dehydroepiandrosterone and its sulfate (DHEAS) are androgen precursor produced by adrenal cortex and represent the most abundant circulating steroids in humans. DHEAS levels peak about thirty, followed by a progressive decrease with age. DHEAS is an effective biochemical marker for adrenal production of androgens.

Aim: Evaluating DHEAS Access imprecision and trueness, and indirect reference intervals verification.

Methods: Tests were performed on Beckman Coulter immunoassay system DxI, according to the manufacturer's instructions. Imprecision was evaluated in 3 pool samples (P1, P2, P3) aliquoted, stored at -20°C and examined 20 times with 3 different calibrations. Trueness was evaluated on 21 routine samples with Steroid Eureka Kit LC-mass spectrometry. Briefly, 400 µL of each sample have been tested with Agilent UHPLC 1290 + Triple Quad 6460 ESI Jet Stream, after deproteinization and incubation with a labeled internal standard. Reference intervals were verified with indirect method.4181 records were selected from our LIS platform during period 2009-2016. Repeated measurement within 3 years and outliers (identified with the iterative 4 SD rule) were excluded.

Results: Imprecision: Mean and CV were evaluated for the three pools and resulted, respectively P1:328 µg/L (7.7%); P2: 1414 µg/L (7.0%); P3: 4243 µg/L (7.4%). Comparison with LC-MS: Passing-Bablock regression showed good correlation (DxI = -11.4+1.16 LCMS) with a moderate overestimate for immunoassay system, as expected. Bland-Altman plot showed a bias of +13% (range +/- 1.96 SD: -13%/+38%). Reference intervals: 3463 patients were evaluated (2500 females ‹50 years, 567 females ≥50 years and 396 males). In females, the 2.5°-97.5° percentile range resulted 381-4077 µg/L in ‹50 years subjects (reference interval 200-4000) and 64-1886 µg/L in ≥50 years (reference interval 100-1800). On the whole, data confirmed the reference intervals currently used. For males, the number of evaluable cases resulted too limited to obtain reliable results.

Conclusions: Our method showed good imprecision and accuracy acceptable for clinical applications. Indirect evaluation of reference intervals in female subjects seemed to confirm the range reported by the manufacturer.

P105
PRIME VALUTAZIONI SUL RAPPORTO ALDOSTERONE RENINA (ARR) NELLO SCREENING DELL'IPERALDOSTERONISMO PRIMITIVO

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Introduzione: L'iperaldosteronismo primario rappresenta la causa più frequente di ipertensione secondaria endocrina, raggiungendo una frequenza > 10% negli ipertesi. Attualmente l'attività reninica plasmatica (PRA) viene dosata con tecniche manuali, con lunghi tempi di esecuzione, che implicano l'utilizzo di radiomarcato (RIA) e con una pre-analitica da tenere sotto stretto controllo. Scopo del presente lavoro è stato quello di confrontare il dosaggio dell'attività reninica attiva (DRC) automatizzata con il dosaggio PRA e di verificare l'utilizzo del rapporto renina diretta/aldosterone (ARR) nei pazienti con sospetta ipertensione. Materiali e metodi : Sono stati analizzati 70 campioni di plasma di 50 pazienti (24 maschi, 26 femmine età media 47 anni) afferenti da vari reparti degli Ospedali Riuniti di Ancona. Sono stati dosati: l'aldosterone (sensibilità <0,9 pg/mL), renina (DRC sensibilità < 0,5 µIU/mL) in chemiluminescenza (Diasorin-Liaison XL), renina (PRA) (RIA, Stratec-SR 300-Pantec, sensibilità 0,1 ng/mL). I range di normalità utilizzati sono stati rispettivamete in ortostatismo e clinostatismo: aldosterone 40-310 pg/mL e 10-160 pg/mL, DRC 4,4-46 µIU/mL e 2.8-40 µIU/mL, PRA 1,5-5,7 ng/mL e 0.2-2.8 ng/mL . ARR >10 per sospetto di iperaldosteronismo primitivo. Risultati e conclusioni: 18 pazienti con sospetta ipertensione (36%) avevano valori nel range di normalità sia per aldosterone (media 85.9 pg/ml) che per renina diretta (media 26.12 µIU/mL) e PRA (media 2.7 ng/mL). In tutti ARR era inferiore al cut-off. 2 pazienti (4%) avevano valori di aldosterone elevato (media 418 pg/ml) e renina diretta e PRA molto bassi (media 0.4 µIU/mL, 0.14 ng/mL) con ARR elevato (range 39-94). Nei 2 pazienti è stata confermata la diagnosi di iperaldosteronismo primitivo. 10 pazienti (20%) avevano valori di renina diretta e PRA elevati (media 131.3 µIU/mL , 8.6 ng/mL) ma ARR bassi. 20 pazienti (40%) avevano valori di PRA bassi (media 0.57 ng/mL) ma aldosterone e DRC nel range di normalità, di questi solo 9 avevano ARR > 10. Nella nostra casistica un ARR aumentato costituisce un test di positività sensibile per l'iperaldosteronismo primitivo e più specifico rispetto al dosaggio PRA in quanto risente meno delle variabilità pre-analitiche.

P106
PROGRAMMA DI SCREENING CENTRALIZZATO DELL'IPOTIROIDISMO CONGENITO IN PUGLIA: ANALISI DEI PRIMI 10 MESI DI ATTIVITA'

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Introduzione: L'incidenza di Ipotiroidismo Congenito (IC) in Puglia, stimata nel triennio 2000-2003 solo su tre province, è di circa 1:2300 nati (dati RNIC). Dal 01/09/2016 l'U.O. Patologia Clinica dell'Ospedale Pediatrico Giovanni XXIII (Policlinico Bari) è istituita come unico Centro di Riferimento Regionale per gli Screening obbligatori. Scopo dello studio è valutare nei primi 10 mesi di attività l'incidenza dell'IC e gli indicatori di processo dello screening stesso.
Materiali e metodi: Dal 01/09/2016 al 30/06/2017 sono stati screenati 31904 neonati con dried blood spot (DBS; cut-off di richiamo ≥6.5 mcU/ml). Nei casi indicati dalla letteratura e da nostre linee guida interne, un ulteriore DBS è stato eseguito a 15 e 30 gg ("protocollo speciale''). Il bTSH (whole blood TSH) è stato determinato con metodo fluoroimmunometrico (GSP PerkinElmer). Nei neonati con bTSH>20 mcU/ml è stata intrapresa terapia con levotiroxina.
Risultati: Per 7383 neonati (23%) è stato necessario eseguire almeno un 2° DBS. Quaranta Ventidue neonati hanno iniziato terapia, 35 diagnosticati su DBS delle 48-72 ore dalla nascita e 5 con "protocollo speciale". Due dei 35 presentavano TSH ≤7 mcU/ml. L'incidenza è stata di 1:798. Il TSH in terza giornata era 3.1±5.7 (range 0.1–266.4) mcU/ml. L'età a inizio trattamento è stata 8.8±0.9 gg di vita.
Conclusioni: L'IC è la più frequente endocrinopatia dell'età evolutiva con incidenza in aumento in tutto il mondo. Seppur stimata in un breve periodo, in Puglia sembra in netto aumento rispetto al passato. I motivi di tale incremento sono molteplici e, almeno in parte, riflettono differenze etniche, apporto iodico, variazioni di cut-off, fattori ambientali. Il cambiamento di incidenza suggerisce di condurre studi a lungo termine, già in atto, per meglio descrivere il fenomeno. Un'analisi aggiornata dei dati ha inoltre messo inoltre in evidenza come l'età media alla diagnosi presa in carico si sia ridotta nel corso degli anni e la revisione del cut-off abbia consentito diagnosi che sarebbero altrimenti sfuggite. Tutto ciò suggerisce che la revisione del programma di screening ha consentito una più precoce identificazione delle alterazioni congenite della funzionalità tiroidea neonatale con conseguente anticipazione dell'intervento terapeutico.

P107
IODOPROFILASSI E VALORI DI TSH NEONATALI

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Obiettivi: La carenza iodica e i disordini che ne derivano, noti come Iodine Deficiency Disorders (IDD), svolgono un ruolo importante nella eziopatogenesi delle tireopatie. Il TSH da screening neonatale è un indice utile per il monitoraggio della iodoprofilassi. Abbiamo valutato la condizione di iodocarenza in 5 macroaree provinciali (Lecce, Brindisi, Taranto, Bari-BT, Foggia) mediante TSH da screening neonatale. Metodi: Criteri di inclusione: nati dal 01/09/2016 al 30/06/2017, peso neonatale 2500-4500 grammi, TSH da cartoncino fra 48 e 72 ore di vita. Campione: 34378 neonati (11895 M, 22483 F), di cui Lecce 4590, Brindisi 2267, Taranto 3264, Bari-BT 10076, Foggia 4344. Abbiamo calcolato la prevalenza di neonati con TSH >5 µU/Ml (indice consigliato dall'Osservatorio Nazionale per il Monitoraggio della Iodoprofilassi in Italia - OSNAMI) per macroarea. Risultati: La prevalenza di TSH >5 µU/ml è maggiore per Brindisi (17.8%), Bari-BT (17.2%) e Lecce (15.4%), seguite da Taranto (12.6%; $p<0.01$ rispetto alle precedenti 3) e Foggia (6.7%; $p<0.001$ rispetto alle altre 4). Il valore assoluto di TSH è risultato inferiore nell'area di Foggia (2.3±6.3 µU/ml) rispetto a Bari-BT (3.1±4.6 µU/ml, $p<0.001$), Lecce (2.9±3.8 µU/ml, $p = 0.001$), Brindisi (3.2±3.6 mcU/ml, $p<0.001$) e Taranto (2.9±6.4 µU/ml, $p = 0.013$). Conclusioni: Uno degli obiettivi della iodoprofilassi proposto dall'OSNAMI è che la prevalenza di TSH >5 µU/ml sia <3%. Questi dati mostrano che la zona di Foggia è quella più vicina a questo obiettivo, ma che le altre 4 sono lontane da questo target. Precedenti dati epidemiologici ottenuti dal dosaggio della ioduria indicavano una percentuale di iodocarenza in Puglia del 48%, decisamente alta. I nostri dati confermano indirettamente la condizione di iodocarenza in regione, suggerendo che nella zona di Foggia la iodoprofilassi è più efficace. La disponibilità di dati sulla vendita di sale iodato e di altri indici proposti dall'ONASMI consentirebbe di analizzare meglio questo fenomeno e di individuare eventuali strategie preventive adeguate.

P108
VALIDATION OF A LABORATORY DEVELOPED METHOD FOR THE ISO15189 ACCREDITATION

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Introduction: The International Standard ISO 15189 describes the requirements for quality and competence for medical laboratories. The verification and validation of examination procedures (EP) are fundamental steps in the accreditation process. Non-standard methods, laboratory developed methods, standard methods used outside their intended scope and validated methods subsequently modified shall be validated. The validation shall confirm, through the provision of objective evidence (in the form of performance characteristics), that the specific requirements for the intended use of the EP have been fulfilled. Aim of this study is to illustrate the validation procedure for a EP developed by laboratory reporting the example of salivary cortisol (sF) determination by a mass spectrometry (MS) based method.
Methods: the following steps have been considered: a) analysis of the available scientific documents; b) evaluation of test intended use; c) identification of performance characteristics to evaluate; d) drafting of validation plan and certificate.
Results: sF is one of the first line test of screening for Cushing's Syndrome. A MS based method was developed in laboratory, considering that the reference methods for the steroids are liquid chromatography coupled to tandem MS methods. The validation plan was prepared on the basis of: the performance characteristics to determine (linearity, limit of quantification, specificity, imprecision, trueness, ion suppression and reference range); the typology of the tests to carry out; the executive time; the evaluation of results related to appropriateness to the intended use (acceptability criterion). A validation certificate was produced reporting the EP typology, the intended use of test, the reference documents used, the obtained results in the evaluation of performance characteristics and the approval to the use in the clinical practice.
Conclusions: The validation procedure followed for the determination of sF is resulted complied with ISO 15189 requirements during the accreditation audit. It can therefore represent a model to be used in other medical laboratories.
ISO 15189:2012 medical laboratories- requirements for quality and competence. Geneva, Switzerland: ISO, 2012.

P109

COMPARING TWO IMMUNOLOGICAL METHODS OF QUICK PARATHYROID HORMONE TEST. PILOT STUDY FOR PROPHYLACTIC CALCIUM THERAPY AFTER TOTAL THYROIDECTOMY

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Backgrounds: Hypoparathyroidism is the main complication of total thyroidectomy (TT). Many authors suggest testing postoperative quick parathyroid hormone (qPTH) to predict postoperative hypocalcemia and then prescribe prophylactic calcium therapy. A cornerstone of this strategy is to use a reliable qPTH method to identify patients with low PTH. The aim of our study is to analyse the agreement between two different methods.
Methods: From January 2015 we collected blood samples taken immediately after TT and we checked qPTH using two different methods: Abbott ® Architect Method CMIA, Intra-assay CV, % 4.1-9, Antibodies used polyclonal goat Healthy reference range 15-68 pg/mL; N-tact Liaison PTH Diasorin® Architect Method CLIA, Intra-assay CV, % 3.9-6.1, Antibodies used polyclonal goat, healthy reference range 14.5-87.1 pg/mL. Statistical analysis: data were described as number, percentage and 95% confidence interval.
Results: We analyzed 102 patients, 23 (23%) male, median age 53 years (22-79). In 3 patients PTH couldn't be determined using Architect. We had 9 patients (9%, 95% CI 4-16%) in normal range for Architect but low for Liaison. In 42 patients both methods were below normal value. In the remaining 48 patients (47%, 95% CI 37-57%) both were over the lower normal value, and Architect always overestimated Liaison, being 34% (±21%) greater.
Conclusions: These two methods analyzing qPTH are not always in agreement. Therefore, we have planned a new study investigating the relationship between each qPTH method and postoperative calcemia as well as duration of postoperative calcium therapy in order to use qPTH for prophylactic calcium therapy.

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DEFINIZIONE DEGLI INTERVALLI DI RIFERIMENTO PEDIATRICI PER METANERFRINE URINARIE SU CAMPIONI DI URINA ESTEMPORANEA DOSATE CON METODICA HPLC-ECD

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Introduzione: l'analisi delle metanefrine urinarie (MTu) è raccomandata in alternativa al dosaggio delle MT plasmatiche con metodo HPLC-MS o con detector elettrochimico (ECD) come analisi di I livello nel sospetto di feocromocitoma o paraganglioma. Gli intervalli di riferimento (IR) nel paziente pediatrico (PP) presenti in letteratura non sono trasferibili a causa della differenza tra metodi utilizzati. Obiettivo: definire gli IR per metanefrina (Met) e normetanefrina (Nmet) rapportate alla creatinina (Crea) in urina estemporanea (Ue) agevolando la raccolta del campione rispetto all'utilizzo dell'urina delle 24h.
Metodo: almeno 20 Ue per sesso e seguenti fasce di età: <1, 1-2, 3-4, 5-6, 7-8, 9-10, 11-12, 13-14, 15-16, 17-18 anni (Y) sono state analizzate, per un totale di 461 campioni. L'estrazione delle MT è stata eseguita con kit ClinRep® (Recipe) e analizzate su HPLC LC-20AT (Shimadzu) associato a ECD (Antec Scientific); la Crea è stata dosata su piattaforma AU680 (Beckman Coulter). L'analisi statistica è stata eseguita con il software MedCalc. La correlazione di MTu con l'età è stata verificata con il test di Spearman, gli outliers con Dixon test e la suddivisione nelle sottoclassi di età sulla base del Mann-Whitney test. Il calcolo del 97,5° percentile e il relativo intervallo di confidenza al 90% sono stati calcolati con il Metodo Robusto. Valori di $p<0,05$ considerati statisticamente significativi.
Risultati: Met/Crea e Nmet/Crea decrescono all'aumentare dell'età (ρ= -0,75 e ρ= -0,72); nessuna differenza è stata riscontrata tra maschi e femmine in ogni intervallo di età considerato. Il 97,5° percentile [90%CI] espresso in mmol/mol Crea è risultato: Met 0-12 mesi (n=59) 0,34 [0,30 – 0,39]; 1-5Y (n=112) 0,22 [0,19 – 0,25]; 6-9Y (n=74) 0,13 [0,11 – 0,15]; 10-18Y (n=207) 0,097 [0,089 – 0,10]. Nmet 0-4 mesi (n=31) 1,76 [1,49 – 2,01]; 5-11mesi (n=28) 0,63 [0,56 – 0,69]; 1-2Y (n=41) 0,47 [0,41 – 0,52]; 3-5Y (n=71) 0,29 [0,26 – 0,32]; 6-9Y (n=74) 0,23 [0,19 – 0,26]; 10-18Y (n=207) 0,15 [0,14 – 0,17].
Conclusioni: sebbene i tumori neuroendocrini siano rari nel PP, la definizione di appropriati IR per MTu permette al clinico di utilizzare il risultato del test con maggiore affidabilità. L'utilizzo di Ue rende più agevole la raccolta del campione anche nel neonato.

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PHARMACOGENETICS OF CYP2D6 IN THE REVOLVING DOOR CONDITION OF PATIENTS WITH PSYCHIATRIC ILLNESSES

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Commonly used psychotropic drugs are mainly metabolized by Cytochrome P450 (CYP) 2D6. Aim of this study is to investigate the role of CYP2D6 polymorphisms in the response to treatment of revolving door (RD) patients with psychiatric illnesses. Sixteen psychiatric patients were selected for their RD condition. Diagnosis of psychiatric illness was made according to the DSM-V criteria. The RD condition was defined according to Kastrup (1987). Social needs were assessed by the "Camberwell Assessment of Need" rating scale. Psychiatric symptoms were evaluated using the Brief Psychiatric Rating Scale Expanded Version 4.0 (BPRS). A response to treatment was defined by a change in BPRS score ≥25% as evaluated at baseline (T_0) and at 1 week (T_1) of treatment: $\Delta BPRS=[(BPRSt_1-BPRSt_0)/BPRSt_0]x100$) according to Leucht et al. A ΔBPRS score <25% suggested a therapeutic failure (TF). Drug-induced extra-pyramidal symptoms were evaluated by means of the Simpson Angus Scale (SAS). An SAS score >0.3 and/or drug-induced metabolic impairment with weight gain according to the NCEP ATP III criteria were considered as adverse drug reaction (ADR). The analysis of 16 functional CYP2D6 polymorphisms was made in by using the INFINITI CYP2D6-I assay according to the manufacturer instruction. The analysis revealed 3 patients as extensive metabolizers (EM) (CYP2D6 genotype *1/*1), one patient as ultrarapid metabolizer (UM) (CYP2D6 genotype *2A/*1XN) and 11 patients as intermediate/poor metabolizers (IM/PM), i.e. carriers of alleles associated to a reduced enzyme activity (*2A, *4, *17, *41). In one patient we observed a new CYP2D6 genetic pattern, also associated to a probably reduced enzyme activity. 13/16 patients (≈80%) showed functional genetic variants influencing CYP2D6 enzyme activity. Excluding patients in whom the RD condition is influenced by social needs, the other patients are readmitted to the hospital because to TFs/ADR, both conditions resulting from defective CYP2D6 EA. Thus, CYP2D6 pharmacogenetics may influence prevalence and frequency of RD condition because to its role in the onset of TFs and ADR.

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EGFR MUTATIONS IN PLASMA OF NON SMALL CELL LUNG CANCER PATIENTS: APPLICATION OF TWO DIFFERENT GENOTYPING TECHNOLOGIES

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Background: Circulating tumor DNA (ctDNA) has emerged as a new promising noninvasive tool to analyze the genetic landscape of several human neoplasia, including lung cancer. EGFR mutation testing is important in the treatment decision for advanced non-small cell lung cancer (NSCLC). Digital polymerase chain reaction (dPCR) represents an affordable and powerful technology for absolute quantification of single target molecules from low-input DNA. We aimed at detecting the genetic alterations of lung cancer in ctDNA, monitoring treatment effectiveness or the potential appearance of acquired resistance, and analyze prognosis value by comparing results obtained from the cobas® EGFR Mutation method and the QuantStudio 3D dPCR System (Thermo Scientific) platforms.

Methods: From April 2017 to June 2017, 37 patients with NSCLC were enrolled in this study. ctDNA samples were extracted from plasma specimens collected before and during a treatment with EGFR-TKI. Cobas® EGFR Mutation method and dPCR reactions were performed by using 20 ng of ctDNA as input. The following EGFR mutations were analysed: G719X (ex18), ex19 deletion, ex20ins, S768I and T790M (ex20), L858R and L861Q (ex21).

Results: Twenty-three out 37 patients (62.1%) carried only wild-type sequence, one patient carried the G719X, one the L861Q and three patients displayed the L858R mutation. Four patients (10.8%) developed a T790M mutation during EGFR-TKIs treatment, two in association with ex19del and two with the L858R. The genetic analysis of ctDNA from these 10 patients were further validated with dPCR, demonstrating the same high sensitivity and specificity for EGFR-sensitizing and for the T790M mutations. Our preliminary results showed a close concordance between the two platforms for EGFR mutations from NSCLC patient plasma.

Conclusion: The cobas® EGFR Mutation and QuantStudio 3D dPCR System platforms provide sensitive, inexpensive, and robust methods for monitoring non-small-cell lung cancer patients' response to TKI, and obviates the need of repeated lung biopsy.

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VITAMIN D GENE POLYMORPHISMS ARE ABLE TO AFFECT DACLATASVIR PLASMA CONCENTRATIONS AT TWO WEEKS AND ONE MONTH OF THERAPY

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Daclatasvir is an inhibitor of HCV NS5A protein and a P-glycoprotein substrate. Nowadays, the expression of several genes involved in xenobiotics transport is known to be modulated by many signals: among these, Vitamin-D is expected to play a role, since its receptor is capable of acting as a transcription factor for many genes, such as ABCB1 one, which encodes the P-glycoprotein of which daclatasvir is substrate. Considering this, several works regarding previous anti-HCV treatments highlighted some significant influence of gene polymorphisms involved in Vitamin-D metabolism and signaling on antiviral drug pharmacokinetics and on the therapeutic outcome or toxicity. In this work, we investigated for the first time the association between daclatasvir plasma concentrations at two weeks and one month of therapy and genetic variants (SNPs) on genes encoding enzymes and receptors involved in vitamin-D pathway.Allelic discrimination has been performed through real-time PCR, whereas daclatasvir plasma concentrations were evaluated through liquid chromatography. 52 patients were analysed from Kineti-C Study. Daclatasvir plasma concentrations were influenced by CYP24A1 rs2248359 T>C polymorphism at two weeks of therapy and VDR Cdx2 A>G at one month of treatment. Linear regression analysis showed that significant predictors of daclatasvir concentrations at two weeks of therapy were baseline body mass index, alanine aminotransferase and hematocrit, while body mass index and hematocrit at baseline, VDR Cdx2 AG/GG and FokI TC/CC genotype polymorphisms were predictive of daclatasvir concentrations at one month of therapy. These polymorphisms could affect vitamin-D serum concentrations, consequently they could regulate the transcription of genes related to daclatasvir metabolism or elimination, such as ABCB1, leading to different plasma drug concentrations at two weeks or one month of therapy.These results showed a possible role of vitamin-D in influencing daclatasvir plasma concentrations, confirming the role of this hormone in regulating the expression of genes involved in drug metabolism and excretion regulation.

P114
A COMMON abcb1 GENE POLYMORPHISM IS ASSOCIATED WITH CHANGES IN LINEZOLID CLEARANCE

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Several factors contribute to the linezolid (LZD) plasma exposure high variability. Very recently, it has been suggested that LZD could be an ABCB1 substrate. The present work was aimed at investigating the role of ABCB1 on LZD pharmacokinetics (PK) through a population pharmacokinetic (POP/PK) approach. Secondary objectives included the evaluation of possible influence of other transmembrane transporters on drug PK. Fourteen patients treated with intravenous LZD (600mg twice daily) were enrolled. Allelic discrimination for abcb1 (c.3435C>T, c.2677G>T, c.1236C>T), abcc2 (-24G>A, c.1249G>A), abcc4 (*879T>C, c.3348T>C, abcg2 (c.421C>A, 1194+928T>C), slc22a6 (-127G>A) and slc22a1 (c.480C>G) genes was performed by real-time PCR. Drug plasma concentrations were measured through an UPLC-PDA validated method and area under the curve over 24 hs (AUC) values were determined by the mixed log-linear rule (Kinetica software). LZD plasma concentrations were analyzed through a nonlinear mixed-effects modeling approach with NONMEM software, together with Xpose and PsN softwares. The nonlinear mixed effect modeling found a significant effect of abcb1 c.3435C>T SNP on LZD clearance, whose values accounted for 11.58±8.50 L/h in wild-type homozygotes and 6.05±3.94 L/h in the remaining individuals. Although that difference was not statistically significant because of the large interindividual variability (57.70%), significant differences were observed for terminal half-life (2.74±0.83 vs. 6.87±5.15 h) and AUC (141.64±75.94 vs. 265.30±124.53 h´mg/L, for c.3435CC and c.3435CT/TT patients, respectively). Simulation according to the final model revealed that the cumulative response fraction for the AUC/MIC parameter was better for individuals carrying at least one c.3435T allele with respect to c.3435CC individuals for MSSA, MRSA and S. pneumoniae species.In conclusions, results from the present study suggest that abcb1 could take part in LZD PK, bringing new interest for pharmacogenetic (PG) analyses in antimicrobial chemotherapy. Although results from the present study should be confirmed in larger trials, PG analyses could be incorporated in therapeutic protocols for precision medicine.

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DOSAGGIO EMATICO DEL LEVETIRACETAM: VALUTAZIONE DELLE PRESTAZIONI ANALITICHE DI UN NUOVO METODO EMIT IN CONFRONTO AL METODO HPLC-UV

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Scopo del lavoro: Il dosaggio del farmaco antiepilettico Levetiracetam su siero viene eseguito nel nostro Laboratorio con cadenza settimanale utilizzando una metodica HPLC-UV (Eureka Lab. Division) dopo deproteinizzazione. In questo lavoro abbiamo valutato le prestazioni analitiche di un metodo EMIT prodotto dalla Ditta ARK ed utilizzato su strumento automatico Dimension EXL-LM.

Risultati: I sieri di 58 pazienti, raccolti nel periodo Aprile-Giugno 2016 (range 8.0-513.8 μmol/L) sono stati dosati con i 2 metodi ed i risultati ottenuti sono stati correlati utilizzando la regressione lineare standard con metodo dei minimi quadrati ($y=0.929x+4.40$, $R^2=0.9523$) e con metodo di Passing e Bablok ($y=0.997x+3.76$, pendenza=0.92-1.06, intercetta=-10.6-5.6).

Del metodo EMIT sono state valutate anche le seguenti prestazioni analitiche:

- Accuratezza tramite Test di Recupero (n=10, Rec %=95.9-109.1) e Test di Diluizione con siero free (n=10, $y=1.041x-9.22$, $R^2=0.9967$)
- Precisione entro la serie e fra le serie a 3 livelli di concentrazione (n=10, CV%<3.8%)
- Sensibilità analitica (3 μmol/L)

Conclusioni: Il metodo EMIT correla con il metodo HPLC-UV e le sue prestazioni analitiche sono comparabili con quelle del metodo HPLC-UV. La sua introduzione avrebbe il vantaggio della completa automazione e della possibilità di esecuzione con cadenza giornaliera, con diminuzione dei tempi di risposta senza diminuzione della qualità del dato. Questo sarebbe auspicabile anche in considerazione del fatto che circa il 15% delle richieste al nostro Laboratorio provengono dai Dipartimenti dell'Emergenza e dalla Terapia Intensiva e rivestono carattere di urgenza.

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STUDIO DEI TEMPI DI ACCESSO ALLE PRESTAZIONI, RELATIVAMENTE ALLA FASE PREANALITICA, NELLA DETERMINAZIONE DELLE SOSTANZE D'ABUSO CON VALENZA MEDICO LEGALE SU LIQUIDI BIOLOGICI – PROPOSTA DI UN PROTOCOLLO

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Nel laboratorio che esegue analisi tossicologiche,risulta fondamentale considerare la parte preanalitca del processo diagnostico, punto sovente più critico, perché legato a fattori esterni al Laboratorio stesso. Risulta quindi necessario uniformare le procedure e standardizzare i metodi considerando che nel caso delle principali sostanze d'abuso, la presenza della sostanza madre nel sangue e saliva è evidenziabile in termini di minuti.Risulta dunque fondamentale la tempistica che intercorre dal momento del fermo della PG al momento del prelievo di materiale biologico.La concentrazione ematica e/o plasmatica della sostanza ricercata,infatti, consente di stabilire o di escludere la recente assunzione ed è direttamente correlabile allo status psicofisico del soggetto al momento del prelievo. Nella nostra esperienza,abbiamo ritenuto indispensabile monitorare la tempistica di prelievo del materiale biologico, analizzando la performance dei nostri due PS, Sarzana e La Spezia, in termini di velocità di risposta alla richiesta di analisi da parte della PG per adempiere agli obblighi derivanti dall'applicazione degli art.186/187 del N.C.d.S.Per far ciò abbiamo valutato l'intervallo che intercorre tra la richiesta scritta della PG e l'allestimento della catena di custodia da parte del personale sanitario del PS.Il delta tempo è stato calcolato basandosi sugli orari annotati sui "Moduli di richiesta esami Urgenti" (art.354 cpp) presentati dalla PG, e il tempo di avvenuto campionamento presente sui moduli di Catena di custodia" che accompagnano i campioni.Su 463 catene di custodia nell'anno 2016 il 63% è stata allestita entro un'ora dall'arrivo della richiesta della PG (34% entro la mezzora e 29% entro l'ora) ,il 90% entro le due ore e mezza e un 10% di risposte superiori alle tre ore.Abbiamo poi suddiviso in 4 fasce orarie le 24h in turni di 6 ore e abbiamo analizzato i tempi di risposta del PS per singola fascia, è emerso che, in generale i tempi sono buoni in tutte le fasce orarie, ma che nel 10% dei casi che superano le tre ore di risposta la fascia oraria più rappresentata è quella dalle 16 alle 22, questo probabilmente dovuto a flussi di maggior affluenza al PS.Dalla valutazione dei dati emersi e dalla analisi critica di questi, abbiamo deciso di introdurre un apposito Codice per l'accesso degli utenti al PS che abbiano necessità Medico Legali. Unitamente ai già preesistenti codici, rosso, giallo e bianco, attiveremo il CODICE VIOLA, che identifica gli utenti, accompagnati dalla PG, che hanno necessità di prelievo di natura medico legale.

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VOLUMETRIC ABSORPTIVE MICROSAMPLING (VAMS) AND LC–MS/MS ANALYSIS FOR SIMULTANEOUS MONITORING OF 16 ANTIEPILEPTIC DRUGS: WORKFLOW DEVELOPMENT AND VALIDATION

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VAMS is a novel sampling technique for collection of capillary blood. VAMS (Mitra™) absorbs a fixed volume of blood (10 or 20 μl) from a drop of blood by dipping an absorbent polymeric tip into it. The resulting blood microsample is dried and analyzed as a whole. VAMS may overcome bias issues associated with dried blood spot (DBS). In this study VAMS were applied to therapeutic drug monitoring (TDM) of 16 different antiepileptic drugs (AEDs). A liquid chromatography–tandem mass spectrometry (LC–MS/MS) workflow for analysis of VAMS sample was developed and validated. Concentration of 16 AEDs on VAMS sampler were then compared to those in venous blood.
Methods: Fresh blood from healthy volunteers was spiked with different amounts of each drug to establish suitable six points calibration curves and three levels QCs and then absorbed on 10 μl VAMS tips. Drugs considered in this study were: levetiracetam, lacosamide, ethosuximide, rufinamide, zonisamide, felbamate, lamotrigine, oxcarbazepine, 10-hydroxicarbazepine, carbamazepine, carbamazepine-epoxide, phenobarbital, primidone, phenytoin, topiramate and perampanel. Desiccated tips were then extracted and subjected to LC–MS/MS assay. HCT bias was measured along five different percentages (19, 30, 35, 55 and 61%) against a reference hematocrit of 48%. Stability issues were examined at different temperatures (-20, 4, 10 and 37°C) after 10 days of storage. Scavenged samples from patients referring to our lab for TDM of AEDs where then compared with routine LC–MS/MS assay on serum samples.
Results: Six point calibration curves demonstrated linear and stable. CVs for three different levels of QCs are consistently under 15%. Recoveries varies from 86% to 106%, no matrix effect was found. HCT bias was tested for the three levels QCs at different hematocrit concentrations but no bias (>15%) was observed. Samples demonstrated stable (% variation less than 15) at different temperatures and times. Scavenged samples from patients had a maximum difference percentage less than +15%.
Conclusions: VAMS were successfully applied for the first time to the therapeutic drug monitoring of 16 different AEDs. LC–MS/MS workflow for analysis of VAMS sample was developed and validated. AED concentrations in whole blood on VAMS device were compared to those in venous blood by routinely used technique with good results.Our results established that VAMS is simple, accurate and delivers the benefits of DBS while overcoming the issues of hematocrit and homogeneity and also overcomes issue on stability.

P118
LC-MSMS METHOD FOR THE QUANTIFICATION OF TEN ANTIEPILEPTIC DRUGS FROM DRIED PLASMA SPOTS ON DRIED SAMPLE SPOTS DEVICE (DSSD) AND THEIR LONG TERM STABILITY IN DIFFERENT CONDITIONS

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A bioanalytical method for the determination of ten commonly prescribed AEDs (levetiracetam, lacosamide, topiramate, ethosuximide, lamotrigine, rufinamide, oxcarbazepine, zonisamide, primidone, monohydroxycarbazepine) was developed, modifying an existing LC-MS/MS method. and a complete short and long term stability evaluation were carried out. Fifty microliters of plasma were distributed on a collection dics device (DSSD) then the filter was dried and stored at room temperature, 4°C, 37°C and #20°C. The analytes were extracted from the device, after the addition of the appropriate deuterated internal standards, using an organic extraction solution. The chromatographic separation was performed on a UHPLC reverse-phase C-18 column and the analytes were quantified using a triple quadrupole mass spectrometer. The method was validated (following EMA guideline) considering the concentration ranges encountered in the routine clinical practice. The assay was linear over the concentration ranges tested. Recovery ranged from 89.1% to 111.4%. Intra-day and inter-day relative standard deviation for all quality control levels ranged from 1.2% to 13.8% and 1.7% to 13.6%, respectively. Intra-day and inter-day accuracies ranged from 97.7% to 108.9% and 88.6% to 113.9%, respectively. No matrix effect was detected. Samples from patients referring to our labs for therapeutic drug monitoring were exchanged between Genova and Milano to assign time and temperature stability in real settings. The dried plasma spots on DSSD allows shipment of samples at room temperature without any risks, therefore the developed and validated method enables an easy and cheap sample shipment for therapeutic drug monitoring and pharmacokinetic studies.

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DOCUMENTO DI CONSENSO GRUPPO TOSSICOLOGI FORENSI - GRUPPO DI STUDIO DI FARMACOTOSSICOLOGIA CLINICA E DOPING SIBIOC SULLE INDAGINI DI LABORATORIO PER LA DETERMINAZIONE DELLE SOSTANZE D'ABUSO

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Le richieste che giungono ai laboratori possono spesso avere una valenza medico-legale come quelle che riguardano le procedure per gli accertamenti sanitari di assenza di tossicodipendenza e di assunzione di sostanze stupefacenti o psicotrope e spesso tale tipologia di richieste, ha contribuito all'adozione da parte del singolo laboratorio di modalità operative personalizzate ed estremamente eterogenee. Lo scopo di questo documento è quello di fornire ai laboratori di farmaco-tossicologia alcuni indirizzi operativi che tengano conto delle migliori pratiche di laboratorio riconosciute a livello internazionale per effettuare analisi precise e accurate delle sostanze d'abuso nelle differenti matrici biologiche, proponendosi ai laboratori che progettano di effettuare o che già effettuano le analisi di tali sostanze, in modo che essi possano far propri i requisiti necessari a erogare un servizio di elevata qualità. Con il presente documento si intende: 1. fornire un contesto operativo comune, riassunto in pochi punti essenziali, ai laboratori che eseguono analisi per le sostanze d'abuso a fini clinici e medico-legali; 2. richiamare e armonizzare le procedure e le linee guida già condivise a livello nazionale, 3. assicurare che le procedure operative messe in atto dal laboratorio producano un risultato accurato e legalmente difendibile; 4. definire, per tutti i laboratori, criteri comuni di assicurazione e controllo della qualità accreditabili da un organismo esterno. Il Documento di Consenso ha individuato pochi punti fondamentali che però devono essere seguiti scrupolosamente da chi si avvicina a queste tecniche di indagine. I punti del Documento di Consenso: 1–requisiti, 2–campionamento, 3–quesito medico-legale, 4–conservazione campioni, 5–refertazione, 6–conservazione referti.

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P120

COMPLETE AUTOMATION IN LC-MS/MS FOR WHOLE BLOOD DRUGS OF ABUSE DETERMINATION WITH FORENSIC PURPOSES

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Aim of the study: Liquid Chromatography coupled to Triple Quadrupole Mass Spectrometry operating in SRM or MRM mode, ensures high sensibility and selectivity, that, together with a sample preparation procedure faster than GC/MS, guarantees higher throughput. All these factors result in a reduction of analysis time, avoiding, for example, derivatization step, typical of GC/MS analysis. These features lead to a growing expansion of LC-MS/MS technique for complex human matrix analysis, like whole blood, achieving LOQ values suitable for Art. 187 (C.d.S.) cut-off.

The aim of the study is to develop an extraction method and a gradient chromatography that allow to get suitable LLOQ levels for the analysis of drugs of abuse in whole blood, especially, on entry-level LC-MS/MS systems. Second step of this study is to evaluate an automatic liquid handler, able to process the sample from primary tube to final results without any manual step and directly connected to laboratory LIS. For these reason, we develop a method for sample preparation, in two steps, that allows more reproducibility in inter-series analysis, reducing the high variability caused by the operator.

This analytical method was thought for minimize matrix effect with two sample preparation steps and a chromatography studied specially for minimize ionic suppression mostly on the early and late eluting substances. The outcomes of this methods are: Working on the chromatography is the only way to separate our analytes of interest to interference peaks. Buffered solution to separate polar compounds. A gradient that provides a column washing step followed by column reconditioning For all these issues, is mandatory the development of methods that meets validation criteria and ensure "robustness" between different operators; in this way, it will be helpful the development of commercial kit to support hospital clinical laboratory dealing with this delicate issue for the requests of police, judiciary and lawyers. As known, the traceability of the sample, despite the chain of custody, is critical when the sample tube is manually handled by the operator for the preparation of the sample and for the injection into the mass spectrometer. B.S.N. srl offers Multitasker®, a liquid handler that, starting from primary tube, is able to read barcode, perform all the preparation steps of the sample and finally inject it directly into the mass spectrometer, thus guaranteeing complete analytical traceability.

P121
VALUTAZIONE DI DUE METODI IMMUNOMETRICI PER LA DETERMINAZIONE DELLA KETAMINA URINARIA E CONFRONTO CON HPLC-MS/MS

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Introduzione: La ketamina (KT) è un anestetico/analgesico dissociativo che trova impiego in campo veterinario, nella terapia del dolore acuto e cronico e in chirurgia pediatrica. Dagli anni '70 si è diffusa in tutto il mondo sia per uso terapeutico che "ricreativo" rappresentando ancora oggi un problema di rilevanza sociale. Pertanto, lo screening tossicologico urinario rimane uno strumento fondamentale per la gestione di questo crescente problema.
Obbiettivo: Valutare le prestazioni di due nuovi metodi di screening automatizzati (Immunalysis Ketamine Urine EIA Kit/Metodo 1 e quantILab DRI Ketamine/Metodo 2) e confrontare i risultati ottenuti con il metodo HPLC-MS/MS e il metodo di screening on-site Instalert One Step Ketamine attualmente utilizzato nella routine di laboratorio.
Metodo: Il Metodo 1 è stato applicato su Vista 1500 (Siemens Healthcare Diagnostics Inc.) e il Metodo 2 su Cobas 6000 c501 (Roche Diagnostics GmbH). La valutazione della precisione è stata effettuata secondo il protocollo CLSI-EP5A2 ridotto (n=10) utilizzando QC commerciali. Sono stati analizzati 101 campioni urinari consecutivi, pervenuti con la richiesta di screening tossicologico, per valutare l'efficienza diagnostica calcolando le relative sensibilità (RSn), specificità (RSp) e accuratezza (RAc) di ciascun dosaggio rispetto al metodo di conferma HPLC-MS/MS.
Risultati: L'imprecisione (CV%, n=40) alle concentrazioni 75-125 ng/mL per il Metodo 1 e 236-400 ng/mL per il Metodo 2 sono <4,4% e <3,3%, rispettivamente. Nel metodo on-site di routine e nel Metodo1 (cut-off 100 ng/mL), la RSp è del 100%, la RSn dell'80% (2 FN) e la RAc del 98%. Per il Metodo 2 (cut-off 300 ng/mL) la RSp è del 93% (6 FP), la RSn del 100%, con RAc del 94%. Inoltre è stato eseguito uno screening mirato con HPLC-MS/MS su tutti i campioni FP e FN. Nei campioni FN era presente solo la KT, mentre nei campioni FP è stata evidenziata la presenza di alcuni farmaci possibili interferenti.
Conclusioni: Nonostante il Metodo 1 presenti maggiori accuratezza e specificità relative rispetto al Metodo2, quest'ultimo è da preferire in quanto non presenta alcun risultato FN. Nel laboratorio di tossicologia clinica, refertare come negativo un campione in realtà positivo, può comportare severe conseguenze legali.

P122
THERAPEUTIC DRUG MONITORING (TDM) OF PIPERACILLIN-TAZOBACTAM (PIP-TAZO) AND MEROPENEM (MEM) IN A COHORT OF PEDIATRIC PATIENTS

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PIP-TAZO and MEM are time-dependent activity beta-lactams. Their effectiveness index is the proportion of time over minimal inhibitory concentration (T>MIC) during a dose interval, that can be evaluated by measuring concentrations before a new administration (trough concentration=TC). Ideally, in presence of severe infections and/or pathogens with reduced sensitivity TC should be at least equal to MIC value or better 4 times above.We reviewed clinical records of patients undergoing TDM during PIP-TAZO or MEM treatment at Istituto Giannina Gaslini in the period 2014-2015. Data were collected on age, antibiotic dosage and isolated pathogens (if any) with MIC. PIP-TAZO was administered at 400 mg/kg/day of PIP divided in 4 doses in cancer or cystic fibrosis (CF), at 300 mg/kg/day of PIP divided in 3 doses in others, at 50-100 mg/kg/day divided in 2-3 doses in preterm neonates. MEM was administered at 60 mg/kg/day divided in 3 doses, in CF at 120 mg/kg in 4 divided doses and in preterm neonates at 20 mg/kg/day divided in 2-3 doses, according to gestational age. TC were measured by liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) from 50 µL plasma samples by using a method developed and validated in our laboratory. TC observed were compared with EUCAST MIC breakpoints for Enterobacteriaceae and P.aeruginosa in case of empirical therapy.In 14 patients, 24 TC measurements were performed, 9 samples for PIP-TAZO TC, while 15 for MEM, in 4 cases TC was measured 2 times and 3 in 1. Median age was 16 years (range 0-39), 6 patients had cancer, 2 CF, 1 kidney transplant, 2 low birth weight neonates and 3 underwent surgery, 8 (57%) were treated in intensive care unit (ICU). We found one documented infection (P.aeruginosa bacteremia). Median TC were 36.6 mg/L (4.3-230) for PIP-TAZO and 2.6 mg/L (0.3-44) for MEM. In our series, mainly represented by children treated in ICU, median PIP-TAZO TC were high and > breakpoint (in 2/3 of samples) for Enterobacteriaceae and P.aeruginosa, while MEM median TC were quite low resulted > breakpoint only in 20% of samples. These preliminary data suggest that beta-lactams' TC monitoring could represent a mandatory practice in critical children for maximizing their efficacy and reducing risk of resistance selection.

P123

EVALUATION OF VAMS (VOLUMETRIC ADSORPTIVE MICROSAMPLING) AS AN ALTERNATIVE SAMPLING STRATEGY FOR THERAPEUTIC DRUG MONITORING (TDM) OF ANTIBIOTICS IN PEDIATRICS

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The measurement of blood levels of antibiotics is of great importance in individualizing antimicrobial therapy, especially in critically ill patients. In pediatrics the blood volume to be drawn is subjected to sample limitations thus the availability of reliable quantitative methods starting from low sample volumes is desirable. Microsampling techniques coupled to LC-MS/MS have thus an emerging role in the quantitative analysis of drugs to help overcoming the limitation to clinical studies and routine therapeutic drug monitoring (TDM) caused by the blood volume to be drawn.Volumetric absorptive microsampling devices (VAMS™) is a promising microsampling which enables the simple collection of an accurate volume of blood (10 µL) without need for haematocrit (HCT) correction. VAMS have been successfully applied to the quantitative measurement of several molecules]. We here show the development and validation (following EMA guidelines) of a VAMS-LC-MS/MS method for the simultaneous quantification of four antibiotics: piperacillin-tazobactam, meropenem, linezolid and ceftazidime in 10 µL human blood. The novel VAMS-LC-MS/MS method has been compared with a dried blood spot (DBS)-based method in terms of impact of HCT on accuracy, reproducibility, recovery and matrix effect. Antibiotics were extracted from VAMS and DBS by protein precipitation with methanol after a re-hydration step at 37°C for 10 min. LC-MS/MS was carried out on a Thermo Scientific™ TSQ Quantum™ Access MAX triple quadrupole coupled to an Accela ™UHPLC system. The VAMS-LC-MS/MS method is selective, accurate precise and reproducible (intra-and inter-assay accuracy: and precision in the range 85-115% and CV %< 15% respectively) with appropriate extraction recoveries in the absence of matrix effects. In contrast to DBS, VAMS allows an accurate quantification without any HCT influence. The method has been applied to samples derived from pediatric patients under therapy and the concentrations obtained have been compared with those of DBS and plasma. VAMS is a valid alternative sampling strategy for the quantification of antibiotics and is valuable in support of clinical PK/PD studies and consequently therapeutic drug monitoring (TDM) in pediatrics.

P124

BIOLOGICAL AND ENVIRONMENTAL MONITORING OF ANTIBLASTIC AGENTS FOR WORKERS SAFETY EVALUATION

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Adverse health events associated with workers exposure to antineoplastic agent are well documented (1). Short-term acute effects and either long-term or chronic effects related to the manipulation of antineoplastic agents have been largely described (2). It is therefore crucial for health care professionals to use validated safety procedures, whose efficiency has to be checked by periodic biological and environmental monitoring. The main problems related to this monitoring activities concern the complexity -in terms of chemical-physical features and chemical-biological stability - of the drug mixtures under investigation. Moreover, the pharmacokinetic behaviours of these drugs are quite different among each other, and it is therefore critical to design protocols allowing evaluating both incidental events and chronic exposures to these chemicals. In that aim, highly selective and efficient analytical methods are required, able to detect and quantify extremely low levels of many compounds in a single step, possibly through a minimal pre-analytical sample treatment. Therefore, we developed and validated an electrospray mass spectrometry/ultra-performance liquid chromatography (UHPLC/MS) based methods for the simultaneous quali-quantitative analysis of 12 widely used antineoplastic drugs; this approach was effective to analyse both urine and blood samples for biological monitoring of workers, and wipes for the environmental monitoring of workspaces. Very promising lower limits of quantitation, ranging from 10 to 50 pg/mL for the different investigated compounds, were achieved. This approach was therefore used to the evaluation of the risk at hospitals in the Salerno province, allowing the improvement of safety protocols in these structures

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P125
IS TDM OF LINEZOLID (LZD) APPROPRIATE?

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Introduction: Linezolid (LZD) is the first oxazolidinone used in the treatment of infection by multidrug resistant gram positive bacteria. Recent data indicates that there is high variability of LZD serum concentration in intensive care patients. This could invalidate the therapeutic success and contribute to develope resistance and drug-related toxicity.
Objective: The aim of our study is to evaluate whether standard dosing of LZD leads to therapeutic serum concentrations in critically ill patients.
Materials and method: In this prospective observational study,20 critically ill adult patients (29% medical, 35% surgical and 35% traumatic) with suspected infections (10 with positive isolation : 3 MRSA; 5 S. Aureus; 1 S.Epidermidis ,1 E.Faecium) in therapy with antibiotics and antiviral drugs, received standard dosing of 600mg LZD intravenously twice a day. Within 48 hours from first administration, and at fourth day, multiple serum samples were obtained from each patient, in order to determine the LZD concentration by chromatographic method (HPLC) in UV.
Results: In 59% of the case there was the resolution of infection. The main of Cmax and Cmin for the first series were 9±3,6mg/L and 0,49±0,76mg/L respectively, for the second series were 5,67±4,3mg/L and 0,76±0,72mg/L respectively.
Conclusions: We find high inter individual variability of Cmax (from 0,18 to 15,50mg/ml in the first series and from 5,2 to 11,7mg/L in the second) and Cmin (from 0 to 3mg/L in the first series and from 0 to 2,4mg/L in the second). We suggest the use of TDM of LZD that can guide to three possible optimizing strategies: more frequent doses, more dosage or continuous infusion. Optimizing the dose and method of administration LZD is essential.

P126
COMPARISON OF BUPRENORPHINE ASSAY BY SIEMENS VIVA vs SIEMENS VISTA

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Introduction: Buprenorphine is a semi-synthetic opioid analgesic derived from opium poppy Papaversomniferum. It is used for the treatment of chronic pain and also in treatment ofopiate addictionand heroin addiction as an alternative to methadone. As the availability of buprenorphine increases, so there is the risk of abuse and addiction, resulting in side effects such as euphoria and respiratory depression. Buprenorphine and its dealkylatedmetabolite are excreted in urine, almost exclusively as glucuronides. The reference test for the quantification of buprenorphine in urine is in the GC/MS or LC/MS; however, qualitative and semi-quantitative immunoassays were developed. In this study the measurements of buprenorphine performed on Viva Siemens are compared to those carried out on Vista Siemens.
Materials and methods: A total of 73 urine samples were tested for buprenorphine by Viva Siemens and Vista Siemens. In this comparison, both analyzers were based on the EMIT method. 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 Slope of 0.7692 (95% CI=0.7230 to 0.8277), Intercept = 0.0854 (95% CI=0.0586 to 0.1745), while at the Bland and Altman analysis the mean differences was -0.905 (±1.96 SD=+2.226 and -4.037).
Conclusions: Comparing the two analyzers, Siemens Vista has a good performance compared to Viva Siemens and the results are superimposable and comparable, especially around the target decision value of positivity and negativity test of the 5 ng/ml.
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P127
METHOTREXATE ASSAY: COMPARISON BETWEEN THERMOFISHER CDX90 AND SIEMENS VISTA

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Introduction: Methotrexate, formerly Amethopterin, is a folic acid antagonist widely used for the treatment of neoplastic disorders, severe psoriasis and adult rheumatoid arthritis. It inhibits the synthesis of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and proteins by binding to dihydrofolatereductase. Methotrexate has the potential for serious toxicity. Patients undergoing methotrexate therapy should be closely monitored so that toxic effects are detected promptly.Indeed, methotrexate can lead to nephrotoxicity, myelosuppression, mucositis, hepatitis and dermatitis.Methotrexate measurements are determined in plasma or serum by enzyme immunoassay, but due to high therapeutic concentrations, sample dilutions are required. In this study, Methotrexate determinations performed by Thermo Fisher CDx90 and Siemens Vista were compared.
Materials and methods: A total of 45 serum samples were tested for methotrexate by CDx90 Thermo Fisher and Vista Siemens after appropriate dilutions. In this comparison, both analyzers were based on the EMIT method. 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 Slope of 1.0708 (95% CI=0.9447 to 1.2099), Intercept = -0.0257 (95% CI=-0.0583 to 0.0088), while at the Bland and Altman analysis the mean differences was 0.708 (±1.96 SD=+7.296 and -5.88).
Conclusions: Comparing the two analyzers, Siemens Vista has a good analytic performance compared to the CDx90 Thermo Fisher. The complete automatic assay performed on Vista instrument allows you to test by reducing the turn around time.

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P128
IMMUNOSUPPRESSANTS (CYCLOSPORINE A, TACROLIMUS, EVEROLIMUS AND SIROLIMUS) TDM BY LC-MS/MS: A FOUR-YEAR EXPERIENCE OF G. GASLINI INSTITUTE

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Therapeutic drug monitoring (TDM) of immunosuppressive drugs (IS): cyclosporine A (Cys A), tacrolimus (Tac), everolimus (Ever), and sirolimus (Sir) is recommended to optimize efficacy and minimize toxicity in individual patients. LC-MS/MS analysis of IS in whole blood is considered the gold standard technique because of high sensitivity and specificity (lack of cross-reactivity with metabolites and endogenous compounds) and low reagent costs. Moreover, LC-MS/MS allows the use of limited sample volumes which is preferable in pediatrics. A pitfall for analysis of IS in whole blood is represented by matrix effect that is usually minimized by using an on-line SPE with a trap column for analyte enrichment and background elimination. In this work we report on a four-year experience of routine TDM of CysA, Tac, Ever and Sir of a tertiary care pediatric institute. Samples tested in the period April 2013-2017 were: 3905 CysA, 3737 Tacr (355 patients), 1160 Sir (113 patients) and 269 Ever (47 patients) belonged to both inpatients and outpatients (age: 1 month-77 years). The LC-MS/MS was based on a commercially available method but chromatographic separation was deeply modified in order to be applicable to our LC-MS/MS system (Thermo TSQ quantum Access max coupled to an Accela HPLC system). The method was then fully validated following EMA guidelines and showed good performances for all the four analytes in the range: 20-2000 ng/mL for Cys A, 0.5-80 ng/mL for Tacr, Sir and Ever. Intra- and inter-assay accuracy and precision were inside the expected ranges. Matrix effect (verified with post-infusion column experiments) was absent in the retention times analyzed. The method showed excellent robustness over time. Analytical runs were always performed following EMA and FDA guidelines and comprised: a blank sample, a zero sample, certified calibration standards at a minimum of 6 concentration levels and at least 3 levels of QC samples. Analytical runs acceptance criteria (in particular: accuracy, calibration curve parameters, retention times monitoring, calibrators and internal standards signal monitoring) were always fulfilled. During the analyzed period proficiency tests (IPT, London, UK) were perfomed monthly and results were always inside the acceptable ranges. Results of incurred samples re-analysed in different runs were always inside the EMA acceptable ranges. In conclusion this work shows that our LC-MS/MS method is suitable for routine analyses of clinical samples with excellent performance.

P129
THERAPEUTIC DRUG MONITORING OF PERAMPANEL IN HUMAN SAMPLES: A NOVEL LC-MS/MS ASSAY COMPARED WITH A FLUORESCENT HPLC ASSAY

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Perampanel (PMP) is a novel non-competitive selective antagonist at the postsynaptic ionotropic alpha-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) glutamate receptor. Studies suggest that AMPA receptor antagonism can lead to reduced overstimulation and anticonvulsant effects, as well as inhibiting seizure generation and spread. The European Medicines Agency and the Food and Drug Administration approved Perampanel (Fycompa, Eisai, Inc) in October 2012 as an adjunctive agent for the treatment of partial-onset seizure with or without secondary generalization in patients at least 12 years of age. In 2015 a second indication has been approved for PMP for primary generalized tonic-clonic seizure in patients with epilepsy who are at least 12 years of age. In this study we compared two different methods for the quantification of PMP in human plasma or serum: an existing commercially available HPLC kit with fluorescent detector and another kit for determination of 18 different AEDs (apart from PMP) based on LC/MS-MS in which we implemented PMP.
HPLC assay: Intra- and inter-batch reproducibility was examined by assessing accuracy and precision using 3 levels QC serum samples. The mean results obtained showed a relative error of 5.4% and 7.8%. The calibration curve across three assay batches was evaluated and found linear over the concentration range from 0.01 to 8 µg/mL with acceptable accuracy. The extraction recovery of PMP from human plasma was consistently above 98%. Extracted samples are stable at least for 2 days at 2-8°C.
LC-MS/MS assay: Chromatographic separation yielded high specificity and sensitivity; peaks are sharp and well separated. Intra- and inter-batch reproducibility was examined by assessing accuracy and precision using 2 levels QC serum samples. Relative CVs are respectively 8.6% and 9.6%. Six point calibration curve was linear (R^2=0.9917) over the concentration range from 0.03 to 2.8 µg/mL. LLOQ was 0.012 µg/mL and ULOQ was 4 µg/mL. The extraction recovery of PMP from human plasma was consistently above 98%. No matrix effect was found. Analytical interferences by other AEDs were not observed. Extracted samples are stable at least for 7 days at 4°C or at 10°C and for 4 weeks at -20°C. Data collected from 99 patients, given PMP as their maintenance antiepileptic therapy and referring to our hospitals for their routine TDM, show a very strong correlation.

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e-GFR E RAPPORTO ALBUMINA URINARIA/ CREATININURIA (ACR): PROPOSTA DI REFERTAZIONE IN EXCEL

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Premessa: Secondo le ultime linee guida KDIGO del 2012, il paziente affetto da insufficienza renale cronica in terapia conservativa così come il paziente trapiantato di rene vengono classificati secondo i parametri di laboratorio e-GFR ed ACR al fine di effettuare una valutazione prognostica del rischio di progressione di malattia. Il nostro lavoro propone un modello di refertazione in Excel in grado di estrapolare i valori di funzionalità renale, di escrezione di albumina ed i relativi commenti così come indicato nelle sopra citate linee guida. Materiali e metodi: I dosaggi di creatininemia e creatininuria e di albumina urinaria (U-ALB), espressi rispettivamente in mg/dL ed in mg/L, si effettuano su COBAS 6000 della ditta Roche. Il referto costruito in Excel calcola il filtrato glomerulare (formula CKD-EPI 2009), il rapporto albuminuria/creatininuria (ACR) ed evidenzia i relativi commenti. Risultati: La valutazione della filtrazione glomerulare si ottiene con una tabella matrice: nella prima colonna è presente uno specifico numero rappresentativo di uno dei sei intervalli di e-GFR e calcolato tramite successivi confronti tra filtrato ottenuto e limiti estremi delle sei categorie; nella seconda colonna si associa il relativo commento (da funzione glomerulare fisiologica o aumentata ad insufficienza renale). Anche la valutazione della escrezione di albumina si ottiene con l'uso di una tabella matrice: nella prima colonna è presente uno specifico numero rappresentativo di uno dei tre intervalli di ACR e calcolato tramite successivi confronti tra ACR ottenuto e limiti estremi dei tre gradi; nella seconda colonna si associa il relativo commento (da escrezione di albumina fisiologica a severamente aumentata). Nel referto è presente inoltre una terza matrice: la prima colonna esprime la somma dei due specifici numeri ottenuti per e-GFR ed ACR mentre nella seconda colonna viene associata la valutazione prognostica del rischio di progressione di malattia. Conclusioni: Nel referto finale si visualizzano i dati anagrafici, il valore della concentrazione della creatininemia, dell'U-ALB e della creatininuria. Si evidenziano inoltre i parametri calcolati quali e-GFR , ACR, le relative note estrapolate ed il rischio di progressione di malattia. Riteniamo che questo tipo di refertazione in Excel (ma anche in Access) possa essere sicuramente di grande utilità al clinico e tranquillamente affiancabile ai sistemi gestionali di laboratorio incapaci di gestire tali specificità.
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P131
UTILIZZO DI STRUMENTI DEL SISTEMA DI GESTIONE PER LA QUALITÀ (SGQ) PER LA CREAZIONE E LA TENUTA SOTTO CONTROLLO DEL PROCESSO DI MOVIMENTAZIONE DEI CAMPIONI FRA LABORATORI

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Introduzione: alla luce della sempre più diffusa riorganizzazione dei Laboratori, con modelli organizzativi basati sulla presenza di Laboratori centrali (hub) a cui afferiscono quelli periferici (spoke), l'attività di conferimento di campioni biologici per l'esecuzione di esami al Laboratorio centrale oppure ad un Laboratorio diverso da quello al quale l'Utente afferisce ("service") assume un ruolo sempre più rilevante. Anche per questa tipologia di prestazioni si deve assicurare la conformità ai requisiti del Cliente e a quelli cogenti applicabili, quindi é utile includere il processo nel SQG del Laboratorio di provenienza.
Scopo: descrivere gli strumenti pratici creati per la realizzazione dell'intero percorso, per garantirne la conformità agli standard ed il monitoraggio continuo.
Metodi: approccio per processi e ciclo PDCA; realizzazione di un software "home made" accessibile a tutto il Personale coinvolto (sia interno che esterno) per la gestione della fase pre-analitica contenente le modalità di richiesta, raccolta, conservazione, preparazione e trasporto del campione presso il Laboratorio che eseguirà l'analisi; realizzazione di un sistema di checklist per agevolare e monitorare tutte le fasi di spedizione; utilizzo di un ulteriore software "home made" per la registrazione e l'analisi delle non conformità; incontri di formazione sul campo per la condivisione delle azioni correttive.
Risultati: l'utilizzo degli strumenti descritti ha permesso di realizzare un processo standardizzato che tiene conto di tutte le attività, di individuare le criticità e implementare le necessarie misure di contenimento, di monitorare il livello della qualità delle prestazioni e di apportare i dovuti correttivi quando necessario.Tutto ciò ha consentito un sensibile miglioramento della qualità erogata e alla riduzione degli errori relativi all'attività di spedizione dei campioni (1.92% vs 16.00%), di quelli relativi al loro trattamento prima dell'invio (0.45% vs 0.60%), ed infine alla riduzione dei referti non pronti per il giorno stabilito (0.34% vs 1.90%).
Conclusioni: l'utilizzo di strumenti SGQ consente di approcciare l'implementazione di un nuovo processo in maniera esaustiva e di ottimizzarlo progressivamente in un'ottica di miglioramento continuo.

P132
BENCE JONES PROTEIN ASSAY COSTING APPRAISAL

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Introduction: The sustainability of the NHS is a goal in professional management: it is increasingly urgent to review diagnostic processes to contain costs maintaining the quality of the health services provided. The possibility to measure analytical parameters together economic indicators allows professional Laboratories to manage diagnostic lines reviews. In this light, the reorganization of Bence Jones protein assays (BJP) at Corelab in Modena is reported. According to the guidelines BJP detection may include an automated phase of Immunonephelometric assay (INA) to screen the negative urine samples. Positive urine samples need further confirmation by urine-immunofixation (uIFE) that is the recommended method but highly operator time-consuming. The aim of this paper is to evaluate the optimal management of samples with BJP request considering costs and resources.
Methods: BJP research was carried out according to 2 working protocols: 1) Determination of INA (Immage 800-Bekman, New Scientific Company antiserum) for the detection of negative samples (k free, L free <10 mg/L), and subsequent confirmation of positive samples by uIFE (anti-GAM anti-k tot, anti-L tot, anti-k free, anti-L free-Sebia). Turnaround time (TAT) 3 days. 2) Determination of BJP in all urine samples with simplified uIFE (anti-GAM anti-K tot, anti-anti-L tot-Sebia). TAT 1 day in 98% of cases. In the 2013, 2014, and until mid-2015, it was used the working protocol 1, while since the second half of 2015 up to now it was applied the working protocol 2.
Results: The BJP accepted in the laboratory and analyzed in 2013, 2014, 2015, 2016 were respectively 12099, 12474, 12787, 12344, while the costs of the reagents were 236392, 256994, 208401, 119962 Euros. The resulting costs per one BJP test were respectively 19.5, 20.6, 16.3, and 9.7 Euros.
Conclusions: The cost-effective results by the application of the working protocol 2 are very encouraging, showing an improvement on the diagnostic accuracy, reduced TAT, and saving of over 50% of costs of each test. It is worthy to note that an overall recovery of around 100,000 Euros a year was obtained. A careful assessment of processes management in specific areas of the Laboratory helps to improve both the quality of obtained data and cost reduction.

P133
DESIGN AND IMPLEMENTATION OF A HOME-MADE SOFTWARE BASED ON MICROSOFT ACCESS FOR TRACING AND ANALYZING THE COMMUNICATIONS OF CRITICAL VALUES AT THE LABORATORY ANALYSIS OF THE SPEDALI CIVILI DI BRESCIA

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Background: Microsoft Access allows to develop applications that meet the needs of traceability and data analysis in a modern Laboratory. Recently, Lippi et al provided an example, implementing a software for pre-analytical errors recording (CCLM 2017; 55 (3)). In this work, we describe how we develop a similar software for managing an important activity of the post-analytical phase: the communication of critical values.
Methods: We performed the following activities: 1) design, aimed at combining the guidelines with our internal organization; 2) development of database elements: tables and their relationships, forms (for registration, communication and search for any previous patient's results), queries, reports, and Visual Basic code modules; 3) population of tables (list of critical limits and their ranges, departments and their telephone numbers, authorized operators for communication). We also install the program as a "split" database (tables on central server and logon forms on the Laboratory clients).
Access features have been exploited to: 1) harmonize recorded data (selection of default entries on forms); 2) facilitate the insertion phase (automatic recording of all event's date and time and, by accessing the software with individual login and password, automatic acquisition of the identity of the operator(s) detecting and communicating the data); 3) prevent the acquisition of incorrect or incomplete records (alarm messages, recording impairment).
Results: The software allows to trace: 1) communication's data (Patient's name and surname, Lab request ID, request's typology, exam and result); 2) time data (date and time of sample arrival in the Laboratory, finding of the critical value and its communication); 3) the operators involved (those that found, communicate and received the informations); read-back of the data (check on dedicated field).
Indicators can be obtained either by using queries or by exporting the records in Microsoft Excel format.
Conclusions: The implementation of software designed and built directly by Laboratory Professionals makes it easier and more reliable to apply recommendations and guidelines developed by our scientific societies.

P134
APPROPRIATEZZA PRESCRITTIVA IN AMBITO OSPEDALIERO: L'ESPERIENZA DELL'AZIENDA POLICLINICO DI BARI

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Un test di laboratorio risulta appropriato se il risultato fornisce una risposta ad un quesito clinico, e rende possibile attuare un intervento diagnostico sul paziente. L'appropriatezza in Medicina di Laboratorio può essere estesa a tre settori di intervento: Appropriatezza nella richiesta di esami; Appropriatezza del processo analitico; Appropriatezza della fase post-analitica. Nel primo caso di inappropriatezza (25 - 40%) possono riguardare: aspetti clinici es. test richiesti a priori, insufficiente conoscenza formale della fisiopatologia, mancanza di compliance con linee guida, bisogno di ridondanza dell'informazione; aspetti organizzativi di laboratorio es.: configurazione di "profili" pre-costituti; ritardo nei tempi di risposta, modulistica con facilità per le richieste di blocchi di esami, mancanza di comunicazione con i clinici, insufficiente attività di consulenza. Scopo del nostro lavoro è stato quello di ridurre l'inappropriatezza prescrittiva. Sono state individuate 67 regole bloccanti classificate in tre gruppi; I) invarianza; intervallo minimo prima che il test possa essere nuovamente richiesto; II) incompatibilità del test in rapporto al risultato precedente; III) incompatibilità del test per sesso. Le regole "Evidence Based", sono state condivise con i clinici prioritariamente e poi implementate nel il software Prometeo (Noemalife). Il blocco ha riguardato solo le richieste di routine per pazienti ricoverati presso la nostra azienda Policlinico. Crono programma attuato: 1) Maggio 2014 studio retrospettivo sui dati storici del 2013; 2) Novembre 2015 Studio Pilota con la UU. OO. di Nefrologia Universitaria , ai fini di valutare la sostenibilità clinica ed informatica del progetto; 3) Gennaio 2016 arruolamento della prima U. O. (Ematologia con trapianto); 4) Marzo 2016 arruolamento del primo D.A.I. (Medicina Interna e Medicina Specialistica costituito da 15 U.U. O.O.); 4) Giugno 2016 arruolamento effettivo dei sei D.A.I. (54 U:U. O.O.); Dicembre 2016 analisi dei dati. Nell'anno 2016 le richieste inappropriate e bloccate dalle regole sono state 55.573, per un risparmio di risorse economiche pari a 169.657,00 euro (valorizzazione secondo tariffario SSN).

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OFFERING AST AS A REFLEX TEST: AN EASY BUT EFFECTIVE WAY TO IMPROVE APPROPRIATENESS OF LABORATORY REQUESTS

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Background: A growing body of evidence is showing that the incremental benefit of routine determination of aspartate aminotransferase (AST), in addition to alanine aminotransferase (ALT), is very limited. Laboratories should rather offer AST determination as a reflex test just in case abnormal ALT results are obtained. Here we report the results of a 6-year experience after the implementation of this approach in our academic hospital (AH) and the recent extension of a similar protocol in an infant-maternity (IM) setting.
Methods: In May 2011, after consultation with clinicians, we deleted from the order entry panel of AH Emergency Department (ED) the AST request and introduced an automatic ALT reflex test when ALT values are ›100 U/L [i.e. higher twice the upper reference limit (URL) in adult population]. In this case, the laboratory middleware activates a query program language (QPL) that automatically adds AST (and the AST-to-ALT ratio calculation) to the specific patient request. If considered clinically appropriate, the laboratory could however be contacted by clinical requestors to support the direct AST request in addition to ALT. More recently, we extended a similar approach to IM hospital, with the reflex test more conservatively activated at ALT values ›40 U/L (i.e. the URL for children and women). The number of requests that generated the reflex test was retrieved by the laboratory information systems.
Results: During the 6-year experience at AH ED, no extra-requests for AST determination to supplement diagnosis were registered and no detrimental situations for patients were reported. Particularly, during the two more recent years, 43,088 ALT measurements were performed, with 1737 AST added by QPL (4% of requests). The strategy resulted in a savings of reagent costs of € 2070 per year. The preliminary experience (7 weeks) at IM substantiated the activation of ALT reflex test in only 5.9% of 693 ALT requests.
Conclusions: Our study shows that the introduction of the automatic ALT reflex test is an effective intervention to improve request appropriateness and reduce the overutilization of laboratory tests. In different clinical settings, a reduction of ≈95% of AST requests can be obtained providing that optimal ALT limits for reflex testing are applied.

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LEAN-SIX SIGMA APPLICATO AL DOSAGGIO HbA1c: IMPATTO ORGANIZZATIVO DEL SISTEMA D-100 Bio-Rad Laboratories

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Introduzione: Le metodologie di processo Lean-Six Sigma, nate per i siti produttivi industriali, sono applicate anche ai laboratori di analisi, per aumentare l'efficienza e la flessibilità dei processi produttivi e decisionali (LEAN: eliminare gli sprechi) e ad aumentare l'affidabilità dei processi stessi (ridurre le variabili, ridurre i costi, incrementare le rese di processo, Six Sigma).
Obiettivi: Valutare, mediante Lean-Six Sigma, l'impatto organizzativo che il nuovo sistema D-100 Bio-Rad Laboratories avrebbe presso l'UOC Servizio Medicina di Laboratorio, per il dosaggio della HbA1c.
Materiali e metodi: In questo studio di Workflow abbiamo analizzato: -la fase pre-analitica, come avviene attualmente e come potrebbe essere; - la fase analitica, confrontando una routine di 200 campioni con il sistema in uso (Menarini 8180) e con il sistema D-100, simulando anche una "routine doppia" per valutare il sistema in casi di emergenza; -la fase post-analitica, intesa come valutazione del rischio clinico, mediante l'utilizzo del software "Mission:Control™" Bio-Rad Laboratories applicato ai risultati del controllo interno di qualità effettuato con i controlli (Lyphochek Diabetes Control).
Risultati: Fase pre-analitica: riduzione di due steps manuali per ogni rack (2 x 25 rack = 50 steps), ed azzeramento della mancata lettura del barcode per errato posizionamento della provetta. Fase analitica: riduzione Lead time tra 20 e 36%, e del Cycle time tra 45 a 48%, consentendo di lavorare con un unico sistema ed un unico tecnico, anche con una routine che preveda un doppio carico di lavoro. Riduzione delle fasi manuali per la manutenzione ordinaria del 71%. Riduzione dei reflui prodotti del 62,5% . Fase post-analitica: la valutazione e monitoraggio del Rischio Clinico mediante i controlli attualmente in uso, evidenzia che la strategia di controllo qualità attuale (ripetizioni ad inizio lavoro e dopo 100 campioni), consente una elevata sicurezza di qualità in quanto il rischio clinico di errore con l'utilizzo del sistema D-100 risulta nullo, con (E(Nuf) < 1 e E(Nuc) < 1.
Conclusioni: Grazie alla valutazione effettuata si è potuto evidenziare che l'utilizzo del sistema D-100 nel nostro laboratorio aumenterebbe l'efficienza globale del processo di analisi dell'HbA1C grazie ad una elevata produttività oraria, alla riduzione dei tempi d'attesa per l'analisi dei campioni, e della manualità richiesta all'operatore e quindi dei costi generali. Inoltre il sistema risulta estremamente affidabile con un rischio clinico calcolato sulla base della strategia di controllo di qualità applicata, praticamente nullo (<1).

P137
GESTIONE DELLA ISO 9001:2015 CON IL SISTEMA DI QUALITÀ INFORMATIZZATO

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La nuova norma ISO 9001:2015 introduce i concetti di risk based thinking ed enfatizza il ruolo della competenza, consapevolezza, comunicazione e formazione. Il Sistema Qualità (SQ) ha lo scopo di migliorare la gestione di un'organizzazione, ma paradossalmente può essere percepito come un intralcio all'attività quotidiana, soprattutto con la nuova ISO che, nonostante la semplificazione documentale, richiede un maggior numero di registrazioni a supporto delle evidenze. L'obiettivo è quindi lo sviluppo di un SQ informatizzato che alleggerisca l'impegno degli operatori e permetta al contempo un controllo reale del processo. Il nostro SQ utilizza database e fogli di calcolo integrati. Ogni operatore accede ad un sito con credenziali personali e attraverso la compilazione di moduli registra informazioni che possono essere visualizzate immediatamente e comunicate agli interessati con modalità di diffusione tracciabile e controllata. Automaticamente si effettua l'analisi dei dati, presentata in grafici, tabelle o cruscotti, che mostrano in tempo reale gli indicatori di processo. Ne risulta una gestione più snella poiché il semplice atto di registrare soddisfa contestualmente i requisiti di archiviazione, visualizzazione, comunicazione e analisi (metodologia lean). Il sistema gestisce i passaggi di consegne, le comunicazioni interne ed esterne, le manutenzioni, i risultati VEQ, le non conformità, i valori critici, nonché la formazione con un modulo dedicato alle competenze professionali che possono essere monitorate con opportuni indicatori. Inoltre abbiamo eliminato le ridondanze e la replicazione delle stesse informazioni su documenti diversi, come l'elenco delle tecnologie, degli analiti o del personale, estraibili così da un solo database per molteplici finalità e la cui revisione risulta agevolata. Il SQ informatico attua un vero controllo di processo, favorisce la rilevazione delle criticità, permette di agire preventivamente sui rischi, ottimizza le risorse e migliora la standardizzazione delle procedure aumentando la consapevolezza degli operatori. L'informatizzazione rappresenta un vantaggio per il raggiungimento dei requisiti ISO e facilita l'uso del SQ, che può essere finalmente percepito come uno strumento necessario al buon funzionamento del laboratorio.

P138
LEAN METHODOLOGY APPLIED TO THE STAT SAMPLES WORKFLOW IN THE CORE-LAB

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The laboratory medicine of the "Ospedale dell'Angelo" (Mestre, Venice, Italy) adopts a shared workflow of STAT and routine samples in the core area. Thus, we applied the Lean methodology (Toyota Production System) to pre-analytical and intra-analytical phases to improve patient safety and guarantee a promptly managing of STAT samples. Aim of this work is to report the results of the Lean experience conducted in the Laboratory Medicine of the "Ospedale dell'Angelo". Methods: The work flow was studied by the Spaghetti Chart, Value Stream Map, duration analysis of processes' tasks recording the times of activities and waiting and the red tag methods, applied to pre and intra-analytical phases. A specific training of the staff was organized to explain Lean concepts and applications. Turnaround Time (TAT) (from sample check-in to results validation) of STAT samples was extracted from Laboratory Information System. Achievements: A preliminary analysis has shown some critical issues related to the congested flow of movements, to the confusion between the urgent and routine samples, to the non-standard conduct of the lab technicians. To simplify the samples workflow and reduce TAT, the layout and work organization changes have focused on the: detailed SOP (standard operating procedures) definition, rotation of the check-in and sorting equipments, centrifuge location and centrifuging duration optimization, replacing of the old coagulation analyzer and outplacement of some minor instruments. The results have demonstrated the effectiveness of the implemented layout changes. TAT, measured in minutes and reported as the 90th percentile (and median value) from December to June, decreased for all the considered analytes: Potassium 51,7-44,3 (23,7-21,3); Prothrombin Time 64,7-57,0 (22,3-17,7); Troponin I 43,0-43,7 (22,3-20,0); Procalcitonin 107,7-93,0 (50,0-45,7); Leukocyte 47,3-40,3 (13,3-12,3). Conclusion: Lean methodology, applied to clinical laboratory workflows, improves their effectiveness and reduces the TAT.

P139
EVALUATION AND USEFULNESS OF OSTEOPROTEGERIN AND PRESEPSIN TITERS IN PATIENTS WITH SEPSIS: OUR PRELIMINARY EXPERIENCE

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Blood culture is the gold standard for the diagnosis of severe sepsis and septic shock, but early diagnosis is the main objective to be pursued to improve patient survival. Osteoprotegerin (OPG), a glycoprotein belonging to the superfamily of tumor necrosis factor receptors (TNFr), is produced from stromal-osteoblastic line cells as well as activated T lymphocytes. OPG is able to inhibit bone resorption playing an important role in regulating bone degradation and it also prevents interaction with the RANK cellular receptor acting as a "fake" receptor activator of nuclear factor-ligand (RANKL). In fact, OPG binds to RANKL mimicking its receptor and decreasing availability for the RANK receptor, with offset of the biological effects of RANKL. During sepsis, OPG concentrations may increase. Presepsin is a protein whose blood concentrations specifically arise septic patients, and its dosage is useful in assessing severity of sepsis and in monitoring clinical response to therapeutic interventions. The purpose of this study was to assess whether a correlation exists between the concentrations of Presepsin and OPG in patients with positive blood culture.
Materials and Methods: From April to May 2017, 15 patients having positive blood culture (11 males and 4 females) were selected (median age: M 58, F 78 ys). The presepsin (plasma lithium-heparine) assays were performed on the PathFast® analyzer (Mitsubishi Gepa) with chemiluminescence immunoenzymatic method. The OPG titer (serum) was determined on the DXSX System (TECHNO GENETICS) analyzer with ELISA method.
Results: Linear Regression Line Parameters for Positive Specimens: log (y) = 0.79 + 0.000075 x; r = 0.74 (95% CI = 0.36 to 0.90) p <0.001.
Conclusions: Our preliminary data show that, in patients with positive blood culture, the OPG concentration increase as well as of the Presepsin. During sepsis, there is on involvement of all tissues an increase of OPG concentration also suggest an involvement of bone tissue in particular inhibit bone resorption. Therefore, further studies with a larger groups of patients are needed to better understand the effective role of the OPG in sepsis.

P140
DISTRIBUZIONE DEL GENOTIPO DEL VIRUS DELL'EPATITE C IN PAZIENTI CON COINFEZIONE HIV/HCV

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Introduzione: L'infezione da virus dell'epatite C (HCV) è la principale causa di epatopatia cronica in tutto il mondo. L'HIV e l'HCV condividono le medesime vie di trasmissione, per cui la coinfezione da HIV/HCV sta aumentando di anno in anno e si stima che interesserà circa 10 milioni di persone in tutto il mondo. L'infezione da HIV accelera la progressione naturale dell'infezione da HCV, pertanto la co-infezione con HCV è diventata la causa più comune di morte nei pazienti affetti da HIV in trattamento con terapia antiretrovirale.
Scopo: Scopo di questo lavoro è di studiare la prevalenza dei genotipi HCV nei pazienti coinfettati da HIV/HCV nel nostro territorio dal 2012 al 2016 in 82 pazienti del reparto Malattie Infettive.
Materiali e metodi: Nel nostro studio sono state analizzati i genotipi e i sottotipi HCV le mediante tecnica ibridazione inversa, previa estrazione ed amplificazione del DNA, in 82 pazienti provenienti dal reparto di Malattie Infettive e HIV-RNA positivi/HCV-RNA positivi.
Risultati e discussione: Di tali pazienti 69 erano maschi e 13 erano femmine, con una età media di 45 anni. Il genotipo maggiormente osservato è stato il genotipo 1, con 47 casi totali (53.7%), di cui 28 pazienti con genotipo 1a (34,1%), 15 con genotipo 1b (18,3%) e 4 non specificati. Sorprendentemente, invece, il genotipo 3 è stato il secondo più frequente con 27 casi osservati (32,9%), dei quali 10 (12.3%) con genotipo 3a. Il genotipo 2 è stato riscontrato solo in 3 (3,7%) pazienti, mentre il genotipo 4 in 4 (4,9%) casi.
Conclusioni: Il presente studio dimostra che il genotipo maggiormente presente tra i pazienti con coinfezione HIV/HCV è il genotipo 1, con una prevalenza maggiore del sottotipo 1a rispetto ad 1b, in disaccordo con i dati di prevalenza Italiana e locale che vede una più alta presenza, in assoluto, del sottotipo 1b. Il genotipo 3 è risultato il secondo più rappresentato con una frequenza maggiore del 30% rispetto a circa un 3% della popolazione solo HCV. Il dato da noi riscontrato sembra essere in accordo con i dati epidemiologici che indicano il genotipo 3 come quello più frequente tra gli utilizzatori di droghe per via endovenosa, anche se non è stato per il momento possibile determinare se la platea oggetto dello studio, appartenga a tale categoria.

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COMPARISON BETWEEN OLD AND NEW SEPSIS DIAGNOSIS MARKERS

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A huge number of markers have been proposed to diagnose the sepsis: this deals with the complex physio-pathology of the syndrome. C-reactive Protein (CRP) and Procalcitonin (PCT) are the commonly used markers, while the more recently introduced ones are the Presepsin (PSP) and the Proadenomedullin (pADM). Presepsin (PSP) is the soluble N-terminal fragment of the protein CD14, a receptor of the complex formed by the bacterial lipopolysaccharide and the bonding protein which is able to provide precise prognostic information for septic patients, since their very admission in the ER. MR-proADM is a proteic fragment, deriving from the proADM molecule, with a many hours half-life; in the clinical practice, it has been identified as a prognostic marker for infected patients with an organ damage.
Aim of the study: Evaluating the early diagnostic and the short/long term prognostic role (28 and 70 days) of the Presepsin and the Proadrenomedullin with reference to the C-reactive Protein and the Procalcitonin for sepsis or septic shock affected patients.
Results: It is a prospective and observational study. 31 adult patients, entering in the Policlinico ER in Bari, were enrolled and then classified in: 4 patients with infection (12.9%), 24 septic patients (7.4%) and 3 patients with septic shock (9.6%) (sec. Sepsi-3). At day 28 the mortality rate was 39.2%, while the global mortality rate at day 70 was 46.4%. Samples for the sepsis markers were collected at hospitalization day 1, 2 and 7. Among the markers, the initial PSP value is the only mortality predictive result (values >800 pg/ml: at day 28 AUC 0.72, at day 70 AUC 0.78; significance according to Kaplan-Meyer p=0.03 at day 28, p=0.004 at day 70). If at day 7 proADM value is persistently >1.56 ng/ml, it is a negative prognostic (AUC 0.75 and 0.80 at day 28 and 0.70 respectively).
Conclusions: Presepsin is the only marker giving us clear indications since the ER admission about the septic patient severity and mortality risk, while Proadrenomedullin provides information about the clinical picture developed some days after its occurrence, even when the germ seems to have been finally eradicated.

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CEREBROSPINAL FLUID ANALYSIS IN AUTOMATED MICROSCOPY: PRELIMINARY RESULTS

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Introduction: The analysis of cerebrospinal fluid (CSF), particularly the white blood cell count (WBC) and cell differentiation in mononuclear (MN) and polymorphonuclear (PMN) cells, represents an useful tools in the diagnosis and follow-up of a several disorders of the central nervous systems (CNS). Microscopic analysis has been the gold standard for determination of the (differential) WBC and RBC counts in fluids, but there are several disadvantages of microscopic evaluation as (high imprecision, time consuming). Automated cell-counting analyzers provide to answer to improving both the accuracy and the productivity of CSF analysis. In recent years, numerous automated cytometry analyzers are available to CSF cells counting. In this study, we evaluate the performance of Sedimax Lite Menarini analyzer compared to Sysmex XE-5000 hemocytometer and to optical manual microscopy.
Material and Methods: A total of 18 CSF samples were evaluated. All the samples were examined on the Sedimax Lite Menarini analyzer, XE-5000 hemocromocytometer and manual optical microscopy counting RBCs and WBCs by differentiating polymorphonucleates from mononucleates. Sedimax Lite Menarini analyzer dispenses sample into the cuvette, performs centrifugation and takes the cuvette to the micosope. To differentiate white cells in polymorphonucleates or mononucleates the samples were pre-treated by Türk's solution. Images taken are then evaluated by the automatic evaluation module for red and white cell.
Results: Sedimax Lite Menarini analyzer when compared to optical manual microscopy to count RBCs showed at the Passing-Bablok analysis a slope 1.0639 (95% CI 0.5029 to 4.3267) and intercept: -9.8785 (95% CI -118.2133 to 4.1486). The comparison with Sysmex XE-5000 was not performed due to a lack of results below 1000 elements. Sedimax analyzer when compared to manual microscopy and XE-5000 to count WBC or differenzial WBC showed a good concordance K statistic = 0.89 and 0.92 respectively.
Conclusions: If the results obtained in this preliminary study are confirmed, the Sedimax Lite analyzer is proposed as a very useful instrument for the study of elements in CSF, enabling greater standardization of the process maintaining the benefits of optical microscopy by storing all images of elements cellular.

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MIDREGIONAL PRO-ADRENOMEDULLIN (MR-ProADM): REFERENCE VALUES

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Introduction: The midregional proadrenomedullin (MR-proADM) is the 45-92 amino acids protein fragment, as well as adrenomedullin (ADM), the 95-146 amino acids protein fragment, derives by post translational processing from the precursor pre-pro-adrenomedullin (preproADM). ADM also may have utility as early marker of disease and would be helpful in the diagnosis, monitoring, and prognosis of these various diseases. The reliable measurement of ADM release in the circulation is difficult due to its short half-life and analytic variability, while MRproADM, secreted in equimolar amounts to ADM, is more stable peptide. The circulating levels of MRproADM are an index of adrenomedulin concentrations in the blood. MRproADM concentrations increase with the commonly encountered severity disorders in emergency medicine. The aim of this study was to determine and establishing the reference values of MR-proADM in healthy population.
Material and Methods: One hundred and two (52 M and 50 F) randomly selected anonymous blood serum samples were collected from healthy blood donors, aged ranged between 19 and 63 years, at the Careggi Hospital, Florence, in July 2016. MR-proADM serum concentrations were measured in an automated Kryptor analyzer, using TRACE technology without any pre-analytical treatments. One-Sample Kolmogorov-Smirnov Test and Rho Spermean correlation test was used.
Results: MRPro ADM was normally distributed in the studied population. The reference values, estimated by the 2.5 and 97.5 percentiles, were 0.26 and 0.51 nmol/L respectively. There was no significant difference (P=0.64) for MR-proADM values between males (0.36 nmol/L; SD=0.06) and females (0.36 nmol/L SD=0.05), while there was correlation between MR-proADM and age (R^2 =0.173; P<0.005.
Conclusions: Reference values of MRproADM can be determined for the evaluation of sepsis conditions, acute dyspnea, lower respiratory tract infections as it is a more early marker of procalcitonin. Furthermore, it is a powerful predictor of adverse outcome in patients after myocardial infarction and cardiovascular events. Substantially this study confirmed the previous references values obtained using other method.

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HIGH-SENSITIVITY CARDIAC TROPONIN I ASSAY: CALCULATING DELTA CHANGE VALUES

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Background: High-sensivity cardiac troponin (hs-cTn) is the gold standard assay for acute myocardial infarction (AMI) diagnosis. International guidelines suggest the use of hs-cTn delta change of 50%, derived by the mean from values included between 30 and 85% calculated from Biological Variation (BV). Small delta change values over the 99th upper reference limit (URL) could be due to clinical conditions, even non-cardiological, and also to analytic variation. A clinically significant change of hs-cTnI values may still be below the 99th URL. Since the c-TnI has marked biological individuality (index of individuality < 0.6), the aim of this study was to determinate our delta value by calculating Reference Change Value (RCV) in order to well-interpreting significant changes in serial results of hs-cTnI.
Methods: Analytic imprecision expressed as Coefficient of Variation (CV_A) of hs-cTnI was evaluated throughout the use of three-levels Abbott quality control (QC) and third-part commutable human serum Bio-Rad QC, daily performed with Abbott Architect i2000SR. During 18 months we collected data, calculated monthly CV_A and monitored by an inter-laboratory quality control program (Unity Real Time, Bio-Rad). To define our RCV we used the average CV_A of Bio-Rad QC and within-individual BV derived from Westgard database available on-line on www.westgard.com.
Results: The mean concentration of QC Abbott (Lev1, 2, 3) were 20.2, 202.2, 16085 ng/L, with CV_A 5.02, 4.18, 3.30% respectively. The mean concentration of QC Bio-Rad (Lev 1, 2, 3) were 19.5, 1398, 7010 ng/L and CV_A 4.91, 4.98, 4.08%. The average CV_A for QC hs-cTnI was 4.49% and used to calculate our RCV. Our RCV of hs-cTnI was 40.7 % (z = 1.96 for bidirectional changes; p < 0.05).
Conclusions: Our CV_A, kept constant during 18 months, showed optimal analytical performances and then we could define our RCV. In our Hospital the clinicians can use the RCV to improving the diagnostic specificity for AMI, considering significant hs-cTnI values exceeding our RCV 40.7% but not yet over the delta changes of 50% suggested by international guidelines, also when results not exceeding the 99^{th} URL.

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SUBCUTANEOUS DEFIBRILLATOR SHOCK EFFECT ON CARDIAC BIOMARKERS AND CARDIOVASCULAR HOMEOSTASIS: THE ROLE OF COPEPTIN

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The use of transvenous implantable defibrillator (ICD) guaranteed a significant increase of survival in high-risk population as a mean of primary and secondary prevention in unexpected tachyarrhythmic death.Few years ago, has been introduced a new system with subcutaneous electro-catheter (S-ICD). The main benefits are that S-ICDs are easy to be implanted and explanted; they do not need fluoroscopes and can be used in children, young and athletes.Effectiveness and reliability of S-ICDs are verified by an induction test, which consists in a50-Hertz continuous current stimulus followed by a shock at 65 Joule.The aim of our study was to analyse myocardial effects induced by electric currents released by S-ICD through the evaluation of cardiovascular homeostasis.15 patients which needed S-ICD implantation because of arrhythmogenic cardiomyopathy were investigated. After the surgical procedure the operativity of the device has been verified with a ventricular fibrillation induction test with 50 Hz continuous current followed by effective shock at 65 Joule. For each patient a complete laboratory evaluation and dosage of myocardic injury and cardiovascular homeostasis index were made.Our data showed that the shock at 65 Joule does not seem to cause myocardial damage, but only e temporary alteration of the cardiovascular hemodynamic homeostasis.The copeptin should be validated as an early prognostic index of acute cardiovascular disease. The biological markers dosage of cardiac damage and cardiovascular homeostasis is an innovative tool to evaluate the effects of the electric discharge by S-ICD and it allowed us to consider the shock deliver by S-ICD as effective and safe.

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PREVALENCE OF VITAMIN D DEFICIENCY IN PATIENS WITH ACUTE MYOCARDIAL INFARCTION

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Introduction: Hypovitaminosis D, increasing in developed countries, is emerging as a risk factor for developing of atherosclerosis and acute myocardial infarction (AMI). Primary source of vitamin D is its cutaneous synthesis under sunlight exposure.AIM: We aimed to investigate vitamin D status in patients with AMI.
Methods: Vitamin D status was measured in 27 AMI inpatients (21 males and 6 females, mean age 70±10 years) recruited from our Clinical Cardiology Department (Ospedale del Cuore G. Pasquinucci, Massa). Patients were enrolled from January 21 to April 12, 2017. Blood samples were collected in lithium heparin tubes and samples sent to the laboratory of Fondazione G Monasterio (Pisa) for 25-hydroxyvitamin D analysis by DiaSorin 25(OH)D assay.
Results: The mean plasma 25-hydroxyvitamin D concentration was 16 ± 9 ng/mL. Levels of 25-hydroxyvitamin D did not change significantly during the first 48 hours after onset of symptoms (time course evaluated in 15 patients), and did not differ according to gender. However, there was an inverse correlation between 25-hydroxyvitamin D and age ($r = -0.42$, $p<0.05$).Only 3/27 patients (11%) had 25-hydroxyvitamin D normal values (≥ 30 ng/mL), and 24 (88%) had 25-hydroxyvitamin D < 30 ng/mL. Specifically, three patients/27 (11%) had Vitamin D insufficiency (between 20 and 30 ng/mL) and 21/27 (78%) patients had 25-hydroxyvitamin D deficiency (<20 ng/mL). Moreover, 30% of the overall patients (8/27) presented severe deficiency (25-hydroxyvitamin D <10 ng/mL). The seasonal effect of 25-hydroxyvitamin D variations among AMI patients was observed, with the lowest levels in the beginning of the year (January-March) and highest levels in patients hospitalized in April ($p<0.01$).
Conclusions: We observed very high prevalence of hypovitaminosis D among subjects with AMI. Vitamin D supplementation may be considered in AMI patients, especially in elderly subjects and during seasons when sun exposure is low.

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COPEPTIN AND HIGH SENSITIVITY CARDIAC TROPONIN IN THE TRIAGE OF PATIENTS WITH SUSPECTED ACUTE CORONARY SYNDROME

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Aims: We sought to the test safety and the safety and effectivness of a fast track strategy for the instante rule out of non ST segment elevation myicardial infarction (NSTEMI) based on the single sampling combined assessment of high sensitivity cardiac troponin I (hs-cTnI) and ultra–sensitive copeptin (us-Cop)compared with 2015 ESC 0-1 algotithm.
Methods: Us-Cop and hs-cTnI levels were misured at presentation to the Emergency Departement for acute non-traumatic chest pain within 24 hours from symptoms onset in 1236 patients. The diagnostic performance for ruling-out of NSTEMI diagnosis of the dual-marker single-sampling strategy with hs-cTni and us-Cop on admission was compared to the 0-1 ESC algorithm in reference to the adjudicated posto-discharge diagnosis,by comparing sensitivity,negative predictive values,the percentage of patients corretly assigned to the rule-out zone and the incidence of the composite endpoint of cardiac rehospitalization,PCI,MIor death.
Results: The exsperimental algorithm (0 h Copeptin < 10 pmol/L and 0h hs-cTnI <27 ng/L and low risk ECG) yielded a NPV of 97,2% and a sensitivity of 95% resulting non inferior to the ESC 0-1 algorithm (NPV 98,9 % p=0,130;sensitivity:98,1% p=0,18). From the comparison of the percentages of patients corretly assigned to the rule-out zone the specificity of the experimental algorithm (44,5 %) was not inferior to that the 2015 ESC 0-1 algorithm (41,0%;p=0,14). After an average follow-up of 12 months, the incidence of the combined endopoint of rehospitalization, death, PCI, MI or death was similar for both algorithms (long-rank test 0,520).
Conclusions: The instante rule-out stategy of combined us-Cop and hs-cTnI was non inferior to the 0-1 h serial hs-cTnI sampling in ruling-out NSTEMI, and may allow to an earlier and safe discharge of patients with suspected NSTEMI.

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IMMATURE PLATELET FRACTION AND THE EXTENT OF CORONARY ARTERY DISEASE: A SINGLE CENTRE STUDY

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Platelets represent a key player in the pathogenesis of coronary artery disease (CAD), involved in endothelial dysfunction, the development of atherosclerotic lesions and its thrombotic complications. Growing attention has been recently addressed to immature platelet fraction (IPF). IPF represents the quote of younger and larger sized circulating platelets, a potential marker of platelet reactivity and major cardiovascular events. Aim of the preset study was to assess the relationship between IPF levels and the prevalence and extent of CAD in patients undergoing coronary angiography.
Methods: A cohort of consecutive patients undergoing coronary angiography in a single center were included. Significant CAD was defined as at least 1 vessel stenosis >50%, while severe CAD as left main and or three-vessel disease. IPF levels were measured at admission by routine blood cells count (A Sysmex XE-2100).
Results: We included 1789 patients, divided according to quartiles values of IPF. IPF levels were directly related to active smoke (p=0.02), and non-acute coronary syndrome as indication to angiography (p<0.001), higher levels of haemoglobin and uric acid (p<0.001, respectively) and lower platelet count (p=0.003). Angiographic features did not significantly differ according to quartiles values of IPF, but for a lower degree of TIMI flow in patients with a higher percentage of reticulated platelets (p=0.01) and a higher rate of lesions involving bifurcations (p=0.05). IPF levels did not affect the prevalence of CAD (77% vs 82.2% vs 79.1% vs 75.6%, p=0.34, adjusted OR[95%CI]=0.93[0.82-1.05], p=0.22), and neither of severe left main/three-vessel CAD (28.5% vs 34.4% v 32.2% vs 33.1%, p=0.27; adjusted OR [95%CI]=0.99 [0.90-1.1], p=0.88).
Conclusion: The present study shows that among patients undergoing coronary angiography, the immature platelet fraction (IPF) is not associated to the prevalence and extent of coronary artery disease, and therefore should not be overlooked as a marker of coronary atherosclerosis.

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ASSOCIATION BETWEEN HIGH-RESIDUAL PLATELET REACTIVITY IN PATIENTS RECEIVING DUAL ANTIPLATELET THERAPY, VITAMIN D PLASMA LEVELS AND VITAMIN D BINDING PROTEIN RS7041 POLYMORPHISM

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Background: Ex-vivo platelet function evaluation investigates (a) platelet disfunction and (b) the efficacy of antiplatelet therapy. Optimal antithrombotic therapy is crucial for the management of acute coronary syndromes (ACS). An association between high residual platelet reactivity (HRPR) on dual antiplatelet therapy (DAPT) and increased risk of recurrent ischemic events and stent thrombosis has been previously shown. Among clinical conditions enhancing platelet reactivity are vitamin D deficiency and rs7041 genetic polymorphism in the Vitamin D Binding Protein (VDBP), that accounts for a significant variability in vitamin D levels. Aim of this study was to investigate the role of vitamin D plasma levels and of rs7041 polymorphism on platelet reactivity in patients on DAPT.
Methods: We measured platelet function by Multiplate® (Roche Diagnostics AG), and VDBP genetic status by polymerase chain reaction and restriction fragment length polymorphism technique in 400 patients treated with DAPT (ASA and clopidogrel or ticagrelor) for an ACS at 30-90 days post-discharge. Fasting samples were obtained for main chemistry parameters and vitamin D levels.
Results: 187 patients received clopidogrel and 213 ticagrelor. The genetic polymorphism rs7041 (T/G) was observed in 318 patients, (79.5%), in 38.7% of them in homozygosis. Main clinical and chemistry features did not significantly differ according to genetic status, but for a higher rate of ACE-inhibitors and beta-blockers use among the carriers of the G allele (p=0.04 and p=0.01, respectively). VDBP genetic status did not affect the rate of HRPR with ADP-antagonists. However, the rate of HRPR with ADP-antagonists was influenced by severe hypovitaminosis D (< 10 ng/ml) only in patients carrying the G allele, especially in homozygosis (T/T: 25.9% vs 26.1%, p=0.99; G carriers: 22.1% vs 35.3%, p=0.02, $p_{interaction}$=0.019; adjusted OR[95%CI]=1.93[1.11-3.34],p=0.02 for G carriers).
Conclusion: Platelet reactivity and the rate of HRPR among patients receiving DAPT rs741 was no affected by polymorphism of Vitamin D Binding Protein. The carriage of the G allele could condition the impact of hypovitaminosis D on the response to antiplatelet agents, by increasing the occurrence of HRPR especially in homozygotes.

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IMPACT OF HIGH-DOSE STATINS ON VITAMIN D LEVELS AND PLATELET FUNCTION IN PATIENTS WITH CORONARY ARTERY DISEASE

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Background: A correlation between hypovitaminosis D, high-residual platelet reactivity and increased risk of cardiovascular disease has been suggested. Statin administration raises the circulating levels of 25-OH vitamin D in patients with dyslipidaemia or diabetes. Aim of this study was to assess the impact of a high-intensity statin therapy on vitamin D levels and platelet function in patients with coronary artery disease. Methods. Patients discharged on dual antiplatelet therapy and high-intensity statins after an ACS or elective PCI were scheduled for main chemistry and vitamin D levels assessment at 30-90 days post-discharge. Vitamin D (25-OHD) was measured by chemiluminescence (LIAISON® Diasorin Inc). Platelet function was assessed by Multiplate® (multiple platelet function analyser; Roche Diagnostics AG). Results. 142 patients were discharged on a new statin therapy or with an increase in previous dose (Inc-S), while 104 were already receiving a high-dose statin at admission, that remained unchanged (eq-S). Median follow-up was 75.5 days. Inc-S patients were younger (p=0.01), smokers (p<0.001), with a lower history of hypercholesterolemia (p=0.05), diabetes (p=0.03), hypertension (p=0.02), or previous cardiovascular events (p<0.001). Higher total circulating calcium was observed in the Inc-S group (p=0.004), while baseline vitamin D levels were similar in the 2 groups (p=0.30). A significant reduction in circulating low-density lipoprotein (LDL) cholesterol was observed in the Inc-S group. Vitamin D levels increased in the Inc-S patients but not in the eq-S group (delta-25OHD: 23.2±20.5% vs 3.1±4.7%, p=0.003), with a linear relationship between the magnitude of vitamin D elevation and the reduction of LDL cholesterol (r=-0.17, p=0.01). Platelet reactivity was slower in the Inc-S patients, when evaluating aggregation with different platelet activating stimuli (arachidonic acid, p=0.02, collagen, p=0.004, thrombin-activating peptide, p=0.07, ADP, p=0.002). Conclusions. In patients with coronary artery disease, the addition of a high-intensity statin treatment, besides the lipid-lowering effects, is associated to a significant increase in vitamin D levels and lower platelet reactivity, potentially providing explanation of the "pleiotropic" benefits of statins therapy in cardiovascular disease.

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ABDOMINAL AORTIC ANEURYSM CALCIFICATION: CAN A SIMPLE AND ECONOMIC BLOOD TEST SUCH AS COMPLETE BLOOD COUNT PREDICT THE CALCIFICATION GRADE?

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Objective: Abdominal aortic aneurysms are a major cause of death in developed countries and calcification of aneurysms have been linked to increased complications. This study was conducted in order to verify if classical risk factors for abdominal aortic aneurysms and cell blood count parameter could help in the identification of calcification progression of the aneurysm. Design. Risk factors were collected and a cell blood count performed in 149 patients with abdominal aortic aneurysms. Patients were analysed for the presence of aorta calcification using Ct angiography.
Results: We found no association of calcification grade with risk factors for abdominal aortic aneurysms but we found a strong association between MCV, MCH and calcification grade (p=0,0172 and 0,0168 respectively) . Instead, no association was found with the other parameters that we analyzed.
Conclusions: This study suggests that MCV and MCH could be a useful biomarker to assess the evolution of calcification and could be used in triaging patients to identify those who should undergo a rapid imaging, thus allowing prompt initiation of treatment or rule-out suspicious patients from non-essential imaging repetition.

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LABORATORY ASSESSMENT OF SUDDEN SENSORINEURAL HEARING LOSS: A CASE-CONTROL STUDY

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Objectives/Hypothesis: Sudden sensorineural hearing loss (SSHL) is an otologic emergency that affects 5 to 30 subjects per 100,000/year. SSHL is defined as a rapid onset of hearing loss of at least 30 dB in three consecutive frequencies in the pure-tone audiogram within 72 hours or less. The cause of SSHL remains unknown or uncertain in 70% to 90% of cases, and treatment decisions are usually made without knowing the etiology.
Methods: One hundred thirty-one idiopathic SSHL patients were recruited from January 2014 to June 2015 in concordance with the Statements of Clinical Practice Guideline and were grouped by the degree of disease severity [High Severity-SSHL (HS-SSHL, n=61) vs. Low Severity-SSHL (LS-SSHL, n=70)]. A complete clinical laboratory assessment comprised of serological, viral, and autoimmune assays, biochemical assays and coagulation assays was completed on blood samples collected from SSHL patients and control subjects (healthy blood donors, n=77).Multivariable regression analysis was performed to investigate the association between laboratory data and SSHL basis.
Results: Only a few SSHL patients were positive for autoimmunity or viral infection. Statistically significant (P <.05) higher levels of blood glucose, glycated hemoglobin (HbA1C), lipoprotein (a), and factor VIII were found in SSHL patients compared to controls. Furthermore, blood glucose, HbA1C, uric acid, factor VIII, and homocysteine were significantly associated to disease severity.
Discussion: The etiology of SSHL is one of the much-discussed topics in otologic literature and there is an open debate on the usefulness of laboratory tests in these patients. In our group of patients acute viral infections and autoimmunity cannot be considered as relevant causes of the SSHL. The vascular hypothesis is a proposed mechanism for sudden deafness. Our data reinforce this hypothesis showing a relationship of some metabolic parameters (Lp(a), blood glucose, HbA1C, uric acid, factor VIII and homocysteine) related to vascular damage and thrombophilic risk with the occurrence of SSHL and with its severity.

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VON WILLEBRAND FACTOR AND SEVERE AORTIC VALVE STENOSIS. THE IMPACT OF THE MODERN TRANSCATHETER AORTIC VALVE IMPLANTATION (TAVI)

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Background: An association between the acquired von Willebrand syndrome (aVWS) and aortic valve stenosis (AS) has been established in the past and surgical aortic valve replacement (SAVR) shown to lead to factor recovery. Severe forms of aVWS are associated with loss of high-molecular-weight multimers (HMWM). This study sought to investigate the prevalence of abnormal von Willebrand multimers in patients undergoing transcatheter aortic valve implantation (TAVI) and the impact of TAVI on aVWS.
Methods: We enrolled 28 patients with severe aortic stenosis and high surgical risk admitted for elective TAVI. All patients were successfully treated by TAVI, using only transfemoral approach. In every patients we evaluated von Willebrand factor (VWF) antigen (VWF: ag), VWF activity with ristocetin cofactor (VWF: Rco), coagulation factor VIII (FVIII) and VWF multimer analysis. Blood samples were collected at time 0 (T0) before the treatment and 24 hours (T1) and 48 hours (T2) after valve implantation.
Results: VWF:ag value was significantly increased compared to baseline at T1 ($p<0.0042$) and T2 ($p<0.0017$). Also VWF:rco value was significantly increased compared to baseline at T1 ($p<0.0012$) and T2 was significantly ($p<0.0002$). Western Blot analysis showed a reduction of HMWM at baseline and confirmed the increase of HMWM expression after TAVI.
Conclusions: This study shows that, similar to surgery, acquired von Willebrand syndrome due to aortic valve stenosis can successfully be corrected by TAVI. The molecular analysis of von Willebrand multimers showed that after aortic valve implantation HMWM increase, reducing the risk of bleeding.

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h-FABP AS AN EARLY BIOMARKER FOR MYOCARDIAL INFARCTION DIAGNOSIS

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Background: Cardiac troponin (cTn) is the gold standard biomarker for myocardial infarction (MI) diagnosis and its evaluation is critical for the early triage of patients presenting to the emergency department (ED) with chest pain. However, some patients are cTn-negative on admission to ED, becoming positive after several hours. Heart-type fatty acid binding protein (h-FABP) is a small protein abundant in the cytoplasm of cardiomyocytes. It appears in the blood 1.5 h after MI onset, peaks at approximately 6 h, and returns to baseline values within 24 h. The aim of this study was to compare the diagnostic performances of h-FABP with hs-TnI in a group of MI patients and in a group of subjects admitted to ED with chest pain suggestive of MI but discharged without a MI diagnosis.
Methods: All consecutive individuals presenting with chest pain, from July 2015 to October 2015, to the ED of Policlinico P. Giaccone of Palermo and a final diagnosis of MI were considered for the study. Age- and sex-matched individuals admitted to the ED with chest pain suggestive of MI, cTnI negative on admission and discharged after excluding MI diagnosis were included in the study as controls. hs-TnI and h-FABP were assessed on admission by Architect STAT High Sensitive Troponin-I assay, i-100 analyzer and by an immunoturbidimetric assay (Randox Laboratroies) on a fully automated Modular P800 unit (Roche Diagnostics), respectively. All statistical analyses were performed by SPSS 22.0.
Results: 28 MI and 28 controls were included in the study. Among MI patients, 55% were positive for h-FABP and 34.6% were positive for hs-TnI ($p=0.015$), thus 21% were positive only for h-FABP. The diagnostic accuracy was assessed by ROC curve. h-FABP showed a higher sensitivity but lower specificity than hs-TnI.
Conclusion: These preliminary findings reveal a possible role for h-FABP in AMI rule-in/rule-out within the ED context. Indeed, h-FABP could identify MI patients initially negative for both cTnI and hs-TnI early presenting to the ED.
Tanaka T, Hirota Y, Sohmiya K, et al. Serum and urinary human heart fatty acid- binding protein in acute myocardial infarction. Clin Biochem 1991;24:195-201.

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VALUTAZIONE DI UN NUOVO SISTEMA POINT OF CARE TESTING PER LA MISURA DELLA CTNI CHE UTILIZZA CAMPIONI DI SANGUE VENOSO E CAPILLARE

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Philips Minicare cTnI è un metodo POCT che utilizza un sistema immunometrico tipo sandwich. Il campione da analizzare viene aggiunto alla cartuccia e discioglie la matrice contenente l'anticorpo monoclonale anti-cTnI coniugato a particelle paramagnetiche. La cTnI umana si lega all'anticorpo anti-cTnI sulla fase solida, mentre l'anticorpo coniugato alle particelle paramagnetiche reagisce con vari siti antigenici sulle molecole di cTnI umana. Il sistema si avvale di cartucce monouso per il dosaggio e di uno strumento portatile. Il valore misurato del campione è fornito dallo strumento dopo 7 min. Il sistema accetta sia campioni plasmatici che campioni di sangue venoso (siero o plasma) e capillare. I valori di cTnI misurati con la metodica Minicare sono stati confrontati con quelli ottenuti con un altro metodo POCT, Alere Triage Troponin I test e con il metodo STAT highly Sensitive cTnI che utilizza la piattaforma ARCHITECT i1000SR (Abbott Diagnostics). Sono stati confrontati 100 campioni di plasma eparinizzato freschi (analizzati entro 2 ore dal prelievo) di soggetti ospedalizzati presso la Fondazione Toscana G. Monasterio (Pisa) e 22 campioni di controllo di qualità distribuiti nel programma di Valutazione esterna dalla ditta QualiMedLab. Le analisi di correlazione sono state effettuate dopo trasformazione logaritmica dei valori ottenuti. Il metodo Minicare cTnI presenta una buona correlazione con l'altra metodica POCT (LogY= 0,9710 LogX + 0,12; R= 0.9251), e non è stata riscontrata una differenza significativa tra i valori medi di cTnI misurati [Minicare cTnI: media (ds) 612.7 (1490.1) ng/L e mediana (25°-75° percentile) 45.0 (10.0-350.0) ng/L; Alere Triage Troponin I test: media (ds) di 639.5 (1923.6) ng/L e mediana (25°-75° percentile) di 40.0 (25.0-250.0) ng/L]. Minicare cTnI presenta una buona correlazione anche con la metodica STAT highly Sensitive cTnI della ditta Abbott (LogY= 0,9065 LogX + 0,1018 R= 0,9079) e non è stata riscontrata una differenza significativa tra i valori medi di cTnI misurati [Abbott: : media (ds) 994.4 (3387.9) ng/L e mediana (25°-75° percentile) 53.8 (14.1-446.0) ng/L]. Questi risultati preliminari indicano che la metodica MINICARE presenta risultati ben correlati anche un metodo ad alta sensibilità nel range dei valori di cTnI all'intorno del valore decisionale. Inoltre presenta il vantaggio di poter utilizzare sia sangue venoso che capillare, il che risulta molto utile in regime di urgenza, in pazienti ambulatoriali e in età pediatrica.

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EVALUATION OF ANALYTICAL PERFORMANCE OF A NEW HIGH SENSITIVITY IMMUNOASSAY USING DXI PLATFORM FOR THE MEASUREMENT OF CARDIAC TROPONIN I

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Introduction: The study aim was to evaluate and compare analytical performance and clinical results of the chemiluminescent immunoassay for cardiac troponin I (cTnI), named Access hs-TnI, using DxI platform, not yet commercially available, with those of Access AccuTnI +3 immunoassays, and with the highly sensitive (hs) cTnI method using the ARCHITECT platform. Materials and Methods: The limits of blank (LoB), detection (LoD), and quantitation (LoQ) at 20% CV and 10% CV were evaluated according to the international standardized protocols. For the valuation of analytical performance and comparison of clinical results, both heparinized plasma samples, collected from healthy subjects and patients with cardiac diseases, and quality control (QC) samples distributed in External Quality Assessment (EQA) program were used. Results: The LoB, LoD, and LoQ at 20% CV and 10% values of the Access hs-TnI, method were: 0.6 ng/L, 1.2 ng/L, 1.9 ng/L, and 4.8 ng/L, respectively. The reproducibility of the Access hs-cTnI method was evaluated using two heparinized plasma samples with mean cTnI concentrations of 3.5 ng/L (within-run CV: 11.2%, between-runs CV: 15.0%) and 18.3 ng/L (within-run CV: 3.8%, between-runs CV: 7.2%), respectively. These data indicate that the Access hs-cTnI method shows analytical performance significantly better than that of the Access AccuTnI+3, and very similar results to those of ARCHITECT cTnI method. Moreover, the cTnI concentrations measured with Access hs-TnI method showed very close linear regressions with those of the old Access AccuTnI+3 immunoassay (R= 0.9738, N= 173), and the ARCHITECT hs-cTnI method (R=0.9926, N=202). Conclusions: The results of the present study suggest that the Access hs-TnI, method should be considered a highly sensitive cTnI method. However, the results of the present study should be confirmed by multicenter studies using large reference populations, divided for age, gender, and ethnic origin.

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SERUM TUMOR MARKERS IN SCREENING OF PANCREATIC MUCINOUS CYSTIC LESIONS ASSOCIATED WITH MALIGNANT CHANGES

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Background: Serum cancer antigen 19-9 (CA19-9) is not a sensitive and specific marker of mucinous cystic pancreatic neoplasms (MPN). However, its determination could provide additional information within the diagnostic work-up since a positive result would be associated with the presence of an invasive carcinoma.
Aim: This study was undertaken to assess both CA19-9 and carcinoembryonic antigen (CEA) serum concentrations in consecutive patients affected by MPN and other chronic benign and malignant pancreatic diseases. We also evaluated whether serum CA19-9 and CEA determinations provide additional information such as the presence of invasive carcinoma in MPN patients.
Methods: Serum CA19-9 and CEA from 91 patients with pancreatic diseases were tested by commercially available kits (VITROS Ortho-Clinical Diagnostics, High Wycombe, UK) at the time of diagnosis. The upper reference limit of serum CA19-9 was 37 U/mL and that of serum CEA was 3 ng/mL.
Results: Thirty-five patients was diagnosed with chronic pancreatitis (CP), 32 with MPN, and 24 with pancreatic ductal adenocarcinoma (PDAC) confirmed histologically. Surgery was carried out in 5 CP patients, in 10 MPN patients (7 of them had severe dysplasia), and 9 PDAC patients. Serum CA19-9 activity was high in 12 (34.3%) CP patients, in 7 (21.9%) MPN patients, and in 12 (50.0%) PDAC patients (P=0.089). High serum CEA concentrations were noted in 6 (17.1%) CP patients, in 6 (18.8%) MPN patients, and in 12 (50.0%) PDAC patients (P=0.010). In the 7 MPN patients associated with histologically confirmed severe dysplasia, 3 (42.9%) patients had elevated serum activity of serum CA19-9, and 2 (28.6%) patients had high levels of CEA.
Conclusion: Serum determination of traditional oncological markers such as CA19-9 and CEA does not provide any useful information for screening MPN patients with malignant changes.

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SERUM PANCREATIC AMYLASE AND LIPASE LEVELS BE USED AS DIAGNOSTIC MARKERS TO DISTINGUISH PANCREATIC DISEASES

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Background: Serum pancreatic enzymes are mainly used to diagnose acute pancreatitis or a flare-up of chronic pancreatitis (CP). In CP patients without pain, the behavior of serum pancreatic enzymes is extremely variable and it is not related to the exocrine pancreatic insufficiency evaluated by the secretin-cerulein test. Their role in patients with pancreatic cancer has also been explored in the past; it was found that measurement of pancreatic enzymes is of limited usefulness in diagnosing pancreatic cancer. Objective. This study aimed to assess the presence of pancreatic hyperenzymemia in patients with pancreatic cystic lesions, a new pancreatic disease group, recognized in the last 20 years, as compared to other chronic diseases of the pancreas.
Methods: Ninety-one patients were studied: 32 had mucinous cystic lesions,35 had chronic pancreatitis (CP), and 24 had pancreatic ductal adenocarcinoma (PDAC). Surgery was carried out in 10 of the 32 patients with mucinous cystic lesion (7 of them had severe dysplasia), in 5 patients with CP, and in 9 patients with PDAC. Serum pancreatic amylase and serum lipase were assayed in all patients at the time of diagnosis using commercially available kits: Sentinel Ch for pancreatic isoamylase and Ortho-Clinical Diagnostics (Vitros) for lipase, respectively.
Results: Abnormally high serum pancreatic isoamylase activity was present in 11 (34.4%) patients with mucinous cystic lesions, in 14 (40.0%) patients with CP, and none in patients with PDAC (P = 0.002); whereas serum lipase activity was abnormally high in 8 (25.0%) patients with mucinous cystic lesion, in 17 (48.6%) patients with CP, and in 3 (12.5%) patients with PDAC (P = 0.009). In 7 patients with mucinous cystic lesions and histologically confirmed severe dysplasia, abnormally high levels of both serum pancreatic amylase and lipase were present in 3 (42.9%) patients.
Conclusions: High serum concentrations of pancreatic amylase and lipase were found in no more than half of the patients with mucinous cystic lesions. High levels of pancreatic enzymes were not associated with a greater risk of malignancy.

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ENDOCANNABINOIDS AND ASSESSMENT OF PAIN IN PATIENTS WITH PANCREATIC DISEASES

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Background: Pain is the main symptom of chronic pancreatitis (CP) and its treatment represents the main therapeutic option since pain severely impairs the quality of life of these patients. In pancreatic cancer, the pain is usually persistent and the therapeutic approach is not based on a pathophysiological mechanism because the interaction of pancreatic cancer with nerves and substances is not completely understood. Aim. To analyze N-arachidonoylethanolamine (AEA), N-oleoylethanolamine (OEA), linoleoyl ethanolamide (LEA), α-linoleoyl ethanolamine (α-LNEA), N-palmitoylethanolamine (PEA) and N-stearoyl ethanolamine (SEA) in two groups of patients having chronic pancreatic diseases.
Patients and methods: Twenty-six patients with chronic pancreatitis (CP), 26 patients with pancreatic ductal adenocarcinoma (PDAC) and 36 healthy subjects were studied. The visual analogic scale (VAS) was used for assessing pain immediately before the venipuncture to obtain blood in all subjects. All participants had blood samples taken on admission to the study and immediately frozen after collection and stored at -80 °C until analysis. All samples were analyzed in duplicate and the six serum endocannabinoids were assayed by HPLC-MS/MS using the previously reported method (J Chrom B. 2014; 958:83-89). The ROC curves were evaluated in order to assess the best cutoff value for differentiating subjects with CP and PDAC having pain as compared to those without. Results: Only OEA, LEA and PEA serum levels were significantly higher in patients with pain as compared to those without. Using the cutoff values of ROC curves, the sensitivity and specificity of the various endocannabinoids in evaluating pain in patients with CP and in those with PDAC were: 44.2% and 95.6% for AEA, 83.7% and 73.3% for LEA, 88.4% and 91.1% for LNEA, 81.4% and 82.2% for OEA, 81.4% and 88.9% for PEA, 86.0% and 88.9% for SEA, respectively.
Conclusion: Endocannabinoids are not useful in assessing pain in patients with chronic pancreatic diseases and they cannot replace a simple method such as VAS for assessing the pain and its intensity. But our results point toward some use of endocannabinoids, especially LNEA circulating levels, as research parameters in the next future.

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DIAGNOSTIC AND PROGNOSTIC ROLE OF NICOTINAMIDE N-METHYLTRANSFERASE IN CUTANEOUS MALIGNANT MELANOMA

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Melanoma represents the deadliest form of skin cancer and accounts for approximately 50,000 deaths annually worldwide. Current treatment of cutaneous malignant melanoma involves surgical excision and chemo- or immunotherapy. However, despite advances in therapeutic strategies, the 5-year overall survival rate of patients affected with late stage melanoma remains below 15%. In this light, a major limit is the lack of efficient biomarkers capable of detecting disease prior to clinically evident metastasis and monitoring cancer progression over the course of treatment. In this work, we focused on the enzyme nicotinamide N-methyltransferase (NNMT), which catalyses the N-methylation of nicotinamide by using S-adenosyl-L-methionine as methyl donor[1]. NNMT overexpression has been reported in several neoplasms, including renal, bladder, oral and lung cancer. To date, no study has been performed to evaluate NNMT expression in cutaneous malignant melanoma. To address this issue, enzyme levels have been explored by immunohistochemistry in 34 melanomas and 34 nevi, used as controls. Subsequently, statistical analyses were performed to explore the correlation between tumor prognostic parameters and NNMT expression levels.Results obtained showed that melanoma samples exhibited significantly ($p<0.05$) higher NNMT expression compared with that detected in controls. Moreover, a significant ($p<0.05$) inverse correlation was found between enzyme levels and prognostic parameters, such as Breslow thickness, Clark level, the presence/number of mitoses and ulceration. Data reported in the present study seem to suggest that NNMT may be considered a molecular marker for melanoma, thus highlighting its promising role as a diagnostic and prognostic determinant for this neoplasm.

1. Pozzi V, et al. Cell Physiol Biochem 2015;36:784-98.

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A RELIABLE RESOURCE FOR CANCER RESEARCH: REGINA ELENA BIOBANK

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Background: A biobank may be defined as the collection, processing and long-term storage of biological samples for research purposes. The availability of well annotated, high-quality human samples linked to accurate diagnostic and clinical information is essential in the research and development of new biomarkers and drugs in the overall goal of personalized medicine. This is also important for successful introduction of several non-invasive multi-marker tests into the clinic such as detecting DNA from circulating tumor cells or fragments of DNA shed by tumor cells into the bloodstream. We are currently engaged in establishing and developing an Institutional Biobank (BBIRE) whose essential function is to collect tissue (T) and body fluids (LB) samples in accordance with standardized criteria and cryoconserve them in order to provide biological material for approved cancer research projects. BBIRE includes a Steering Committee and an Operating Group. A broad informed consent has been drawn up, submitted to the Ethics Committee, subsequently validated and added to the medical record. Methods: In order to meet the requirements of quality and traceability of biological samples, different protocols are utilized for the samples crioconservation, using tubes with two-dimensional codes and dedicated IT infrastructure (software, barcode readers) which is able to manage the transportation, handling and information storage of biological samples and to update follow-up and associated data. Results: The possibility to send body fluids for storage to the BBIRE-LB is available to the various departments and cancer outpatient units within the Institute, thanks to the creation of an electronic page through which doctors can make a request via Computer Order Entry System (DNWEB). Blood samples are sent to the BBIRE-LB within one hour of collection. After checking and testing for biomaterial conformity, the samples are processed and stored according to the established procedures. More than 7200 aliquotes of whole blood, serum, EDTA plasma and citrate plasma from patients with soft tissue sarcoma and bone tumors, timoma, breast cancer and melanoma, have been collected in the BBIRE-LB taken at first diagnosis and at different therapeutic stages. Conclusions: An improvement of such relevant core facilities in term of the development of standardized methodologies for the acquisition of the most appropriate samples required for new approaches to research such as “liquid biopsy”, targeted therapy and biomarkers validation is required.

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COMPARISON OF PLASMATIC BILE ACIDS PROFILE IN PATIENTS WITH BILIARY TRACT CANCER AND HEALTHY SUBJECTS

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Background and aim: Cholangiocarcinoma (CCA) is a rare but devastating type of cancer, accounting for 15% of all primary liver malignancies. Its diagnosis is challenging since there are no sensitive and specific biomarkers to accurately differentiate benign and malignant biliary disease. Since some studies recently suggested that bile acids (BA) may have a pathogenic role in cholangiocarcinogenesis, this study was aimed to compare the plasma profile of BA in patients with CCA and healthy controls using an in-house developed liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay.
Methods: A panel of 12 BA were quantified in 20 patients with CCA (10 men, mean age: 68 years) and 20 healthy controls (13 men, mean age 67 years). The biliary tract cancer group included 10 perihilar, 5 intraepatic and 5 distal tumours. LC-MS/MS analysis was performed using a Nexera X2 series UHPLC (Shimadzu, Kyoto, Japan) coupled to 4500 MD triple quadrupole MS detector (ABSciex, Darmstadt, Germany). Three deuterated internal standards (IS) were used for quantification. Partial-least squares discrimination analysis (PLS-DA) was performed to explore the predictive capability of our model and receiver operating characteristics (ROC) curve analysis was used to assess accuracy. Analyses were performed using Metaboanalysit 3.0 software.
Results: PLS-DA data revealed a statistically significant separation ($p<0.0001$) of patients and controls. Leave-one-out cross validation of PLS-DA model showed a predictive capability of 0.80. Sensibility, specificity and classification rate were found to be 90%, 95% and 92.5%, respectively. The area under the curve (AUC) was 0.99 (95% CI:0.96-1.00). Among the 12 BA analyzed, those with the major discriminatory capability (i.e., variable importance in the projection value > 1) were deoxycolic acid (DCA), taurocholic acid (TCA) glycocholic acid (GCA), taurochenodeoxycholic acid (TCDCA) and hyodeoxycholic acid (HDCA).
Conclusions: Our study shows that plasma BA profile alone can help diagnosing biliary tract cancer, with good predictive ability. Further studies are needed to establish if it may also represent a reliable approach for differentiating malignant and benign biliary diseases.

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ROLE OF HEAVY/LIGHT CHAIN RATIO IN MYELOMA PATIENTS ACHIEVING COMPLETE RESPONSE AFTER FIRST LINE THERAPY

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Heavy and light chains (HLC) and HLC ratios may be particularly useful for monitoring the presence of monoclonal component in oligo-secretory MM or when it migrates in the β region, as frequently observed in IgA MM. The International Myeloma Working Group (IMWG) has published in 2016 new consensus criteria for assessing response and minimal residual disease (MRD) in MM, outlining the potential role of HLC assay in this setting and the need of its further investigation, particularly in patients achieving complete response (CR) after treatment. We conducted a single center, prospective study of HLC ratio, in comparison with free light chain (FLC) ratio, for the evaluation of MRD and its prognostic role in MM patients achieving CR after first line treatments including novel agents. Twenty-five consecutive patients were evaluated. With a median follow-up of 52 months. Overall survival (OS) of the entire cohort was 61 months (95% CI 52-80) and progression-free survival (PFS) was 26 months (95% CI 12-38). Ig HLC pairs (IgGk/IgGλ and IgAk/IgAλ) and FLC were analyzed on serum samples at diagnosis and at the time of immunofixation negative CR (according to 2006 IMWG criteria), using Hevylite and Freelite commercial kits, respectively, on a SPAplus analyzer (Binding Site); IgGk/IgGλ, IgAk/IgAλ and k/λ ratios were then calculated. At CR time, we found seven (28%) samples still showing abnormal HLC ratio and fourteen samples (56%) with abnormal FLC ratio. Discrepancies between the two assays occurred in 11 patients. FLC assay normalization in CR was significantly associated with better PFS (43 months, 95% CI 14-45) respect to patients with persistent abnormal FLC ratio (12 months, 95% CI 9-35, p=0,049). In contrast, normalization of HLC ratio had no impact on PFS (26 months, 95% CI 10-38, vs 20 months, 95% CI 10-34, p=0,51), even selecting IgA MM. Notably, in 9 patients, the negative effect of abnormal FLC ratio at CR on PFS was not mitigated by concomitant normalization of HLC ratio (19 months, 95% CI 4-35; p=0,022). Neither FLC, nor HLC affected OS. There were no differences between patients who received AuSCT and those who did not. While our preliminary data confirm the prognostic usefulness of FLC in this setting, currently they do not support a role for HLC as putative biomarker of MRD.

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DEVELOPMENT OF A CIRCUIT TO HOLD THE CIRCULATING TUMOR CELLS: STUDY IN VIVO AND EX VIVO

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Background: The number of circulating tumor cells' (CTCs) correlates with prognosis for many solid tumors as breast and colon. However, the challenge remains to eliminate CTCs from bloodstream to avoid metastasis process. Our study focused on a system that could retain this metastasis progress. About this, CTCs behavior and interaction during hemodialytic treatment (HD) is not reported in literature. The aim of this study was to evaluate interaction between part of HD components and CTCs in vivo and ex vivo by the development of a circuit that allows to wash the dialytic filter. Moreover, we evaluate what are the membrane filter features to hold CTCs.

Methods: Our study was subdivided into 2 parts: a) identification of patients with solid neoplasia (4 K mammary and 1 K colon) undergone chronic hemodialysis for end stage renal disease and neoplastic patients in HD for acute renal damage. Blood drawings were performed pre and postHD. ADNAtest was used for CTCs' isolation; b) ex vivo circuit construction to reproduce in vivo HD. The circuit was filled with PBS, albumin and electrolytes solution in which CTCs derived from cell cultures HCT-15 were injected. HD was performed for 30'. At the end, the circuit was extracted and were collected the eluate of lines and filter. The presence of CTCs was evaluated by expression of tumor markers in RT-PCR from HTC-15 RNA.

For filter cleaning in both cases, a circuit has been built in the laboratory and, through a vacuum pump, the filter was emptied and washed with a PBS solution. The eluate was treated as sample to evaluate markers expression. The filters used were FiltryzerB3 (PMMA, $1m^2$), F5HPS (Polysulfone, $1m^2$), HFT03 (DIAPES, $0.3\ m^2$).

Results: In one of 3 patients with metastasis, CTCs were detected in the preHD and in filter but not in the post-HD sampling. In the other 2 subjects, CTCs were detected within the dialysis filter but not in pre and postHD. In 2 patients without metastasis, the presence of CTCs in filter and in preHD was detected. Finally, the ex vivo study showed a greater intake of neoplastic cells in the PMMA filter than the other filters used.

Conclusions: CTCs interact with dialysis membranes and probably the filter retains cells through an adsorption mechanism. The various dialysis membranes have different efficacy in capturing CTCs. A protective effect on the remote metastasis process is not excluded.

P165
HIGH-THROUGHPUT-TRANSCRIPTOME PROFILING OF COLORECTAL CANCER AND DISTANT PAIRED NORMAL TISSUES

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Colorectal cancer (CRC) is one of the most frequent malignant tumor and the commonest cause of cancer death worldwide. The identification of specific biomarkers should be of great benefit for early diagnosis and develop of new targeted therapies to decrease the CRC mortality. Next-Generation Sequencing (NGS) techniques give us the complete genomic structure of neoplastic tissue and permits the identification of changes in the tumor pathogenesis.In this study, we performed high-throughput transcriptome sequencing on CRC and normal colon tissue (NCT). Total RNAs were extracted from 16 paired primary CRC and NCT. Whole-transcriptome analysis was performed using the Illumina TruSeq Stranded mRNA Library Prep Kit and the Illumina HiSeq3000. The RNAseq data were analyzed using the TopHat and Cufflinks protocols using GRCh37/hg19 as a reference.The transcriptome analysis revealed cancer-specific differentially expressed genes (DEGs) and differential alternative splicing. A total of 1378 DEGs were identified in CRC: 611 and 767 were significantly up and down-regulated, respectively. Gene Ontology analysis revealed that CRC overexpressed DEGs were enriched in pathways involved in the cell cycle checkpoint, E2F transcription factor network, DNA damage response, WNT/beta-Catenin. While CRC downregulated DEGs affect Respiratory electron transport, Mitochondrial Fatty Acid beta-Oxidation, Phase II conjugation, Cytokine Signaling in Immune system.12684 genes were found mutated. KRAS and NRAS mutations were identified in 56% of CRC. Interestingly, mutations were identified in BRAF, TP53, PTEN, SMAD4 and in FOX-O, ERB-B, AKT1, EGFR, CDKN1A genes, whose proteins are known members of pathways such as colorectal cancer, miRNAs in cancer and PI3K/AKT, respectively. RNA-sequencing technology revealed the variation landscape of CRC transcriptome. Our data raise the knowledge of the expression differences that underlying malignancy and revealing useful genes that may be used as diagnostic or prognostic markers.

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SERUM HUMAN EPIDIDYMIS PROTEIN 4 (HE4) vs. CARBOHYDRATE ANTIGEN 125 (CA125) IN OVARIAN CANCER (OC) FOLLOW-UP

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Background: HE4 has been proposed as an alternative to CA125 for assessment of OC. Here we sought to compare HE4 and CA125 in a series of patients undergoing OC monitoring.
Methods: We enrolled OC patients requesting serial CA125 measurements from March 2014 to February 2017, measuring HE4 on the same serum samples (Roche Cobas e601). Cut-offs were 35 kU/L for CA125, and 70 or 100 pmol/L for HE4 in women younger or older than 55 years, respectively. Reference change values (RCV) (CA125, 25.8%; HE4, 33.5% or 18.3% in premenopausal or post-menopausal women, respectively) were considered for assessing significant marker changes vs. baseline concentrations. Multiple regression models (MRM) were used to test determinants influencing marker concentrations in patients free of active disease.
Results: We studied 43 patients (58.9±12.9 years old; 79% post-menopausal) followed for 3.5±2.6 years. Serous OC was prevalent (53.5%), with 58% of patients diagnosed at late stages. 18.6% of patients had two marker measurements, 30.2% three and the remaining ≥4. The overall case series was partitioned into three groups according to the outcome: A) steady state/remission (n=13; 30.2%); B) relapse (n=26; 60.5%); C) worse outcome and death (n=4; 9.3%). According to the cut-offs, positive CA125 vs. HE4 results were found in 11/38 (29%) vs. 13/38 (34%) of group A, in equal number (72/104, 69%) in group B, and in 8/9 (89%) vs. 7/9 (78%) of group C (always P›0.05). Using RCV, a significant increase for CA125 and HE4 was detected in 2/13 (15%) and 3/13 (23%) of patients in group A, and in 13/26 (50%) vs. 11/26 (42%) in group B (always P›0.05). However, by comparing biomarker trends in individual subjects, CA125 and HE4 disagreed in 17/43 (39.5%) patients. In six of those patients, the HE4 trend appeared to be related to a simultaneous serum creatinine increase. In group A, MRM showed that the HE4 behaviour was significantly different among individual patients, values being higher in younger (P≤0.03).
Conclusions: By applying correct interpretative criteria (cut-offs or RCV), there was no difference between the two evaluated markers in OC follow-up. However, their time trends were different in ›1/3 of monitored OC. The clinical significance of this difference remains to be determined.

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METODOLOGICAL APPROACH FOR UGT1A1*28 POLYMORPHISM IN PHARMACOGENETICS ANALYSIS

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Introduction: Irinotecan is widely used in gastrointestinal cancers but the presence of SNPs (Single nucleotide polymorphisms) in the phase II enzyme UGT that converts SN-38, the active metabolite of irinotecan, to its corresponding inactive glucuronide can result in toxicity. The most important genetic variant of this isoform is UGT1A1*28: patients with homozygote genotype UGT1A1*28/*28 shows a reduced enzymatic activity which leads increases of the side effects, thus UGT1A1 genetic testing is actually strictly recommended.
Aim: This study aims to the evaluation of the results over a 3-years use period of the homemade, sequencing based, analytical protocol for the individuation of UGT1A1*28 and of the analytical performances of three CE-IVD methods with higher throughput than sequencing for an implementation of the laboratory procedures for routine diagnostic purpose.
Methods: Three CE-IVD methods have been applied to the genotyping of ten DNA samples with known genotype as obtained from direct sequencing: the Easy® UGT1A1 kit (Diatech Pharmacogenetics), the Therascreen UGT1A1 Pyro® kit (Qiagen), and the Miriapod® ADMET UGT1A1*28 TSER 28bp VNTR Amplification Reagents (Diatech Pharmacogenetics).
Results: In relation to a 3-years activity and to the analysis of 385 patients, Sanger-sequencing polymorphism analysis for the TA repetitions evidenced the presence of 170 (44,15%) patients (TA)6/6 (UGT1A1*1/*1), 166 (43,12%) patients (TA)6/7 (UGT1A1*1/*28), 48 (12,47%) patients (TA)7/7 (UGT1A1*28/*28) and one patient(TA)5/7 (UGT1A1*38/*28).The methods selected for comparison showed a variable concordance with Sanger sequencing evidencing peculiar characteristics in terms of specificity, type of report and turn-around time.
Conclusions: Among the three CE-IVD evaluated kits, the Real Time PCR showed high specificity, rapidity, and good affordability. Pyrosequencing, despite the higher throughput, requires a more expensive effort and specialized operators. Finally, the agarose gel based approach provides a simple to use protocol, but the preparation and interpretation represent a limiting step to a routine analysis, affecting the reproducibility of the data.

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EVIDENZA DELLA POSITIVITÀ DFS-70 MEDIANTE UTILIZZO DI SUBSTRATI DI CELLULE HEP-2 DFS70 KNOCK OUT

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Razionale: Il test di screening per ANA (IFA), anche se gestito con sistema esperto automatizzato, necessita della validazione dell'operatore su quadri di difficile interpretazione. Tra questi, l'antigene DFS-70 ha un pattern quasi sovrapponibile al quadro Omogeneo e, anche se la sua correlazione clinica con le MAIS non è ad oggi accertata, necessita di esserne distinto. Si presenta come quadro Granulare con positività delle mitosi. Abbiamo valutato un kit ANA HEp2 DFS70 KO (30% cellule Hep-2 che non esprimono il DFS-70) che in presenza di DFS-70 evidenzia un quadro simile al pattern PCNA.
Materiali e metodi: Sono stati valutati con il kit ANA HEp2 DFS70 KO, 70 sieri precedentemente processati su cellule HEp2, (inclusi possibili quadri DFS-70+), con pattern noti: 14 Granulari, 8 Granulari con Mitosi Positive, 26 Omogenei, 3 deboli pos. 80, 3 Nucleolari, 2 Citoplasmatici, 1 Midbody, 1 FND, 1 Centriolo e 11 Negativi. La lettura è stata eseguita in doppio cieco da 2 operatori. Eventuali positività per DFS-70 sono state confermate in Immunoblot.
Risultati: È stata evidenziata piena concordanza su 14 sieri Granulari,11 negativi, 3 positivi deboli 80, 2 Citoplasmatici, 1 Midbody, 1 FND, 1 Centriolo. Sugli 8 campioni Granulari con mitosi positive 1 è risultato positivo per DFS-70; dei 26 sieri Omogenei: 19sono risultati concordanti, 3 positivi al DFS-70, e 4 Granulari. Si sono confermati 1/3 campioni con pattern Nucleolare. Tutti i campioni positivi per DFS70 su Hep-2 DFS70 Knock Out hanno confermato la positività DFS70 in Immunoblot.
Conclusioni: I nostri risultati preliminari evidenziano la capacità del kit ANA HEp2 DFS70 KO di riconoscere il pattern specifico: 4/70 sieri (5,7 %) sono risultati DFS-70 positivi vs risultato Omogeneo o Granulare con mitosi positiva su HEp2 di altra ditta, e confermati in Immunoblot. La discordanza riscontrata nel gruppo di campioni con pattern Omogeneo è dovuta a diversa evidenza alla espressione delle cellule in mitosi sui 2 substrati. Il kit valutato potrebbe migliorare il riconoscimento di DFS-70 in particolare nella gestione della routine favorendo il RULE OUT dei campioni e riducendo il numero dei test di conferma di secondo livello. È tutttuttavia necessario ampliare la casistica valutata al fine di confermare il dato ottenuto.

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UTILITÀ CLINICO-DIAGNOSTICA DEL DOSAGGIO DEGLI ANCA NELLA DIAGNOSI DELLE VASCULITI AUTOIMMUNI. ANALISI RETROSPETTIVA

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Introduzione: Gli ANCA sono autoanticorpi diretti contro le proteine contenute nel compartimento lisosomiale dei neutrofili e dei monociti (proteinasi 3 e mieloperossidasi). Essi sono strettamente correlati con le vasculiti ANCA-associate. In presenza di manifestazioni cliniche, gli ANCA hanno un'elevata sensibilità e specificità per queste malattie, con un alto valore predittivo.
SCOPO: Obiettivo di questo studio è verificare la frequenza con cui sono state riscontrate positività sieriche per gli ANCA ed effettuare un'analisi retrospettiva al fine di verificare l'utilità clinico-diagnostica di tale dosaggio per porre diagnosi nei pazienti esaminati con quadri clinici complessi e/o indistinti.
Materiali e metodi: sono stati esaminati 2107 campioni di siero inviati alla nostra U.O.C. di Patologia Clinica dal 2011 al 2016. La determinazione degli ANCA è stata eseguita con metodica di Immunofluorescenza Indiretta (IFI). La ricerca di anti-PR3 e anti-MPO è stata effettuata con metodica immunoezimatica ELISA e lettura fluorimetrica.
Risultati e discussioni: Nei sieri esaminati sono state riscontrate complessivamente 40 sieropositività, così distribuite: C-ANCA 45% del totale e P-ANCA (55%). È stata osservata una prevalenza del sesso femminile. La positività C-ANCA è associata a quella PR3 nel 39% dei pazienti, mentre la positività P-ANCA associata ad MPO si osserva al 64% dei pazienti. Sia per i C-ANCA che per i P-ANCA è stata riscontrata una prevalenza di positività a medio titolo. Il reparto con il maggior numero di casi positivi è quello della Nefrologia.
Conclusioni: La percentuale relativamente bassa di sieropositività riscontrata (1,9%) suggerisce che le richieste non sono sempre supportate da un rilevante sospetto clinico, ma rientrano in un modello routinario di richieste relative anche ad altri autoanticorpi. Inoltre sono state riscontrate positività ANCA in condizioni cliniche non vasculitiche, in cui i test antigene-specifici MPO e PR3 sono risultati negativi. La più alta percentuale di positivi è stata riscontrata nei pazienti ricoverati in Nefrologia, con quadro clinico classico di sindrome renale-polmonare, nei quali il riscontro di anticorpi P-ANCA con specificità MPO è stato di ausilio per la diagnosi di poliangioite microscopica localizzata al rene.

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PERCORSO ANALITICO DI LABORATORIO E VALUTAZIONE DEGLI ASPETTI EPIDEMIOLOGICI NELLA DIAGNOSTICA DELLA MALATTIA CELIACA

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Lo spettro clinico della malattia celiaca è estremamente eterogeneo,con sintomatologia tipica, atipica, extraintestinale o del tutto silente. I test di laboratorio sono fondamentali perchè in grado di individuare soggetti celiaci con segni clinici non facilmente interpretabili; scopo del lavoro è valutare l'incidenza di nuovi casi in una popolazione afferente al nostro laboratorio con diagnosi di sospetta celiachia, attraverso il classico percorso analitico di laboratorio.
Materiali e metodi: Dal Gennaio 2014 ad Aprile 2017 sono stati esaminati 9850 pazienti (età 1-67 anni), ricercando gli anticorpi Anti Transglutaminasi IgA (test CLIA Zenit RA t-TG IgA, Menarini Diagnostics) e utilizzando come test di conferma gli anticorpi anti Endomisio IgA con IFI (test EMA, Menarini Diagnostics); nei bambini con età inferiore ai 2 anni e in caso di deficit di IgA totali si è testata la presenza di anticorpi anti Gliadina Deaminata IgA e IgG (test Zenit RA AGA IgA e IgG, Menarini Diagnostics).
Risultati: 255 pazienti (2.5%) sono risultati positivi agli anticorpi t-TG IgA ed EMA con una media di 6.2 nuovi casi al mese, con rapporto 2:1 F/M (174 donne, 68 %, 81 uomini, 32 %). Sotto il profilo delle fasce d'età,si riscontra una maggiore incidenza di nuovi casi tra la popolazione pediatrica (0-12 anni) con 162 casi (64%) rispetto agli adulti, 93 casi (36%); inoltre nella fascia pediatrica solo 1/3 dei casi positivi sono da attribuirsi ai bambini al di sotto dei 3 anni (60 casi), per 2/3 i pz appartengono al range 4-12 anni (102 casi). Nella popolazione adulta l'età media di prima diagnosi è 25 anni; 18 casi positivi si sono presentati alla nostra osservazione con più di 40 anni d'età, la fascia d'età 19-40 prevale (44%) rispetto al range 13-18 anni (36%). Sono emersi 98 casi di pazienti affetti da deficit di IgA totali (1%),di cui 11 positivi alla ricerca degli anticorpi AGA IgG.
Conclusioni: Sottoporre il paziente ai test specifici non sottovalutandone la sintomatologia,consente di diagnosticare la malattia celiaca anche in età adulta,con percentuale superiore al 30% tra i nuovi casi.
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ROLE OF FECAL CALPROTECTIN FOR ASSESSMENT OF AUTOIMMUNE RHEUMATOID DISEASES ACTIVITY IN PATIENTS WITHOUT INTESTINAL SYMPTOMS REPORTED

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Background: Currently, some laboratory tests are used for prediction of systemic autoimmune disease activity including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and leucocyte count. Because some studies show a correlation between inflammatory rheumatic diseases and a variety of gastrointestinal manifestations, fecal calprotectin, that is a sensitive and specific marker for the presence of inflammatory bowel disease (IBD) and other intestinal illnesses, has been used to assess gut inflammation in these patients. Aim of our study was to evaluate the clinical utility of fecal calprotectin for diagnostic as well as assessment of autoimmune rheumatoid diseases in patients without intestinal symptoms reported.

Methods: We enrolled eighteen patients, twelve females and six males (mean age of 63.3 ± 9.2), with a new diagnosis of rheumatic arthritis, polymyalgia rheumatica or psoriatic arthritis according to 2010 ACR/EULAR Classification Criteria for Rheumatoid Arthritis and not showing gastrointestinal manifestations at recruitment. The control group included eighteen age- and sex-matched healthy subjects (mean age of 63.6 ± 13.7, p=0.943). We excluded patients presenting IBD, acute or chronic kidney disease, any form of current acute or chronic infection and patients receiving antibiotic therapy and/or corticosteroid and estrogenic therapy. Fecal calprotectin was evaluated on Phadia® 100 with EliA™ Calprotectin (Thermo Scientific) kit. Statistical significance was calculated with Student's t test. A p-value of <0.05 was considered significant.

Results: We observed a significant increased level of fecal calprotectin in patients compared to control group (p=0.0223), as well as of ESR (p=0.0001), CRP (p=0.0185) and leukocytes count (p=0.0234).

Conclusion: We suggest that fecal calprotectin can be associated to systemic autoimmune rheumatic diseases independently from the presence of gastrointestinal manifestations as well as other laboratory test already used as biomarkers, thus it could be considered as a safe and non-invasive test for diagnostic and assessment of this kind of pathological conditions even in patients without intestinal symptoms reported.

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COMPARISON OF TWO SYSTEMS FOR THE DETECTION OF ANTI-CARDIOLIPIN AND ANTI-β2–GLYCOPROTEIN ANTIBODIES IN AN ITALIAN COHORT WITH THROMBOTIC PREDISPOSITION

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Background: Antiphospholipid syndrome (APS) is a heterogeneous autoimmune disease characterized by recurrent arterial/venous thrombosis, and/or pregnancy morbidity, with the presence of antiphospholipid antibodies (aPL). The classification criteria for definite APS include anti-cardiolipin (aCL) and anti-β_2–glycoprotein (β_2GPI) antibodies of IgG and or IgM isotype in medium or high titer. However, the commutability of aPL results is limited, mostly due to the lack of standardization of commercial assays. Main factors contributing to the variability include differences in the cut-off values, the calibration system as well as the antigen used. In the present study we evaluated the concordance between a chemiluminescent immunoassay (CIA) (QUANTA Flash) with an addressable laser bead immunoassay (ALBIA) (Bioplex™ 2200) for the detection of IgG/IgM aCL and IgG/IgM β_2GPI antibodies.

Methods: Sera from 134 patients fulfilling the Sydney criteria for APS were tested for aCL and anti-β_2GPI of IgG and IgM isotype using the QUANTA Flash CIA (Inova Diagnostics) and Bioplex 2200 ALBIA (BioRad). Total percent agreement and cohen's kappa were used to assess the agreement between the methods.

Results: For aCL IgG/IgM, 27/24 and 21/13 samples were positive by CIA and ALBIA, respectively. For β_2GPI IgG/IgM, 20/17 and 17/25 samples were positive by CIA and ALBIA, respectively. Almost perfect agreement was found between CIA and ALBIA for β_2GPI IgG and aCL IgM with a total qualitative agreement of 97.8% (kappa=0.91) and 96.3% (kappa=0.88), respectively. On the other hand, there is moderate level of agreement between the two platforms for aCL IgG and β_2GPI IgM assays with a total agreement of 88.1% (kappa=0.57) and 89.6% (kappa=0.53), respectively. When analyzing antibody levels, most results were outside the analytical measuring range (AMR) by ALBIA, but not by CIA.

Conclusion: The agreement between QUANTA Flash and Bioplex™ 2200 for the detection of aPL as an aid in the diagnosis of APS depends on the assay. Overall, good agreement was observed. Only CIA allowed for a quantitative assessment of aPL levels as most samples were outside the AMR on ALBIA.

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DETERMINATION OF DRUGS AND ANTI-DRUG ANTIBODIES IN RHEUMATIC PATIENTS: A COMPARISON OF TWO LABORATORY METHODS

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Background: A reliable identification of anti-drug antibodies (ADA) represents a fundamental tool in the follow up of patients under treatment with anti-tumor necrosis factor alpha (TNFα). Even if several assays are currently commercially available in routine diagnostic laboratory, testing drug concentrations and ADA is hindered by lack of assay standardization. We perform a comparative analysis of two commercially available ELISAs for determination of drugs and ADA.
Methods: The study was performed in 102 samples from patients with rheumatic diseases, (36 under treatment with Adalimumab, 30 with Infliximab and 36 with Etanercept). All drugs and ADA were detected by two different bridging Enzyme-linked immunosorbet assay (ELISAs), manufactured by Promonitor (Progenika) and Lisa Tracker (Theradiag) following manufacturers' instructions.
Results: The correlation between drugs levels of both assays showed a good correlation (Adalimumb, Infliximab, Etanercept Pearson r = 0.90, 0.70, 0.71 respectively). Bland-Altman plots showed a good agreement for all drugs tested.Comparison of ADA to Adalimumab showed that out of 36 samples, 17 were negative by Progenika and Theradiag, 1 was negative by Theradiag but low positive by Progenika, 16 were positive by Theradiag and negative by Progenika, 2 were positive by both assays with a total percent agreement of 52.8 %. Comparison of ADA to Infliximab showed that out of 30 samples, 22 were negative by Progenika and Theradiag, 0 was negative by Theradiag and positive by Progenika, 7 were positive by Theradiag and negative by Progenika, 1 was positive by both assays with a total percent agreement of 76.7% . Comparison of ADA to Etanercept showed that out of 36 samples 24 were negative by Progenika and Theradiag, 12 were negative by Progenika, but positive by Theradiag with a negative percent agreement of 66.7%.
Conclusion: The lack of standardization of routinely assays used in clinical setting and the absence of a reference technique result in discrepancies, especially in the antibody detection, with difficult interpretation and unfavorable effects on patient care. Antibody results must always be interpreted in the context of drug levels and methodology.

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RILEVAZIONE DEGLI ANTICORPI ANTI-MITOCONDRIO SU CELLULE HEp-2

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Scopo dello studio: Gli anticorpi anti-mitocondrio (AMA) sono il marcatore immunosierologico più sensibile e specifico di cirrosi biliare primitiva (CBP). Gli AMA sono rilevabili con l'immunofluorescenza indiretta (IFI) su triplo tessuto (LKS) murino e su linee cellulari HEp-2. Oltre all'IFI, gli AMA con specificità anti-M2, che hanno la maggiore rilevanza diagnostica nella CBP, possono essere rilevati con metodi immunometrici o immunoblot. Poiché l'indagine in IFI presenta, rispetto a queste metodiche, una maggior sensibilità nello screening, nella nostra realtà noi utilizziamo l'IFI come test di primo livello per la diagnosi di CBP e, in caso di quadri fluoroscopici compatibili con AMA, un metodo immunofluoroenzimatico o l'immunoblot per la conferma degli anti-M2. In particolare come substrato utilizziamo il triplo tessuto, considerato il gold standard per la rilevazione degli AMA perchè l'analisi contestuale dei tre tessuti consente in genere una corretta interpretazione del quadro sierologico. Tuttavia, anche quando ricerchiamo gli anticorpi anti-antigeni intracellulari (ANA) sul substrato HEp-2, rileviamo occasionalmente pattern mitocondriali. Scopo di questo lavoro è stato quello di valutare la concordanza tra i pattern mitocondriali rilevati su cellule HEp-2 e quelli rilevati LKS.
Metodi: Nel periodo gennaio-giugno 2017 sono stati raccolti 16 campioni di siero con pattern mitocondriale sulle cellule HEp-2 e analizzati su LKS (Zenit, Menarini Diagnostics). Gli anticorpi anti-M2 sono stati determinati con il test EliA-M2 IgG (Phadia, Friburgo, Germania).
Risultati: Campioni positivi su LKS e positivi per M2: 7/16; campioni negativi su LKS e negativi per M2: 6/16; campioni negativi su LKS e positivi per M2: 3/16.
Conclusioni: Sulle cellule HEp-2 il riscontro di un quadro di fluorescenza citoplasmatica granulare può suggerire la presenza di AMA, che deve essere confermata con metodi più specifici L'IFI su triplo tessuto, sebbene rappresenti il gold standard per la rilevazione degli AMA non è un buon metodo di conferma perché vi sono sieri che risultano positivi solo con test più specifici e negativi su tessuti murini.

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PITFALLS AND BENEFITS OF BIOLOGICAL THERAPEUTIC MONITORING IN AUTOIMMUNE DISEASE

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Autoimmune diseases treatment was revolutionized by the advent of biological therapy, however there is still a significant number of patients with loss of response because of the development of anti-drug antibodies (ADAb). ADAb can form immunocomplexes with the target drug and can influence drug efficacy and pharmacokinetic. As to prevent these complications, the need of individualized treatment regime is becoming important and it lead to design different therapeutic drug monitoring (TDM) consisting in measuring drug serum level and ADAb. Although its benefits, from laboratory point of view, TDM may present potential pitfalls. 1) type of assay: there are different measurement methods with advantages and disadvantages and it is important to choose the best assay for each specific laboratory 2) target drug levels: the timing of drug level sampling is important and many studies show that “trough levels” correlate best with activity for most drug. Consequentially, therapeutic range for trough levels are different according to the different autoimmune diseases and phenotypes, the treatment end-point, the gender, the body mass 3) ADAb cut-off: it is important to use assays with a validated ADAb cut-off. Transient or not-neutralising ADAb as well as possible cross-reaction could complicate the interpretation of TDM results. TDM benefits: 1) it can help clinicians to understand the causes of loss of response or side effects 2) it may lead to cost saving selecting patients to switch out of biologicals class or need optimization of therapy 3) It may be used to adjust anti-TNF# therapy dosing for stable patients on maintenance therapy. In conclusions, different TDM algorithms should be set-up in strict collaboration with clinicians and optimize for each Operative Units. Even if there is active discussion about the real utility of TDM in clinical practice, we believe that TDM could be a useful tool for the management of patients with autoimmune disease treated with biological drugs.

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USEFULNESS OF FREE LIGHT CHAINS AND IgG SUBCLASSES IN MYASTHENIA GRAVIS

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Background: In approximately 85% of the patients with Myasthenia Gravis (MG), antibodies are directed towards the nicotinic acetylcholine receptors (AChR) while a smaller portion present antibodies towards muscle specific tyrosine kinase (MuSK) or low density lipoprotein receptor-related protein 4. The management of MG patients is not easy for the heterogeneity of the disease and because there are no circulating serum biomarkers that correlate with the disease state between patients or different MG subtypes. Polyclonal FLCs have been analyzed as suitable biomarkers of immune activity in chronic conditions and MG represents a good model of chronic autoantibody production.
Objectives: The aim of this study was to evaluate serum FLC concentrations in a group of MG patients together with serological parameters of specific auto-antibody (auto-Ab) and nonspecific, total IgG subclasses, in comparison to a control group of patients with systemic autoimmune rheumatic diseases (SARD) and a group of healthy blood donors (HBD).Methods: We collected 78 sera from 40 MG patients: 48 sera were from 25 patients with auto-Abs directed against AChR and 30 from 15 patients with immunoreactivity to MuSK. Sera from 16 SARD and 18 HBD represented the controls. Each sample was tested for FLCs and the four IgG subclasses (IgG1, IgG2, IgG3 and IgG4).
Results: Our data demonstrate a statistically significant increase in free κ chains in both AChR and MuSK groups, as well as in SARD, when compared to HBD, while λ levels were increased only in AChR group and in SARD patients. Regarding IgG subclasses, different mean levels and distribution among AChR/MuSK groups, SARD and HBD were found, but only for IgG1 in SARD the levels were significantly higher than in HBD and above the range of normality.
Conclusions: In conclusion this study shows that serum FLC may represent a new marker of B cell activation in MG, likely useful to monitor response to treatment.

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RUOLO DEL SISTEMA MIT3 NELLA DETERMINAZIONE DEGLI ANTICORPI ANTI MITOCONDRIO

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Introduzione: La Colangite Biliare Primitiva (CBP) è una patologia autoimmune cronica caratterizzata da colestasi intraepatica, danno infiammatorio ai piccoli dotti biliari e positività sierologica agli anticorpi anti mitocondrio (AMA). Gli AMA sono il marcatore sierologico più specifico e sensibile di CBP essendo riscontrabili ad alto titolo nel 90-95% dei pazienti con una specificità quasi assoluta. Gli AMA sono rilevabili con il metodo di Immunofluorescenza Indiretta (IFI, gold standard) su sezioni di triplo tessuto (stomaco/rene/fegato) di roditore e su cellule HEp-2. Inoltre gli AMA possono essere rilevati con metodi lineblot o immunoenzimatici (ELISA e CLIA).
Scopo: Valutazione delle performance analitiche del sistema MIT3 in chemiluminescenza per la determinazione degli AMA (Inova Diagnostic, San Diego USA). L'antigene ricombinante M2 (MIT3) contiene gli epitopi immunodominanti dei complessi PDC-E2, BCOADC-E2 e OGDC-E2.
Materiali e Metodi: Sono stati analizzati 230 campioni di siero di soggetti afferenti al Servizio di Medicina di Laboratorio dell'Azienda Ospedaliera di Padova con richiesta di AMA. In ciascun campione sono stati determinati gli AMA in IFI su triplo tessuto e su cellule HEp-2 e con il sistema MIT3. I campioni AMA positivi IFI sono stati confermati con metodo Immunoblot.
Risultati: 35 AMA IFI positivi con diagnosi di CBP, 4 AMA IFI negativi ma con diagnosi di CBP, 40 AMA IFI positivi con altre patologie, 151 AMA IFI negativi. Il sistema MIT3 in chemiluminescenza è risultato positivo in 34/35 campioni CBP positivi, in nessuno dei 4 campioni CBP positivi AMA negativi, in 29/40 dei soggetti AMA positivi con altre patologie ed in 1/151 dei pazienti AMA negativi.
Conclusioni: Il sistema MIT3 presenta analoghe caratteristiche di sensibilità e specificità dei test classici in IFI (sensibilità 87.2% e specificità 98,7%). L'analisi della curva ROC evidenzia un'area sotto la curva di 0.927 (95% CI: 0.868-0.987). L'agreement tra metodi è risultato ottimale (K Cohen = 0.97).Considerando le performance analitiche e i numerosi vantaggi del metodo in chemiluminescenza si può ipotizzare l'introduzione di questo sistema per la determinazione degli AMA.

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TRATTAMENTO DI PAZIENTI EPATOTRAPIANTATI CON RECIDIVA DI EPATITE C: NUOVI APPROCCI TERAPEUTICI

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L'infezione primaria da HCV cronicizza nell'80-85% dei casi e può rimanere asintomatica, da un punto di vista clinico, anche per decenni. In una quota variabile di soggetti portatori del virus, l'epatite C cronica può evolvere verso la cirrosi epatica e l'epatocarcinoma. Scopo del lavoro è valutare efficacia e tollerabilità della terapia antivirale con Sofosbuvir e Ribavirina in pazienti epatotrapiantati con recidiva di epatite C e sottoposti a terapia antirigetto.
Metodi: Lo studio è stato condotto su 29 pazienti sottoposti a trattamento antivirale con Sofosbuvir e Ribavirina in cura presso l'A.O.R.N. "A. Cardarelli" di Napoli nel periodo Giugno 2014 – Febbraio 2016. Il monitoraggio quali/quantitativo di HCV-RNA è stato eseguito con il test COBAS®AmpliPrep/ COBAS®TaqMan® HCV v2.0 (Roche Diagnostics), la determinazione del genotipo di HCV è stata ottenuta mediante metodica di ibridazione inversa (VERSANT®HCV Genotype 2.0 Assay - LiPA) (Siemens) laddove il genotipo dei polimorfismi dell'interleuchina 28B (IL28B) è stato eseguito mediante LightMix® Kit IL28B (Roche Diagnostics). Durante il trattamento sono stati monitorati i parametri biochimici ed ematologici. Il grado di fibrosi è stato determinato mediante Fibroscan (Echosens, Parigi).
Risultati: I pazienti esaminati (25 M e 4 F, età media 63 anni, tutti di nazionalità italiana) presentavano un'infezione con diversi genotipi di HCV: 25 pazienti con genotipo 1b, 2 pazienti con genotipo 1a, 2 pazienti con genotipo 3. L'analisi del polimorfismo per il gene IL28B ha evidenziato 3 pazienti con genotipo C/C, 21 con genotipo T/C e 5 con il genotipo T/T. Tutti i pazienti hanno completato regolarmente il trattamento. Al termine del protocollo terapeutico 28 soggetti sono risultati HCVRNA negativi e 1 ha avuto una risposta di tipo breaktrough. Tra i 28 soggetti HCVRNA negativi, 22 hanno ottenuto una risposta virologica sostenuta (SVR) e 5 hanno ottenuto il relapse. Il genotipo di HCV, l'esposizione a precedenti trattamenti, il genotipo dell'IL28B, la carica virale all'inizio della terapia e il farmaco immunosoppressore assunto non hanno influenzato i tassi di SVR.
Conclusioni: La terapia è risultata efficace in termini di SVR (il tasso di SVR complessivamente è risultato pari al 76%) ed è stata in genere ben tollerata (solo il 7% ha manifestato effetti collaterali di rilievo durante il trattamento).

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MARCATORI BIOCHIMICI DEL METABOLISMO DEL RAME IN SOGGETTI PORTATORI DI MALATTIA DI WILSON

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Introduzione e scopo: La malattia di Wilson (MW), trasmissione autosomica recessiva, è caratterizzata da difetto di eliminazione del rame (Cu) e dalla sua ridotta incorporazione nella ceruloplasmina (Cp), con accumulo dello stesso nel fegato e conseguente danno epatocellulare. Secondo lo score diagnostico di Ferenci, impiegato nella MW, che valuta parametri clinici e laboratoristici, valori di Cp inferiori a 0,2 g/L e di cupruria (Cuu) superiori a 50 µg/24h sono suggestivi di MW. In letteratura pochi sono i dati riferiti a soggetti portatori di MW. Popolazione studiata: Abbiamo valutato i livelli di Cp, Cuu e cupremia (Cus) in 27 soggetti portatori della MW (confermata da presenza di mutazioni nel gene ATP7B), 14 uomini e 13 donne, età media 52 e 48 anni rispettivamente.
Risultati: Il livello medio osservato per la Cp = 0,20 ± 0,03 g/L è posizionato sul cut off inferiore dei valori di riferimento (vr) 0,2 – 0,6 g/L, mentre i valori medi per Cuu = 17,86 ± 11,69 µg/24h (vr 0 – 50) e per Cus = 87,23 ± 20,36 µg/dL (vr 69 – 122) rientrano a pieno nell'intervallo di riferimento. Dividendo la popolazione femminile (pf) e maschile (pm), le stime medie della Cp ricadono nel range per le donne (0,21 ± 0,03 g/L) e al di fuori per gli uomini (0,18 ± 0,03 g/L). I valori medi di Cus e Cuu rientrano nei vr per entrambi i gruppi, pm: Cus = 78,98 ± 19,41 µg/dL, Cuu 20,74 ± 14,18 µg/24h; pf: Cus = 96,11 ± 18,05 µg/dL, Cuu = 14,49 ± 7,07 µg/24h. Sono stati inoltre valutati marcatori di funzionalità epatica che sono risultati nella norma.
Discussione: La mutazione del gene ATP7B su singolo cromosoma non sembra alterare i marcatori del metabolismo del rame e di funzionalità epatica; solo la Cp risulta essere al di sotto dei vr nel 51,85% della popolazione studiata. I livelli di Cp e Cus sono risultati significativamente differenti tra pm e pf, p-value di 0,0061 e 0,0258 rispettivamente; nella pf il valore medio di Cus più elevato e quello di Cuu più basso potrebbero dipendere dai livelli di estrogeni che incrementano i valori di Cp, di conseguenza, di Cus. Esiste, inoltre, correlazione positiva tra livelli di Cp e Cus con una ρ di 0,88. EASL Clinical Practice Guidelines. J of Hepatology,2012,56,671-85. Vieira J.et al. Digestive and liver disease,2012,44,323-27

P180
PROGNOSTIC TOOLS OF COMORBIDITY AND MORTALITY IN HCV INFECTION

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Background: The clinical spectrum of Hepatitis C virus (HCV) cryoglobulinemia largely varies from an asymptomatic presentation to severe vasculitis and lymphoma. Since even limited amounts of cryoglobulins (CGs) may be pathogenic and significant in certain clinical context, are responsible for severe renal and neurological complications, leading to high morbidity and mortality. Serum levels of Free Light Chain (FLC) were altered in the majority of HCV-CGs patients, completely confirming the value of these serum proteins as markers of HCV-related Mixed Cryoglobulinemia, even in patients without symptoms and with an early-stage disease.
Objectives: The aim of this pilot study is therefore to find a panel of biomarkers, easy to assess, in HCV positive patients with low amounts of CGs. The possibility of identifying subpopulations at risk may open scenarios for target treatment strategies for HCV patients which have a good cost/benefit ratio for clinical management.
Methods: Serum samples from 44 untreated patients with chronic HCV infection were examined for CGs, FLC, C3, C4 and Rheumatoid Factor IgG, IgM (RF-IgG/IgM). Positive CG patterns were divided in type III and microheterogeneous, according to Brouet's CG classification.
Results: Differences in the FLC k/λ ratio and sum were significant between type III CGs patients, microheterogeneous CGs patients and HCV naïve negative patients ($p<0.01$). Moreover, also RF-IgG concentrations between groups were significantly different with a p-value of 0.016.Concerning C3, C4, RF-IgM amounts, no statistically significant differences were observed.
Conclusions: HCV patients with/without CGs showed different level of RF-IgG and FLC clear indication of a multiple-stage disease toward a clonal selection of antibodies due to chronic stimulus. This pilot study hypothesizes that increased FLC levels and the presence of CGs is associated with more severe and active disease, especially in terms of mortality and comorbidity. Furthermore, high level of RF-IgG may present at an early stage of autoimmune disease.

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HIGH RESOLUTION MELTING ANALYSIS TO SCANNING MUTATION OF TNNT2 GENE EXONS IN DM PATIENTS

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Background: Myotonic dystrophy (DM) is a genetic disorder caused by nucleotide repeats expansion. Sudden death represents the main cause of mortality in DM patients, caused by the onset of severe arrhythmias: ventricular tachycardia, ventricular asystole, ventricular fibrillation or electromechanical dissociation. The aim of this study is to investigate the TNNT2 gene exons in DM patients with alteration in serum cTnT levels.
Methods: Case-control study included 59 DM patients and 22 hearty controls. An additional group of 62 controls with similar cardiac defects to DM were enrolled. High Resolution Melting (HRM) technology was used to identify genetic variation in the TNNT2 gene.
Results: NT-proBNP, hs-cTnT and CK levels were significantly increased in DM patients compared to healthy subjects ($p=0.0008$, $p<0.0001$, $p<0.0001$). Hs-cTnT levels were significantly higher in DM compared to control group with cardiac defects ($p=0.0003$). Western blot analysis, on muscle tissue samples, with anti-cTnT, suggests that there is not skeletal muscle involvement. Positive correlation was found between hs-cTnT and hs-cTnI in both DM patients and controls ($p=0.019$, $p=0.002$). Independently from the age, the risk of DM disease was positively related to an increase in hs-cTnT ($p=0.03$). On the contrary, the risk of DM was not related to hs-cTnI, but was evidenced a role of PR interval ($p=0.03$) and CK ($p=0.08$). Finally, in the first 21 scanned DM patients, HRM analysis did not show any variation on 9, 10 and 11exons in the TNNT2 gene, respectively.
Conclusions: The levels of hs-cTnT were significantly higher in DM patients. Analysis, with anti-cTnT, shows that this increase might be linked to heart problems and from preliminary HRM results no mutation of TNNT2 gene exons seems to be correlated with this increase.

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SCREENING OF HEMOCROMATOSIS GENE MUTATION: A PREDICTIVE MARKER OF LIVER INJURY?

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Hemocromatosis is a pathology characterized by a progressive accumulation of iron in the body.
The most common genetic form, called type 1, is due to mutations in the HFE gene, located on chromosome 6; the most investigated mutations in molecular diagnosis are: C282Y, H63D, S65C (1).
The C282Y mutation is present in about 6% of the Caucasian population and represents the most common genetic variant in this population (2).
To evaluate the frequency of mutations in the HFE gene, in the section of Hematology of the UOC Clinical Pathology of the "San Giovanni di Dio and Ruggi D'Aragona" University Hospital in Salerno, we have studied 249 patients (45± 25 years) with signs and symptoms related to hemochromatosis.
43.4% was positive for one or more mutations typical of the disease.
The H63D mutation was found in heterozygosity in 64.8% and 18.5% in homozygosity. Heterozygous and homozygous for C282Y were found in 7.4% and 1.85%, respectively. Double Heterozygous H63D/C282Y was found in 3.7%, S65C heterozygous in 1, 8%, H63D / S64C double heterozygous and E60X heterozygous in 0.9%.
Ultrasound and hematochemical findings were conducted in order to verify anatomical and/or functional alterations in H63D and C282Y mutant carriers.
The rate of the two main mutations of the HFE, C282Y and H63D mutations showed a prevalence of H63D mutation (87.2%) versus C282Y (12.8%), according to data available in literature (3).
The association between C282Y mutation and the development of hepatic disease, including non-alcoholic hepatic steatosis, was statistically significant ($p <0.05$, OR: 2).
In contrast, H63D mutation carriers have less probability of developing hepatic complications ($p> 0.05$, OR: 0.9).
In conclusion our study suggests that a screening for mutation in the HFE gene may be a predictor of hepatic injury.
Moreover, better knowledge of HFE and/or associated mutations genes, its pathogenetic role in liver disease, and the development of new drugs targeting could provide a better outcome for patients.

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IMPROVEMENT IN THE DIAGNOSIS OF PATHOGENIC SUBCHROMOSOMAL COPY NUMBER ALTERATIONS BY NEXT-GENERATION SEQUENCING-BASED PREIMPLANTATION GENETIC SCREENING

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Introduction: The main purpose of preimplantation genetic screening (PGS) is the identification of chromosome copy number variants (CNVs). Aneuploidies represent the most relevant causes of implantation failures and miscarriages. Deletions and duplications smaller than 5 Mb do not usually affect embryo implantation or subsequent fetus development, but can be associated with developmental delay and intellectual disability in the newborns. Despite the fact that these alterations are well characterized and diagnosed in pre- and post-natal period, their identification with the current methods used in PGS has not been evaluated to date. In the present study we assess the accuracy of NGS protocols currently performed for PGD for the identification of subchromosomal CNVs, ranging from 3 to 20 Mb.

Materials and Methods: We selected seventeen samples from patients affected by different neurological disorders, previously analyzed by a-CGH.High-resolution a-CGH analyses were performed using the Human Genome CGH Microarray kit 4X180K (Agilent Technologies), with an average space of 13 Kb and allowing an average resolution of 25 Kb, according to manufacturer's protocols. NGS analyses including libraries preparation and sequencing was carried out following manufacturer instructions (Illumina).

Results: NGS-based analyses allowed identification of CNVs previously identified with a-CGH with an almost complete concordance: only in a single case, associated to mental disability, we observed a duplication about 7 Mb in length in chromosome 3 by a-CGH whereas NGS identified an alteration of 2.4 Mb, smaller than the selected cut-off.

Discussion and Conclusions: These data demonstrate a good sensitivity of NGS-based identification of subchromosomal CNVs (5-20 Mb) with the possibility to introduce these analyses also in PGS protocols; generation of comprehensive databases for the complete characterization of the clinical significance of CNVs is the next challenge to improve genetic test and apply these techniques in the PGS diagnostics. We observed a single discordance that is probably due to a technical artifact or a possible mosaic sample. These results show the need to extend this comparison to a larger population to better evaluate NGS-based methods for genomic analysis.

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IMPLEMENTATION OF 3 LARGE PANELS OF CARDIOPATHY-RELATED GENES FOR MUTATIONAL SCREENING IN A DIAGNOSTIC WORKFLOW

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*,** equal contributors. Inherited cardiopathies are a heterogeneous group of genetic diseases, including primary myocardial disorders and heart rhythm alterations, that can predispose to sudden cardiac death (1). Given cardiopathy genetic heterogeneity, their clinical variability and reduced penetrance, and the genetic and phenotypic overlap among different cardiopathies, the correct setting of genetic tests for the identification of mutation carriers is crucial to ensure a rapid diagnosis and consequent timely treatment (2). Thus, the implementation of a next generation sequencing (NGS) screening based on large gene panels becomes necessary. To address this issue, we analyzed 160 unrelated subjects using 3 large gene custom panels: a 111-genes panel (MyoNext) to identify myocardial diseases, a 75-genes panel (CanalPlus) for channelopathies, and a 138-genes panel (SuddenDeath) constituted by all genes related to sudden death, including those present in the previous two panels. NGS libraries were prepared using the HaloPlex Target Enrichment System (Agilent) and sequenced using the NextSeq (2x151 PE) systems (Illumina). Data were analyzed using the SureCall software (Agilent). Thirty per cent of patients analyzed with MyoNext, 13% of patients analyzed with CanalPlus and 44% of patients analyzed with the SuddenDeath panel carried pathogenetic mutations. Most mutations occurred in the MYBPC3 and MYH7 genes. Notably, however, mutations were found in at least 10 genes not usually tested for myocardial disease. This finding suggests that molecular screening for myocardial disease should be extended to many other genes beyond the conventional myocardial genes. In conclusion, this faster and more extensive molecular screening versus traditional Sanger sequencing of only the most common disease-related genes, in a routine diagnostic workflow, increases diagnostic sensitivity and will lead to a more accurate heart disease risk assessment in patients and their families. Lastly, the method we describe can be used to detect other inherited cardiomyopathies-related genes thereby shedding light on myocardial disease genetics.

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SUDDEN CARDIAC DEATH IN ATHLETES: A MULTI-GENE PANEL AMELIORATE THE RISK ASSESSMENT

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Sports activity is associated with an increased risk of sudden cardiac death (SCD) in adolescents and adults with clinically silent cardiovascular disorders. The spectrum of cardiac alterations is wider in young athletes and includes inherited (i.e., cardiomyopathies and channelopathies) and congenital (i.e., anomalous origin of coronary arteries) structural heart diseases [1,2]. To determine the frequency of inherited cardiac mutations in athletes with signs or symptoms of cardiac disease or with familial positive history, we developed a next-generation sequencing (NGS)-based analysis using a gene panel of 138 SCD-related genes. Thirty-seven athletes were recruited from the Italian Olympic Committee (Rome) and from patients observed at the Cardiology Unit of the Hospital Monaldi (Naples). The athletes were affected by overt disease or were suspected to have an inherited heart condition, such as primary myocardial and/or cardiac rhythm alterations. Our custom-designed gene panel explored 3,009 target regions for a total of 1.302 Mbp. Targeted enrichment was carried out using the HaloPlex system (Agilent Technologies). Three sequencing runs were optimized with the NextSeq and MiSeq systems (Illumina). We used Agilent's SureCall software v.3.5.1.46 for data analysis. We performed two runs on the NextSeq system (Mid Output, 150x2) to analyze 35 samples, and obtained about 40 Gb per run (70% of Q Score >30, 80% of Clusters Passing Filters) with about 55 million reads per run. The remaining two samples were sequenced on the MiSeq system and yielded 7 million reads (90.6% of QScore>30, 83% of Clusters Passing Filters). Causative or possibly-causative variants, identified via bioinformatics analysis of the reads obtained, were confirmed by Sanger sequencing. We found some causative or possibly-causative variants in several genes, which are not generally used in genetic cardiopathy screening. Our data assess the efficacy of enlarged genetic screening for the early detection of DNA variants in genes which may represent an increased risk of SCD to correctly identify “at risk” athletes.

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P186
GENOME-WIDE DNA METHYLATION ANALYSIS IN BLOOD CELLS FROM PATIENTS WITH WERNER SYNDROME

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Background: Werner Syndrome is a progeroid disorder characterized by premature age-related phenotypes. Although it is well established that autosomal recessive mutations in WRN gene is responsible for Werner Syndrome, the molecular alterations that lead to disease phenotype remain still unidentified.
Results: To address whether epigenetic changes can be associated with Werner Syndrome phenotype, we analyzed genome wide DNA methylation profile using the Infinium MethylationEPIC beadchip in whole blood from 3 patients affected by Werner Syndrome compared with 3 age- and sex-matched healthy controls. Hypermethylated probes were enriched in glycosphingolipid biosynthesis, FoxO signaling and insulin signalling pathways, while hypomethylated probes were enriched in PI3K-Akt signaling and focal adhesion pathways. Interestingly differentially methylated regions identified CERS1 and CERS3, two members of the ceramide synthase family and 22 out of 47 of the differentially methylated genes belonging to the enriched pathways resulted differentially expressed in a publicly available dataset on WS fibroblasts.
Conclusions: DNA methylation changes in peripheral blood from WS patients provide new insight in the pathogenesis of the disease, highlighting in some cases a functional correlation of gene expression and methylation status.

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A RARE CASE OF SEVERE HYPERTRIGLYCERIDEMIA DUE TO VARIANTS IN LMF1 GENE

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Introduction: Severe Hypertriglyceridemia (HTG) is a rare disease characterized by levels of triglycerides (TG) higher than 10mmol/L associated with eruptive xanthomas and pancreatitis. Transmission is autosomal recessive and several genes are involved i.e. those encoding Lipoprotein Lipase (LPL), Apolipoprotein A-V (APOA5), Apolipoprotein C-II (APOC2), Glycosyl-Phosphatidyl-Inositol-anchored HDL-Binding Protein (GPIHBP1), and Lipase Maturation Factor-1 (LMF1). Only few families with HTG due to LMF1 variants have been described. The LMF1 protein is required for the LPL folding and dimerization.
Methods: The proband is a male patient aged 44 with TG levels of 15.9 mmol/L, eruptive xanthomas and mild hepatic steatosis. The severe HTG phenotype is also present in one out of 5 siblings. The genetic screening was performed by direct sequencing of the coding region and the exon-intron junctions of the above genes. SALSA MLPA kit was used to search for large rearrangements in the LPL gene.
Results: Two rare variants were found in LMF1 gene in the proband, whereas only common variants were found in LPL, APOA5, APOC2 and GPIHBP1 genes. The proband carries the variants c.157delC and c.410C>T (p.Arg53Glyfs*5 and p.Ser137Leu). The analysis of proband relatives confirmed that the 2 variant are present on the 2 alleles (compound heterozygosis). The first variant is a deletion producing a frameshift leading to a truncated protein lacking of the most part of LMF1 protein. The missense variant was never associated with HTG but was previously found during the exome sequencing of the Exome Aggregation Consortium (ExAC) with a MAF=0.000027. In silico predictions suggest a pathogenic role of the variant. The LPL mass in the proband serum was 229 ng/mL that correspond to 81.7% of healthy subjects. The proband brother with severe HTG is compound heterozygote like the proband whereas, the analysed unaffected relatives are heterozygotes.
Conclusions: The proband and his brother are compound heterozygote for LMF1 variants. Both variants can be considered pathogenic as confirmed by the decreased LPL mass in the serum. Genetic diagnosis could help to identify the disease etiology and to enlarge the screening to the relatives allowing an accurate monitoring.

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BLOOD LEVELS OF PROTEASOME AND ACYL-PEPTIDE HYDROLASE (APEH) ACTIVITIES IN ALZHEIMER DISEASE

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Neurodegenerative diseases, such as Alzheimer's disease (AD), are characterized by the presence of protein aggregates due to alterations in protein-quality control systems. The proteasome complex is a major regulator of intracellular protein homeostasis. Acyl-peptide hydrolase (APEH), an ubiquitous bifunctional enzyme exhibiting exopeptidase activity towards N-acyl-peptides and endoprotease activity towards oxidized proteins, was also reported to play a key role in protein degradation machinery in coordination with proteasome. This study aimed to investigate the activity and the transcript blood levels of proteasome and APEH in AD patients, in comparison to those of healthy controls (HC). Fifty-two participants were recruited and divided in two groups based on their clinical profiles: 26 participants with probable AD and 26 cognitively HC. Venous blood samples were used to obtain erythrocyte hemolysates and measure the chymotrypsin-like (CT-like) proteasome (β5-subunit) and exopeptidase APEH activities by spectrophotometric/spectrofluorimetric analyses, using specific substrates. The endopeptidase APEH activity was determined by RP-HPLC, using as substrate an oxidized peptide projected ad hoc. The activity levels of proteasome and APEH were also measured in samples prepared by an optimized batch-based purification protocol. mRNA levels of β5 and APEH genes were determined by qPCR. Results obtained from hemolysates correlated well with those from purified samples carried out to confirm the enzyme activity data in the crude extracts. Proteasome, exopeptidase / endoprotease APEH activities, as well as proteasome gene expression, were significantly reduced in AD patients respect to HC, whereas no significant differences were observed in APEH gene expression. Moreover, exopeptidase and proteasome activities displayed a significant correlation only in HC group, suggesting that AD also affects the functional cooperation of the two enzymes involved in the degradation machinery. This preliminary study demonstrated, for the first time, the existence of a relationship between the blood APEH-proteasome levels and AD, laying the foundations for a possible use of this enzyme system in the diagnosis and therapy of neurodegenerative disorders.

P189
KFLC INDEX IN MULTIPLE SCLEROSIS DIAGNOSIS

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Multiple sclerosis (MS) is one of the most common neurological diseases of the young adults, often causing deficits since its early stages, by altering memory, view, attention and executive functions. The free light chains (FLCs) determination might be a sensitive alternative to identify the presence of intrathecal synthesis in cerebrospinal fluid (CSF), respect to the detection of oligoclonal bands (OCBs) currently used. We performed an automated nephelometric assay for FLCs detection in CSF/serum, based on specific monoclonal antibodies against epitopes that are hidden in intact immunoglobulins.We analyzed serum and CSF of 176 patients, with different neurological disorders, admitted at the Neurology Clinic of the Tor Vergata University Hospital of Rome and at IRCCS Neuromed Institute in Pozzilli. CSF was collected and stored at #80 °C, following standard preanalytical procedures; immunoglobulin and albumin concentrations were measured by nephelometry in CSF and serum samples, while OCBs were determined by immunofixation. The 176 patients were divided into three groups based on neurological diagnosis. We obtained a cut off of 12.3 for kFLC Index with a sensitivity and specificity of 93% and 100% respectively. In this study we increased the number of patients compared to our previous work. The results obtained confirm the importance of kFLC Index for the diagnosis of MS. A kFLC Index cut off higher than 12,3 could be an help for clinicians in suspected or unclear cases to diagnose MS. The use of kFLC Index takes into account the function of the blood-CSF barrier and it represents an attempt to increase the diagnostic accuracy of kFLC determination avoiding false positive. Our data confirm that the kFLC Index is a valid tool in the diagnosis of MS and could replace the OCBs diagnostic test. Moreover, it could be useful for evaluating treatment response.

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ON THE POSSIBLE ROLE OF KAPPA FREE LIGHT CHAIN INDEX (KFLCi) IN THE INITIAL SETTING FOR THE DIAGNOSIS OF MULTIPLE SCLEROSIS

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Introduction: Diagnosis of Multiple Sclerosis (MS) includes clinical and imaging findings to demonstrate lesions dissemination in space and time and to exclude other diseases. The contribution of biochemical assays of cerebrospinal fluid (CSF) is marginal. However, the kappa free light chain index (KFLCi) has emerged as alternative marker, with high sensitivity and low costs.
Methods: 85 patients were enrolled in this preliminary study: 54 had a suspicion of MS (sMS) and 31 of non MS Inflammatory Disease (sNMSID) within the central nervous system. MS diagnosis was based on the 2010 McDonald's criteria (MD). In addition to albumin and IgG, also KFLC have been measured on serum and CSF by nephelometry. The results were expressed and analyzed as Link index and KFLC index (KFLCi). All the samples were also investigated for the presence of oligoclonal bands by isoelectrofocusing.
Results: 34 sMS patients (63%) fulfilled both the MD criteria at clinical presentation and had a KFLCi above cut-off values (>5); all of them were diagnosed as MS. Despite 20 sMS patients (37%) did not fulfill MD criteria, a final diagnosis of MS was confirmed in 13 of them (65%). In the 92% of this latter group, KFLCi was indeed above the cut-off value. About 23% of sNMSID patients fulfilled the MD criteria at the time of rachicentesis and had a KFLCi higher than 5; for all of them a final diagnosis of MS has been confirmed. Despite 74% of sNMSID patients initially did not fulfill MD criteria, MS was confirmed in 26% of them, and KFLCi was indeed above the cut-off value in 86% of them.
Conclusion: These findings strongly suggest that KFLCi must be included in the initial setting to improve diagnosis of MS.

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THE WORLD OF ASD BIOMARKERS: LOOK FOR THE NEEDLE IN THE HAYSTACK AND DISCOVER NEW INTERESTING INDICATIONS FOR FUTURE RESEARCHES

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Autism spectrum disorders (ASD) are a heterogeneous neurodevelopmental and behavioral disorder, which symptoms usually occur in the first three years of life. Although the etiology of ASD is largely unknown, genetic, epigenetic, nutritional and environmental factors may be responsible for the disorders (1). In particular, the genes involved in the folate/homocysteine/transulfuration pathways (one-carbon cycle), which downstream regulate/affect oxidative status and DNA methylation, are considered risk factors for ASD (2). Moreover a high rate of comorbidity is associated with ASD including gastrointestinal (GI) dysfunctions, epilepsy, attention deficits, allergies, oxidative stress and mitochondrial disease, immune dysregulation, metabolic as well as microbiota alterations. In the last four years a growing body of literature data has evidenced a clear connection between gut microbiota composition and ASD also via the production of short chain fatty acid (SCFA) and 5'-HT (3). We have studied 20 ASD children, focusing on oxidative stress, some one-carbon cycle-related polymorphisms, SCFA and some amino acids levels. In addition, in order to better investigate on the individual metabolic phenotype, we performed both whole exome and intestinal microbiome analysis (3-4). Our data, confirming the presence of a mosaic of metabolic phenotypes among ASD children, indicate that: i) oxidative stress, the altered levels of some vitamins (B6-B12 and D in particular), the enteric microbiome anomalies and its related products, the state of the GI tract, have to be considered in close relationship with ASD, and need to be evaluated before the dietary planning, the pharmacological or probiotic therapies and prebiotic supplementation; ii) the immense intrinsic potential of exome analysis and its flexibility, allowing us to know the bases on which the metabolic expressions are settled, modulated or not by some possible epigenetic interventions; iii) the needing of an individualized approach in studying and treating ASD children.

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KFLC INDEX AS AN ALTERNATIVE TEST TO OLIGOCLONAL BANDS IN DIFFERENTIAL DIAGNOSIS BETWEEN MULTIPLE SCLEROSIS AND DIFFERENT NEUROLOGICAL DISORDERS: OUR EXPERIENCE

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Background: The diagnosis of multiple sclerosis (MS) is still based on clinical criteria, MRI flags and the detection of oligoclonal bands (OCBs). Recently the Kappa free light chains (KFLCs) have been proposed as a diagnostic biomarker in patients with clinically isolated syndrome (CIS) and MS. The aim of our study was to identify kFLC Index cut-off for improving in our laboratory the validation between MS-CIS patients and different neurological disorders (ONDS).

Patients and Methods: We analyzed serum and cerebrospinal fluid (CSF) of 100 patients from Neuroscience Department ,so subdivided: 50 affected by MS and CIS, 27 affected by non-infective ONDs and 23 with infective ONDs. The OCBs were determined by immunofixation (Hydragel 9 CSF kit isofocusing, Sebia Italia). KFLCs were perfomed by turbidimetric assay (the Binding Site Ltd, UK).

Results: We obtained a kFLC Index cut-off of 11.9 for MS-CIS diagnosis with sensitivity and specificity of 78% and 80% respectively, with ROC analysis of all calculated kFLC Index. MS-CIS patients: 37 patients (OCBs positive) had kFLC Index=123.2 and 13 patients (OCBs negative) had kFLC Index =25.8 (reference values $\leq$ 5.9). kFLC Index in infective ONDs (n=23) and non-infective ONDs (n=27) patients were respectively 44.7 and 3.8.

Conclusions: Our result in MS-CIS patients suggest that the kFLCs assay might be an useful and new diagnostic biomarker for these diseases, even if it is mandatory to evaluate this cut-off with Mc Donald's criteria for a clear diagnosis. In fact it is pointing up that the our found kFLC Index of MS-CIS (OCBs negative) is lower than observed kFLC Index in infective ONDs (OCBs negative, but sometimes with a supernumerary band) where the conclusive diagnosis is done by microbiological CSF tests. Although the measurement of CSF KFLCs is a rapid and quantitative assay and CSF analysis is no longer a mandatory part of diagnostic criteria in MS, since the diagnostic sensitivity and specificity is almost equal but not superior to OCB assay, further studies are necessary to evaluate the accuracy of this test for neurological differential diagnosis.

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KIDNEY STONES DISEASES AS A MANIFESTATION OF THE MITOCHONDRIAL DNA m.3243A>G MUTATION

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Introduction: Mitochondrial diseases (MDs) are a heterogeneous group of genetic disorders characterized by primary defects in mitochondrial oxidative phosphorylation. These genetic disorders are clinically heterogeneous, can occur at any age and can manifest with a wide range of clinical symptoms, characterized by the simultaneous presence of a many clinical features in different combinations, mostly affecting tissues with high-energy demands, including the brain, eye, skeletal muscle, heart, and rarely kidney. Here we report the clinical and biochemical findings in a Italian family (mother and two sons S1 and S2) affected by kidney stones disease with maternal inheritance.
Methods: Genetic investigation were performed by polymerase chain reaction-restriction fragment length polymorphism analysis for common mtDNA mutations and subsequently direct sequencing. The stones were analyzed both by stereoscopic microscopy and infrared spectroscopy.
Results and discussion: Genetic investigations in the mother and two sons identified m.3243A>G mutation in the MTTL1 gene. This mutation is responsible for several reported clinical syndromes including mitochondrial encephalopathy lactic acidosis and stroke like episodes (MELAS), maternally inherited diabetes and deafness (MIDD) and progressive external ophthalmoplegia (PEO). Many patients harbouring m.3243A>G exhibit a clinical phenotype that does not fall within accepted criteria for the currently recognized classical mitochondrial syndromes. The renal stones analysis of two sons showed:S1:90% calcium oxalate monohydrate, 10% calcium oxalate dihydrate;S2:85% calcium oxalate dihydrate, 10% carbapatite, 5% calcium oxalate monohydrate. This report expands the phenotypes of m.3243A>G-associated disorders and supports the role of mitochondrial dysfunction in the pathogenesis of chronic kidney disease. To better explain the role of this mutation in the pathogenesis of chronic kidney disease we are performing a metabolomics studies of this family. Considering clinical variability among individuals with this mutation, due to chameleon nature of the MDs, the diagnosis could be missed if clinicians considered the diagnosis only in terms of a 'classic syndromes' and without extensive laboratory and biochemical investigations.

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ASSOCIATION BETWEEN ADAM10 POLYMORPHISMS AND AGE OF ONSET OF MOVEMENT DISORDERS IN HUNTINGTON'S DISEASE

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Huntington's disease (HD) is an inherited neurodegenerative disorder characterized by motor, cognitive and behavioral disturbances. The expansion of a trinucleotide CAG repeats in the HTT gene is the only known genetic cause and the main determinant of the disease's development. The length of the expanded CAG repeat inversely correlates with age of onset (AOO) of symptoms, which however may be also influenced by other genetic and/or environmental factors [1]. HTT protein allows neurulation and rosette formation by regulating activity of ADAM10 metalloprotease on N-cadherin cleavage [1]. As mutant HTT affects the N-cadherin pathway, we considered ADAM10 a potential modifier gene in HD.To test the possibility that ADAM10 genetic variations influence variations in AOO of motor disturbances also in relationship to the CAG repeat number, we analyzed four ADAM10 SNPs (rs2305421A>G, rs4775083T>C, rs514049A>C and rs653765A>G) in a cohort of 55 Italian HD patients with known CAG repeat expansion, assessed by using PCR-based methods [2]. Twenty-three patients showing motor signs were then divided in 3 groups, based on AOO of motor signs and CAG repeat number. Group G1.1 includes patients with 45-48 CAG repeats and AOO<40 years; group G1.2, 45-48 CAG and AOO>40; group G2, 40-45 CAG and AOO>40. Allele and genotype distribution was analyzed within each group (c2 test) and compared among groups.In the cohort of 55 patients, allele frequency of the analyzed SNPs perfectly matched to that reported for Europeans. Statistical analysis revealed that only rs514049 genotype distribution significantly varies in G1.1 ($p<0,005$), with prevalence of the A allele ($p<0,007$). Therefore, allele A of SNP rs514049 could to be considered a worsening factor of HD. However, observational comparison among group G1.1 (early onset) and G1.2 (late onset) revealed that haplotype AC (rs653765-rs514049), which is associated to a lower level of ADAM10 protein [3], is prevalent in G1.2, suggesting it likely exerts a protective effect. Our preliminary results suggest a possible correlation between ADAM10 variants and onset of motor disorder in HD.

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P195
THE IMPORTANCE OF TNF GENETICS IN SPONDYLOARTHRITIS

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Background: Spondyloarthritis (SpA) are chronic inflammatory diseases resulting of a complex interplay among environmental factors and an inherited and permissive genetic background (mainly HLA-B27). TNF is primarily involved in these processes, supporting the beneficial effects of anti-TNF therapy.
The aim of this work was to investigate whether polymorphisms (SNPs) of the autoinflammatory genes MEFV and TNFRSF1A, or SNPs in the promoter region of TNFA are associated with SpA and/or response to treatment.
Aims: The aim of this work is to investigate whether polymorphisms (SNPs) in the promoter region of TNFA, or in TNFRSF1A and MEFV genes (involved in autoinflammatory diseases), concur with HLA-B27 in enhancing the risk of SpA and/or in predicting the response to anti-TNFα treatment.
Methods: 223 controls and 91 SpA (55 with Psoriatic Arthritis-PsA and 36 with Ankylosing Spondylitis- AS; 65/91 under TNFα inhibitor therapy) from the Veneto Region (Italy) were studied. TNFA polymorphisms (-1031T>C;-857C>T;-376G>A;-308G>A;-238G>A) and HLA-B27 were assayed by RT-PCR. Direct sequencing of MEFV (exons 2,3,5 and 10) and TNFRSF1A (exons 2,3,4 and 6) genes were performed.
Results: HLA-B27 was correlated with AS (χ^2=120.1; p=0.000). None of the studied TNFA SNPs was singly associated with SpA, while the haplotype C/G, resulting from -1031T>C/-308G>A combination, was significantly associated with a reduced risk of SpA (OR: 0.63, CI: 0.40-0.99; p= 0.047). Two SNPs were identified in TNFRSF1A, the R92Q (MAF=0.034) and c.625+10A/G (MAF=0.479). None of them was associated with SpA (p>0.05). The TNFRSF1A c.625+10 G allele was associated with late response to anti-TNFα therapy (p=0.031). Twenty-one SNPs were identified in MEFV gene, 10 with a known potential functional significance. Variant alleles were extremely rare in our population (Minor allele frequencies-MAF<0.025) except for R202Q (MAF=0.27). None was associated with SpA diagnosis (p>0.05).
Conclusions: TNFRSF1A and MEFV gene SNPs are not associated with SpA in the north-East of Italy. AS risk appears to depend not only on HLA-B27, but also on the TNFA haplotype -1031C/-308G. The TNFRSF1A c.625+10A/G impacts on the response to anti-TNFα therapy.

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VALUTAZIONE DEL VALORE DEL FILTRATO GLOMERULARE CALCOLATO (eGFR) NELLA POPOLAZIONE BOLOGNESE

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Un indicatore molto accurato dell'efficienza funzionale del rene è il filtrato glomerulare, poiché la sua misura è direttamente correlata al numero di nefroni integri e fisiologicamente attivi. Da alcuni anni sono state studiate formule matematiche che permettono una stima (eGFR) del filtrato glomerulare che ben si presta ad una campagna di screening per la malattia renale cronica (MRC). Esistono in Italia pochi studi di popolazione. Lo scopo di questo lavoro è di analizzare i dati di eGFR su un campione indicativo della popolazione bolognese.
Materiali e metodi: È stato studiato un campione di 1848 pazienti (691 di sesso maschile e 1157 di sesso femminile) con un'età compresa tra 18 e 70 anni; I parametri presi in considerazione per lo studio sono età, sesso, creatinina, eGFR (calcolato secondo la formula MDRD e CKD-EPI), glicemia ed emoglobina glicata. Questi ultimi due parametri sono serviti per individuare i soggetti con patologia diabetica, individuati con i criteri diagnostici della Società Italiana di Diabetologia. Per le analisi statistiche si è utilizzato il programma Stata versione 10.0 con un livello di confidenza α=0,05. Per le variabili in scala numerica abbiamo usato i test parametrici: t per campioni indipendenti e t per dati appaiati; per le variabili in scala nominale il test non parametrico del χ2. Risultati: Sono stati individuati 102 soggetti diabetici, gli altri 1746 individui (94,5%) sono classificati come non diabetici. La popolazione è stata stratificata per 5 fasce d'età: <30; 30-39; 40-49; 50-59 e >a 59 e per sesso. Per ogni fascia d'età si è valutato lo stadio della MRC, sulla base delle linee guida internazionali esistenti. Sono poi stati elaborati i dati sui soggetti diabetici.
Conclusioni: La stima del GFR è statisticamente diversa utilizzando le due equazioni, soprattutto nello stadio 2 della MRC. Data l'alta prevalenza di questa patologia nella popolazione generale uno screening di questo genere è in grado di incidere sulla programmazione sanitaria con un impatto positivo sull'outcome del pazienti e sui costi a carico del SSN.
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ALTERAZIONI DEL TRACCIATO ELETTROFORETICO IN PAZIENTI SOTTOPOSTI A TRAPIANTO RENALE

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Introduzione: Nei pazienti sottoposti a trapianto renale è frequente il riscontro di anomalie del tracciato elettroforetico, in particolare nella zona delle immunoglobuline. L'immunosoppressione e il conseguente aumentato rischio infettivo rivestono un ruolo importante nell'insorgenza di situazioni di alterato equilibrio immunologico. Lo studio del tracciato elettroforetico, analisi prevista nel follow-up di questi pazienti, può aiutarci nell'identificazione e classificazione di questi disequilibri.
Materiali e metodi: sono stati selezionati 78 pazienti di età compresa tra i 23 e i 76 anni (mediana 57) di cui 43 M (55%) e 35 F(44%), sottoposti a trapianto renale presso la UO Trapianti d'Organo del Policlinico Umberto I di Roma, anni 2015-16, con un follow-up di almeno 6 mesi. L'elettroforesi è stata eseguita su gel di agarosio con strumento INTERLAB G26. Nei tracciati sono state analizzate alcune alterazioni: presenza di addensamenti e/o bande, ipoprotidemia, ipogammaglobulinemia, ipergammaglobulinemia policlonale.
Risultati: I risultati evidenziano un'alta percentuale (62,8%) di anomalie nel tracciato elettroforetico di pazienti con esordio ad almeno 5 mesi dal trapianto; ad 11 mesi più della metà dei pazienti sviluppa alterazioni nella zona γ. Dei 78 pazienti 20 (25,6%) mostrano un'alterazione apparentemente monoclonale in γ di cui 4 (20%) presentano una discreta banda (probabile MGUS) e 16 (80%) un addensamento. Dei rimanenti 58 pazienti: 29 (50%) presentano un tracciato elettroforetico normale, 14 (24%) registrano ipoprotidemia, 11 (19%) presentano ipogammaglobulinemia, 3 (5%) ipergammaglobulinemia policlonale, 1 (2%) ipoalbuminemia. L'alterazione si riconferma nel tempo in 26 (53%) dei 49 pazienti, in 13 (26,5%) il tracciato si normalizza stabilmente, in 10 (20,5%) si ha una situazione discontinua.
Conclusioni: La costante terapia immunosoppressiva e la maggiore vulnerabilità di questi pazienti alle infezioni possono essere corresponsabili dell'alta percentuale di alterazioni riscontrate nel tracciato elettroforetico. Una analisi attenta di queste alterazioni è auspicabile per monitorarne l'eventuale evoluzione, in particolar modo per le anomalie non transitorie che dovranno essere sottoposte ad ulteriori indagini per essere tipizzate.

P198
INDIPENDENZA DALLA CONCENTRAZIONE DELLE URINE NELLA DETERMINAZIONE DEL RISCHIO DI AKI CON NEPHROCHECK

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Introduzione: Nephrocheck è un test per la valutazione precoce del rischio di danno renale acuto (AKI). L'esame si basa sulla valutazione contemporanea dell'inibitore tissutale della metalloproteinasi 2 (TIMP-2) e della proteina 7 legante il fattore di crescita simil-insulinico (IGFBP7) proteine espresse dalle cellule tubulari renali al momento dell'arresto del ciclo cellulare in fase G1. Il suo uso è previsto per pazienti ad elevato rischio di AKI (post Cardiochirurgia, ammissione in Terapia Intensiva, ICU) al fine della valutazione di tale rischio entro le successive 12 ore. Nephrocheck ha dato prova di fornire migliori sensibilità e specificità, rispetto a tutti i marcatori attualmente disponibili (Kashani et al. Critical Care 2013, 17:R25).Il dosaggio di sostanze nelle urine tuttavia è spesso condizionato dallo stato di idratazione del paziente. Scopo di questo lavoro è stato di valutare la relazione tra concentrazione di creatinina urinaria e AKIRisk.
Materiali e Metodi: Sono stati valutati 1312 campioni di routine pervenuti nel nostro laboratorio dal 1 giugno 2016 al 1 giugno 2017 per valutazione del rischio di AKI. In tutti i campioni sono stati dosati sia Nephrocheck (ASTUTE 140TM) che la creatinina urinaria (Dimension Vista, Siemens). Le stesse determinazioni sono state eseguite anche su un gruppo di 20 volontari sani.
Risultati: Nel gruppo di controllo (n=20) la correlazione tra AKIRisk e creatinina urinaria, mostra un andamento direttamente proporzionale (r= 0.85, p=0.001). L'AKIRisk è risultato inoltre positivo in 14 soggetti su 20. Nei 1312 pazienti provenienti da terapia intensiva e cardiochirurgia invece non è possibile dimostrare alcuna correlazione. Analizzando i pazienti suddividendoli in 3 classi, negativi (<0.3 , (ng/ml)2/1000) (710/1312) intermedi (tra 0.3 e 2.0 ng/ml)2/1000) (436/1312)e positivi (>2.0 (ng/ml)2/1000) (165/1312), solo nel primo gruppo si nota un lieve trend nella correlazione tra creatinina urinaria e nephrochek, mentre negli altri due gruppi non vi è correlazione.
Conclusioni: La concentrazione urinaria non ha relazione significativa con la stima dellAKIRisk ottenuta con il calcolo del Nephrocheck. Questo dato supporta l'uso del marcatore in ogni condizione di idratazione e capacità di filtrazione renale. Le positività nel gruppo di controllo risultano di difficile interpretazione, anche se già precedentemente descritte. Verosimilmente i pazienti in ICU e post Cardiochirurgia presentano caratteristiche particolari di idratazione indotta, tali da spiegare come i livelli decisionali debbano essere riferiti esclusivamente a questa particolare popolazione di pazienti.

P199
URINARY BIOMARKERS TIMP-2 AND IGFB7 IN HIV PATIENTS: PILOT STUDY

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Background: Human immunodeficiency virus (HIV) and antiretroviral therapy (ART) can damage the kidney. In this regard, HIV-infected patients are still at higher risk for both acute kidney injury (AKI) and chronic kidney disease (CKD). AKI is more frequent in HIV-patients than in the general population and the causes are many (opportunistic infections, kidney hypoperfusion and ischemia, rhabdomyolysis, urinary tract obstruction, drugs). Therefore, a baseline evaluation of renal function should be performed on the diagnosis and initiation or change of pharmacological therapy. We have first evaluated the combination of urinary tissue inhibitor of metalloproteinases-2 (TIMP-2) and insulin-like growth factor binding protein 7 (IGFBP7), Nephrocheck test, on HIV patients for the identification of patients at risk of AKI at 12 hours compared with serum creatinine.
Methods: We enrolled 8 HIV adult patients (5 men and 3 women) admitted to Department of Infectious Diseases, University Hospital of Palermo. Patients with preexisting chronic kidney disease (CKD) and hospital acquired AKI were excluded. AKI was defined on the basis of the KDIGO critera. Blood samples were collected at admission for the determination of biochemical parameters. Urine sample were also taken for AKIRisk score measurement.
Results: The results showed a mean [TIMP-2] x [IGFBP7] concentration of 0.20 (SD 0.16) $(ng/ml)^2/1000$ and mean creatinine concentration of 0.79 (SD 0.32) mg/dl. No patient developed AKI during the hospitalization period. No change in the AKIRisk was observed with use of ART.
Discussion: In the validation study, a cut-off of AKIRisk score >0.3 $(ng/ml)^2/1000$, identify patients at high risk for developing moderate to severe AKI within 12 hours. In our study no HIV patient developed kidney failure during the hospitalization period, as demonstrated by the AKIRisk values. The main limitation of this study is the small number of subject included, so further researches are needed in order to clarify the usefulness of AKIRisk score in the HIV adult patients.
Reference: Booth JW, Post FA. HIV and the kidney in the acute medical unit. Clin Med (Lond) 2015;15(6):571-6.

P200
QUANTIFICATION OF URINARY TIMP-2 AND IGFBP-7 AS EARLY BIOMARKERS IN CRITICALLY ILL PATIENTS

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Background: Acute kidney injury (AKI) is one of the most common organ dysfunctions in critically ill population, associated with increased risk for end-stage renal disease and significant morbidity and mortality. The risk stratification remains very difficult, mainly due to available diagnostic tests for AKI. In the 2014, the combination of two new markers, tissue inhibitor of metalloproteinases-2 (TIMP-2) and insulin-like growth factor binding protein 7 (IGFBP7), was approved by the US Food and Drug Administration (FDA) to identify patients at high risk of developing AKI.
Methods: We enrolled 28 critically ill adult patients admitted to the ICU , University Hospital of Palermo, 21 men (75%) and 7 women (25%) with median age of 67.5 years (IQR 49-78). Severity of illness was determined using tha Simplified acute physiology score II (SAPSII) with median and the Sequential Organ Failure Assessment (SOFA) score. AKI was defined on the basis of the Kidney Disease: Improving Global Outcomes (KDIGO) critera. Blood samples were collected at admission for the determination of biochemical parameters. Urine sample were also analyzed for TIMP-2 and IGFBP7 using a clinical immunoassay (NephroCheck test , VITROS 5600®,Ortho Clinical Diagnostics). Nephrocheck measurements and were performed at admission (H_0) and 4 (H_4), 12 (H_{12}), 24 (H_{24}), 36 hours (H_{36}) later for blood determination of creatinine (sCR).
Results: The study population consisted of 28 patients. Five patients (18%) experienced AKI, whereas twenty-three (82%) had no AKI. In the AKI group AKIRisk score was higher at the 4th hour (1.11 vs 0.29 $[(ng/ml)^2/1000]$, $p = 0.045$) than at the admission (0.96 vs 0.42 $[(ng/ml)^2/1000]$, $p = 0.15$) in comparation with the no AKI patients.
Discussion: In recent studies, a cut-off of AKIRisk score >0.3 $(ng/ml)^2/1000$, identify patients at high risk for developing moderate to severe AKI within 12 hours. Our results highlight the importance of investigate the AKIRisk's kinetics for tackle the acute kidney injury.
Daubin D, Cristol JP, Dupuy AM, et al. Urinary Biomarkers IGFBP7 and TIMP-2 for the Diagnostic Assessment of Transient and Persistent Acute Kidney Injury in Critically Ill Patients. PLoS One 2017;12:e0169674.v

P201

IL TRATTAMENTO IPOCOLESTEROLEMIZZANTE RISTABILISCE I LIVELLI DI METILAZIONE GLOBALE DEL DNA IN PAZIENTI CON MALATTIA RENALE CRONICA (CKD)

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La gestione dell'ipercolesterolemia rappresenta un obiettivo importante per ridurre il rischio di CVD in pazienti CKD. L'uso combinato di simvastatin/ezetimibe nella terapia, riduce lo stato infiammatorio e la concentrazione dei markers plasmatici di disfunzione endoteliale attraverso una riduzione dello stress ossidativo (OS). [1] Lo scopo del lavoro è stato quello di valutare la concentrazione di mCyt nel DNA di pazienti CKD sottoposti a diverse terapie ipocolesterolemizzanti e verificare se il miglioramento dello OS durante il trattamento farmacologico fosse associato ad una modifica del pattern di metilazione del DNA. Sono stati reclutati 30 pazienti CKD (età 60.2±10.5 anni), randomizzati in 3 gruppi sottoposti a trattamento di 12 mesi con: 40mg/die di sim (gruppo 1, n=10), eze/sim 10/20mg/die (gruppo 2, n=10) o eze/sim 10/40mg/die (gruppo 3, n=10). I pazienti sono stati valutati al reclutamento dopo 4, 8 e 12 mesi di terapia. È stato inoltre reclutato un gruppo di controllo di 30 soggetti (età 59±10anni, 19 maschi).È stato osservato un significativo miglioramento nel profilo lipidico in tutti i gruppi, e una riduzione di parametri di OS come la Malondialdeide (MDA) e il rapporto Allantoina/Acido Urico (All/UA). Nei pazienti CDK è stata riscontrata una riduzione di mCyt rispetto ai controlli sani (4.06±0.20% vs. 4.27±0.17%, p=0.0001). Il trattamento farmacologico ha determinato un aumento nei livelli di mCyt in tutti i pazienti (4.06±0.04% al basale; 4.12±0.03% a 4 mesi; 4.17±0.03% a 8 mesi; 4.20±0.02% a 12 mesi) portando i livelli della metilazione globale verso concentrazioni simili a quelle presenti nei controlli sani dopo 12 mesi (4.20±0.02% vs 4.27±0.17%, p›0.05). L'aumento maggiore è stato riscontrato nel gruppo 3 (+5.2% dopo 1 anno). Il trend in crescita della metilazione durante la terapia risultava significativamente correlato alla riduzione della concentrazione della MDA (r=–0.987, p=0.013) e del rapporto All/AU (r=–0.983, p=0.017). I dati ottenuti suggeriscono che la terapia ipocolesterolemizzante nei pazienti CKD ristabilisce un pattern di metilazione del DNA simile a quello osservato in soggetti sani. L'incremento della mCyt è associato ad una concomitante riduzione dei parametri di OS.

1. Zinellu A, Sotgia S, Mangoni AA, et al. J Pharm Biomed Anal 2016;129:383-8.

P202

EFFETTI DEL RAMIPRIL E DEL TELMISARTAN SULLE CONCENTRAZIONI PLASMATICHE DEI TIOLI A BASSO PESO MOLECOLARE, DEI TIOLI PROTEICI E DEI VALORI DI IMT CAROTIDEA IN PAZIENTI CON MALATTIA RENALE CRONICA (MRC)

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Numerosi studi hanno dimostrato che il mantenimento di adeguati valori pressori rappresenta la chiave per prevenire esiti avversi in pazienti con malattia renale cronica [1]. Le linee guida indicano che tale controllo può essere esercitato sul paziente nefropatico mediante trattamento con inibitori del sistema renina-angiotensina (RAS): sia attraverso l'impiego di inibitori dell'enzima di conversione dell'angiotensina (ACEI) sia con antagonisti del recettore per l'angiotensina II (ARB). Obiettivo di questo studio è stato quello di valutare e comparare gli effetti di un trattamento farmacologico della durata di 6 mesi con ARB con quelli di una combinazione ARB/ACEI sui valori di spessore medio-intimale (IMT) della carotide e sui livelli plasmatici dei marker di stress ossidativo (LMW thiols, Hcy, Cys e PSH) in pazienti ipertesi con MRC non sottoposti a dialisi. 24 pazienti sono stati randomizzati in due gruppi e trattati con telmisartan (80 mg/die) e con una combinazione telmisartan/ramipril (40/5 mg/die). Al basale i pazienti presentavano elevati valori plasmatici di Hcy e Cys; l'IMT della carotide era inversamente correlato alle concentrazioni di PSH (r= –0.42, p= 0.039). Al termine del trattamento è stata osservata una riduzione dei valori pressori nei due gruppi. Entrambi i trattamenti non hanno avuto nessun effetto sulla concentrazione dei LMW thiols, sia in forma ossidata sia ridotta, così che lo stato redox degli stessi non ha subito modificazioni. Le concentrazioni di PSH sono aumentate nel gruppo telmisartan/ramipril (mediana baseline: 3.59 μmol/g prot; mediana post-trattamento: 4.66 μmol/g prot, p= 0.015), con miglioramento del quadro ossidativo, mentre sono rimaste invariate nel gruppo telmisartan (mediana baseline: 4.61 μmol/g prot; mediana post-trattamento: 4.43 μmol/g prot). L'IMT della carotide è diminuito in entrambi i gruppi. Dato che al baseline il PSH era l'unico parametro associato all'IMT, abbiamo ipotizzato che cambiamenti nell'IMT possano essere mediati, almeno in parte, dalla riduzione dello stress ossidativo. Ulteriori studi su coorti più estese di pazienti sarebbero necessari per confermare tale ipotesi.

1. Turnbull F, et al. Lancet 2003;362:1527-35.

P203
UNA PROPOSTA ALTERNATIVA PER GESTIRE L'ESAME MORFOLOGICO DEL SEDIMENTO URINARIO (EMSU) E AUMENTARNE L'APPROPRIATEZZA

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Introduzione: L'EMSU rimane un ausilio fondamentale nella diagnostica delle patologie renali. L'approccio tradizionale ne prevede l'abbinamento con l'esame chimico-fisico (ECF), con ricadute sui carichi di lavoro e conseguente ricerca di totale automazione nella sua esecuzione. Nel considerare l'EMSU come un esame specialistico, accuratamente eseguibile solo con una specifica professionalità, dal 2005 il nostro laboratorio ne ha deciso l'esecuzione ai pazienti degenti solo in seguito a richiesta specifica. A distanza di 11 anni, abbiamo voluto analizzare l'impatto a lungo termine di questo approccio sul servizio fornito.
Metodi: Sono stati valutati i risultati nel periodo 2009-2016. In questo periodo, il nostro ospedale non ha subito modifiche sia nel numero dei letti di degenza che nel case-mix. Abbiamo anche inviato un questionario di gradimento (QG) ai reparti richiedenti.
Risultati: Nel periodo 2009-2013 sono stati richiesti una media di 2264 EMSU/anno (intervallo: 1825-2802). Se confrontato con il numero degli ECF richiesti nello stesso periodo (10.204/anno; intervallo: 9678-11.168), questo corrispondeva a un rapporto medio del 22,2%. Dal 2014, a seguito di una nuova modalità di accettazione informatica dell'EMSU, con il suo spostamento nella pagina degli esami di II livello, si è registrata un'ulteriore diminuzione delle richieste (media/anno: 923), che non si associava a una diminuzione di ECF (media/anno: 9811) (media EMSU/ECF, 9,3%). Le richieste di EMSU pervenivano principalmente da Pediatria (47%), Nefrologia (20%) e Reumatologia (18%). Per quanto riguarda i risultati ottenuti, solo il 10,4% era refertato come "nulla di patologico", dimostrando l'impiego del test come esame mirato. I quadri prevalenti erano: "1-5 eritrociti/campo" (14.5%), "1-5 leucociti/campo" (13%) e presenza di batteri (11,2%). I QG hanno valutato il servizio fornito in modo soddisfacente, mettendo d'altro canto in luce alcune criticità di tipo preanalitico a carico di alcuni campioni pervenuti oltre 2 ore dalla raccolta.
Conclusioni: La modalità di gestione dell'EMSU da noi implementata riduce drasticamente il numero delle richieste e aumenta l'appropriatezza della richiesta. I risultati del QG indicano che ciò è ottenuto senza alcun impatto negativo sulla cura dei pazienti.

P204
EFFECTS OF GENETIC BACKGROUND ON CARDIOVASCULAR RISK FACTOR MODIFICATION AFTER TRAINING AND COMPETITION IN WATER POLO PLAYERS

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Recent studies showed that intense physical activity induces oxidative stress dependently on type, intensity, volume, and duration of muscle contraction, and may increase the susceptibility to adverse cardiovascular outcomes. Oxidative stress, as well as hyperhomocysteinemia (HHcy), another established cardiovascular risk factor, develop more easily in subjects having an unfavourable genetic background. Here we aimed to assess the effects of single nucleotide polymorphisms (SNP) in genes encoding for the enzymes methylenetetrahydrofolate reductase (MTHFR C677T, A1298C), paraoxonase 1 (PON1 Q192R), superoxide dismutase2 (SOD2 A16V), catalase (CAT -844 G>A), and glutathione peroxidase-1 (GPx-1 rs1800668 C>T), on the modification of oxidative stress and cardiovascular risk markers in 28 elite water polo male players prior to and after a routinely programmed friendly match. The mean plasma concentrations of derivatives of reactive oxygen metabolites (dROMs), advanced oxidation protein products(AOPP), homocysteine (Hcy), lactic dehydrogenase (LDH) activity, creatine kinase (CK) activity, CK-MB, and myoglobin, were above the reference range in resting athletes, and significantly increased after competition, while blood antioxidant potential (BAP) and total free thiols were significantly decreased, in comparison with those measured before exercise. Water polo players with either SOD heterozygous AV16 or homozygous mutated VV16 genotype exhibited a significant increase of post-exercise plasma concentrations of AOPP, LDH, CK, and myoglobin in comparison with wild-type athletes. Athletes with either CAT -844 GA or GPx1 CT genotype showed a significant increase of post-exercise plasma levels of dROMs and, respectively, of GPx and CAT enzyme activities in comparison with wild-type subjects. The highest Hcy and AOPP values were found in athletes with either MTHFR CT/AC or TT/AA genotype, and PON1 QR192 genotype, respectively. CK and CK-MB increased at a higher extent in CT/AC or TT/AA subjects than in athletes having other MTHFR genotypes.Our preliminary results suggest that the screening for these gene variants in water polo players is useful to assess individual susceptibility to cardiovascular disorders and should be included in prevention programs.

P205
MEASUREMENT OF PLASMA METANEPHRINES AND NORMETANEPHRINE BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC-MS/MS) IN RECREATIONAL ATHLETES

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Background: Although comprehensive data are available on the behaviour of several biochemical markers of physiological function following strenuous exercise, less is known on kinetics of new adrenergic test such as metanephrines and normetanephrine in athletes performing a sub-intensive aerobic physical exercise, such as a half-marathon run. Measurements of metanephrines in plasma are the tests of first choice because they have higher diagnostic accuracy than catecholamines or other metabolites due to long half-life in plasma. The aim of this study was to evaluate adrenergic responses in peripheral blood of recreational running athletes by using a new Liquid chromatography-Mass Spectrometry (LCMS) for measuring plasma metanephrines.
Materials and Methods: The study population consisted of 16 healthy trained Caucasian athletes (9 males and 7 females) who successfully concluded a 21.1 km half-marathon at 75–85% of their VO2max. Blood samples were collected before the run (T0), immediately after (post) (T1), 6 hour (T2), and 24 hour after (T3). The metanephrines and normetanephrines were evaluated on the new LCMS AbSciex 4500 with the new kit Recipe ClinMass® Complete Kit.
Results: A significant post-run (T1) increases were observed for plasma metanephrines and normetanephrine ($p<0,005$). The median and interquartile range for metanephrines and normetanephrine at different time point were: 138 pmol/L (127-164) at T0, 389 pmol/L (238-428) at T1, 96 pmol/L (89-129) at T2, 124 at T3 and 523 pmol/L (498-610) at T0, 2925 pmol/L (2522-3481) at T1, 841 pmol/L (668-1056) at T2, 657 pmol/L (522-728) at T3 respectively.
Conclusions: These results, which can be interpreted as a physiologic reaction under our experimental conditions, describe the biological variation of biogenic amines in recreational athletes.

P206
VALUTAZIONE DELL'ATTENDIBILITA' DELLA MISURA DELL'EMOGLOBINA TOTALE IN POCT

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La diffusione dei POCT ha permesso di soddisfare le esigenze del clinico in regime di urgenza, di avere esami in tempi rapidi e di qualità, per una corretta diagnosi ed un'efficace terapia. Con lo scopo di valutare l'attendibilità della misura del parametro Emoglobina Totale effettuata nei reparti dell'Azienda USL di Livorno, parte dell'area Nordovest, attraverso l'emogasanalizzatore mod. GEM Premier 4000 (Instrumentation Laboratory - Werfen) sono stati raccolti dalla normale routine in duplicato 1651 campioni in quattro reparti (U.O. di Pronto Soccorso di Cecina, Livorno, Piombino e Portoferraio) ed analizzati con l'emogasanalizzatore mod. GEM Premier 4000 in dotazione e con l'analizzatore di ematologia in Laboratorio mod. XE-2100 (DASIT Sysmex). L'analizzatore GEM Premier 4000 fornisce la misura quantitativa dell'Hb totale attraverso CO-Ossimetro integrato (metodo spettrofotometrico), mentre il sistema XE 2100 Sysmex di Laboratorio utilizza metodo spettrofotometrico a 555 nm. Tutti i campioni di sangue intero sono stati prelevati utilizzando siringhe eparinate per emogasanalisi per la determinazione su sistema GEM Premier 4000 in reparto e con provette K2-EDTA per l'analisi con sistema XE-2100 (DASIT Sysmex) in Laboratorio, rispettando tempistiche di pochi minuti tra i due prelievi e le norme per una corretta fase preanalitica. L'analisi statistica attraverso il calcolo della regressione lineare e il modello non parametrico di Passing-Bablock dei risultati ottenuti ha mostrato un'ottima correlazione tra i due strumenti, nonostante metodologie di misura differenti. La regressione lineare ha mostrato uno slope di 0,97 con intercetta di 0,36 e un indice di correlazione R^2 pari a 0,93. L'analisi con Passing-Bablok ha individuato uno slope pari a 1, con valore dell'intercetta di 0. Il numero di dati statisticamente rilevanti, la distribuzione dei valori su un'ampia scala di lettura (da 4,5 a 19,6 g/dL) e l'analisi dei campioni di reparto su quattro emogasanalizzatori differenti (mod. GEM Premier 4000) permette di affermare che le misure di tHb ottenute in reparto sono affidabili e perfettamente allineate con quelle del Laboratorio centrale.

P207
SCREENING DELLE BATTERIURIE: IL RUOLO DEI CONTAMINANTI NELLA DEFINIZIONE DEI CUT-OFF DI POSITIVITÀ

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Introduzione: L'U.O.ha attivato uno screening delle batteriurie con cut-off dedicati alla popolazione afferente e alla valutazione di possibili contaminanti.Scopo:Lo studio ha visto due momenti di valutazione:1)definizione di cut-off di positività e negatività per lo screening delle batteriurie; 2) valutazione dei campioni risultati falsi positivi e/o contaminati.
Materiali e metodi: Nella prima fase dello studio sono stati valutati 554 campioni di urine di pazienti sia esterni che ospedalizzati. Tutti i campioni sono stati prima analizzati con SEDIMAXconTRUST e in seguito seminati mediante ansa calibrata da 10 μl sui terreni CNA e CPS. Dalla valutazione combinata dei parametri bact, wbc, yeast e epi in associazione all'esame colturale sono stati definiti cut-off specifici di popolazione [Bact 34-110 WBC<5 neg, Bact<34 WBC>50 pos, Bact>110 pos, YEAST 3 pos]. In questa fase il 20% dei campioni sono risultati essere falsamente positivi e/o contaminati alla verifica colturale. Nella seconda fase dello studio, intercorsa fra Agosto-Settembre 2016, sono stati presi in esame ulteriori 179 campioni per meglio definire i falsi positivi e/o i contaminati dai reali positivi. Anche in questo caso sono stati confrontati i parametri bact, wbc, yeast e epi in associazione all'esame colturale. Da questa seconda analisi è emerso come il cut-off di positività Bact<34 e WBC>50 sia gravato dal un alto numero di falsi positivi, la modifica del parametro WBC>110 ha determinato un recupero del 10% dei falsi positivi. Altro elemento importante di valutazione sono state le epi >=17 quale elemento utile nella definizione di campione contaminato.
Conclusioni: Gli elevati standard e la rapidità di esecuzione in associazione all'esame microbiologico ha permesso di definire cut-off di positività, negatività e contaminazione specifici per la popolazione afferente all'AUSSL 9 Bussolengo. L'attenzione rivolta a fattori interferenti ha portato ad una meglio definizione dei reali positivi e a una migliore performance operativa. Tale studio ha permesso di esprimere al laboratorio una valutazione di idoneità del campione, in fase di screening ,riducendo i costi diagnostici e le risorse umane con un TAT ridotto ed appropriato.

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COMPARAZIONE NELLA MISURA DEGLI ELETTROLITI (SODIO E POTASSIO) E GLUCOSIO TRA L'EMOGASANALIZZATORE IN POC E LA MISURA IN LABORATORIO CENTRALE

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L'emogasanalisi svolge un ruolo fondamentale nella gestione dei disturbi dell'equilibrio acido-base di origine respiratoria o metabolica, in particolare nei pazienti critici. Scopo di questa analisi è confrontare le determinazioni di parametri fortemente raccomandati in Pronto Soccorso come Sodio, Potassio e Glucosio eseguiti in reparto con l'EGA in uso mod. GEM Premier 4000 (Instrumentation Laboratory - Werfen) e le determinazioni ottenute in Laboratorio. GEM Premier 4000 fornisce la misura di Sodio e Potassio mediante elettrodi Ione-selettivi a potenziometria diretta, e del Glucosio con metodo amperometrico. Le performance analitiche dell'EGA sono state comparate con il sistema di riferimento in uso nei Laboratori in chimica clinica mod. ARCHITECT c8000 (Abbott), con metodo a potenziometria indiretta per la misura di Na+ e K+ ed esochinasi - G6PDH per la determinazione del Glu. Sono stati analizzati più di 2300 campioni provenienti dalla normale routine dei Pronto soccorso dell'Azienda USL di Livorno, parte dell'area Nordovest, e analizzati dai laboratori di pertinenza dei diversi P.O. I campioni sono stati prelevati in provetta con litio eparina per gli esami di laboratorio e in siringa eparinata per l'esecuzione dell'EGA eseguita direttamente in reparto. I prelievi sono state eseguiti in contemporanea per i campioni destinati ai due strumenti. La valutazione dell'agreement è stata effettuata su base grafica e su base statistica (Passing-Bablok). I dati ottenuti evidenziano un'ottima concordanza tra i due analizzatori, gli indici di correlazione R^2 risultano per Na + pari a 0,90, K+: 0,81 e Glu: 0,96, e l'analisi di Passing-Bablok evidenzia uno slope pari a 1 per Na+ e K+, e pari a 1,009 per Glu. Inoltre la media delle differenze dei valori di tutti i risultati ottenuti con GEM Premier 4000 e ARCHITECT risulta per il parametro Sodio pari a - 0,41 e per Potassio pari a – 0,032, consistente con un bias dovuto ai diversi metodi (potenziometria indiretta e diretta). L'analisi dei nostri risultati ha mostrato un'ottima correlazione tra i due strumenti, confermando l'attendibilità dei valori ottenuti con l'EGA mod. GEM Premier 4000 in POCT.

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HOMOCYSTEINE DETERMINATION: POSSIBLE INTERFERENCE FROM METHIONINE

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Background: Homocysteine (Hcy) is an aminothiol; its concentration increases both in pathological and physiological conditions. Deficiency of vitamins B6 and B12 and folate also cause an increase of Hcy concentration that will restore with vitamin supplementation. Hcy determination can be performed both fasting and after methionine loading; methionine administration allows to detect about 40% more patients with defective Hcy metabolism than those detectable by the basal Hcy level.
Aim: Verify the possible interference of methionine in a method for determination of Hcy.
Materials and methods: 136 samples from routine, withdrawn both fasting (n=125) and after methionine loading (n=11), were immediately centrifuged and processed (undiluted and diluted 1/10 with saline) with Roche reagent. The samples were measured undiluted also with another reagent (Sentinel). We performed paired t-test to verify the significance of the difference between results without and with dilution, and we compared the results obtained with the different methods by linear regression (Passing - Bablok); furthermore we calculated the agreement by Bland Altman plot.
Results: Mean Hcy concentration in fasting samples: 15.8 µmol/L (undiluted samples) and 16.2 µmol/L (diluted 1/10); paired t-test: p = 0.068. Mean Hcy concentration after methionine loading: 37.3 µmol/L (undiluted samples) and 50.7 µmol/L (diluted 1/10); paired t-test: p = 0.001. Linear regression, undiluted fasting samples (125): Roche= 0.96 Sentinel + 2.2; post-methinine loading samples: Roche= 0.67 Sentinel + 7.4. Post-methionin loaging samples diluted 1/10: Roche = 1.03 Sentinel – 1.28. Bland Altman plot: undiluted samples determined with Roche reagent versus undiluted samples with Sentinel reagent showed a bias = -11.2 µmol/L; samples diluted 1/10 determined with Roche reagent versus undiluted samples measured with Sentinel reagent showed a bias = 0.3 µmol/L.
Conclusion: Methionine is an intermediate product in the reaction scheme of Roche method (but not in Sentinel method). Its excess may slow down the reaction explaining the negative methionine interference, that can be overcome by a preliminary sample dilution 1/10 with saline. Our indirect demonstration needs to be confirmed with direct studies.

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EFFETTI PRE-ANALITICI DELLA MOVIMENTAZIONE DELLE PROVETTE SULLA LATTATO DEIDROGENASI PLASMATICA

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L'utilizzo di gel separatore nelle provette per campioni di sangue determina la convinzione che il materiale possa essere maneggiato liberamente dopo l'avvenuta centrifugazione. Noi abbiamo osservato come sia facilmente ottenibile una risospensione parziale degli eritrociti con conseguente incremento spurio dei livelli di Lattato Deidrogenasi (LAD) anche a seguito di movimentazioni modeste. Abbiamo quindi valutato il confronto di questi potenziali artefatti tra il gel separatore SST II (Becton Dikinson, Milano, I) ed il recente polimero Barricor dello stesso fornitore, entrambi su provette eparinate del formato 13x75 mm.
Materiali e metodi: ad un gruppo di 35 soggetti volontari sono stati prelevati da personale esperto con ago retto 6 ml di sangue, suddivisi nelle due tipologie di provette contenenti eparina di litio. Dopo una centrifugazione standard di 10 minuti a 3000g è stata misurata la LAD (analizzatore AU 5800 Beckman Coulter, Cassina de Pecchi, I). Un gruppo di coppie di provette ha successivamente subito un movimento singolo omogeneo che le ha portate a 90° (orizzontali) e riportate subito dopo verticali; un altro gruppo ha invece subito un singolo rovesciamento completo a 180 gradi. Una seconda determinazione di LAD è stata eseguita immediatamente dopo la movimentazione. E' stata pure valutata la movimentazione automatica con due diversi sistemi pre-analitici automatizzati. Risultati: i valori di LAD misurati hanno mostrato i seguenti risultati di incremento della media dei singoli campioni: provette SST II a 90° +12,5%, a 180° +14,2%; provette con Barricor a 90° +6%, a 180° +5,5%. Il confronto tra le singole coppie di risultati (basali e dopo movimento) è stato significativo nei 4 casi ($p<0,01$). Anche il confronto tra le due tipologie di materiale sintetico separatore è risultato significativamente diverso con risultati spuri più contenuti per il Barricor ($p<0,01$). Abbiamo anche dimostrato che il fenomeno non è dovuto ad emolisi degli eritrociti.
Conclusioni: 1- anche movimentazioni limitate pregiudicano la corretta determinazione di LAD; 2- il nuovo materiale Barricor limita comunque questo effetto indesiderato.

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DETECTION OF BENCE JONES PROTEIN AND PROTEINURIA IN A SINGLE IMMUNOELECTROPHORETIC DIAGNOSTIC LANE

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Introduction: The research of Bence Jones protein (BJP) is indicated in patients with plasma cell dyscrasias. The recommended method for detection of BJP is urinary immunofixation (uIFE). An antiserum consisting of a mix of anti-K total, anti-L total, anti-K free, anti-L free, produced by BIOCI (Torino, Italy), with a single immunoelectrophoretic lane can detect a monoclonal band (MB) in a urine sample, to be lately confirmed by uIFE. The BIOCI antiserum is also composed by a mix of anti-alpha1-microglobulin, anti-albumin and anti-immunoglobulins, respectively indicators of tubular proteinuria and of selective and non-selective glomerular proteinuria, providing overall indications of renal damage.The aim of this work is to evaluate the diagnostic accuracy of BIOCI antiserum to detect MB in urine samples, and to assess its ability to provide qualitative information on different types of proteinuria of nephrological value.
Methods: 234 consecutive non-concentrated urine samples were collected with the request for both BJP and urinary electrophoresis (UPE) assay. Samples were analysed using simplified uIFE (anti-GAM, total anti-K, anti-L total antisera - Sebia) and UPE with HR-AGE (Sebia), SDS (Sebia) and with BIOCI on agarose gel (Sebia).
Results: The results of MB assay were the following: 162 urine samples were negative by uIFE, and 167 by BIOCI; 72 urine samples were positive by uIFE, and 67 were positive by BIOCI. The samples uIFE+ and BIOCI- were 7, while the uIFE- and BIOCI+ were 2. BIOCI Sensitivity was 90.3%, Specificity 98.8%, Kappa = 0.91, McNemar p = 0.18.The proteinuria assessment, for each type of test, provided the following results: positive and negative proteinuria samples were respectively for BIOCI 106 vs 128; for HR-AGE 72 vs 162; for SDS 72 vs 162.
Conclusions: BIOCI is an appropriate screening test to detect MB-negative urine samples while only positive ones must be submitted for further confirmation. The McNemar test confirm a non-statistical significant difference between uIFE and BIOCI test. Compared with the other methods, BIOCI test provide strong suggestion of proteinuria of nephrological relevance, by using antisera against specific protein markers even in non-concentrated urine samples.

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INDAGINE DELLE PRESTAZIONI ANALITICHE DELLO STRUMENTO PER IMMUNOCHIMICA ADVIA CENTAUR XPT

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Introduzione: Tra i compiti del Laboratorio clinico è di centrale importanza la valutazione, indipendente e “on the field”, di metodiche e strumentazioni presenti sul mercato della diagnostica. Il Laboratorio è tenuto a seguire procedure e norme professionali in ambito clinico, soprattutto quando si tratta di metodi analitici immunochimici, in generale non ben definiti sul piano metrologico.Obiettivo del lavoroScopo di questo lavoro è la comparazione tra due sistemi analitici di immunochimica al fine di valutare se il nuovo metodo consente uguali o migliori prestazioni analitiche del metodo in uso, definendo, se necessario, nuovi intervalli di riferimento e nuovi livelli decisionali.
Materiali e Metodi: Campioni di siero, di pazienti afferenti al Servizio Analisi Chimico-Cliniche nel marzo 2017, sono stati testati con due diversi sistemi analitici di immunochimica (Immulite 2000 XPi e ADVIA Centaur XPT) e i risultati ottenuti (concentrazione di estradiolo, folato, FT3, FT4, FSH, LH, la Prolattina, Testosterone, TSH e Vitamina B12) sono stati confrontati mediante curve di correlazione e diagrammi di differenza (Method Verification Protocol V4.0.6).
Risultati: La relazione esistente tra i risultati ottenuti, in termini entità e direzione, è espressa mediante coefficiente di correlazione di Pearson (r). Gli indici di correlazione ottenuti sono i seguenti: 0.988 per estradiolo,0.994 per TSH, 0.976 per FSH, 0.965 per prolattina, 0.953 per vitamina B12,0.951 per LH, 0.941 per testosterone, 0.917 per folato, 0.858 per FT4 e 0.797 per FT3.
Discussione: Dalla comparazione dei risultati ottenuti, si evince che il nuovo sistema analitico consente di migliorare drasticamente le tempistiche di refertazione per la minore durata di esecuzione dei test e per la maggior linearità dei metodi. Infatti in tutti i metodi esaminati, a parte il dosaggio di FT3, i range analitici sono più ampi su ADVIA Centaur XPT o sovrapponibili. Inoltre, con il nuovo sistema, il volume di campione necessario risulta inferiore per tutti i dosaggi tranne per Estradiolo e Folato e la stabilità a bordo dei reattivi risulta paragonabile nei due sistemi valutati. In conclusione, il nuovo sistema apporterebbe una riduzione dei tempi di esecuzione dei dosaggi e una maggiore sensibilità funzionale, molto importante soprattutto nel caso di pazienti di giovane età e nelle pazienti sottoposte a procreazione medico-assistita.

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CONFRONTO DI METODI PER LA DETERMINAZIONE DEL CORTISOLO URINARIO

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Solo una piccola parte del cortisolo plasmatico è biologicamente attivo, non legato alle proteine di trasporto. Il cortisolo libero filtra attraverso i glomeruli ed è eliminato con le urine. La sua misura nelle urine correla con la frazione libera del sangue. Diversi metodi immunometrici estrattivi e non estrattivi sono disponibili per la determinazione del cortisolo libero urinario (CLU). L'estrazione della molecola con solventi come il diclorometano dovrebbe ridurre le interferenze dovute alla presenza dei numerosi metaboliti del cortisolo in grado di cross-reagire con l'anticorpo impiegato nel dosaggio e rendere la matrice urinaria più simile a quella sierica. Le procedure di estrazione sono però laboriose, espongono a rischio chimico gli operatori e introducono un'importante variabile pre-analitica. L'analisi dei report delle VEQ del College of American Pathologists degli anni 2015 e 2016 sembra tuttavia suggerire che metodi estrattivi e non estrattivi siano comparabili ed intercambiabili.
Obiettivo: valutare la confrontabilità tra metodi estrattivi e diretti anche su campioni di urine reali.
Materiali e metodi: sono stati analizzati 80 campioni di urine 24/h con 2 metodi estrattivi (Liaison DiaSorin e COBAS Roche) e 2 metodi diretti (ADVIA Centaur Siemens e Architect Abbott). Le estrazioni sono state condotte con diclorometano secondo le raccomandazioni del produttore (procedure diverse per i due metodi).
Risultati: coefficiente di correlazione, intercetta e pendenza, bias e limiti di agreement sono stati, rispettivamente: COBAS vs Liaison 0.695; -7.99; 1.12; Centaur vs Liaison 0.538; 12.78; 4.45; Architect vs Liaison 0.649; 2.01; 0.78; Architect vs Centaur 0.649; 2.02; 0.78; COBAS vs Centaur 0.299; -6.94; 0.22; COBAS vs Architect 0.308; -14.83; 1.63. Sono stati classificati sopra cut-off rispettivamente 4 campioni per Liaison, 2 Cobas, 9 Centaur, 0 Architect.
Conclusioni. Le misure su campioni umani non confermano la confrontabilità delle misure tra i metodi. Pur avendo analizzato campioni in prevalenza di soggetti senza patologie surrenali, marcate differenze si sono osservate tra i metodi estrattivi e non estrattivi. I metodi estrattivi non correlano tuttavia a sufficienza nemmeno tra loro, determinando significative differenze nella classificazione dei pazienti. Ciò fa supporre che la variabile preanalitica dell'estrazione rappresenti una importante fonte di possibile errore. L'agreement rilevato dai programmi di VEQ è verosimilmente determinato da una matrice priva di sostanze interferenti. La standardizzazione dei metodi è dunque un problema critico e soluzioni alternative come la spettrometria di massa o l'uso di una matrice diversa come la saliva potrebbero essere la soluzione.

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VALUTAZIONE DI UN NUOVO METODO IMMUNOTURBIDIMETRICO PER LA MISURA DELLA CALPROTECTINA FECALE IN AUTOMAZIONE

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Introduzione: La calprotectina fecale (CF) è una proteina il cui dosaggio ha un ruolo importante in gastroenterologia per la diagnosi e il monitoraggio delle malattie infiammatorie intestinali di origine organica, sia in soggetti adulti che pediatrici. In questo studio abbiamo valutato un nuovo metodo immunoturbidimetrico [particle enhanced turbidimetric immunoassay (PETIA)] automatizzato su analizzatore Beckman Coulter AU480.
Metodi: Il metodo PETIA (fCAL Turbo, Bühlmann Laboratories) impiega particelle di lattice rivestite con anticorpi anti-CF umana (MRP8/14). fCAL è stato confrontato con il metodo POCT Quantum Blue Calprotectin High Range (Bühlmann Laboratories) in uso presso il nostro laboratorio, analizzando 23 campioni di feci. L'estrazione del materiale fecale era eseguita utilizzando i dispositivi Bühlmann Smart prep per il metodo POCT e Calex cap per quello fCAL. La concordanza diagnostica dei due metodi è stata valutata utilizzando il cut-off di 200 µg/g feci precedentemente definito (Clin Chim Acta 2012;413:350). La linearità del metodo è stata valutata eseguendo diluizioni scalari (da 1:2 a 1:10) di un campione a concentrazione elevata di CF con soluzione fisiologica.
Risultati: 3 campioni su 23 (13%) presentavano risultati discordanti, due essendo positivi solo con POCT (255 vs. 94 µg/g; 345 vs. 157 µg/g) e uno con fCAL (112 vs. 217 µg/g). Il coefficiente kappa di concordanza era pari a 0,638 (IC95%: 0.48-0,80). Le prove di linearità di fCAL mostravano un recupero medio del 94,1%.
Conclusioni: Compatibilmente con l'imprecisione del metodo POCT, che intorno al cut-off ha un CV ≈20%, e con l'impiego di differenti dispositivi di estrazione, i risultati ottenuti dimostrano una buona concordanza tra POCT e fCAL. Il metodo fCAL ha inoltre un intervallo di misura più esteso (20-8000 µg/g), con il vantaggio di consentire una migliore caratterizzazione della malattia investigata e un minore numero di diluizioni dei valori molto alti. Inoltre, i risultati sono disponibili in soli 10 min in completa automazione, garantendo la totale tracciabilità del dato, essendo possibile trasferire i risultati automaticamente al sistema informatico di laboratorio e da qui al reparto richiedente.

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VALUTAZIONE DI UN NUOVO METODO AUTOMATIZZATO PER LA DETERMINAZIONE DELLA CONCENTRAZIONE DELL'INIBITORE C1 ESTERASI NEL SIERO

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Introduzione: L'inibitore della C1 esterasi (C1-INH) è la principale proteina che regola l'attivazione dei mediatori della permeabilità vascolare. La sua carenza è tipica dell'angioedema ereditario, rara malattia caratterizzata da episodi edematosi ricorrenti a carico di cute, mucose e organi interni. Il dosaggio di C1-INH nel siero è quindi d'ausilio alla sua diagnostica. In questo lavoro abbiamo valutato un nuovo sistema di misura immunoturbidimetrica delle concentrazioni sieriche di C1-INH (Optilite, The Binding Site) confrontandolo con il metodo immunoturbidimetrico in uso nel nostro laboratorio (Sentinel Ch, implementato su analizzatore Beckman AU480).

Metodi: Lo studio di comparazione è stato condotto analizzando 27 campioni di siero (conservati in congelatore fino al momento delle misurazioni), di cui 2 da soggetti con totale carenza di C1-INH. L'imprecisione del metodo Sentinel è stata stimata misurando un materiale di controllo liquido-congelato (BioRad Liquichek Unassayed Chemistry Control Level 2) in un periodo di 10 mesi consecutivi (n=144). Per il metodo Optilite, l'imprecisione è stata valutata misurando un pool di sieri congelato lungo due mesi consecutivi (n=25).

Risultati: Il confronto tra metodi ha evidenziato uno scostamento proporzionale tra i metodi molto significativo (P <0,0001) (regressione di Deming: Optilite = 1,81Sentinel - 0,01), con differenze medie assoluta e relativa, rispettivamente, di 0,12 g/L (IC95%: 0,109-0,137) e 72,2% (IC95%: 67,3-77,1). Esisteva, tuttavia, una buona correlazione nei risultati (r^2=0,938), essendo entrambi i metodi anche in grado di evidenziare l'indosabilità di C1-INH nei due soggetti carenti. L'imprecisione (CV totale) era 4,2% (media C1-INH, 0,17 g/L) per il metodo Sentinel e 2,8% (media C1-INH, 0,31 g/L) per Optilite.

Conclusioni: Nonostante i metodi utilizzati abbiano mostrato simile specificità, i dati ottenuti evidenziano la mancanza di confrontabilità dei risultati. Questo probabilmente origina dalla diversa riferibilità metrologica dei sistemi (Optilite verso uno standard interno e Sentinel verso la preparazione RPPHS del College of American Pathologists). Nell'impiego clinico dei metodi è quindi necessario adeguare gli intervalli di riferimento al metodo utilizzato.

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ANALYTICAL PERFORMANCE OF THE NEW AUTOMATED ISYS N-TERMINAL PROPEPTIDE OF TYPE I COLLAGEN (P1NP®)

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Background: Serum biomarkers of bone turnover were proven useful for monitoring efficacy of treatments, predicting fracture risk and for the accurate assessment of clinical progression of disease. The measurement N-terminal propeptide of type I collagen (P1NP®) is a highly sensitive indicator of bone formation and may be helpful to assess efficacy of several new anabolic bone drugs. Accordingly recommendation of the Medical Accreditation Standard (ISO 15189), clinical laboratories should verify the quality specifications of new tests according to a defined protocol. The aim of this study was to verify the analytical performance of the new P1NP® automated immunoassay assayed on iSYS (Immunodiagnostic Systems, Boldon, UK).

Methods: The two-site chemiluminometric assay employs a two-point calibration in triplicate (top calibrator values around 135 μg/L), quality control materials in duplicate, and 20 μL of sample in single. Three serum samples were prepared to check the analytical sensitivity, imprecision intra and inter-assay.

Results: The within- and between-run coefficients of variations (CVs) of P1PN® samples with low (26,9 mg/L), medium (51,2 mg/L) and high (125,3 mg/L) vales were 2,8%, 3.9%, 3.5% and 2,8%, 3.4%, 5,4%, respectively. The assay was linear in a range of P1NP® concentrations between 4,3-212,4 mg/L, as confirmed by linear regression analysis (y=1.02x + 0.93) and correlation coefficient (r=0.99; p, p<0.001). The functional sensitivity was calculated at different concentrations by measuring six consecutive 1:2 scalar dilutions in P1NP sample buffer (i.e., from 1:2 to 1:64) of a routine serum sample with a P1NP value of 106,1 The assay showed average recoveries of between 95 % and 101%.

Conclusion: We conclude that the analytical performance and the technical features of new iSYS P1NP® make it a suitable assay for rapid quantification of P1NP in routine clinical laboratories.

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ANALYTICAL PERFORMANCE OF THE NEW AUTOMATED ACCESS AMH®

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Background: Several parameters such as Follicle-stimulating hormone (FSH), Luteinizing hormone (LH), estradiol, Inhibin B, antral follicle count (AFC), and ovarian volume (OV) have been used to assess ovarian function and support physicians in protocol selection and patient counselling . Serum anti-Mullerian hormone (AMH) has proven to be better than other markers for assessing ovarian reserve and has so become the reference biomarker for assessing the number of remaining oocytes in ovary.This study was aimed to compare the performances of the new Access AMH® automated immunoassay assayed on Access2 (Beckman Coulter Inc., Brea CA, USA) compared to the previous Beckman Coulter AMH Gen II ELISA on Triturus Analyser (Diagnostics Grifols, S.A., Barcelona,Spain). Methods. Two serum aliquots were assayed with Beckman Coulter AMH Gen II ELISA assay and the novel Access 2 AMH automated immunoassay.Results.The within- and between-run coefficients of variations of Access 2 AMH at low (5,08 ng/mL) and high (20,38 ng/mL) concentrations were 1,11-2,7% and 0,52.2,5%, respectively. The assay was linear in a range of Access AMH® concentrations comprised between 0,07-20,46 ng/mL (y = 1.009x + 0.103; r=0.999, p<0.001). Results of serum samples (n=89) were compared with those of Gen II ELISA. The median values (2.5-97.5 percentiles) were 1,92 ng/mL (0,10-9,67 ng/mL) with Access AMH® and 1,97 ng/mL (0.02-11,50 ng/mL) with AMH Gen II ELISA, respectively. The nonparametric regression of Passing & Bablok and the Spearman's correlation showed excellent performance of Access AMH® (Access AMH® = 0,79 x AMH Gen II ELISA + 0,44; r= 0.98, p<0.001). Conclusion. The analytical performance and technical features of new Access AMH® make it a suitable assay for reliable, rapid and automatic analysis of AMH in clinical laboratories.

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COMPARISON BETWEEN SAMPLE QUALITY OBTAINED USING THE NEW BD BARRICOR PLASMA TUBE WITH RESPECT TO THE BD PST II PLASMA TUBE

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Backgrounds: The new BD Barricor Plasma tubes (BD Barricors) not only have an innovative non-gel separation method, which is supposed to improve sample quality, but also have a faster turnaround time thanks to faster centrifugation.
Aims: The aim of this study was to compare the quality of plasma samples obtained using BD Barricors (BD, Italy) at different centrifugation rates, with respect to that obtained utilizing BD PST II plasma tubes (BD, Italy).
Materials and methods: Four BD Barricor and one BD PST II samples were obtained from 40 donors at fasting conditions. BD PST II was centrifuged at 1300g/10min (C0), while BD Barricors were centrifuged at 1800g/10min (C1), 4000g/3min (C2), 4000g/7min (C3) and 4000g/15min (C4). Plasma quality was evaluated by measuring White Blood Cells (WBC), Red Blood Cells (RBC) and Platelets (PLT) by ADVIA 2120 (Siemens Healthineers, Italy). Wilcoxon matched-pairs and non-parametric trend tests were used to identify significant statistical differences.
Results: WBC, RBC and PLT median counts, obtained from plasma of BD PST II at C0 were: 0.38 (10^9/L), 0.0291 (10^{12}/L) and 113.5 (10^9/L), respectively. The BD Barricor plasma showed a significant reduction trend from C1 to C4 for WBC, RBC and PLT (p<0.001). Differences among plasma conditions were then expressed as the median bias percentage, considering the BD PST II at C0 as reference. BD Barricors WBC demonstrated a significant reduction in all conditions (p<0.01), being the median biases: 63.9% (C0-C1), 69.9% (C0-C2), 75.0% (C0-C3) and 82.7% (C0-C4). Significant WBC differences were found between C1 and C4 (p<0.01). BD Barricor RBC reductions were all statistically significant (p<0.01), being the reduction media bias percentage: 29.7% (C0-C1), 33.8% (C0-C2), 39.6% (C0-C3) and 66.4 (C0-C4). RBC at C4 was different from those at C1, C2 and C3 (p<0.01). PLT reductions were: 1.6% (C0-C1,p=ns), 1.2% (C0-C2, p=ns), 27.1% (C0-C3, p<0.01) and 46.6% (C0-C4, p<0.01). PLT at C3 and C4 were different from those at C1 and C2 (p<0.01).
Conclusions: Plasma quality obtained using BD Barricors progressively improved with increasing centrifugation times. However, already at 4000g/3min, BD Barricor allowed a significant quality improvement compared to BD PST II, guaranteeing a faster centrifugation rate.

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AN INNOVATIVE AND SPECIFIC METHOD FOR MDA QUANTITATIVE ANALYSIS IN HUMAN SERUM

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Malondialdehyde (MDA) is the final product deriving from lipid peroxidation process and is commonly used as an index of oxidative stress in many health problems such as cancer, chronic obstructive pulmonary disease, asthma and cardiovascular diseases.
Although thiobarbituric acid (TBA) assay is the most commonly used method for the determination of MDA in biological fluids, this method presents several limitations:
- Lack of specificity in TBA reactivity on MDA
- Low stability of MDA in biological samples
- TBA cross-reactions with other aldehydes.

Our main hypothesis is that MDA-TBA assay is not able to provide valid analytical data for biological samples due to its high reactivity and possibility of various cross-reactions with co-existing biochemicals.
In biological matrices, MDA exists in free (f-MDA) and bound (b-MDA) forms, mainly linked to proteins.
We developed a simple and sensitive method for the detection of f-MDA and t-MDA in human serum based on the derivatization of MDA with 2,4-dinitrophenylhydrazine (DNPH) and subsequent HPLC separation.
This method offers several advantages such as high sensitivity with a limit of detection (LOD) of 3.5 pmol/ml and limit of quantification (LOQ) of 10 pmol/ml. Recoveries of MDA from spiked matrices (R%) reached 98.1±1.8 and 96.51±1.8 for t-MDA and f-MDA, respectively.
First, we tested our method on 17 healthy control volunteers with no disease evidence and correct life style in their medical history.
In the second phase of our research we will test our method on patients with proved oxidative disease to optimize derivatization conditions in DNPH medium solution, reaction time and temperatures.

P220

FAECAL CALPROTECTIN: COMPARISON OF AN AUTOMATED TURBIDIMETRIC WITH AN ELISA ASSAY

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Background: Faecal calprotectin (fCal) allows to rule-out or rule-in intestinal inflammation among patients with abdominal symptoms and to monitor the disease phase (active/remission) among patients with inflammatory bowel diseases. In medical laboratories, a significant increase in the number of fCal assays has been observed in the last decade; therefore, theavailability of high-throughput and cost-effective methods is advisable. The aim of this study was to compare anautomated turbidimetricmethod with and ELISA fCal assay.
Methods: fCal was measured in 96 faecal samples randomly selected from the laboratory routine series by means of an ELISA procedure (IDK Calprotectin, Immundiagnostik, Germany), automated on DSX ELISA processing system (Dynex Technologies) and of a turbidimetric assay (BÜHLMANN fCALTM turbo, BÜHLMANN Laboratories, Switzerland) automated on Cobas Module c501 (Roche).
Results: The upper measurement limit of IDK was 2100 mg/g, while that of fCALTM turbo was 8000 mg/g. The results obtained with the two methods were correlated (R^2=0.787). The Bland-Altman analysis demonstrated a bias of 119 (95%CI: -963 to 1201) mg/g, while Deming regression underlineda non-constant bias. The lack of concordance between the two methods was mainly due to samples with very highfCal levels (n=6), because the fCALTM turbo assay performs an automated dilution step for values higher than 2058 mg/g; instead, the IDK assay requires a repeated analytical set-up of manually diluted samples. After the exclusion of 6 results with values above 1500 mg/g, the Bland-Altman analysis documented a bias of 0.02 (95%CI: -195 to 195)ug/g. The Deming regression confirmed the absence of proportional or constant bias (slope 95%CI: 0.9176-1.092; intercept 95%CI: -26.80 to 25.37).
Conclusions: The two studied methods for fCal measurement have comparable performances for values covering the clinical relevant range. The fCALTM turbo assay presents the advantages of an automated turbidimetric platform, such as decision criteria for control rules before the set-up of the analytical series and the independence form any analytical batch. Moreover it is faster (about 30 minutes for 96 samples) than an automated ELISA (about 3 hours).

P221
AMINO ACIDS PROFILE BY UPLC-MS IN PATIENTS WITH MITOCHONDRIAL DISEASES

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Introduction: Mitochondrial diseases (MDs) are heterogeneous genetic disorders caused by impairment of the oxidative phosphorylation system that represent a considerable diagnostic challenge for the multitude of clinical features. Therefore, there is a need for sensitive, specific and quantitative biomarkers for the diagnosis and assessment of progression in MDs. The aim of this study is to evaluate the utility of a semi-target metabolomics approach that focuses on a specific class of metabolite (amino acids) in diagnosing and understanding these diseases, as well as screening and monitoring of patients.
Methods: We studied all consecutive adult patients affected by MDs referred to our Neuromuscular Unit, during 6-mounth period.50µL sample were mixed with 100µL 10% (w/v) sulfosalicylic acid containing 50µM IS were and centrifuged at 10000 g for 15 min. 10 µL of the supernatant was transferred to a vial and 70 µL of borate buffer with the addition of NaOH, provided with AccQ Tag reagents kit was added, vortexed for 10 sec and heated at 55°C for 10 min.The chromatographic separation was performed by ACQUITY H-Class using an ACQUITY CORTECS C18 column eluted at a flow rate of 500 µL/min with a linear gradient (9 min) from 99 to 1 water 0.1% formic acid in acetonitrile 0.1% formic acid. The mass spectrometry was a ACQUITY QDa single quadrupole.
Results and discussion: The glutamic acid and alanine were increased in patients with MDs. We hypothesize that this phenomenon is driven by excessive muscle protein breakdown, accompanied by upregulation of alanine amino transferase. The increase in circulating glutamate in patients suggested two possible scenarios: it could result from increased muscle protein breakdown, leading to excessive glutamate production and release or from defective muscle uptake of glutamate. Furthermore, we observed a perturbed catabolism of the branched chain amino acids. Other metabolites are currently being studied. The preliminary results of our study show that semi-target metabolomics approach is a successful tool for generating new information on the biochemical pathways in MDs. In addition this combination of biomarkers could fulfill the purpose to monitor the disease progression and disclose the biological consequences of these complex diseases.

P222
ANALYTICAL PERFORMANCE EVALUATION OF A NEW ASSAY FOR URINARY FREE CORTISOL ASSESSMENT IN LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Background and aim: According to current guidelines, urinary free cortisol (UFC) is the first-line biochemical test for screening endogenous Cushing syndrome. Due to its higher selectivity and specificity compared to radioimmunoassays (RIA), liquid chromatography-tandem mass spectrometry (LC-MS/MS) has become the reference technique for UFC assessment in clinical practice. Aim of the study was to evaluate the analytical performance of a new UFC LC-MS/MS assay and to compare results with those obtained with a RIA method currently used in our laboratory.
Methods: The LC-MS/MS assay and analytical system were the ISBN UFC kit and a Nexera X2 series UHPLC (Shimadzu, Kyoto, Japan)-4500 MD triple quadrupole MS detector (ABSciex, Darmstadt, Germany) respectively.
Results: The LC-MS/MS method was linear up to 5000 nmol/L. The limit of detection (LOD), defined as the lowest concentration generating a signal to noise ratio (S/N) >3, was 3 nmol/L. The limit of quantification (LOQ), defined as the minimum concentration of cortisol measurable with an imprecision of at least 20%, was 6 nmol/L. Within-run and between-run coefficients of variation were <6% and 10% over a broad range of values (5 sample pools, 10 repetitions). Linear regression analysis between RIA and LC-MS/MS on 50 samples yielded a correlation coefficient of 0.88. The RIA method showed a positive proportional bias of 56% compared to the HPLC-MS/MS assay. Results obtained from two external quality assessment schemes (UK NEQAS) showed excellent agreement (mean specimens bias <15%), with mean values from 44 laboratories using the same method.
Conclusion: The new commercial UFC LC-MS/MS assay display optimal analytical performance in terms of precision, sensitivity and accuracy, and seems hence suitable for routine use as replacement of RIA assays.
Fassnacht M, et al. Management of adrenal incidentalomas: European Society of Endocrinology Clinical Practice Guideline in collaboration with the European Network for the Study of Adrenal Tumors. Eur J Endocrinol 2016;175:G1-G34.

P223
ANTI-ACHR ANTIBODIES DETECTED BY ELISA METHOD USING AN AUTOMATED SYSTEM (SKYLAB 752): EVALUATION OF ANALYTICAL PERFORMANCES ACCORDING TO CLSI EP15-A

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Antibodies against the acetylcholine receptor (AChRAb) are currently detected in the clinical laboratory by using the radioimmunoassay which uses receptors extracted from human muscles, identified and quantified by means of labelling with ^{125}I -α-bungarotoxin. For the first time, Hewer et al. developed a new enzyme–linked immunosorbent assay (ELISA) based on the use of purified foetal and adult AchR. The aim of the study was to verify the performances for precision and trueness of anti-AChRAb ELISA test claimed by the company (RSR Ltd, Cardiff, United Kingdom), on the automatic instrumentation SkyLAB 752 (DASITGroup, Cornaredo, Italy) according to Clinical and Laboratory Standards Institute (CLSI).Two commercial control sera, high positive (C1, 7.5 nmol/L) and low positive (C2, 1.6 nmol/L), provided by the manufacturer were assayed in triplicate, in five independent runs for within-run, between-day and within-laboratory precision. The four calibrators at different concentration level included in the diagnostic kit (E1, 0.5 nmol/L; E2, 1.0 nmol/L; E3, 6.5 nmol/L; E4, 20 nmol/L) were tested in duplicate in five consecutive days for the trueness study. The within-run CV% , between-day CV% and the within-laboratory CV% were 7.9% (95% CI, 0.61 to 1.52), 8.4% (95% CI, 0,00 to 2,98) and 11.5% (95% CI, 0.92 to 3.12) to the mean concentration measured=11.2 nmol/L (C1) and 5.6% (95% CI, 0.13 to 0.32), 1.9% (95% CI, 0.00 to 0.33) and 6.0% (95% CI, 0.15 to 0.39) to the mean concentration measured =3.2 nmol/L (C2).The total repeatability, expressed as Standard Deviation (SD) (within laboratory), resulted 1.27 for C1 and 0.19 for C2, more less than the verification value (1.37 and 0.49, respectively) obtained on the basis of the repeatability declared by the manufacturer [SD (C1) = 0,95874 and SD (C2) = 0,3589].The analytical performance obtained in "routine activities" has confirmed the specifications produced in the experimental stage and has shown that the reliability of the test persists even when the assay is performed in automation. The good precision and accuracy of the test and its availability on an automated system featuring ease of use and rapid response lead us to conclude that the test is satisfactory for routine use and it is potentially suitable for the long-term monitoring of AChRAb.

P224
CALPROTECTINA FECALE: CONFRONTO TRA METODICHE QUANTITATIVE E SEMIQUANTITATIVE

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Introduzione: La calprotectina (Cal) è una proteina di 36,5 KDa, formata da 3 subunità, presente in alte concentrazioni nel citoplasma dei granulociti neutrofili e monociti. La proteina è stabile nelle feci (fCal) e l'escrezione fecale correla con il quadro istologico ed endoscopico delle principali patologie infiammatorie intestinali, acute o croniche, quali rettocolite ulcerosa, morbo di Chron, neoplasie dell'apparato gastroenterico ecc., permettendo una diagnosi differenziale con la sindrome dell'intestino irritabile. Scopo: Scopo del lavoro è quello di confrontare le performances analitiche di tre differenti kit per la determinazione della fCal.
Metodi: La fCal è stata testata su 42 campioni fecali selezionati casualmente dalla routine, pervenuti presso il laboratorio di Patologia Clinica della AOUS, utilizzando tre diverse metodiche di cui due immunoenzimatiche quantitative (ELISA): Chorus calprotectin (Diesse, Siena) e Calprest (Eurospital, Trieste, considerato test di riferimento), e un test immunocromatografico semiquantitativo: Calfast (Eurospital, Trieste) in uso presso l'AOUS. La concordanza statistica tra i metodi è stata valutata mediante il K di Cohen. Risultati: Per l'analisi statistica, avendo i tre metodi range di riferimento leggermente diversi, abbiamo valutato la concordanza tra i risultati positivi e negativi, considerando i valori compresi nella grey zone come positivi. Il confronto tra i due metodi ELISA (Chorus vs Calprest) mostra un positive agreement con un K di Cohen pari a 0,76. Il test rapido semiquantitativo Calfast presenta un K di 0.72 vs il Calprest e un K di 0.72 vs il Chorus , in entrambi i casi con positive agreement.
Discussione: La buona concordanza dei dati ottenuta indica che i tre metodi presi in esame, hanno performances paragonabili nel range di valori di rilevanza critica per la patologia considerata. Il test Fast ha il vantaggio di essere rapido per discriminare la presenza della patologia, ma non utile ai fini del follow up. Il metodo Chorus Calprotectin ha il vantaggio di poter essere utilizzato anche come monotest ed adatto anche a laboratori medio-piccoli.

P225
TOSOH HLC-723 G11 PERFORMANCE EVALUATION: A MULTICENTER STUDY

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Background: Glycated hemoglobin (HbA1c) measurement provides the most important medium to long-term marker of time averaged glycemic status. Its relationship to clinical outcome in diabetes has been convincingly demonstrated for both type 1 and type 2 diabetes. Main HbA1c measuring methods are: ion-exchange chromatography electrophoresis and isoelectric focusing; affinity chromatography and immunoassay. We evaluated the analytical performance of Tosoh HLC-723 G11 analyzer in the VAR mode and compared it with: Sebia Capillarys 2 Flex Piercing, Bio-Rad D-100® and Bio-Rad VARIANT™II Hemoglobin Testing System.
Materials and methods: Whole blood samples, drawn by venipuncture, were collected in K2-EDTA tubes and processed according to the manufacturer's instructions. Precision assessment (based on CLSI-EP5) was performed using: two levels (high and low) Tosoh control, two levels (high and low) Randox control and three patient samples. We analyzed samples stability within a day (0; 2; 4; 6; 8; 24 h) and carryover. Tosoh HLC-723 G11 linearity was evaluated using Bio-Rad Lyphocheck HbA1c Linearity Set. Methods comparison was realized using 750 blood samples (randomly chosen healthy and diabetic subjects) analyzed for routine glycemic testing. Statistical analysis was carried out using Analyse.it and SPSS software.
Results: We tested repeatability and reproducibility of Tosoh HLC-723 G11 obtaining values in agreement with goals of NGSP (< 2%) and IFCC (< 2.8%). The Linearity exhibited good results ranging (15-182 mmol/mol). There was no carryover effect and samples were stable at room temperature for at least one day. We observed correlation between methods and the Passing Bablok regression analysis was reported: Tosoh G11 vs Sebia Capillarys (y = 4.9138 + 0.90 x; r = 0,994), Tosoh vs Bio-Rad D-100® (y = 1.17 + 0.96 x; r = 0,997) and Tosoh G11 vs Bio-Rad VARIANT™ (y = -0.66 + 0.97 x; r = 0,993).
Conclusion: The new Tosoh HLC-723 G11 is user-friendly, fast throughput, with good precision. The short analytical TAT (1 min/analysis) did not result in increased variability of the results or carry-over between samples; moreover, our data showed a good correlation between new Tosoh results and those obtained using other clinical routine analyzers.

P226
PTH WHOLE: IT'S TIME FOR A CHANGE?

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Introduzione: il Paratormone (PTH) è un ormone secreto dalle paratiroidi come polipetide di 84 amminoacidi e regola il metabolismo del calcio e del fosforo. Oltre al PTH attivo, in circolo, sono presenti frammenti di PTH inattivo, frammenti del dominio medio e C-terminale che sono eliminati dal rene e avendo una maggiore emivita rispetto al PTH attivo tendono ad accumularsi nei pazienti con insufficienza renale. Il PTH (7-84) che manca dei residui N-terminali 1-6 è un frammento presente in circolo ma non ha attività biologica. Le metodiche di seconda generazione non riescono a discriminare questo frammento dal PTH intero e quindi portano ad una sovrastima della reale concentrazione del PTH in circolo.
Scopo: lo scopo del nostro lavoro è stato la valutazione di un metodo di terza generazione in confronto al metodo di seconda generazione in uso nel nostro laboratorio.
Materiali e metodi: Sono stati analizzati di 100 campioni di siero con valori di PTH distribuiti in tutto il range analitico, in particolare nell'intervallo di riferimento, con Intact PTH Advia Centaur XPT (Siemens) e successivamente con Whole PTH Lumipulse G (Fujirebio).
Risultati: L'analisi dei risultati ottenuti con la regressione lineare di Passing Bablok (y=3,28+0,42x; R^2=0,99) evidenzia la presenza di un errore sistematico proporzionale e costante. Il grafico di Bland-Altman mostra un incremento della differenza dei due metodi all'aumento della concentrazione dell'analita. L'analisi dei risultati ha consentito di evidenziare un valore asintotico del rapporto Fujirebio/Siemens pari al 39% confermando molteplici evidenze sperimentali riguardante la scarsezza di frammenti di PTH per valori fisiologici dello stesso e l'incremento della loro concentrazione all'aumentare dei livelli dell'ormone. Tuttavia già alla concentrazione di 72 pg/mL, valore soglia della metodica Siemens, il contributo dei frammenti risulta essere pari al 49% del totale. Infine, il K di Cohen ha evidenziato una buona concordanza (0,79) tra le due metodiche.
Conclusioni: la metodica di terza generazione risulta essere più accurata essendo in grado di discriminare i frammenti. Dalla nostra esperienza risulta che l'interferenza di questi frammenti per valori bassi di PTH non è significativa; per i valori vicini al limite superiore di riferimento risulta essere di circa il 50%, assumendo rilevanza clinica nei pazienti con PTH molto elevato come quelli affetti da insufficienza renale.

P227
INTERFERENZA DA COMPONENTE MONOCLONALE NELLA DETERMINAZIONE DELLA CDT

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La transferrina carboidrato carente (CDT) è considerata un marcatore biochimico di abuso alcolico cronico estremamente specifico. I metodi comunemente utilizzati nella routine di laboratorio per la determinazione della CDT sono l'elettroforesi capillare (CE), la cromatografia ad alta prestazione (HPLC) e l'immunonefelometria. Le tecniche separative CE sono di facile applicazione e utili nella gestione di grosse routine. Tuttavia, non sono in grado di risolvere alcuni casi dovuti alla presenza di proteine interferenti. Nel tracciato elettroforetico delle sieroproteine la transferrina migra in zona beta, pertanto le componenti monoclonali (CM) migranti in tale zona possono interferire nella determinazione della CDT. Scopo di questo lavoro è valutare la capacità discriminante della CE e dell'HPLC nella determinazione della CDT in soggetti con picchi monoclonali in zona beta. Sono stati selezionati 42 campioni di siero di pazienti affetti da discrasie plasmacellulari caratterizzate da un picco monoclonale in zona beta, tipizzato con tecniche di immunosottrazione e immunofissazione (Sebia). La determinazione quantitativa della CDT è stata effettuata utilizzando il metodo di elettroforesi capillare MINICAP CDT (Sebia) ed il metodo separativo cromatografico HPLC (Bio-Rad su analizzatore Agilent 1200). Le componenti monoclonali tipizzate sono risultate essere: 28 IgA (11κ e 17λ); 9 IgG (7κ e 2λ); 5 IgM (4κ e 1λ); la concentrazione delle componenti monoclonali variava da 0.2 a 5.7 g/dL. Il metodo CE è stato in grado di determinare il valore della CDT in 9 casi; il successivo pretrattamento dei campioni con soluzione CDT/IS ha permesso la determinazione di altri 4 casi. Gli altri 29 sono rimasti irrisolti. Il dosaggio in HPLC ha permesso la quantificazione di 38 CDT su 42. I 4 campioni non determinabili avevano una CM $\geq$ 3.9 g/dL o la presenza di catene leggere libere, evidenti all'elettroferogramma. CE e HPLC generano tracciati di separazione simili e la CE può generalmente essere impiegata in questo dosaggio. Tuttavia il metodo CE viene considerato meno affidabile poiché utilizza una lunghezza d'onda non specifica per la transferrina (200-210 nm) alla quale assorbono anche altre proteine potenzialmente interferenti. Le componenti monoclonali con migrazione in zona beta, sono tra queste possibili proteine interferenti. L'HPLC misura l'assorbanza del complesso ferro-transferrina a 460-470 nm e questo lo rende un metodo specifico, utile non solo per confermare i risultati positivi alla CE, ma anche per risolvere interferenze analitiche dovute alla presenza di CM di lieve e media entità.

P228
COMPARISON BETWEEN TWO METHODS FOR THYROGLOBULIN: COBAS-ROCHE vs CHORUS TRIO DIESSE

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Introduction: Thyroglobulin (Tg) is a secretory protein synthesized in the thyrocyte endoplasmic reticulum (ER), where it acquires N-linked glycosylation and conformational maturation (including formation of many disulfide bonds), leading to homodimerization. Its primary functions include iodide storage and thyroid hormonogenesis. Tg consists largely of repeating domains, and many tyrosyl residues in these domains become iodinated to form monoiodo- and diiodotyrosine, whereas only a small portion of Tg structure is dedicated to hormone formation. Production of Tg is stimulated by TSH, intrathyroidal iodine deficiency and the presence of thyroid-stimulating immunoglobulins. Serum Tg measurement has greatly facilitated the clinical management of patients with differentiated thyroid cancer, in the post-operative follow-up and a variety of other thyroid disorders. The purpose of this study is to evaluate the immunoenzimatic method using Chorus Trio Diesse through comparison with Cobas Roche.
Material and Methods: Ninety-eight serum samples recovered from the daily clinical routine were tested for Tg on Cobas-Roche and then on Chorus Trio Diesse. The Cobas-Roche's technology is based on chemiluminescence immunoassay detection, while that of Chorus Trio Diesse on an immunoenzymatic method. The results obtained were examined by statistical analyzes Bland-Altman and Passing-Bablok performed with SPSS version 11.0.
Results: Linearity trials on Chorus Trio Diesse performed on 10 replicates with serial dilution showed a y = 0.9844x - 0.1495 R2 = 0.9863. LOQ was 0.1 ng/ml. By comparison of the Chorus Trio Diesse method with that of Cobas-Roche, the results at the Passing-Bablok analysis showed r=0.92; Intercept = -0.0078 (95% CI= -0.0332 to 0.0196), while at the Bland and Altman analysis the mean differences was 0.431 (+1.96 SD= 3.269 and -1.96 SD= -2.408).
Conclusions: Chorus Trio Diesse has a good analytical performance. The measurements obtained by Chorus Trio Diesse are comparable and similar to those of Cobas-Roche, especially for concentrations ranged between 0.5 and 1 ng/ml, this result represent a good analytic target for automated immunoenzymatic method.

P229
COMPLETE AUTOMATION OF LIBRARY PREPARATION FOR NEXT GENERATION SEQUENCING (NGS)-BASED MOLECULAR TESTING FAMILIAL HYPERCHOLESTEROLEMIA (FH) BY OMNIA LH100 AND LH60 (MASMEC) SYSTEMS

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Introduction: FH is a common genetic cause of premature coronary heart disease. The inherited genetic variants occur several genes as LDLR, APOE, APOB and PCSK9. NGS technology represents a robust approach for applications involving simultaneous targeted re-sequencing of multiple genes. In the NGS workflow one of the most critical and time-consuming steps is the library preparation. Our objective was to report a rapid, automated solution to prepare Multiplicom-NGS based protocol for Illumina MiSeq® for FH molecular test and to analyze the performance of NGS in terms of number of sequences/run, coverage uniformity and number of variants detected. A total of 40 samples were used to evaluate the automated preparative process.
Methods: Library preparation was performed on OMNIA LH 100 and LH 60 automated workstations designed and produced by MASMEC Biomed (Modugno, Bari) using ADH MASTR v2 kit (Multiplicom) an amplicon-based gene panel targeting all exons of LDLR, PCSK9 and APOE, together with exon 26 of APOB. 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 60 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). Moreover, as the two workstations can work contemporaneously, the library preparation time has been considerably reduced.
Discussion: This study describes a new automated solution for fast and reproducible library preparation for NGS-based molecular testing FH using a robotic workstation. The results obtained are comparable to those from a standard manual library preparation. The throughput of our pipeline was high positively improved by introducing these machines in our routine workflow.

P230
FT3 AND FT4 ASSAYS: A THREE METHOD COMPARISON

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Introduction: The measurement of the "free" fraction of T3 (FT3) and T4 (FT4) is useful for better understanding of physiologically active thyroid hormone levels for a more accurate diagnosis of thyroid disease. In this comparative study on FT3 and FT4 analysis methods, were evaluated the performance of the Chorus Trio Diesse compared to Vista Siemens and Diametra.
Material and Methods: Eightyseven random sample serum, enrolled by the daily clinical routine, were assayed for FT3 and FT4 by Chorus Trio Diesse, Diametra and Vista Siemens. Chorus Trio Diesse and Diametra are based on an immunoenzymatic method, while the Vista Siemens immunoassay is a homogeneous chemiluminescent method based on LOCI® technology.
Results: The results obtained by comparing the three methods for FT3 are the following:Comparing the Chorus Trio Diesse with Vista Siemens, for FT3 Passing-Bablok analysis showed a slope 0.9140 (95% CI 0.8175to 1.0107); Intercept = 0.2813 (95% CI= 0.0968 – 0.5000), for FT4 showed a slope 1.0403 (95% CI 0.8056 to 1.2941); Intercept = 0.2224 (95% CI -0.0318 – 0.4408). Bland and Altman analysis showed mean differences for FT3 of 0.074 (±1.96 SD = 0.871 and -0.724), and FT4 of 0.199 (±1.96 SD= 0.823 and -0.425).Comparing the Diametra with Vista Siemens, for FT3 Passing-Bablok analysis showed slope 0.7501 (95% CI 0.6060 to 0.9216); Intercept = 0.5684 (95% CI= 0.1314 – 0.8904), for FT4 showed a slope 0.9447 (95% CI 0.8177 to 1.1156); Intercept = 0.2382 (95% CI= 0.0446 – 0.3747), while at the Bland and Altman analysis the mean differences was for FT3 -0.131 (±1.96 SD = 1.207 and --1.469); and FT4 0.072 (±1.96 SD = 0.658 and -0.513).Comparing the Chorus Trio Diesse with Diametra, for FT3 Passing-Bablok analysis showed a slope 0.8165 (95% CI 0.6815 to 1.0015); Intercept = 0.3576 (95% CI= -0.1016 to – 0.6930), for FT4 showed a slope 1.0204 (95% CI 0.8314to 1.2202); Intercept = 0.0716 (95% CI= -0.1897– 0.3071),while at the Bland and Altman analysis the mean differences was for FT3 of -0.205 (±1.96 SD= 0.947 and -1.358) and for FT4 of 0.126 (±1.96 SD= 0.536 and -0.283)
Conclusions: The Trio Diesse FT4 and FT3 assays compared to Siemens Vista and Diametra showed good analytical performance. This automated immunoenzymatic method is reliable for small laboratories.

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FSH, LH AND PROLACTIN COMPARED METHODS: CHORUS TRIO DIESSE vs SIEMENS VISTA

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Introduction: The measurement of FSH, Lh and PRL hormones is widely used today in clinical practice for fertility evaluation, such as screening and follow-up of gonadal disorders and pituitary gland. Both in males and females, FSH, LH and PRL secretion is necessary for normal sexual function and is regulated by the interaction of positive and negative feedback mechanisms that relate to secretion of pituitary hormones, hypothalamus and gonads. In this study we assessed the performance of the Chorus Trio Diesse by comparison with Vista Siemens.
Material and Methods: FSH, LH and PRL were measured in 99, 134 and 99 respectively serum samples of patients arriving in our laboratory for routine analysis using both Siemens Vista and Chorus Trio Diesse methods. The dosage method to evaluate Chorus Trio Diesse is an immunoenzymatic method compared to the homogeneous chemiluminescent method based on LOCI® Technology of Siemens Vista method. The results obtained by the two methods were subjected to analysis Bland-Altman and Passing-Bablok. The statistical analyses were performed with SPSS version 11.0.
Results: Comparing the two methods, the results at Passing-Bablok analysis was for FSH, r=0.97; Intercept = -1.5189 (95% CI= -2.3317 to -0.7650), for LH r=0.94; Intercept = -0.2225 (95% CI= -0.4926 to -0.0118), r=0.98; Intercept = 20.5245 (95% CI= 3.4095 to 34.4452).Bland and Altman analysis showed mean differences for FSH 4.359 (+1.96 SD= +29.292 and -1.96 SD= -20.573), for LH, -0.994 (+1.96 SD= +11.165 and -1.96 SD= -13.154), for PRL, 62.157 (+1.96 SD= +540.094 and -1.96 SD= -415.78).
Conclusions: Based on the results obtained by comparing the two methods for the three analytes, the Chorus Trio Diesse generally has good analytical performance compared to Siemens Vista, but the best and overlapping results are obtained at low concentrations. Indeed, at high concentrations, Chorus Trio Diesse tends to be more imprecise. The possible use must be supported by the reference values of the healthy population and in different physiological conditions in relation to the new method.

P232
IMPROVEMENT OF URINARY STONE ANALYSIS BY MORPHOLOGICAL DATA

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Background: The identification of crystalline species of urinary stones is of great importance because they relate with particular biochemical alterations. Infrared Spectroscopy Fourier Trasform (FTIR) is the most appropriate technique for stones analysis. The microscopic analysis (MA) of composition of different area (core, middle layer and surface) of urinary stone, permits to understand urinary stone genesis and to know the origin of this event allowing a more targeted clinical intervention to reduce the risk of recurrence.The aim of our study is to analyze urinary stones both with FTIR and MA to underline the usefulness to introduce the analytical methodology known as morphocostitutional analysis which permits a more accurate urinary stone stratification.
Methods: We analyzed 100 urinary stones both by MA (following the classification criteria of professor Daudon) and FTIR, from 100 patients from our Divisions of Nephrology and Urology.On the basis of FTIR analysis, the 100 calculi were divided into three groups: pure stone (????=50), mixed stone (????=46), and pure stone with substances in trace (????=4). Results of each group were compared with those obtained with MA.
Results and discussion: The comparison between the two methods showed a wide agreement in all groups, indeed we have only 6%, 25%, and 26% of partial agreement for pure stones, pure stones with substances in trace and mixed stones respectively. In addition we never found disagreement. FTIR technique shows a high sensitivity and allows an accurate identification of stone composition but it can not discriminate in different subtype. The advantage of adding a stone morphology examination to infrared analysis is to provide a rapid, inexpensive orientation to specific pathological conditions that could not be readily identified when extensive metabolic investigations are not systematically performed.No analytical technique taken individually is able to provide all this information then a specialized center in the analysis of urinary calculation must combine morphological analysis to determine the morphological characteristics and the precipitation sequence of components, and biochemical analysis with the FT- IR for the determination and quantification of the individual components.

P233
EVALUATION OF LONG TERM IMPRECISION OF PHOTOMETRIC HEMOLYTIC INDEX DETERMINATION ON ABBOTT ARCHITECT c16000

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Background: Automatic photometric determination of hemolytic index (HI) provides estimate of free hemoglobin in serum and plasma samples to establish interference on laboratory test. When HI is above the established cut-off for interference, results for that specific analyte may suffer of a significant bias and undermine clinical reliability of such test. Despite its undeniable importance for patient safety, the analytical performance of HI estimate, as well as that of other interference indices, is usually not checked in clinical laboratories. For this reason, we decided to evaluate the long term imprecision of the measurement of HI on our Abbott Architect c16000 platforms (c16-1 and c16-2).
Methods: HI is measured on the Architect c16000 through the dilution of samples with saline solution and the polychromatic photometric detection of HI. The mathematical calculation to determine the interferent concentration uses an equation for the addition of absorbance measurements at four specific wavelength pairs (primary/secondary) covering the hemolysis spectrum, each one multiplied by a specific constant. From January 2016 to May 2017, we collected data from weekday photometric determination of HI on a frozen serum pool with a predetermined value of ≈1 g/L of free hemoglobin, performed on both c16-1 and c16-2. Monthly and cumulative CVs were calculated.
Results: During 17 months, 248 and 254 measurements were performed on c16-1 and c16-2, respectively. Monthly CVs ranged from 1.0% to 2.7% on c16-1 (mean CV, 1.6%) and from 0.8% to 2.5% on c16-2 (mean CV, 1.7%). Average HI values on the two platforms (mean of the means, 100 on c16-1 and 102 on c16-2, respectively) were essentially equivalent, confirming their interchangeability in estimating HI.
Conclusions: In our opinion, the determination of interference indices in laboratories should be considered like any other spectrophotometric measurement, the accuracy of which can potentially affect patient outcome. This implicates the implementation of dedicated quality control programs. Even though no quality specifications are available to date, our study shows that the HI measurement on Architect c16000 has nice repeatability that could be considered in establishing the state of the art of the measurement.

P234
VALIDAZIONE DI UN NUOVO METODO HPLC-UV PER IL DOSAGGIO PLASMATICO DEL MITOTANE

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Introduzione: Il mitotane, è un farmaco approvato per il trattamento dei pazienti affetti dal carcinoma delle ghiandole surrenali e viene utilizzato per la soppressione di nuove insorgenze in quanto è in grado di ridurre forme recidive nel 25-30% dei casi. Come ogni farmaco, la sua efficacia terapeutica è strettamente legata al raggiungimento di determinate concentrazioni plasmatiche; tuttavia, essendo un farmaco ad elevata tossicità con effetti collaterali rilevanti, risulta fondamentale il suo monitoraggio terapeutico. Il metodo fino ad ora utilizzato in laboratorio si basava sulla preparazione del campione con una metodica di estrazione liquido-liquido. Materiali e metodi: E' stato sviluppato e validato un nuovo metodo HPLC-UV con una nuova metodica di preparazione del campione mediante estrazione per precipitazione delle proteine e i risultati ottenuti sono stati comparati con quelli della vecchia metodica. La validazione è stata effettuata secondo le linee guida CLSI EP15-A3. Le analisi sono state eseguite con un sistema HPLC Chromsystems dotato di una pompa isocratica, un autocampionatore e un detector UV-VIS. La colonna utilizzata è una C8 a fase inversa (Chromsystems). La fase mobile è costituita da una miscela CH_3CN:H_2O (85:15) con 0.05% di H_3PO_4. La velocità di flusso è 0.6 mL/min e la λ utilizzata è 218 nm. Risultati: Si è ottenuto una ripetibilità con CV% compreso tra 1,9 e 6,8; una riproducibilità con CV% nel range tra 2,1 e 6,1. Il metodo risulta lineare tra 0,5-50 μg/mL con coefficienti di correlazione r compresi fra 0.998 e 0.999. Il recupero medio è risultato essere intorno al 98%. L'accuratezza, BIAS (%) medio, compresa tra -0,4% e -0,96%. Il Limit of Detection (LOD) è risultato essere 0.15 μg/mL ed il Limit of Quantification (LOQ) pari a 0.47 μg/mL. La regressione lineare calcolata confrontando i dati ottenuti con le due metodiche mostra una slope di 1,04 con un R^2 pari a 0,98, mentre i risultati dell'analisi di Bland-Altman mostrano una differenza media di 0,16 con un intervallo di confidenza del 95% da -0,20 a 0,53. Conclusioni: I risultati soddisfacenti dei vari parametri misurati nella validazione del nuovo metodo e l'ottima correlazione con il metodo precedentemente utilizzato, ha permesso l'implementazione della metodica nella routine clinica.

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STUDY OF BRONCHOALVEOLAR LAVAGE (BAL): STANDARDISATION AND PREPOSITION OF NEW ASSESSMENT PARAMETERS

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The BAL is an artificial biological liquid prepared by the pulmonologist during the bronchoscopy. They insert some aliquots of 50 ml of sterile physiological solution in the lung that are aspirated immediately. The procedure can be repeated 4 times (maximum) with an introduction of 200 ml. The aspiration of the aliquots is collected in a container on which laboratory investigations are performed, as there are several cells and solutes coming from the depths of the lung. Then, the content is filtered with a single layer of gauze and spun for 8 minutes at 3000 RPM'S at 4C°. Afterwards, the supernatant is removed, the body botton is described and 2 ml of PBS are added. This is the sample called BCC (Bal cell collection) on which flow cytometric and microscopic investigations are performed. The lymphocyte typing is performed with BD FACSCanto II and Sample Prep Assistant II on both peripheral blood and BCC and as lymphocyte antiserum is used BD Multitest 6-color TBNK, a reagent based on a six color immunofluorescence reaction to determine the percentage and the total count of T,B and NK cells and the subpopulations CD4 and CD8. The cell count per ml is performed in a Burker's chamber with Turk's solution, the cell viability with Trypan Blue solution, follows the cytospin and the slide staining with May Grunwald-Giemsa. Basic statistical data of 36 patients arrived at the Central Laboratory from 1/2017 to 5/2017: Cell count per ml has the following range: minimum 8.4 x 105, maximum 1,03 x 108. The percentage of Macrophages: 2 to 92; Lymphocytes: 2 to 88; Neutrophils: 0 to 57; Eosinophils: 0 to 48. As regards the lymphocyte subpopulations: CD3+: 61.4 to 98.5; CD4+: 11.3 to 79.1; CD8+: 4.1 to 79.6; CD19+: 0 to 8.8; CD16+56: 1.4 to 34.5 and the ratio CD4/CD8: 0.14 to 18.02. Nine patients have a ratio CD4/CD8 > 4 and this indicates a probable sarcoidosis condition. Total proteins and albumin, contained in the supernatant of Bal, have been dosed in order to support the presence of biological substances such as proteins and enzymes.

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GLI INDICATORI DI QUALITA' DELLA FASE PREANALITICA

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La medicina di laboratorio ha un ruolo importante nel processo decisionale clinico. L'evoluzione in campo tecnologico ha migliorato la qualità della fase analitica. Non è così per la qualità extra-analitica che rimane un problema a causa delle difficoltà di attuare un'adeguata standardizzazione. Scopo di questo lavoro è quello di analizzare gli indicatori di qualità preanalitica. A tal fine abbiamo:1) Rilevato le non conformità (NC) dei campioni nell'anno 2016; 2) Raccolto i valori degli indici di emolisi dei campioni di plasma pervenuti nell'arco di 2 mesi ed eseguiti dallo strumento Roche Cobas 8000. Il numero delle NC è stato 462. La NC più frequente è rappresentata dal campione coagulato (55%), campione insufficiente (8%), campione inquinato (8%), errori di identificazione (7%), provette errate (3%) e campioni giunti a temperatura non adeguata (0.4%). I dati relativi alla statistica descrittiva degli indici di emolisi (espressi in mg/dL) sono:numero di campioni=16383; valore minimo=0; valore massimo=940; mediana=9 (intervallo di confidenza da 9 a 9). Il campione è stato suddiviso in 8 gruppi in base all'analogia dell'area specialistica di provenienza del prelievo e, prendendo come riferimento il gruppo ambulatoriali, è stato applicato il test di Wilcoxson per dati non appaiati. Tutti i confronti tra i gruppi (ad eccezione del gruppo oncologia) sono risultati statisticamente significativi ($p<0.0001$). Considerando come normale un indice di emolisi <=20mg/dL, 14072 campioni (86%) rientrano in questo ambito. L'interferenza dell'emolisi sulla determinazione degli analiti è variabile. Relativamente a LDH(cut-off 20mg/dL) i prelievi ripetuti sono stati il 6%. Per AST e K (cut-off 100 mg/dL), la percentuale dei prelievi ripetuti è stata rispettivamente del 2.4% e 2%. Conclusioni.Gli indicatori di qualità rappresentano un elemento oggettivo per documentare la fase preanalitica, monitorarla e fornire elementi per azioni correttive. La bontà del prelievo riveste molta importanza ed è condizionata dall'area di proveneinza del paziente. Oggi, grazie alla determinazione strumentale degli indici del siero è possibile utilizzare un criterio oggettivo per valutare l'emolisi del campione e quindi ripetere il prelievo in base ad un dato puramente quantitativo.

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DOCTOR G: A GRAPHIC MEDICINE PROJECT PROMOTING STATISTICAL LITERACY

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In everyday life we have to make choices, which involve a degree of uncertainty and risk. However many doctors fail to understand the advantages and the limitations of the available medical evidence. One of the main reasons for this deficiency is statistical illiteracy, that is, the inability to understand the risk and the probabilities that lie behind the decisions mainly due to poor reporting of information.A statistical culture provides physicians the skills to understand numbers and it applies to every decision, from requesting a test to properly interpreting its result. However, even though doctors know exactly tests' performance and the prevalence of a given disease, are not able to infer from this information the probability that a patient found positive actually has the disease.For example, since 1st December, the HIV self-test is on sale in the italian pharmacies. The problem about HIV self-testing is that also people at low risk of infection might think to do the test. Imagine a blood donor, without risk behavior, worried about a positive HIV self-test result, asking you the following question: What is the probability I'm HIV-infected? 100%, 99%, 50%, or maybe less? You are up-to-date and you know that the HIV test performed by the patient has a sensitivity of 100% and a specificity of 99.8%. Moreover, HIV prevalence in his population is 0.01%. The answer is 4,7%, that is 21 persons will test positive but only 1 will actually be infected. This is the positive predictive value of the HIV test, a basic statistical concept that every professional should handle.To communicate numbers and probabilities in a simple and efficient way we recently published "Doctor G", a 132 pages graphic novel containing health statistics episodes. The story deals with the importance of being informed while making decisions and gives many examples of how numbers can be misleading. Inspired by true medical events and important people (doctors, statisticians, etc.) in the field of medicine, the book aims at imparting the readers the message that medicine is not an exact science.Statistical thinking is not for few chosen people, but rather a tool for everyone and doctors don't have to be afraid of statistics but be able to use it to make proper decisions.

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NEUTROPHIL-LYMPHOCYTE (NLR), LYMPHOCYTE-MONOCYTE (LMR), AND PLATELET-LYMPHOCYTE RATIOS (PLR) AS PREDICTORS OF ANASTOMOTIC DEHISCENCE IN COLORECTAL SURGERY

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Anastomotic dehiscence is one of the most challenging complications in modern colorectal surgery; it is associated with greater short-term mortality, poorer oncological outcomes and higher costs. Poor data exist regarding the role of inflammation indexes like neutrophil to lymphocyte (NLR), lymphocyte to monocyte (LMR), and platelet to lymphocyte ratios (PLR) in predicting anastomotic dehiscence in colorectal surgery. The archives of four surgical units were searched and 44 patients with anastomotic dehiscence after colorectal surgery operated on from 2012 through 2016 were enrolled. Subsequently, a control group of further 44 patients with as much as possible similar characteristics in terms of sex, age, localization and stage of the disease, ASA score, BMI, and surgical approach was retrieved. The NLR, LMR, and PLR before surgery and at the 1st and 4th postoperative days were calculated. Statistical differences between groups were compared using unpaired Student's t-test or Mann-Whitney rank sum test, as appropriate. Multiple comparisons were performed by one-way ANOVA. The ability of studied parameters to predict anastomotic leakage was analyzed using receiver operating characteristics (ROC) curve analysis. At the 4th postoperative day, the NLR and the PLR were significantly higher in patients with anastomotic damage. The analysis of the trends of the median values of all the factors studied revealed in day four a tendency to return to the preoperative values; nevertheless, the NLR and PLR remained significantly high in the dehiscence group. The Area Under the Curve (AUC) was 0.697 (95% CI: 0.581 - 0.797) and 0.653 (95% CI: 0.535-0.759) for the NLR and PLR respectively. Considering a cut-off NLR value at 8.7, sensitivity and specificity were 52% (95% CI: 33.5 - 69.2) and 88% (95% CI: 74.9 - 96.1) respectively. The corresponding figures were 82% (95% CI: 64.5 - 93.0) and 56% (95% CI: 39.9 - 70.9) respectively, when a PLR cut-off value at 189 was tested. Our findings suggest that the NLR and PLR evaluated at the 4th postoperative day are useful markers in predicting anastomotic dehiscence in patients who underwent colorectal surgery.

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EVIDENCE THAT THE EFFECT OF DARK CHOCOLATE ON PLATELET FUNCTION IS MEDIATED BY FLAVAN-3-OL METABOLITES

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Aim: Cocoa is rich in #avan-3-ols, a particular class of flavonoid. Increasing body of research now suggests that consuming flavan-3-ol-rich foods can positively modulate hemostasis, through mechanisms that directly target platelet function. Aims of this study were exploring the effect of dark chocolate on platelet function and investigating the relationship between this effect and flavan-3-ol bioavailability.
Methods: The study population consisted of 16 healthy male volunteers (36±10 years) who voluntarily ingested 50 g of 90% cocoa chocolate within 3-5 min. Blood was drawn early in the morning, immediately before chocolate ingestion (T0) and 4 h afterwards (T1). Platelet Function Analyzer (PFA)-100 closure time was assessed in sodium citrate anticoagulated whole blood, using collagen/adenosine-5'-diphosphate (COL/ADP) and collagen/epinephrine-(COL/EPI) cartridges. The concentration of plasma flavan-3-ol metabolites was measured in EDTA samples by using liquid chromatography coupled to a triple quadrupole mass spectrometer (UHPLC-ESI-QqQ-MS/MS). Significance of differences between T0 and T1 was evaluated with paired Wilcoxon's signed-rank test and the correlation between values was assessed with Spearman's test. The level of statistical significance was set at $p<0.05$.
Results: A significant increase of COL/ADP-induced PFA-100 closure time (113.0 vs. 98.5 sec, $p=0.039$), but not COL/EPI, was observed 4 h after ingestion of dark chocolate. Total plasma flavan-3-ol metabolites concentration was also significantly increased at T1 (1.0 vs. 1.9 µmol/L, $p=0.04$). Notably, the percentage increase of total plasma flavan-3-ol metabolites was significantly correlated with percentage increase of COL/ADP closure time ($r=0.63$, $p=0.009$).
Conclusions: Our data confirm that the potential beneficial effect of dark chocolate on primary hemostasis may be mediated by the circulating metabolites derived from its flavan-3-ols.

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NASAL CITOLOGY: A EARLY TEST TO EVALUATE INFLAMMATION IN WORKERS EXPOSED TO FORMALDEHYDE

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Formaldehyde (FA) is a very common precursor of many materials and chemical compounds. Pathology and Anatomy laboratory workers may be exposed to formaldehyde that is a known carcinogenic compound. An evaluation of the early signs of damaging effects of this substance is mandatory. The study aim was to assess whether nasal cytology (NA) was able to reveal any alteration of nasal mucosa in workers exposed to FA compared to unexposed subjects. A further aim was to find if a specific pattern of alterations correlated with years of exposure in order to evaluate long-term occupational exposure effects. The study included a group of 15 workers exposed to FA and a group of 10 healthy controls (no smoker and no allergic subjects). All subjects underwent clinical examination, research of inflammatory parameters (blood count, C-Reactive-Protein and erythrocyte sedimentation rate level), followed by scraping of nasal mucosa. Slides were coloured using MGG protocol and analysed by optical microscope (100 X oil immersion lens). All exposed workers was affected by an infiltration of neutrophils and muciparus cells related to exposition years. Moreover, the 33% of exposed subjects showed a chronic non-allergic inflammatory condition, "minimal persistent flogosis", characterized by a persistent infiltration of neutrophils and few eosinophils. Statistical analysis showed a significative increasing of neutrophils and mucous-secreting/ciliated cells ratio in workers with an average exposure of 15 year compared to controls ($p<0.001$); however, there is not correlation between nasal mucosa alteration and systemic inflammatory parameters analysed. Our data showed that NA is an early marker of local inflammation and could be a promising tool for FA exposed workers safety. An early detection of local inflammation could avoid the development of severe systemic pathologies.

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QUANTIFICAZIONE DELLA PROTEINURIA DI BENCE JONES: DUE METODI A CONFRONTO

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La proteinuria di Bence Jones (BJ) è uno dei marcatori utilizzati nel monitoraggio delle patologie plasma-proliferative con significato prognostico. 38 campioni di urina da raccolta di 24h (proteine totali (UPT) range <0.05-2.11g/L) positivi per proteinuria di Bence Jones (22 kappa e 16 lambda; metodo qualitativo di immunofissazione su gel di agarosio (Hydragel 2 IF/BJ)) sono stati selezionati per l'analisi quantitativa della BJ con due diversi metodi: lettura densitometrica su gel di agarosio della elettroforesi urinaria (U-ETF) (campione non concentrato) (Hydragel HR) e quantificazione in elettroforesi capillare (U-CETF) (Sebia-Capillary/minicap-urine). In 13/38 campioni la BJ è risultata quantificabile con entrambi i metodi: (11/13 UPT 0.33-2.11g/L). Regressione di Passing-Bablok: U-CETF=0.49U-ETF +0.01 (95% CI alfa da -0.11 a 007, beta da 0.08 a 0.95); r Pearson =0.50. Dei rimanenti 25 campioni che non hanno fornito risultati interpretabili con U-ETF, in 15 (UPT range 0.10-1.28g/L) U-CETF ha permesso la quantificazione della BJ; nei rimanenti 10 (UPT=0.05-0.17g/L) la BJ di troppo lieve entità ed il rumore di fondo del tracciato elettroforetico, non hanno consentito una accurata valutazione. I due metodi valutati presentano notevoli differenze. Nella fase pre-analitica il trattamento del campione è più oneroso per U-CETF poiché prevede due cicli di dializzazione/concentrazione del campione. Dal punto di vista analitico, invece, l'esecuzione con U-CETF seguita dall'immunotipizzazione avviene in completa automazione, diversamente da U-ETF e immunofissazione su gel di agarosio. La fase post-analitica d'interpretazione dei tracciati, richiede in entrambi i casi personale esperto, ma l'interpretazione di U-CETF può risultare più difficoltosa quando la BJ risulta di lieve entità. D'altra parte U-CETF ha una maggiore sensibilità, consentendo di quantificare la BJ anche per proteinuria <0.3g/L, diversamente da U-ETF. La scarsa comparabilità dei risultati relativi alla BJ quantificata con entrambe le metodiche (attribuibile sia alle diverse tecniche impiegate che al diverso pre-trattamento del campione), dimostra che la quantificazione deve essere effettuata sempre con lo stesso metodo.

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ALTERAZIONE DEL METABOLISMO DELLE ARGININE IN SOGGETTI AFFETTI DA BPCO LIEVE E MODERATA

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La broncopneumopatia cronica ostruttiva (BPCO) è una patologia respiratoria caratterizzata da ostruzione non completamente reversibile delle vie aeree, persistente tosse produttiva, muco in eccesso e dispnea. È noto che lo stress ossidativo svolge un ruolo di rilievo nella fisiopatologia delle malattie respiratorie. In particolare nell'asma [1] e nella fibrosi cistica sono stati riportati livelli plasmatici elevati dell'arginina dimetilata asimmetrica (ADMA). Poiché gli enzimi coinvolti nella sua produzione e degradazione sono redox sensibili, lo scopo di questo lavoro è stato valutare le concentrazioni di arginine nei pazienti affetti da BPCO. Lo studio è stato effettuato su 43 soggetti affetti da BPCO, suddivisi in lievi (n = 29) e moderati (n = 14), e su 43 controlli sani appaiati per età e genere. Sono stati valutati in tutti i soggetti biomarcatori di stress ossidativo come le sostanze reattive all'acido tiobarbiturico (TBARS), antiossidanti come i gruppi –SH delle proteine (PSH), la taurina e la paraoxonasi umana 1 (PON1). Sono stati inoltre misurati i livelli plasmatici di arginina, ADMA e dimetilarginina simmetrica (SDMA). I risultati ottenuti mostrano come i livelli di PSH diminuiscono con la gravità della patologia (6.69±1.15 vs 6.04±0.85 vs 5.33±0.96 μmol/gr prot, rispettivamente nei controlli, nei pazienti lievi e in quelli moderati, $p<0.0001$), mentre aumentano i valori di TBARS (mediana 2.93 vs 3.18 vs 3.64 μmol/L, $p<0.0001$). È stato osservato anche un aumento del rapporto ADMA/Arginina con la progressione della patologia (mediana 0.0067 vs 0.0075 vs 0.0100, $p<0.0001$). Attraverso analisi di regressione logistica multipla, solo il rapporto ADMA/Arginina (OR 1.72, 95% CI 2.27–13.05; $p = 0.02$) e i valori di PSH (OR 0.44, 95% CI 0.25–0.77; $p = 0.0045$) sono risultati indipendentemente associati con la progressione della BPCO.Questi risultati suggeriscono che l'insorgere e il progredire della BPCO siano associati sia con lo stress ossidativo che col rapporto ADMA/Arginina. L'aumento di tale rapporto è dovuto in particolare ad una riduzione della concentrazione di arginina, la cui causa potrebbe essere legata all'incremento dell'attività dell'arginasi dovuta alla presenza di stress ossidativo.

1. Scott JA, et al. Chest 2013;144:367–8.

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A SYSTEMIC AND TRASPARENT FRAMEWORK TO SUPPORT INFORMED DECISIONS FOR DIAGNOSTIC TEST IN LABORATORY SETTING

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Background: Decision-makers sometimes neglect important criteria supporting their judgments about the adoption of diagnostic test that could benefit patients. A trasparent and explicit approach based on evidence criteria is an efficient tool to guide decision-making process. We developed a multidimensional pathway based on GRADE Evidence to Decision(EtD) frameworks in order to define a valid process to assess the adoption of a diagnostic test and the consequences of a decision about a test.

Methods: The framework includes three sections reflecting the main steps of GRADE EtD: formulating the relevant question, making an assessment of the evidence, and drawing conclusions.We systematically searched in bibliogrphic databases and websites of scientific societies and regolatory agencies. We included systematic reviews, guidelines and HTA reports assessing the diagnostic accuracy of various molecular methods for the diagnosis of sepsis in neonates. We collected data about purpose, inclusion criteria, essential results and authors conclusions, and qualitatively summarised the main dimensions.

Results: The EtD framework presented evidence concerning the molecular diagnosis of sepsis in neonates and and included seven dimensions: (1) furmulating the question; (2) assessment of diagnostic test accuracy; (3) certanty of the evidence; (4) effects of test on main patient outcome; (5) belance between the desiderable and undesiderable effects; (6) resource use; (7) equity, acceptability and feasibility. Reviewers completed each dimension with relevant informations extracted. Several factors could influence the final decisions: importance of problem, the diagnostic accuracy values, effects of test on main patient outcome and the feasibility. Others such as resource and undesiderable effects were less consistently reported.

Conclusions: The EtD framework consists of a comprehensive decision aid model to ensure that all important criteria are considered to explain a judgment. This approach could help health professionals to use the best available research evidence in a structured and trasparent way to inform decisions in the context of laboratory medicine. Advantages of EtD framework include: trasparent process, explicit consideration of outcomes evaluation, use of reproducible approach.

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NUOVI MARCATORI PER LA GESTIONE IN PRONTO SOCCORSO DEL PAZIENTE CON SOSPETTA INFEZIONE: IL RUOLO DELLA PRESEPSINA NELLA STRATIFICAZIONE DEL RISCHIO

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La sepsi è una delle condizioni più comuni nel paziente critico, la cui corretta gestione prevede il tempestivo riconoscimento e la pronta somministrazione di una adeguata terapia. Scopo dello studio è valutare la capacità diagnostica di due biomarcatori tradizionali, la proteina C-reattiva (PCR) e la procalcitonina (PCT), e di due emergenti, la presepsina (PSP) e la copeptina. Lo studio si propone inoltre di confrontare l'accuratezza diagnostica delle nuove molecole con quelle della PCT, e di definire la capacità di PCR, PCT, PSP e copeptina nel predire l'outcome del paziente settico. Dal 15/04/2016 al 15/07/2016 sono stati arruolati 51 pazienti (pz) afferenti al Pronto Soccorso dell'Azienda Ospedaliera di Padova, sottoposti a misurazione di conteggio leucocitario, PCR e PCT nel sospetto di infezione/sepsi/shock settico. Per ogni pz sono state determinate in aggiunta PSP e copeptina, e sono stati raccolti i dati relativi a parametri vitali, terapia, dati biochimici, e diagnosi di dimissione. I pz sono stati suddivisi in 3 gruppi di diagnosi: no infezione/infezione/sepsi. L'età mediana è risultata di 68 anni, con un trend in aumento all'aumentare della gravità clinica (p=0.211). Per PCR e copeptina, la differenza nei tre gruppi di diagnosi è ai limiti della significatività statistica (p=0.052 per entrambe). L'associazione fra PCT e diagnosi di dimissione è risultata statisticamente significativa (p=0.008). Esiste una differenza statisticamente significativa nei livelli di PSP nei tre gruppi (p=0.02), così come fra i pz senza infezione vs sepsi (p=0.010); al contrario, non vi è significatività statistica nelle differenze fra pz non infetti vs infezione semplice (p=0.070) e fra pz con infezione vs sepsi (p=0.333). Le curve ROC dei 4 biomarcatori non presentano differenze statisticamente significative. La PSP è risultata associata alla durata del ricovero in modo statisticamente significativo (p=0.020). Dai risultati descritti, si può concludere che la PSP aumenta il valore diagnostico della PCT nell'identificare il pz settico, con maggiori sensibilità (100% per la PSP vs 50% per la PCT), e VPN (100% per PSP vs 83% per la PCT) e rappresenta invece un marcatore con prestazioni prognostiche superiori alla PCT per la stratificazione del rischio in Pronto Soccorso.

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PROCALCITONINA E PRESEPSINA IN PAZIENTI RICOVERATI IN TERAPIA INTENSIVA PER ARRESTO CARDIACO: EFFICACI PREDITTORI DI OUTCOME?

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L'arresto cardiaco è un evento con mortalità e morbidità elevate; meno del 50% dei pazienti (pz) rianimati con successo sopravvive senza sequele neurologiche. Dopo una rianimazione efficace i pz possono sviluppare la Sindrome post-arresto cardiaco (PCAS), caratterizzata da una risposta infiammatoria diffusa, dovuta al danno da ischemia/riperfusione. La risposta sistemica conseguente è analoga a quella osservato in corso di shock settico: per tale motivo, i marcatori di infiammazione potrebbero rappresentare potenziali strumenti utili al riconoscimento e alla stratificazione di gravità di malattia. Sono state valutate quindi la capacità diagnostica e di stratificazione della gravità della PCAS, di presepsina (PSP) e procalcitonina (PCT), e la loro capacità nel predire l'outcome a 12 mesi, nei pz che hanno presentato un arresto cardiaco. Sono stati arruolati 277 pz, per i quali era disponibile almeno 1 prelievo ematico eseguito all'ammissione e/o a 24-48 o 96 ore (h) dall'ingresso in Terapia Intensiva (TI). Le determinazioni di PSP e PCT sono state eseguite su ogni campione, ed è stato valutato l'esito neurologico ad 1 anno dall'evento. I livelli di PSP e PCT sono risultati significativamente più elevati nei pazienti con outcome neurologico negativo in tutti i tempi analizzati. L'aumento di concentrazione di PCT nelle prime 24 h è significativamente maggiore nei pz con outcome negativo ($p<0.001$). La PSP raggiunge il picco all'ammissione, ma la sua cinetica di concentrazione non differisce tra i pz con vs i pz senza sequele neurologiche. PCT e PSP hanno dimostrato una correlazione inversa e statisticamente significativa con il valore di pressione media delle 48 h ($p\leq0.001$). L'AUC della PCT per l'associazione con l'outcome sfavorevole aumenta durante la permanenza in TI, con un picco pari a 0.755 a 96 h. La PSP è invece più accurata al momento dell'ammissione (AUC=0.717). Per la capacità di predire la morte in TI, il valore di AUC della PCT è maggiore di quello della PSP. In un modello statistico contenente le notizie cliniche il solo valore di PCT a 96 h è risultato un indice prognostico indipendente di outcome neurologico negativo a 12 mesi. I valori di PSP in questo preciso contesto clinico non rappresentano un fattore prognostico indipendente di outcome.

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GASTROESOPHAGEAL REFLUX AND IDIOPATHIC PULMONARY FIBROSIS: THE ROLE OF BAL

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Interstitial lung diseases (ILDs) form a heterogeneous group of nonmalignant, non-infectious processes of the lower respiratory tract that are characterized by alveolo-interstitial inflammation and fibrosis on histological examination. They are also referred to as pulmonary interstitial diseases or diffuse infiltrative pneumopathies, and they are the most common cause of chronic non-obstructive pulmonary disease. The most common type of ILD is idiopathic pulmonary fibrosis (IPF), a disabling chronic fibrosis with progressive and fatal outcome within 2-3 years after diagnosis. Its etiology is still unknown and available therapies show little results.
Among the possible triggers of inflammation, uninhibited autoimmune disorders, viral infections and gastroesophageal reflux (GER) have been proposed. GER is a risk factor for microaspiration, which contributes to the development of chronic pulmonary disorders and the progression of IPF. The accumulation of lipids into the cytoplasm of alveolar macrophages is considered to be an evidence of aspiration of fatty acids. Such accumulation can be quantified and expressed as lipid loading index (lipid-laden index LLI), which takes into account the number of macrophages containing lipids, and the amount of lipid into each macrophage. Broncho-alveolar lavage (BAL) is an important method to investigate the so-called "deep lung" facilitating the diagnosis of various pulmonary disorders. Cytological analysis allows to define the size and profile of alveolar inflammation and to identify the presence of inhaled contaminants and respiratory pathogens. The aim of our study was to define the possible role of BAL in assessing microaspiration and its role in the etiopathogenesis of idiopathic lung disorders.

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CHOLECALCIFEROL TREATMENT IN PATIENTS WITH PAGET DISEASE OF BONE AND VITAMIN D DEFICIENCY: EFFICACY AND SAFETY

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Paget's disease of bone (PDB) is the second most frequent metabolic bone disorder after osteoporosis worldwide. As such, vitamin D status should be evaluated and the vitamin D deficiency corrected in PDB patients, which also are at increased risk of nephrolithiasis (NL).
Aim of this study was to evaluate the efficacy and the safety of cholecalciferol treatment in PDB patients with vitamin D deficiency [i.e. 25OH vitamin D (25OH-D) <50 nmol/L, normal values > 70 nmol/L].
We retrospectively examined the medical records of 82 PDB patients treated with oral cholecalciferol for 8 weeks at a cumulative dosage of 400.000 UI. The 25OH-D serum levels and the metabolic risk factors for NL, including the super-saturation indices of calcium-oxalate (APCa-Ox) and calcium-phosphate salts (APCa-P), were evaluated before and after cholecalciferol treatment, to evaluate the efficacy and safety of this treatment, respectively.
Cholecalciferol treatment induced a significant increase in 25OH-D levels in all patients (31.5 ± 18.3 vs. 91.8 ± 29.2 nmol/L) and more than 90% of them (76/82) reached 25OH-D levels ≥70 nmol/L. The increase in 25OH-D levels significantly reduced PTH (5.80 ± 2.40 vs. 4.50 ± 2.13 pmol/L) and ALP (133.2 ± 46.7 vs. 101.9 ± 45.9 U/L) levels. No significant changes were observed in serum and urinary levels of calcium, phosphate, magnesium and uric acid, in urinary levels of magnesium, oxalate and citrate, and in urinary pH after cholecalciferol treatment. Also the APCa-Ox and APCa-P indices not showed significant changes after cholecalciferol treatment.
In conclusion cholecalciferol orally administered significantly increased 25OH-D levels in PDB patients, correcting the vitamin D deficiency in the large majority of them. The correction of vitamin D deficiency not induces significant changes in serum and urinary NL metabolic risk factors.

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DETERMINATION OF BIOMARKERS IN VITREOUS HUMOR OF CORPSES FOR ESTIMATION OF THE POSTMORTEM INTERVAL

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Introduction: A precise estimation of the postmortem interval (PmI) is one of the most important topics in forensic pathology. However, the PmI estimation is based mainly on the visual observation of cadaveric phenomena that remain relatively imprecise. The main objective of the study is to evaluate the concentration of new biochemical indicators in vitreous humor in order to be able to develop a protocol for reliable definition of the age of death.
Materials and methods: A total of 12 cadavers were selected from the Department of Health Science, with known time and cause of death. Cadavers with pre-existing pathological conditions that may alter the concentration of electrolytes in vitreous were excluded. Vitreous humor was removed from both eyes of the corpses and the following parameters: sodium, potassium, chlorine, magnesium, urea nitrogen, uric acid, glucose, and creatinine were determined. The vitreous humor samples were obtained after median 47.5 [range 2.75 - 79.75] hours of death and 1.8 (range 1 - 4.9) hours between different eyes. They were collected and stored at -80 ° C and analyzed together in one time by Vista instrument Siemens.
Results: The results show a positive correlation of potassium (0.699;p<0.005), urea nitrogen (0.283; p=0.181) and uric acid (0.530;p=0.006); a negative correlation of sodium (-0.612;p<0.005) and chlorine (-0.784;p<0.005); no magnesium correlation with the time of death. Both the glucose and the creatinine measurements were below the instrumental sensitivity limit. There are no significant differences between the mean of biochemical parameters analyzed for vitreous humor obtained from different eyes.
Conclusions: These preliminary results are promising to evaluate the PmI when it is unknown. In order to properly evaluate the PmI, relying on the concentration of biochemical parameters, and with the help of an algorithm, it is necessary to confirm the results on a larger sample. There are no significant differences between the parameters in the two vitreous humor of the corpse perhaps due to the short time between the first and second samples. The study of new analytics and the re-evaluation of classics with up-to-date methods may give new inputs since there is still no gold standard techniques for the ascertainment of PMI.

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INTESTINAL PERMEABILITY IS INCREASED IN CHILDREN WITH IRRITABLE BOWEL SYNDROME: A CASE CONTROL STUDY

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Background and Aim: Intestinal permeability (IP) seems to play a pivotal role in Irritable Bowel Syndrome (IBS) pathogenesis. Aim of this work was to assess IP in a pediatric population with IBS.Methods: We recruited a prospective consecutive cohort of children diagnosed with IBS according to the Rome IV Criteria, and a cohort of healthy controls, at a tertiary care center. All patients performed L/M ratio test to assess IP, and underwent Lactulose Breath Test (LBT) for the diagnosis of Small Intestinal Bacterial Overgrowth (SIBO).
Results: 30 children (19 males, age range 7-16) were enrolled. 41 healthy patients (29 males, age range 8-14) constituted the control group. IP was significantly increased in IBS compared to controls (IBS 0.03±0.03 vs controls: 0.77±0.95; p=0,002, fig 2). Among IBS children, 16 (53%) had a diarrhea predominant subtype; these showed a significantly higher IP (IBS-D: 1.19±1.01 vs IBS-Other 0.48± 0.40; p=0,04 fig 3). IP did not differ between SIBO positive (66%) and SIBO negative (34%) children (SIBO+ 0.84±1.05 vs SIBO- 0.63± 0.76; p=0.8 fig 4 a and b), neither among patients who followed different therapies in the 3 months prior to the visit (No therapy L/M 1.04±1.22; VSL#3 L/M: 0.46±0.43; Rifaximin L/M: 0.79±1.06. No therapy vs VSL#3 p=0,4; No therapy vs Rifaximin p=0,8; VSL#3 vs Rifaximin p=0,6 fig5).
Conclusions: IP is increased in children with IBS, especially if diarrhea predominant. In our series, IP did not seem to be modified by antibiotics and probiotics, suggesting a background role in IBS pathogenesis. Further studies on larger series are needed to clarify how IP can act in the pathogenesis of functional gastrointestinal disorders.

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PATIENT GROUPING FOR PERSONALIZED MEDICINE BY SERUM MALDI PROFILING

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Obesity is a severe condition that hinders everyday life as well as health of 600 million of adults and 100 million of children worldwide. De facto this physical status has a huge impact over health by increasing the risk of developing cardiovascular diseases, type II diabetes, non-alcoholic fatty liver disease and cancer. This common condition, which is one of the preventable causes of premature death, is associated with three major points: exaggerate food intake, scarcity of physical activity and genetic predisposition. The first two causes are, to a certain degree, “manageable”, whereas the genetic predisposition creates heterogeneity among patients particularly on disease progression, regression and severity making advisable for patients a targeted therapy. Changes in circulating intact proteins, including their post translational modifications and splicing variants, have been investigated analyzing serum proteomic profiles, from low and high body mass index (BMI) male and female subjects, with the purpose of identifying new biomarkers for patient grouping and gender differentiation.Sera samples were subjected to immunodepletion using MARS Hu14 column, that removes the 13 most abundant proteins in serum, and the unbound low abundant fractions were profiled by MALDI mass spectrometry. Differences in acquired spectra were evaluated using software ClinProTools v.2.2.Results showed that subjects can be gender-based grouped by their serum protein profiles using Principal Component Analysis (PCA). The comparison between different gender groups with low BMI showed 23 best separating peaks (p-value<0.01; C.V.<20%). Conversely, high BMI patients showed a lower variability between males and females, with 15 best separating peaks (p-value<0.01; C.V.<20%). These data strengthen the idea of the usefulness of this method to discriminate patients in view of their pharmacological treatments differentiation.For the identification of best separating peaks, serum protein extracts were separated by SDS-PAGE, in-gel digested and analyzed by LC-MS/MS. Validation of the identified dysregulated species was performed by western-blot analysis. This strategy provides hints to clarify gender related changes and paves the way for targeted therapies and personalized medicine.

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CLASSIFICAZIONE DI LEUCEMIE ACUTE MIELOIDI E LINFOIDI MEDIANTE LO STUDIO DI VOLUME, CONDUTTIVITÀ E SCATTER CELLULARE TRAMITE ANALIZZATORE AUTOMATICO UNICEL DxH 800

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Introduzione: La diagnosi di leucemia acuta mieloide (LAM) o linfoide (LAL) si esegue integrando citofluorimetria, biologia molecolare, morfologia e citochimica. Questo iter è gravato da limiti organizzativi per la necessità di un'alta specializzazione, raramente reperibile in tutti i presidi e attiva sulle 24 ore, mentre un'accurata distinzione tra LAM e LAL è auspicabile già all'esordio per una migliore gestione clinica. I moderni analizzatori per l'esame emocromocitometrico dispongono di molteplici parametri per definire le caratteristiche di ogni cellula e un'opportuna analisi consente lo sviluppo di algoritmi per una pre-classificazione delle leucemie già al primo accesso.
Metodi: Sono stati analizzati retrospettivamente i risultati dell'esame emocromocitometrico eseguito con l'analizzatore UniCel DxH 800 (Beckman-Coulter) sul sangue periferico di 57 pazienti con leucemia acuta all'esordio (45 LAM, 12 LAL). Sono state considerate la media e la deviazione standard di 7 parametri (derivati da impedenziometria, radiofrequenza e scatter di luce laser) relativi a varie popolazioni cellulari, per un totale di 108 variabili. Mediante curve ROC, sono state definite le variabili in grado separare le due classi di leucemie ($p < 0.05$) e selezionate solo quelle per cui l'area sotto la curva era > 0.70. Attribuendo, ad ogni paziente, una serie di punteggi incrementali (0 -1) in base al valore di ciascuna rispetto a un cut-off (1), è stato ottenuto uno score complessivo.Risultati Venti variabili rispettavano i criteri fissati; tra queste, ne sono state scelte arbitrariamente 10 (5 più rappresentative di LAM e 5 di LAL) per calcolare lo score che ha classificato correttamente 53 su 57 casi (93%; area sotto la curva: 0.96; potere predittivo: 96% LAM e 83% LAL).
Conclusioni: In attesa di validare e migliorare le performance dello score con una casistica prospettica, lo studio mostra come le tecnologie presenti nelle strumentazioni attualmente disponibili possono essere utilizzate per ottenere una pre-classificazione dei casi di sospetta leucemia in un contesto di routine anche in regime di urgenza, a supporto di una migliore assistenza al paziente, in attesa della diagnosi definitiva.
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VERSO LE LINEE GUIDA: 11 ANNI DI ESPERIENZA NELLA DIAGNOSTICA CITOFLUORIMETRICA DELL'EMOGLOBINURIA PAROSSISTICA NOTTURNA IN UN SINGOLO CENTRO

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Introduzione: L'Emoglobinuria Parossistica Notturna (EPN) è una rara malattia derivante dall'espansione di un clone di cellule staminali emopoietiche carenti di un'ancora glicolipidica sulla membrana, con conseguente mancanza di proteine di superficie. L'EPN è classificata in tre forme cliniche (1) a cui corrispondono di solito diverse dimensioni del clone stimate in citofluorimetria. Al fine di standardizzare la procedura analitica e migliorarne l'accuratezza diagnostica sono stati nel tempo introdotti reagenti sempre più specifici e linee guida (2,3). Lo scopo del lavoro è valutare retrospettivamente gli effetti apportati sul piano diagnostico dagli aggiornamenti metodologici.
Metodi: Sono stati analizzati i referti di 704 fenotipizzazioni effettuate tra ottobre 2006 e aprile 2017 presso un unico laboratorio su campioni di sangue periferico di 591 pazienti. La ricerca del clone è stata eseguita marcando, con combinazioni di anticorpi monoclonali, granulociti, monociti (panello a 5 fluorescenze che include dal 2016 l'aerolisina FLAER) ed eritrociti (3 fluorescenze), aggiornate nel corso degli anni secondo le linee guida.
Risultati: Un clone EPN è stato rilevato 98 volte (14%) in 23 pazienti distinti (4%), 14 dei quali poi sottoposti a un follow-up, variabile da 2 ad un massimo di 18 determinazioni. Le dimensioni dei cloni sono risultate comprese tra lo 0.1% e il 99.9% (9 cloni <1%, 39 cloni 1%-30%, 50 cloni >30%). La taglia dei cloni è risultata correlata in modo lineare tra le diverse popolazioni cellulari, ma ha evidenziato una tendenza alla diminuzione nel corso degli anni, giustificata da un miglioramento dell'accuratezza analitica. L'analisi dei casi in follow-up ha dimostrato che questa riduzione non ha determinato una cambiamento nella classificazione dei pazienti.
Conclusioni: L'adeguamento alle linee guida ha garantito maggiore specificità nella quantificazione del clone grazie all'esclusione di cellule difettive per cause diverse da EPN. Le complessità tecnologiche intrinseche al metodo analitico, così come quelle interpretative dei risultati, devono trovare un corretto approccio secondo linee guida proprio perché il clone può evolvere nel tempo, spontaneamente o dopo terapia.
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