Special Supplement 2 SS2

BC biochimica clinica

RIASSUNTI 53° CONGRESSO NAZIONALE SIBioC

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

BIOMEDIA
La condivisione del sapere

ISSN 0393 - 0564
e-ISSN 0392 - 7091

volume 45

sommario

S2

biochimica clinica

Rivista fondata da Norberto Montalbetti
e già diretta da Carlo Franzini

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

membro di

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

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

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

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

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

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

Volume 45
Special Supplement 2

Autorizzazione del Tribunale di Milano
n. 40 del 2.02.1987

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

Stampa
GRAFICA BRIANTEA srl
20040 Usmate (MI)

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

Prezzo di un fascicolo Euro 5,16

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

Associato all'USPI
Unione Stampa Periodica Italiana
Tiratura di questo numero 2000 copie
2000 copies of this issue have been printed
ISSN 0393-0564
e-ISSN 0392-7091

53° Congresso Nazionale della Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica (SIBioC - Medicina di Laboratorio)

Vertual Edition, 11-13 ottobre 2021

Riassunti Sessioni Scientifiche

Codice Abstract	Titolo Sessione Scientifica
• **SS01-01, SS01-02, SS01-03, SS01-04**	Problematiche extra-analitiche nella diagnostica decentrata
• **SS02-01, SS02-CO01, SS02-CO02**	Il monitoraggio delle terapie anticoagulanti orali durante la pandemia da COVID-19
• **SS03-01, SS03-02 SS03-03**	Medicina di Laboratorio di prossimità e telemedicina nella malattia diabetica: innovazione tecnologica e nuove prospettive
• **SS04-01, SS04-02 SS04-03, SS04-CO03 SS04-CO04**	Diagnostica ematologica decentrata
• **SS05-01, SS05-02**	Cure primarie, poct e prove di efficacia: la sinergia necessaria
• **SS06-01, SS06-02, SS06-CO05, SS06-CO06**	Big data e valutazione dei risultati prodotti della diagnostica di laboratorio nei percorsi di cura dei pazienti territoriali
• **SS07-01**	La diagnostica molecolare decentrata: “rischi e opportunità”
• **SS08-CO09**	Young Scientists - Evoluzione della Medicina di Laboratorio: strategie e nuove sfide
• **SP01-01, SP01-CO10**	Il ruolo del POCT nella diagnosi delle sindromi coronariche acute
• **SP02-01**	Alleanza per la qualità e miglioramento degli outcome clinici
• **SP03-01, SP03-02 SP03-CO07, SP03-CO08**	Diagnostica decentrata di COVID-19: rischi e opportunità
• **CJ01-CJ03**	Sessione SIBioC Young Scientisis
• **CC01-CC08**	Casi Clinici

Legenda:

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

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

SS01 - Problematiche extra-analitiche nella diagnostica decentrata

SS01-01

THE PREANALYTICAL PHASE IN THE DECENTRALIZED TESTING: DIFFERENCES AND COMPETENCES

G. Bonetti

Laboratory of Clinical Pathology, Esine, Brescia, Italy

Point-of-care testing (POCT) can be used in clinical setting such as hospitals and in areas where decentralized testing is requested (i.e pharmacies, patient homes, healthcare practitioner's office, residences for elderly, blood donation centers, in ambulances, public utility services). ISO 22870:2016, ISO 15189 and Joint Commission indicate that all hospital based POCT must be supervised by the central laboratory; the laboratory director is responsible for standards of performance in all domains of POCT. Decentralized diagnostics are affected by several organizational, environmental, operational and technical challenges. According to CLSI POCT04 (1) nonhospital based POCT programs should have a POCT director who has to develop a process to ensure compliance and implementation of quality testing. The personel responsabilities in nonhospital-based POCT are the same as hospital-based ones. In Italy there isn't a national rule for POCT but different local regional ones. Recently SIBioC WG on POCT produced a recommendation for the use of POCT in hospital setting (2). Laboratory errors may have serious consequences for patient health and outcome. The preanalytical phase is most vulnerable to errors (60-70% of all laboratory errors). Preanalytical errors are quite frequent in decentralized diagnostic because it's usually performed by nonlaboratory personnel or by patients and because the increasing robustness and simplicity of currently available POCT systems can lead to the false perception that no risk or harm to the patient is possible. In CLSI POCT07A the most common preexamination variabiles, potentially source of errors are listed (3). The most common preanalytical errors in decentralized diagnostics are related to patient preparation such as incorrect sampling time, to blood collection as patient identification, to sample handing such as inadequate sample mixing and tube filling, transport and to interferences such as hemolysis. Many constituents have a daily variation and the blood composition undergoes significant changes after food consumption. Sampling should preferably be done after an overnight fast from 7am and 9am and should always be done prior to the potentially interfering diagnostic and terapeutic treatments. If decentralized tests have to be performed at different times it is necessary to record sampling time and time of administration of any therapeutic treatments. For a proper patient identification it is recommended to use barcoding systems. Capillary specimen should be labeled with the patient's first and last name, identification number, date and time of specimen collection, and initials of the person collecting the specimen (4). The recommended order for microcollection is different from venous blood sampling: blood gases (they may be altered if sampling is delayed), EDTA tubes, tubes with other additives and serum tubes (4). Sample clots may cause spurious results, so it's necessary to invert the tubes gently several times. Any anticoagulated sample must be rejected if any detected clot are present. In capillary sampling when blood flow doesn't permit the right sample volume the massage and squeezing around the puncture site may cause falsely decreased concentrations of some analytes due to the dilution of the blood sample with tissue fluid and falsely increased concentration of potassium due to hemolysis. Hemolysis is the most frequent preanalytical error and can affect many assays. Spurious iperkaliemia in whole blood may be found using POCT such as blood gas devices if occult hemolysis is present and no HIL detection system is available.
Most preanalytical errors in decentralized diagnostics can't be detected but can be prevented by standard operating procedures and achieving proper personnel education not only in analytical but also pre and postanalytical phases.

REFERENCES

1 CLSI. Essential Tool for Implementation and management of a Point-of-Care testing program. 3rd ed. CLSI guideline POCT04. Wayne, PA: Clinical and laboratory Standards Institute; 2016.
2. Rampoldi E, Patrucco G, Casati M, Morelli P, Carraro P per il Gruppo di Studio Point of Care Testing (POCT) Implementation and management of the point-of-care testing (POCT): essential indications. Biochim Clin 2021 (ahead of print) DOI: 10.19186/BC_2021.032.
3. CLSI. Quality Management: Approaches to reducing errors at the point of Care: Approved Guideline. CLSI guideline POCT07-A. Wayne, PA: Clinical and laboratory Standards Institute; 2010.
4. CLSI. Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard—Sixth Edition. CLSI document GP42-A6. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.

SS01-02

THE PRE-ANALYTICAL PHASE IN THE POCT MANAGEMENT OF TERRITORIAL DIAGNOSTIC NETWORKS

C. Bellini[1], M. Fantacci[2]

[1]Clinical Chemistry Laboratory Analysis, Misericordia Hospital Grosseto, South East Tuscany USL, Italy
[2]POCT Network Director, Valdichiana Amiata Siena-South Laboratory Analysis Director, South East Tuscany USL, Italy

In recent years, the management of territory-based diagnostic networks has taken on a major role, in order to guarantee an effective and efficient service covering the entire territory. Most recently the need for a territorial response to Covid-19 has further highlighted its centrality. In this context the use of point-of-care testing (POCT) represents a valuable diagnostic opportunity that responds to the need for timeliness and proximity for the management of territorial emergencies and urgencies and to support the health activities of zonal hospitals, first-aid points and remote areas. The area of the USL Toscana Sudest is an example of such complexity, both for the extension covering more than 11,000 km2, with a population of about 800,000 inhabitants (density

72/km2), and for its variety, with 60% of municipalities being mountainous or partially mountainous and 1 island municipality. For several years now an ISO 9001:2015 certified territorial POCT Network has been organised to support and integrate laboratory analysis activities, coordinated by the Laboratories, which guarantees a timely and quality diagnostic service close to the places of care and also at domiciliary.
Technological and analytical advances in POCT equipment, together with careful monitoring of Quality Control (QC), have improved the analytical quality of results. However in order to produce an overall quality result and minimise errors that may jeopardise patient safety, the whole process must be addressed for POCT, as we are used to doing for laboratory results. In detail several pre-analytical issues that can cause many potentially harmful errors, also considering the rapid availability of results, are worth focusing. A risk-based approach to POCT management allows to analyse all steps, to set up monitoring indicators to verify their correctness and to implement corrective and preventive measures to decrease the incidence of errors (1). Traceability from request to report and registration of all operators and devices involved (doctor, nurse, patient, request, sample, batch, instrument) facilitate the workflow management and enable verification of data consistency.
Besides the selection of tests (often already preset for the emergency), the timing of their execution useful for the clinical need and the correct preparation of the patient (e.g. fasting), great attention is paid to the procedure for unique identification of the patient and of the biological sample. Also the collection of the sample in terms of type, volume, device and sampling method, and the eventual treatment or storage before analysis are carefully controlled with shared checklists and operating procedures, as well as other QC on the sample, such as for example the presence of bubbles, clots or haemolysis.
It is therefore essential to act on training and to manage the skills of all the operators involved both in the use of the equipment and in its control in presence and remotely. The multidisciplinary committee is crucial in all aspects of the POCT network from the choice of tests, sites and patients, to the supervision of training and competency, as well as in monitoring performances (2).

REFERENCES

1 Brun M, Füzéry AK, Henschke B, Rozak K, Venner AA. Identifying sources of error and selecting quality indicators for point of care testing. Pract Lab Med. 2021 Mar 21;25:e00216. doi: 10.1016/j.plabm.2021.e00216. PMID: 33869709; PMCID: PMC8042172.
2. Nichols JH, Alter D, Chen Y, Isbell TS, Jacobs E, Moore N, Shajani-Yi Z. AACC Guidance Document on Management of Point-of-Care Testing. J Appl Lab Med. 2020 Jul 1;5(4):762-787. doi: 10.1093/jalm/jfaa059. PMID: 32496555.

SS01-03

IL TRASPORTO DEI CAMPIONI BIOLOGICI: ESPERIENZA DELLA REGIONE CAMPANIA

M. D'Amora

Direttore Dipartimento Integrato Medicina di Laboratorio asl Napoli 1 Centro

Il DCA n. 55 del 30.09.2010 ha disposto il "Piano di Riassetto della Rete Laboratoristica Ospedaliera e Territoriale". Con nota prot. 0406752 del 07.09.2020 è stato istituito il Gruppo di lavoro "Rete della Medicina di Laboratorio" per la revisione del citato Piano. Il GDL ha elaborato un documento tecnico ad oggetto "Modalità Trasporto Sangue e Materiali Biologici" che rappresenta la normativa con cui la Campania ha inteso regolamentare tutte le attività relative al trasporto dei campioni biologici per tutte le prestazioni dettagliate nel documento e ne ha definito,per ciascuna,le modalità di organizzazione per garantire una corretta gestione del processo in tutte le sue fasi attraverso la tracciabilità del percorso e nel rispetto delle procedure e protocolli esistenti (Decreto n.219 del 23/06/2021). Il documento evidenzia come tali attività sono necessarie anche per garantire la qualità delle prestazioni e la sicurezza degli operatori. Sinora non esisteva una norma dedicata in Campania. Il documento è stato elaborato con riferimento alle Linee Guida professionali internazionali e nazionali di riferimento e dispone, tra l'altro, che le AASSLL, le AAOO, quelle Universitarie, gli IRCCS ed il privato accreditato, ove esistano modalità difformi da quanto previsto debbano adeguarsi entro sei mesi dall'emanazione. Nell'allegato al decreto sono trattati i seguenti argomenti: 1. Introduzione 2. Definizioni 3. Finalità 4. Norme per il trasporto 5. Modalità Operative 5.1 Confezionamento Campioni Biologici 5.2 Conservazione e Trasporto dei campioni biologici / Raccomandazioni generali 5.3 Conservazione e Trasporto campioni di Biochimica ed Ematologia/Coagulazione 5.4 Conservazione e Trasporto campioni di Tossicologia 5.5 Conservazione e Trasporto campioni di Anatomia Patologica 5.6 Conservazione e Trasporto campioni di Microbiologia e Virologia 5.7 Conservazione e Trasporto campioni biologici per tutte le indagini Genetiche 6. Procedura trasporto materiale biologico all'interno di un presidio sanitario 7. Scheda di Trasporto 8. Fuoriuscita liquidi biologici 9. Tracciabilità 10. Livelli di responsabilità 11. Tabella esami Chimica Clinica ed Ematologia con tempi e modalità di conservazione per singolo test 12. Tabella esami di Microbiologia/Virologia con tempi e modalità di conservazione per singolo test 13 Bibliografia. Le raccomandazioni contenute nel documento si riferiscono ad ogni determinazione che deve essere eseguita con la finalità di procedere nel modo più affidabile per non compromettere l'accuratezza della sua misura dalla fine del prelievo all'inizio dell'analisi, considerando i seguenti elementi: la temperatura di trasporto; l'eventuale necessità di separare il plasma/siero dalla parte corpuscolata; l'eventuale necessità di congelare il campione (previa centrifugazione della relativa provetta);la distanza temporale dalla fase del prelievo. Conclusioni: Il trasporto dei campioni biologici è oggi un tema strategico. La movimentazione di essi non è più un

evento raro ma prassi frequente, sia per la tendenza delle strutture pubbliche agli accentramenti che di quelle private a consorziarsi. Anche il modello hub e spoke ed il fiorire di centri prelievo vede nella movimentazione dei campioni il fulcro delle attività teso a migliorare il servizio ai cittadini. A livello italiano mancano normative univoche per cui diverse regioni hanno disciplinato in materia ed oggi possiamo annoverare fra queste anche la Campania.

SS01-04
POST-ANALYTICAL PHASE IN DECENTRALIZED TESTING

D. Giavarina

Medicina di Laboratorio, AULSS 8 Berica, Vicenza

As with tests in central laboratories, decentralized analyzes show risks of error in the post-analytical phase. Trying to outline some elements of this problem, it is possible to identify at least three critical elements with different peculiarities, with respect to "centralized" analyzes validation, reporting and, last but not least, clinical interpretation of the result.
We can discuss these items with respect to at least three macro areas: the skills of the personnel dedicated to POCT analysis, the available technologies and methodologies, the standardization of procedures.
The validation of the result is the decision to consider an analytical result "valid", reliable, to make decisions. Results from central laboratory are generally considered valid a priori. In the POCT setting, validation is responsibility of personnel performing the test, usually "non-laboratory" people who do not have the training that laboratory professionals do.
Validation of a result must also take into account at least the correctness of the pre-analytical and analytical phases, the devices functionality, as well as the verification of quality through the control systems. Controlling panic and delta check ranges adds further complexity. We can combat these risks of error with continuous training, such as in the pre-analytical and analytical phases; using updated technologies, which allow self-checks, warning systems, blocking of results in case of non-compliance with quality specifications, etc.; using software for self-validation and decision support. We are near the start of Artificial Intelligence and utilizing big data to prove competency of operators, to prove that meters were giving reliable results, close to the other meters in the hospital and to maintain quality control in devices used outside of the hospital and operated by nurses, emergency medical technicians, and others not laboratorians [1].
Numerous studies have shown that reporting can be a critical element, when the execution of the tests is very far from the control of the central laboratory. The example of carrying out tests at pharmacies is emblematic: for some tests, the analytical quality is now sufficient, but there is “an urgent need to adopt recommended decisional levels and reference ranges updated according to the more recently published clinical guidelines [2]. The incorrect laboratory report is the most relevant issue for the post-analytical POCT phase, and specific quality indicators specific quality indicators could be very useful or even mandatory soon [3].
Specific competence counts in the interpretation of the results. For some tests, such as blood gas tests or thromboelastometry, the skills of specialists who have POCT systems at their points of care are likely to be high, often the highest in the health care system. However, there is evidence for others tests and other clinical contexts (monitoring tests [4] or management of critical results [5]) the situation is very variable and sometimes worrying. The training of operators and the assessment of skills must therefore also concern, and with particular attention, the clinical significance of the results and the actions to be taken in the event of critical or unusual results.

REFERENCES

1. Miller JA. https://www.aacc.org/cln/articles/2021/april/artificial-intelligence-is-poised-to-transform-point-of-care-testing (July, 2021)
2. Zaninotto M, Miolo G, Guiotto A, Marton S, Plebani M. Quality performance of laboratory testing in pharmacies: a collaborative evaluation. Clin Chem Lab Med 2016;54:1745-1751.
3. Brun M, Füzéry AK, Henschke B, Rozak K, Venner AA. Identifying sources of error and selecting quality indicators for point of care testing. Pract Lab Med 2021;25:e00216. doi: 10.1016/j.plabm.2021.e00216
4. Choi S, Choi SJ, Jeon BR, Lee YW, Oh J, Lee YK. What We Should Consider in Point of Care Blood Glucose Test; Current Quality Management Status of a Single Institution. Medicina (Kaunas). 2021 Mar 4;57(3):238. doi: 10.3390/medicina57030238. PMID: 33806620; PMCID: PMC8001912.
5. Nichols JH, Alter D, Chen Y, Isbell TS, Jacobs E, Moore N, Shajani-Yi Z. AACC Guidance Document on Management of Point-of-Care Testing. J Appl Lab Med 2020 1;5:762-787.

SS02 - Il monitoraggio delle terapie anticoagulanti orali durante la pandemia da COVID-19

SS02-01
TELEMEDICINE AND HEMOSTASIS-THROMBOSIS CENTRES

D. Barcellona

Dipartimento di Scienze Mediche e Sanità Pubblica Università di Cagliari, SSD di Emostasi e Trombosi Azienda Ospedaliero - Universitaria di Cagliari

Nowadays, telemedicine has different clinical applications since it is used in almost all medical specialties. The COVID-19 pandemic has created not only major economic and social upheavals but also has an important impact on public health. In this emergency situation, the use of telemedicine has been rise aiming to mitigate the effects of COVID-19 on human health (1).
In Italy, the Hemostasis-Thrombosis Centres (HTCs) belong to the Federation of Centres for the diagnosis of thromboembolic disease and the Surveillance of Anticoagulant therapy (FCSA) and they offer the highest possible quality of assistance to patients treated with Vitamin K Antagonists (VKAs) or Direct Oral

Anticoagulants (DOACs).
Unlike DOACs, VKAs need a close monitoring through the Prothrombin Time (PT) expressed as International Normalized Ratio (INR). The test result allows doctors at HTCs to prescribe the correct dosage of VKAs to maintain the INR in the therapeutic range. This means that patients have to go to their HTC more often than patients treated with DOACs.
Telemedicine has been implemented in the routine clinical practice at HTC many years ago (2) and actually, during the COVID-19 pandemic, this system is of significant aid in the management of this therapy allowing patients to perform the test at home or to self-manage their own therapy.
The system is organised as a centralised net-supported program with a server and PC stations in the HTC and workstations in the peripheral districts. Points-of-Care INR allow patients to easy perform the test on capillary blood and to quickly gain the INR result thus reducing the number of controls that patients would perform at the HCT (3). This aspect is of important value during COVID-19 pandemic since overcrowding should be avoided.
In general, self-management and self-testing have similar safety (RR=1.08, 95% CI 0.81-1.45, RR=0.99, 95% CI 0.8-1.23, respectively) than traditional monitoring as reported by the metanalysis of Sharma et al (4). As regard the efficacy, the authors showed a less incidence of thromboembolism when self-management was used (RR=0.51, 95% CI 0.37-0.69) with a trend versus a significant reduction in all-cause mortality (RR=0.68, 95% CI 0.46-1.01) while self-testing allowed to reach a 4.4% (95% CI 1.7-7.18) increase in time in therapeutic range. In accordance with other economic models, the metanalysis also showed that self-monitoring is cost-effective.
Another advantage of the use of telemedicine in HCT is the patients' satisfaction. In our experience (5) 85% of the patients are satisfied with self-testing at home and the quality of life is improved in 87% of them. The cost of test strips was medium–high for 89% of the patients, and 75% of them stated that it was worth improving their quality of life.
Telemedicine is usefull in managing patients on VKAs and, as suggested by the FCSA (6), it is a safe and efficacy system to guarantee an adequate medical assistance not only routinely but especially during pandemic. Finally, telemedicine could be used also for DOACs patients putting in place a system that may allow patients to attach the PDF file of their laboratory tests and to video-call the doctors at HTC.

REFERENCES

1. Doraiswamy S, Abraham A, MD; Mamtani R, Cheema S. Use of Telehealth During the COVID-19 Pandemic: Scoping Review. J Med Internet Res 2020; 22: e24087.
2. Barcellona D, Fenu L, Cornacchini S, Marongiu F. Telemedicine can improve the quality of oral anticoagulation using portable devices and self-testing at home. J Telemed Telecare 2013; 19: 298-301.
3. Sharma P, Scotland G, Cruickshank M, Tassie E, Fraser C, Christopher Burton C et al. Is self-monitoring an effective option for people receiving long-term vitamin K antagonist therapy? A systematic review and economic evaluation. BMJ Open 2015; 5: e007758.
4. Barcellona D, Fenu L, Marongiu F. Point-of-care testing INR: an overview. Clin Chem Lab Med 2017; 55: 800-805.
5. Barcellona D, Mastino D, Marongiu F. Portable coagulometer for vitamin K antagonist monitoring: the patients' point of view. Patient Prefer Adherence 2018; 12: 1521-26.
6. Poli D, Tosetto A, Palareti G, Barcellona D, Ciampa A, Grandone E et al. Managing anticoagulation in the COVID-19 era between lockdown and reopening phases. Intern Emerg Med 2020; 15: 783-86.

SS02-CO01

NEW ORGANIZATIONAL MODEL FOR DECENTRALIZED MANAGEMENT OF ORAL ANTICOAGULANT THERAPY OF ULSS 6 EUGANEA

E. Gnatta, R. Ghirardo, V. Temporin, A.M. Leo

UOC Medicina di Laboratorio Ospedali Riuniti Padova Sud "Madre Teresa di Calcutta" Monselice (Padova-Italy)

Background: Point of Care (POCT) portable coagulometers allow to shift determination of prothrombin time (PT-INR) from central laboratory to peripheral health facilities. In a broad and complex territory such as that of ULSS 6 Euganea, composed of five local health districts with a population of about 936.000 inhabitants, determining PT-INR in territorial decentralized offices is a key objective in therapy control and patient's life quality improvement.
Aim of the study: description of the organizational model for decentralized management of patients in Oral Anticoagulant Therapy (OAT) involving Anticoagulation Clinic (AC), Integrated Home Cares (IHCs) and Routine Medical Cares (RMCs).
Methods: Starting from October 2020, a bidirectional connection was implemented between Laboratory Information System (LIS) of the Ospedali Riuniti Padova Sud "Madre Teresa di Calcutta" Monselice (Padova, Italy) and peripheral offices, where clinical data are collected and PT-INR is determined by POCT portable coagulometers (Coaguchek® Roche Diagnostics, Germany). Information is sent in real time to central laboratory using IT1000 middleware, where data are validated and historicized. Historicized data can be consulted and downloaded like other laboratory exams. Furthermore, for patients followed by AC, data are sent to OAT monitoring software (GESTAOWEB® Tesigroup Milan). Results: the project involved 2980 patients. Centres to which patients are belonging are located throughout the territory of ULSS 6 Euganea and consist of 7 IHCs, 9 RMCs and one AC, a total of 81 POCT instruments for about 60.000 determinations per year. From January 1st 2021 to June 30th 2021 the average frequency of determinations per patient was about 30 days, while the average number of determinations per patient was about 7,5.
Conclusions: the connection with LIS represents project's focal point, allowing historicization of data, making them available at any time for any connected facility. This model simplifies management of both patients in IHC and followed by RMC, allowing easier access to the determination of PT-INR, with more constant therapy control and significant improvement of life quality.

SS02-CO02

THE ORAL ANTICOAGULANT THERAPY CLINICAL MANAGEMENT DURING PANDEMIA: THE SOLUTION FOUND BY THE FCSA 123 HEMOSTASIS AND THROMBOSIS CENTER IN SAVONA

C. Scarone, V. Dovere, C. Traverso, F. Lillo

S.C. Laboratorio di Patologia Clinica ASL 2 Regione Liguria

Background: The emergency caused by the Covid-19 pandemic has forced the reformulation of the operating methods of the Health System, turning the spotlight on the need for greater interaction between hospital and territory. This aspect is even more evident in patients needing for a more strict followup as those under antithrombotic therapy (TAO), making clear the essential usefulness of digital tools and of new organizative models.
Objectives: Minimize the risks for adverse events in patients on oral anticoagulant therapy with vitamin K inhibitor drugs (AVK) or direct oral anticoagulants (DOAC), maintaining an optimal level of clinical / consulting support, allowing accurate monitoring of compliance and managing both hemorrhagic and thrombotic emergencies remotely, in order to reduce access to the Emergency Room (when possible) and to hospital facilities. Methods: The patients in charge at the Thrombosis Center of the San Paolo Hospital in Savona (FCSA 123) are in total 781: 66% diagnosed with atrial fibrillation (FA), 20% for the presence of mechanical or biological valve prostheses and 14% for thrombotic pathologies such as deep vein thrombosis (DV) and pulmonary embolisms (EP). 560 patients are in DOAC, while 221 patients in AVK. During the pandemic, a dedicated mobile telephone number was activated (twelve hours/day from 8 am to 8 pm) equipped with an instant messaging application service. Each patient was asked for an email address and signed consent to the computerized management of TAO therapy and forwarding of health documents. Results: In order to monitor the expected results, the following indicators were evaluated: number of incoming phone calls; number of treatment plans issued for DOAC; time in range for patients in AVK; number of complications recorded in the period under review (April 2020 -April 2021). The results obtained are respectively: about 9000 phone calls received; 560 treatment plans released; time in range from patients in AVK (TTR) 74%; 22 adverse events including two major hemorrhages. Conclusions: The new organization, based on digital support of clinical monitoring, has received high appreciation from patients and consequently a greater compliance with the therapy protocol. This management model has allowed an effective control both of the number and severity of adverse events, while the reduction of outpatient access has allowed to drastically reduce the infectious risk. In addition, e-mailing of reports and treatment plans allowed an optimization of human resources.

SS03 - Medicina di Laboratorio di prossimità e telemedicina nella malattia diabetica: innovazione tecnologica e nuove prospettive

SS03-01

TELEMEDICINA, CURA A DISTANZA E MODELLI DI INTEGRAZIONE CON LA MEDICINA GENERALE

A. Scatena

Direttore UOC Diabetologia, Ospedale San Donato – Arezzo, ASL Toscana Sud Est

La gestione integrata del diabete mellito in Toscana dall'implementazione del Piano Sanitario regionale (PSR) 2008-2010 si basa sulla Medicina d'Iniziativa, che ha lo scopo di intercettare tempestivamente il bisogno di salute prima dell'insorgere della malattia o prima che essa si complichi, rallentandone il decorso e garantendo interventi adeguati e differenziati in rapporto a livello di rischio (proattività). Tale esperienza prevedeva un livello assistenziale di base gestito dai medici di medicina generale e un livello assistenziale complesso gestito dal diabetologo. Con la Delibera regionale nr 650 del 5 Luglio 2016 si amplia la copertura a tutti gli assistiti e si creano PDTA dedicati alle multicronicità con interventi che tengano conto della differente complessità assistenziale; per la prima volta si introduce il concetto di Piano Assistenziale Individuale (PAI) per i pazienti più complessi, gestito dal medico di medicina generale supportato da tutti i membri del team multidisciplinare e multiprofessionale.
Con la legge regionale Toscana n. 20/2020, il PAI diviene la modalità di presa in carica di ogni assistito e deve tenere conto di tutti gli aspetti che incidono sulla salute del paziente, da quelli strettamente sanitari a quelli sociali, e che preveda l'intervento di più professionisti in forme coordinate, quali un Team multiprofessionale. Parallelamente alla legge regionale, la Commissione Permanente per le Attività Diabetologiche della Regione Toscana ha portato avanti la Delibera n 5 del 7 Gennaio 2020 (Percorso diagnostico terapeutico assistenziale per il Diabete nell'adulto. Modello di gestione integrata tra i medici di medicina generale e servizi di diabetologia. Aggiornamento Delibera GRT n. 108/2011- sostituzione del PDTA nell'adulto), nella quale si tracciano le modalità di condivisione del percorso di cura tra medico di medicina generale e specialista diabetologo, a fronte delle variazioni del compenso glicometabolico, delle complicanze acute e croniche e, più in generale, del cambiamento delle condizioni di salute.
I mesi successivi all'emanazione della legge, sono stati caratterizzati dalla pandemia Covid-19, dalla diffusione della telemedicina, grazie anche alla delibera regionale n 604 del 6 Aprile 2020, e con essa al crescere dell'esigenza della condivisione dei dati da parte del team, tanto che nella delibera n 469 del 4 Maggio 2021 si fa riferimento anche ad una software house/piattaforma regionale dedicata. I dati diventano la base per la continua e tempestiva revisione del PAI, utilizzando anche fonti nuove, come i Point of Care (POC) e modalità di assistenza più rapide, come il teleconsulto.
La nostra esperienza con il teleconsulto ha avuto lo scopo di implementare la delibera n5 del 7 Gennaio 2020, migliorare l'appropriatezza prescrittiva e implementare le nuove linee guida farmacologiche. Sono stati inclusi 65 pazienti, per i quali è stato effettuato un

teleconsulto con il medico di medicina generale per discutere le caratteristiche cliniche, il compenso glicometabolico e la classe di rischio cardiovascolare. La terapia è stata quindi adeguata in modo condiviso e sempre con la modalità del teleconsulto è stata gestita e risolta anche la comparsa di effetti collaterali (7,7%) e la programmazione del follow-up.

SS03-02

HOSPITAL AND TERRITORY: PROXIMITY MEDICINE AND THE ROLE OF THE CLINICAL LABORATORY FOR MONITORING THE DIABETIC PATIENT

A. Ognibene

Dipartimento di Medicina di Laboratorio e Trasfusionale USL Toscana Sudest

The Tuscany regional law n. 20/2020, in the preamble, establishes to "guarantee the assisted person the use of an appropriate assistance path in relation to the ascertained needs for health education, preventive services, social assistance, diagnosis, treatment and rehabilitation, where this requires the " intervention of several professionals in coordinated, integrated and programmed forms, the care of the assisted person must take place on the basis of an Individual Care Plan (PAI), which takes into account all the aspects that affect the patient's health, from those strictly health care to social ones, and which provides for the intervention of several professionals in coordinated forms, such as a multi-professional team ". The clinical problem must be recognized and identified promptly in such a way as to require an overall care of the patient by a multi-professional and multidisciplinary team, for clinical framing and / or monitoring. It is necessary to create a permanent link between the professionals involved and in particular between the general practitioner treating the patient, the outpatient specialist and the Laboratory and Diagnostic Service, between which the exchange of information and opinions must be constant, timely and also through the possibilities offered by "Digital Medicine" such as “the teleconsult”, to define the path. The advantages of the multi-professional and multidisciplinary team and of the second-level pathology / specialty outpatient clinic concern all the actors involved: the patient who performs the diagnostic investigations; the General Practitioner (GP) who sees guaranteed the possibility of obtaining an answer to the clinical question or the scheduled management of controls in a short time; and the specialist doctor who uses all the diagnostic potentials present in the hospital or in a multi-specialist outpatient facility, to formulate, in a short time, diagnoses that require multidisciplinary interventions. The clinical laboratory is part of the team as a determining factor both to clarify the clinical question but also to establish the terms and times of 2nd level specialist intervention. The patient with known diabetes accesses the II level of care, exclusively by being sent by the GP using the priority classes as already indicated for the first visit or through a scheduled visit. The use of priority classes for patients in the care of the GP can take place whenever the current situation constitutes a novelty / variation compared to the previous equilibrium, such as to require an assessment in a short time. Different laboratory parameters represent indicators that, introduced in the priority decision algorithm, can, if communicated in a timely manner, define the path and strategies of specialist therapy in the most appropriate way. In the reality of the USL TSE, a project has been developed that allows to communicate via SMS the results of the laboratory parameters, related to diabetes monitoring, to the patient and the GP when these vary significantly compared to previous controls or represent a clinical state that justifies an intervention by the Team.

SS03-03

THE ROLE OF LABORATORY MEDICINE IN THE MANAGEMENT OF MEDICAL DEVICES FOR SELF-MONITORING BLOOD GLUCOSE

M. Carta[1], G. Bonetti[2]

[1]UOC Medina di Laboratorio, Ospedale S. Bortolo, AULSS 8 Berica, Vicenza
[2]Laboratorio Analisi ASST-Valcamonica, Esine

The self-monitoring of blood glucose is of utmost importance for diabetic patients: it allows patients to evaluate their individual response to the therapy and verify whether the glycemic objectives have been met. All of this must of course be based on the use of one data which accurately reflects the glycemia.
The guidelines recommend the use of a device for the self-measurement of glycemia (POCT) which passed the necessary tests of accuracy and precision and thus presents the CE mark. Some studies, however, have emphasized how a non-negligible number of devices bears the CE mark without actually meeting the minimum criteria of accuracy requested (1).
Even the Italian guidelines (2) highlight the necessity that producing firms clearly declare the analytical features of their devices, particularly in terms of accuracy and precision. The hierarchy of the sources of information has the scientific studies of literature in the first place, with the declarations of producers only ranked second. Lacking explicit and comparable references or in the presence of diverging situations around the analytical performances, diabetic facilities may activate a local evaluation of themselves, with a periodic comparison of the accuracy of the devices being recommended anyway.
The evaluation of the accuracy of POCT systems is very discussed. ADA, FDA, CLSI and ISO 195-2013 (only to mention the most authoritative sources) report different targets. The Italian recommendations revolve around the norms ISO 105-2013 which provide that at least 95% of the measures obtained with the glucometer differ of around 0,8 mmol/L with respect to the measurements obtained with the reference method for concentrations < 5.6 mmol/L and of around 15% for concentrations > 5.6 mmol/L. Moreover, at least 99% of the measures must fall between the areas of error A and B displayed in the Clarke error grid (3). According to the FDA, on the other hand, the 95% of the values must not fall beyond 15% from the reference value and the 99% must be within 20%.
In any case, the accuracy of glucometers is based on the comparison with a reference method, and it is thus necessary to utilize a correct reference method in laboratories.
Much has been discussed around possible variables that may alter the quality of a comparative study of this type

(method used, type of capillary or venous sample, use of first or second drop for POCT measurement etc..) but what undoubtedly plays a major role is the use of procedures which can stop the in vitro glycolysis. Traditionally, sodium fluoride (NaF) is used to stop the glycolysis; this, however, is unable to contrast the glycolysis during the first hour of conservation of the sample. The use of inhibitors that associate the NaF with the citrate buffer thus provoking an early inhibition of the glycolysis has proved more effective in that sense.
This aspect has also been recently highlighted from the IFCC Working Group (5) and assessed in some studies. The use of early glycolysis inhibitors (NaF plus citrate) has proved effective to stabilize samples even up to 15 days and this may allow their use even as control material to evaluate the analytical performances of the glucometers alternatively to the materials currently employed which are based on serum or plasma with an addition of glucose and which may present commutability problems.

REFERENCES

1. Pleus S, Baumstark A, Jendrike N, et al. System accuracy evaluation of 18 CE-marked current-generation blood glucose monitoring systems based on EN ISO 15197:2015. BMJ Open Diabetes Res Care. 2020;8(1):e001067. doi:10.1136/bmjdrc-2019-001067.
2. Carta, M., Testa, R. Raccomandazioni per l'autocontrollo della glicemia nel paziente diabetico: sinossi. Riv Ital Med Lab 10, 122–124 (2014). https://doi.org/10.1007/s13631-014-0050-6.
3. International Organization for Standardization. In vitro diagnostic test systems-requirements for blood glucose monitoring systems for self testing in managing diabetes mellitus. ISO 15197:2013.
4. US Food and Drug Administration. Self Monitoring Blood Glucose Test Systems for Over The Counter Use. Guidance for Industry and Food and Drug Administration Staff. Available at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/self-monitoring-blood-glucose-test-systems-over-counter-use Document issued September 29, 2020, last accessed June 2021).
5. IFCC Working Group GMECC. How should glucose meters be evaluated for critical care. Available at https://www.ifcc.org/ifcc-education-division/emd-committees/c-poct/wg-gmecc/ (accessed June 2021).

SS04 - Diagnostica ematologica decentrata

SS04-01

ESAME EMOCROMOCITOMETRICO: TELEMEDICINA E COMPETENZE

S. Buoro

Centro di Riferimento Regionale per la Qualità dei Servizi di Medicina di Laboratorio
c/o ASST Grande Ospedale Metropolitano Niguarda, Milano

L'evoluzione tecnologica è un elemento fondamentale della ri-progettazione ed organizzazione dei processi della medicina di laboratorio nell'ineluttabilità dei percorsi di riordino e consolidamento, tuttavia le competenze dello specialista di medicina di laboratorio giocano un ruolo centrale nella governance di questo processo evolutivo.
La diagnostica ematologica nella sua globalità, dall'emocromo, alla caratterizzazione immunofenotipica, genetica e molecolare è uno dei settori della medicina di laboratorio che maggiormente ha beneficiato del rapido progresso tecnologico.
L'emocromo è ampiamente richiesto dai clinici perché è un esame necessario per la valutazione dello stato di salute del paziente; è però uno degli esami più difficili da gestire nei processi di riordino e consolidamento dei laboratori. L'emocromo è un esame multiparametrico la cui refertazione prevede l'integrazione dell'analisi automatizzata eseguita dai citometri a flusso con l'eventuale valutazione morfologica dello striscio di sangue periferico, che talvolta porta alla formulazione di un referto commentato.
Postulato che la qualità delle prestazioni, la sicurezza del paziente e un adeguato contenuto informativo del referto devono essere garantite indipendentemente dalla sede di esecuzione dell'esame. Questa può avvenire solo attraverso il governo dell'integrazione tra competenze professionali e tecnologia.
I sistemi di analisi digitalizzata dello striscio di sangue periferico possono supportare il raggiungimento di questo obbiettivo permettendo l'armonizzazione del processo di lettura dello striscio, garantendo la tracciabilità del processo, l'ottimizzazione dei tempi e facilitando la condivisione delle informazioni e delle conoscenze.
L'impiego della morfologia digitalizzata nella pratica routinaria si sta progressivamente diffondendo per gli indiscutibili vantaggi documentati, per le opportunità future di sviluppo di sistemi più performanti, di intelligenza artificiale e di incremento delle piattaforme di big-data.
Tuttavia di controparte solo una minima parte di energie sono spese per promuovere e documentare corretti percorsi di verifica e validazione di questi sistemi di analisi digitalizzata delle immagini, di verifica dell'efficienza ed efficacia diagnostica dei nuovi flussi operativi proposti e di qualificazione del personale deputato alla revisione microscopica digitalizzata.
Infatti, ad ora non è disponibile un documento che definisca limiti e regole di applicabilità della analisi morfologica digitalizzata al pari di quanto avviene per i conteggi in automazione, come pure le competenze che devono avere gli operatori che utilizzano questi sistemi, e i percorsi di verifica della qualità delle prestazioni strumentali e di verifica di qualità esterna
Sicuramente il futuro della medicina di laboratorio e dell'ematologia di laboratorio sarà condizionato e supportato da strumenti innovativi come la morfologia digitalizzata e lo sviluppo di reti neuronali, ma questa evoluzione non può prescindere dalla necessità di garantire il governo della tecnologia da specialisti di laboratorio con adeguate competenze in ambito ematologico e morfologico.

REFERENCES

- Buoro S, et al. Harmonization of laboratory hematology: a long and winding journey. Clin Chem Lab Med 2018;56:1575-8.
- Buoro S, et al. Harmonization of interpretative comments in laboratory hematology reporting: there commendations of the Working Group on Diagnostic Hematology of the Italian Society of Clinical Chemistry and Clinical Molecular Biology (WGDH-SIBioC). Clin Chem Lab Med 2018;57:66-77.
- Da Costa L. Digital image analysis of blood cells. Clin Lab Med 2015;35:105-22.
- Da Rin G et al. Multicentric evaluation of analytical performances digital morphology with respect to the reference methods by manual optical microscopy.J Clin Pathol. 2020 doi: 10.1136/jclinpath-2020-206857. Online ahead of print.
- Kratz A, et al. Digital morphology analyzers in hematology: ICSH review and recommendations. International Council for Standardization in Haematology.
- Int J Lab Hematol. doi: 10.1111/ijlh.13042. Epub 2019 May 2.

SS04-02

POCT IN HAEMATOLOGY: TECHNOLOGY AND NEW OPPORTUNITIES

R. Pajola

U.O.C. Laboratorio Analisi, ULSS 6 Euganea, Ospedali Riuniti Padova Sud "Madre Teresa di Calcutta", Monselice (Pd)

The complete blood count (CBC) is one of the most requested tests, routinely performed in the central laboratory (LAB) by large haematological analysers, useful to diagnose many diseases and manage urgent clinical decisions such as transfusion or administration of chemotherapy and antibiotics.
In recent years, rapid technological improvements led to the spread of point of care testing (POCT), performed outside the LAB and capable of providing CBC in a few minutes, measuring haemoglobin (Hb), red blood cells (RBCs), white blood cells (WBCs) with their differential count of 3 or 5 populations, platelets (PLTs) and many other parameters. Hb remains the most common POCT in haematology, essential to exclude anaemia.
There are two types of technology: small benchtop analysers and portable devices. The former are smaller and fully automated versions of LAB's analysers and, although portable, are not suitable to use at patient's bedside. The latter, some of which use disposable cartridges, do not require start-up procedures, maintenance and calibrations.
The latest generation of portable devices combines advanced digital technology with innovative technologies of viscoelastic focusing and microfluidics and techniques, such as digital microscopy and computer vision, using near infrared spectroscopy and the absorption of light at multiple wavelengths, obtaining CBC results unthinkable until a few years ago.
POCTs' accuracy is influenced by several factors: sample collection, blood type (venous or capillary) and timing. Poor finger prick technique can provide misleading results, it was proved that capillary samples significantly underestimate PLTs overestimating Hb and WBCs, but differences have not clinical relevance when the samples are collected according to standardized procedures.
POCT devices can not differentiate normal cells from pathological ones (e.g., erythroblasts, blasts, etc.). The presence of large platelets can lead to inaccurate PLT counts compared to the impedance method used in LABs. As recommended by the guidelines, due to the inherent risk of preanalytical errors and the standard risk of error during analytical and postanalytical phases, threshold values must be established to repeat CBC in a LAB.
Another consideration is POCTs' cost, which is cumulatively higher than that of a LAB-performed CBC.
Literature suggests that POCTs are not yet the ideal tools to perform CBC for diagnostic purposes, but they are useful in urgent situations such as rapid monitoring of some parameters (e.g., WBCs and Hb). Further studies are needed to confirm the promising results of POCTs and evaluate their performance even at low ranges and in pathological conditions.

REFERENCES

1. Rampoldi E., Patrucco G., Casali M., Morelli B. Carraro P. per il GdS POCT. Principi per l'implementazione e la gestione del point-of-care-testing (POCT): indicazioni essenziali. Biochim Clin 2021; 45: 312-326.
2. Briggs C., Kimber S., Green L. Where are we at with point-of-care testing in haematology?. BJH 2012; 158: 679-690.
3. Mooney C., Byrne M., Kapuya P. et al. Point of care testing in general haematology. BJH 2019; 187: 296-306.

SS04-03

DIAGNOSTICA EMATOLOGICA DECENTRATA: INGEGNERIZZAZIONE E VALUTAZIONE DEL PROCESSO

A. Brioschi

InGeSan (Ingegneri Gestionali in Sanità)
UOC Controllo di Gestione
ASST della Brianza

In un contesto in cui la Medicina di Laboratorio, fondamentale supporto nelle decisioni cliniche, è stata oggetto negli ultimi decenni di sistematiche politiche di contenimento dei costi, la valutazione del ruolo del POCT non può limitarsi ad una semplice valutazione costi/ricavi (che porterebbe a scelte di non utilizzo di tali tecnologie) ma necessita di un sistema di valutazione HTA multi-variabile, sia in fase di valutazione della scelta di implementazione della tecnologia che in fase di valutazione nel corso della vita utile del POCT.
Il Lean Management può fornire approccio e strumenti utili a "giustificare" il costo, in genere superiore a quello dell'analisi di laboratorio, con un outcome atteso migliore o ad un uso più efficiente delle risorse durante l'assistenza riducendo l'utilizzo inappropriato e gli effetti negativi del rischio legato alle attività effettuate da operatori non di laboratorio.

MEDICINA DI LABORATORIO: destinatario “privilegiato” delle azioni di contenimento dei costi

L’affermazione che “il 70-80% delle decisioni cliniche richiedono informazioni di Medicina di Laboratorio” benchè non supportata da valutazioni numeriche sistematiche, è (se non nella %) almeno empiricamente evidente nella clinica quotidiana.

Benchè i costi di laboratorio rappresentino una piccola quota della spesa totale della Sanità, la Medicina di Laboratorio è stata, negli ultimi 10/15 anni, oggetto di sistematiche politiche di riduzione dei costi (forse guidato anche dal fatto che, rispetto ad altre attività di diagnosi e cura, i costi delle attività di laboratorio sono facilmente tracciabili e monitorabili), che si sono sviluppate secondo modalità di approccio progressive:

- un primo approccio di contenimento della spesa definita attraverso “tagli lineari”;
- un secondo approccio finalizzato alla riduzione degli esami non necessari;
- un terzo approccio ha posto il focus sul controllo sistematico delle richieste “ex ante”, in particolare per i test ad alto costo, investito i dirigenti di laboratorio del compito di “cerbero”;
- il quarto approccio prevede la definizione di algoritmi di utilizzo, simili a quelli che vengono definiti nella pratica di prescrizione dei farmaci.

In tale contesto e forse nonostante o favoriti da tali politiche di taglio dei costi si è assistito all’evoluzione del sistema dei laboratori caratterizzato da un doppio fenomeno:

- riorganizzazione con consolidamento e decentralizzazione delle attività di processazione;
- sviluppo di una rete capillare di attività di prelievo distribuita sul territorio; facendo della Medicina di Laboratorio un precursore del processo di spostamento dei servizi da ospedale a territorio cui si sta assistendo in questi ultimi anni, grazie anche alla caratteristica, propria della Medicina di Laboratori e di poche altre specialità, di poter distinguere il momento di “contatto con il paziente” dal momento di effettiva erogazione della prestazione prima dell’avvento delle tecnologie a supporto della Telemedicina.

TELEMEDICINA in laboratorio

“Per Telemedicina si intende una modalità di erogazione di servizi di assistenza sanitaria, tramite il ricorso a tecnologie innovative, in particolare alle Information and Communication Technologies (ICT), in situazioni in cui il professionista della salute e il paziente (o due professionisti) non si trovano nella stessa località”
(definizione del Ministero della Salute)

Pur essendo oggetto di sviluppo già da un ventennio con l’iniziale utilizzo di POCT nelle attività di gestione dell’emergenza/urgenza ospedaliera, nell’analisi delle linee guida ministeriali del 2008 sulla telemedicina, per la Medicina di Laboratorio viene riportato un unica valutazione:

“Riorganizzazione della diagnostica di laboratorio e diagnostica per immagini

Il rilevante impatto economico ed organizzativo delle iniziative di sviluppo del settore della diagnostica di laboratorio (incluse le tecniche diagnostiche avanzate) e della diagnostica per immagini ha reso indispensabile attivare iniziative finalizzate, tra l’altro, ad assicurare l’interoperabilità dei sistemi ospedalieri e delle ASL (intra e inter-regionali), con particolare riferimento al Teleconsulto (second opinion)”

POCT: come valutare le reali opportunità per la Medicina di Laboratorio

“Per POCT si intende l’analisi eseguita vicino o al punto di cura del paziente, con il presupposto che il risultato sia disponibile immediatamente o in un lasso di tempo molto breve al fine di permettere ai clinici una diagnosi immediata e/o un’immediata decisione terapeutica”
(definizione da “Raccomandazioni per l'implementazione e la gestione del “point-of-care testing” (POCT)”)

Il costo per esame del POCT generalmente eccede quello del laboratorio clinico, di contro la remunerazione della prestazione, (ove prevista e applicabile anche alle prestazioni POCT), è pari al valore riconosciuto per l’attività effettuata in laboratorio, a fronte di un aumento del livello di rischiosità legato alla esecuzione da parte di personale non appartenente o comunque professionalmente non formato nei settori del laboratorio clinico.

In un’ottica di mera valutazione costi/ricavi il POCT risulterebbe essere una soluzione organizzativa cui preferire una gestione di “movimentazione fisica” del campione biologico, sulla quale sono stati fatti anni di efficientamento per la gestione della logistica.

La valutazione non può prescindere da altre dimensioni di analisi rispetto a quelle di costo/ricavo, considerando il POCT come modalità integrativa e non sostitutiva, da attivare quando le attività del laboratorio centrale non sono accessibili o non risultano tempestive in rapporto alla condizione clinica.

I maggiori costi devono essere “giustificati” bilanciati da un outcome atteso migliore (legato alla tempestività della cura) o ad un uso più efficiente delle risorse durante l’assistenza, (come il il contenimento delle giornate di degenza e un uso più mirato di farmaci) mettendo in atto tutte le possibili attività volte a ridurre l’utilizzo inappropriato che comporta un non giustificato incremento della spesa sanitaria nonché a ridurre gli effetti negativi del rischio legato alle attività effettuate da operatori non di laboratorio.

La valutazione HTA diviene quindi un imprescindibile strumento di programmazione nell’ambito iniziale di valutazione dell’acquisizione della tecnologia, ma deve proseguire durante tutta la vista utile del POCT con la finalità governo clinico, finalizzato a perseguire il miglioramento continuo della qualità delle prestazioni sanitarie rese, ricercando in particolare la riduzione del ricorso al POCT inappropriato ed il monitoraggio, valutazione e riduzione sistematica dei rischi.

HTA DURANTE LA VISTA UTILE DEL POCT: approccio Lean Management

L’organizzazione LEAN della produzione deve tendere in modo continuo

- ad AUMENTARE il VALORE erogato al CLIENTE tramite la riduzione sistematica degli SPRECHI
- all’ANALISI dei PROCESSI per l’eliminazione degli SPRECHI e la valorizzazione delle PERSONE

Si passa quindi da un approccio “storico” in cui il miglioramento della performance è basato sulla razionalizzazione degli «input» (risorse umane, tecnologie) ed il controllo degli «output» (volumi di produzione, tempi di attesa) arrivando alle prime analisi di «outcome» (mortalità) senza un preciso focus su come gli input siano combinati per ottenere output/outcame, ad un approccio di “miglioramento continuo” in cui l’analisi del processo di erogazione dei servizi è finalizzato ridurre ciò che non aggiunge valore per il paziente (gli sprechi),

ridurre la variabilitá evitabile ed il sovraccarico (entrambi fonti di errore).
Nella valutazione del processo di utilizzo del POCT devono essere, attraverso la valutazione del processo in ogni singola azione e nelle modalità in cui le azioni si combinano e si sequenziano, individuate e ridotte tutte le fonti di "spreco"

- sovrapproduzione: produrre più del necessario per avere a disposizione ciò che è utile
- attese: i tempi morti che si generano tra le diverse fasi del processo quanto non si susseguono in modo sincronizzato
- trasporti: spostamenti dei pazienti/clienti o dei materiali quanto non siano necessari, evitabili, senza valore aggiunto per il processo
- operazioni inutili: errato utilizzo delle risorse durante le attività che in genere si associano a ridondanza di operazioni, senza valore aggiunto
- scorte: surplus di materiali immagazzinati che aspettano di essere utilizzate, con conseguente costi di gestione, obsolescenza, inutilizzo
- movimenti: spostamenti inutili degli operatori per attività non a valore aggiunti (reperimento materiale, postazione di lavoro in altra sede)
- difetti: di prodotti o di servizi che danno luogo a un risultato «non conforme» e ad un conseguente ritardo nelle attività di processo e ad una riprocessazione
- competenze: professionalità poco valorizzate, carichi di lavoro non valutati in base ai profili e alla capacità, formazione carente

Puntare alla "perfezione" è l'obiettivo del Lean Thinking, intesa come MIGLIORAMENTO CONTINUO. L'approccio in contrapposizione al concetto di innovazione radicale tipico «occidentale» è quello di cercare un miglioramento moderato ma continuo: individuare uno spreco, attivarsi per ridurlo ed eliminarlo, consolidare il risultato e… non accontentarsi! individuare un altro spreco, attivarsi per ridurlo ed eliminarlo, consolidare il risultato e… individuare uno spreco, attivarsi per ridurlo ed eliminarlo……
In tal senso strumenti propri della LEAN quali

- l'utilizzo dei sistemi di "analisi delle cause radice" finalizzate ad individuare le vere cause dei problemi e individuarne conseguentemente la soluzione, senza arrivare alla soluzione (in genere errata o incompleta) prima di aver individuato l'effettivo problema;
- la definizione di indicatori di processo per misurare il sistema "AS IS" e definire il sistema "TO BE" per guidare ogni azione di miglioramento, necessari a definire il raggiungimento di un obiettivo;
- l'esplicitazione della necessità di una fase di "mantenimento" dei risultati raggiunti attraverso azioni programmate ed esplicite di monitoraggio, verifica ed audit, formazione e informazione, senza la quale in genere la qualità della prestazione tende a ridursi;

possono fornire gli strumenti su cui definire il percorso di ricerca/mantenimento/miglioramento della performance di un POCT.
Il lean thinking non punta alle soluzioni "preconfezionate", ma a far sì che siano le persone a sviluppare le soluzioni; questo per un POCT, un sistema "lontano" dal laboratorio, è coerente con la necessità di coinvolgimento attivo del personale esterno al laboratorio nella ricerca del miglioramento in ottica di riduzione del ricorso inappropriato e della minimizzazione degli errori.
In tal senso ben si coniuga con l'approccio Lean quanto previsto dalle "Indicazioni essenziali" SIBIOC in termini di attori da coinvolgere e fasi di processo da valutare.
Le indicazioni infatti prevedono la costituzione di un comitato multidisciplinare, finalizzato a facilitare la progettazione e la pianificazione di tutte le attività, facendo emergere le necessità dei vari gruppi e raccogliendo proposte, indicazioni correttive e possibili soluzioni.
In tale comitato trovano il proprio ruolo tutti gli attori principali di un progetto Lean:

- Direttore Sanitario – sponsor del progetto e facilitatore nei momenti critici;
- Responsabili delle diverse fasi del processo (direttore del laboratorio, direttore di farmacia, responsabile servizio di ingegneria clinica, responsabile dei sistemi informativi, referente di UO)
- Personale effettivamente coinvolto nelle fasi di processo (coordinatore POCT con funzioni di POCT Manager di laboratorio, tecnico di laboratorio, consulente infermiere; ecc.)

Nello stesso documento vengono identificate alcune delle fasi del processo di implementazione delle attività di un POCT (controllo del processo, manutenzione della strumentazione, controllo elettronico, controllo di qualità esterno, compatibilità dei risultati, analisi degli indicatori di qualità, ecc) e le attività di sviluppo e mantenimento delle competenze del personale (verifica dei fabbisogni formativi, formazione, re-trainig).

REFERENCES

- "Lo spirito Toyota. Il modello giapponese della qualità totale. E il suo prezzo" 1988 – Taiichi Onho
- "La logica diagnostica di laboratorio ed il Point of care testing" 2001 - Paolo Rizzotti - Riv Med Lab - JLM
- "Raccomandazioni per l'implementazione e la gestione del "point-of-care testing" (POCT)" 2011 - Francesca Di Serio, Tommaso Trenti, Paolo Carraro – SIBioC Documents "TELEMEDICINA Linee di indirizzo nazionali" 2012 - Ministero della Salute
- "Contenimento dei costi nel laboratorio clinico: razionamento o razionalizzazione?" 2015 - Mario Plebani – SIBIOC Documents
- "Principi per l'implementazione e la gestione del point-of-care-testing (POCT): indicazioni essenziali" 2021 - Erica Rampoldi, Giovanna Patrucco, Marco Casati, Benedetto Morelli, Paolo Carraro – SIBioC Documents

SS04-CO03

HEMATOLOGICAL DIAGNOSTICS: THE TELEMEDICINE PROJECT FOR THE LABORATORY SPREAD ACROSS A LARGE TERRITORY

M. Lorubbio[1], F. Baldelli[1], E. Bromo[5], G.P. Caldarelli[4], C. Donnini[1], S. Fabbroni[1], A. Fanelli[1], M. Fantacci[2], L. Gasbarri[1], M. Mazzi[4], A. Periccioli[3], P. Pugliano[4], C. Rapini[2], A. Rebuffat[3], A. Sereni[1], M. Sorini[1], E. Tripodo[1], A. Ognibene[1]

[1]Clinical Chemical Analysis Laboratory, San Donato Arezzo Hospital
[2]Clinical Chemical Analysis Laboratory, Montepulciano Valdichiana Reunited Hospital
[3]Clinical Chemical Analysis Laboratory, Hospital Alta

Valdelsa
[4]Clinical Chemical Analysis Laboratory, Misericordia Grosseto Hospital
[5]Dip. del Farmaco, Misericordia Grosseto Hospital

Introduction The territory of the USL TUSCANY SOUTH EAST (TSE) has an area of 11,557 sq km with 850,446 inhabitants. The use of information and communication technologies (ICT) and E-Health can contribute to a reorganization by moving the focus of health care from the hospital to the territory. The aim of the present project is to evaluate of the complete blood count (CBC) test and the peripheral blood smear through digital images, shared and available to the team of the 12 TSE laboratories. During implementation, the image analyzers and the staining adopted were compared, together with the quality indicators (QI) to support the new flows implemented between the peripheral laboratories and the Hub laboratory. Materials and methods The connection network between all TSE Laboratories was guaranteed by the DMS (DASIT Management System) WEB application, for XN (Sysmex) instruments and, Caresphere TMXQC for the management of CQI. The cellular digital images were shared through digital online archives, managed using the DI60 and and DC-1 (CellaVision™). 135 peripheral blood smears were performed using SP10 (HUB) and HemaPrep (Spoke), staining May-Grünwald Giemsa (Sysmex) and Romanowsky-type (RAL Diagnostics), respectively. For study QI, 26 CBC performed in the peripheral laboratories were reanalyzed after 4-6 h at the Arezzo Laboratory. Statistical analysis was performed using SPSS and GraphPad Prism. Results The Passing-Bablok and Bland-Altman plot analysis performed for comparison of all elements of the blood count test, provided excellent results between the technologies and the different cell staining used (data not shown). The MCV and PLT parameters of the CBC, performed in the spoke laboratories and reperformed in the Hub laboratory after 4-7 hours, showed a statistical significative differences of 3.18(±0.66) fL and 10.69 (±17.83) 103µL respectively. Conclusions The organization proposed in the project improves the analytical quality, harmonises the reporting and interpretation of analytical data, promotes uniform training, preparing for continuous professional comparison.This approach represents a model that contrasts with centralization, favoring the concept of widespread laboratory medicine, while maintaining an advantageous cost/benefit ratio.

SS04-CO04

LEUCOCYTE DIFFERENTIAL AND MORPHOMETRIC PARAMETERS WITH MINDRAY BC-6800 PLUS: A POSSIBLE PREDICTIVE TOOL TO DIAGNOSE SEPSIS AND SARS-COV-2 INFECTIONS.

M. Pelagalli[1,2], A. Giovannelli[1,2], C. Calabrese[1,2], S. Sarubbi[1,2], M. Minieri[1,2], M. Nuccetelli[1,2], M. Pieri[1,2], S. Bernardini[1,2]

[1]Department of Laboratory Medicine, "Tor Vergata" University Hospital,Rome, Italy
[2]Department of Experimental Medicine, University of "Tor Vergata",Rome, Italy

Background: Sepsis is an infectious disease (the etiology can be viral or bacterial) with hight mortality, threatening human health. Clinicians need to diagnose the patient's infection in time and look for pathogens in order to develop an effective treatment plan; therefore, a quickly and early screen to diagnose sepsis has become an urgent problem in clinical laboratories. Different inflammatory factors are used to diagnose the sepsis; CRP, IL-6, PCT, ADM, lactate, D-dimer etc., but they also have limitations such as insufficient sensitivity and specificity and requiring additional examination cost. The aim of this study is to use leucocyte counts (neutrophils and monocytes that are activated from pathogenic virus or bacteria) and others morphological change with Mindray BC-6800-plus platform to diagnose sepsis early, quickly, conveniently and at low cost.
Methods: A total 957 EDTA-k2 anticoagulant venous whole blood samples were collected: 70 control patients (blood donors) with a normal complete count blood and negative VES, and 887 samples hospitalized at the emergency department with symptoms attributable to sepsis with PCT request. All data was divided in 4 groups: control group, group where sepsis cannot be confirmed, group with confirmed sepsis diagnosis and a group with sepsis from SARS-CoV-2 infection. Morphometric and numeric parameters are reported with Mindray BC-6800 plus: blood count like positional parameters X, Y, Z of neutrophils, lymphocites and monocytes, PLT, NLR (neutrophil lymphocyte ratio) and IMG (index of immature granulocytes). For statistical analysis was used Shapiro Wilk test for distribution analysis and the non parametric Kruskal Wallis test to evaluate significative differences among the groups ($p < 0.05$) and also examined ROC curve analysis.
Results: There is a statistically significant difference between control group and sepsis group for haematological parameters: positional parameters (Neu X, Y, Z; Mon X, Y, Z and Lym X, Y, Z), IMG, NLR, PLT. The roc curves highlight acceptable sensitivity and specificity values for some haematological parameters and suggest a possible cut-off. Conclusions: The BC-6800 plus can help the diagnosis of sepsis upon the admission to the emergency department using some morphological positional parameters.

SS05 - Cure primarie, poct e prove di efficacia: la sinergia necessaria

SS05-01

EBLM: ITS VALUE AND ROLE IN DECENTRALIZED DIAGNOSTIC AND PROXIMITY MEDICINE"

V. Pecoraro

Dipartimento integrato di Medicina di Laboratorio e Anatomia Patologica, Modena

Evidence based laboratory medicine (EBLM) focuses on the use of diagnostic tests to improve patient outcomes. Principles of EBLM, integrating the best research and the clinical expertise, could be applied also for point of care testing (POCT) (1). POC are tests conducted near the site of patient care, outside of the laboratory, usually performed by patients or clinical personnel not trained in laboratory medicine. POCT require smal sample volumes, minimize pre-analytical errors, and reduce

alterations of labile analytes. However, when used appropriately, could improve the patients outcomes by providing faster results and earlier therapeutic strategies (2). Instead, its over or incorrected use could leads a patient risk and potential increase of healthcare costs. We assessed, throught a systematic review of the recent scientific letterature, the accuracy of the POCT on troponin, procalcitonin, C-reactive protein, parathyroid hormone, INR and d-dimer, and evaluate the impact of faster results on patient management. Studies measuring PCT, PTH and d-dimer reported a limited impact on diagnostic decisions. Instead, studies on CRP claimed a significant reduction of antibiotic prescription. Several authors evaluated troponin and INR reporting faster decision-making without any improvement in clinical outcome. Faster results are often translated in better outcomes, without evidence to support this conclusion. So, it is important that the POCT practice is evidence-based looking for evidence of whether POCT confers any advantage in clinical decision making in different scenarios. In some settings, such as rural enviroment, a rapid availability of cardiac troponins or other analytes can help clinicians to rule out or rule in disease, without tranfer patient in other center, avoiding unnecessary costs (3). Likewise, in Emergency Department, availability of more rapid results with POCT help clinicians to refere patients, but does not always translate into shorter stays (4). The satisfactory analytical performance, together with an excellent practicability, suggest that the POCT represents an important technologycal advance in patient care, but, the lack of evidence about the patients outcome invite healthcare workers to use it with judgement.

REFERENCES

1. Price CP. Point of care testing. BMJ 2001; 322: 1285–1288.
2. Pecoraro V, Banfi G, Germagnoli L, Trenti T. A systematic evaluation of immunoassay point-of-care testing to define impact on patients' outcomes. Ann Clin Biochem. 2017 Jul;54(4):420-431.
3. Herd GCE, Musaad SMA. Point-of-Care Testing in Rural and Remote Settings to Improve Access and Improve Outcomes: A Snapshot of the New Zealand Experience. Arch Pathol Lab Med. 2021 Mar 1;145(3):327-335.
4. Alter DN. Point-of-Care Testing for the Emergency Department Patient: Quantity and Quality of the Available Evidence. Arch Pathol Lab Med. 2021 Mar 1;145(3):308-319.

SS05-02

EBLM AND BLOOD GAS ANALYSIS PARAMETERS: WHICH IS MANDATORY TO MEASURE.

E. Rampoldi

Coordinatrice del Gruppo di studio POCT per SIBioC

Arterial and venous blood gas analysis reveals oxygenation and acid-base status of the body. The analysis usually includes: pH, PaO2, PaCO2 and a wide range of different analytes, besides other several derivated (calculated) parameters, as HCO3-, BE, anion gap.

Arterial pO2 (PaO2) is the most important variable to assess the oxygenation status and can't be substituted by venous blood or capillary measures of O2 (1).

American Association for Respiratory Care (AARC) updated Recommendations on blood gas analysis (BGA) and hemoximetry (2). The clinical practice guideline is based on 237 clinical trials, 54 reviews, and 23 meta-analyses searched in MEDLINE, CINAHL, and Cochrane Library database. Hemoximetry is recommended to determine the impact of dyshemoglobins on oxygenation. Some calculated values may be in error, e.g. calculated SaO2 may overestimate oxyhemoglobin saturation in the presence of carboxyhemoglobin or methemoglobin. Moreover, the presence of high concentration of fetal hemoglobin may also be a problem if blood gas analyzer does not detect it, as instrument assumes hemoglobin to be of the adult type, and therefore the calculated blood gas oxygen saturation values are underestimated.

In critically ill patients many other analytes have been used to estimate the severity of disease and try to prognosticate morbidity and mortality. No measurements can encompass the complexity of a disorder, but lactic acid can approach that goal (3) Indeed lactic acidosis is the most frequent metabolic acidosis and many causes are reported for lactate increase, not only hypoxia: the higher the lactate concentration, the worse the outcome. The initial values have a prognostic significance, but serial measurements are more valuable for prognosis. Conductivity-based Hematocrit (Ht) estimations have limitations. Abnormal protein concentration will change plasma conductivity. Low protein concentration, resulting from dilution of blood with protein-free electrolyte solution during surgery, will result in erroneously low Ht value. Conductivity-measured hematocrit during and after surgery could produce inaccurate results when Ht are lower than 30%, and, therefore, result in unnecessary red cell transfusions in some patients. In any situation, to correctly interpret BGA results history should be always considered: reasons for presentation, information concerning events, environment, trauma, medications, poisons, toxins and an accurate physical examination should be carefully collected.

REFERENCES

1. Acute respiratory distress syndrone: the berlin definition, Ranieri MV et al 2012 Jun 20; 307(23):2526-33.doi: 10.1001/jama.2012.5669.
2. AARC Clinical Practice Guideline: Blood Gas Analysis and Hemoximetry: 2013 Michael D Davis, Brian K Walsh, Steve E Sittig, and Ruben D Restrepo Respir Care 2013; 58(10):1694 –1703.
3. N Engl J Med Lactic Acidosis 2014; 371:2309-19. DOI: 10.1056/NEJMra1309483.
4. Conductivity-based Hematocrit measurement during cardiopulmonary bypass. Steinfelder-Visccher J et al. 2007; J Monitoring and Computing, 27:7-12.

SS06 - Big data e valutazione dei risultati prodotti dalla diagnostica di laboratorio nei percorsi di cura dei pazienti territoriali

SS06-01

INTELLIGENZA AUMENTATA E IL VALORE DELL'INNOVAZIONE: I MODELLI CHE SPIEGANO E PREDICONO

N. Musacchio

Gli algoritmi di apprendimento automatico, o machine learning, si sono dimostrati molto efficaci nel prevedere il comportamento dei fenomeni rappresentati nei dati biomedici.
Gli algoritmi di machine learning più comunemente utilizzati, come ad esempio le reti neurali artificiali, producono risultati cosiddetti a "scatola nera", ovvero:

- un insieme complesso di equazioni matematiche che non possono essere interpretate da persone che non abbiano specifiche competenze di tipo matematico;
- modelli predittivi che non forniscono nessuna spiegazione sulla motivazione sottostante alla previsione stessa.

Quando si applica il machine learning a dati come le immagini, gli algoritmi black box non sono un problema, poiché il valore del modello risiede nella sua accuratezza nel rilevare la presenza di determinati pattern, riconducibili, per esempio, alla presenza di un tumore. Tuttavia, se lo scopo è comprendere in maniera più approfondita uno specifico fenomeno, è fondamentale capire, per esempio, perché il modello predittivo ha classificato un particolare paziente come 'a rischio' o 'non a rischio', elicitando le caratteristiche associate a questa classificazione.
Una specifica tecnica di ML, il “metodo a generazione di regole” di Rulex, costruisce modelli basati su regole intelligibili che consentono di ricavare importanti conoscenze sulle variabili incluse nell'analisi e sulle loro relazioni rispetto agli esiti del fenomeno analizzato.
L'intelligenza artificiale "Clear box" apre a entusiasmanti scenari d’implementazione di una vera e propria intelligenza aumentata, in cui uomini e macchine creeranno una sinergia - controllata dall'esperto – che potrà concretamente migliorare la qualità delle cure.
Ultimo, ma non meno importante, secondo il GDPR i dati personali devono essere trattati in modo trasparente e qualsiasi cittadino europeo ha il diritto alla spiegazione qualora una decisione che lo riguardi si sia basata su algoritmi di machine learning: la AI 'clear box' permette di operare nel pieno rispetto della legislazione europea.

SS06-02

THE RISE OF THE UBIQUITOUS LAB

G. Giannella

Healthcare is the one of the largest success stories of our times. Technology is another of the largest success stories of our times. People expectations about healthcare+technology is at its absolute peak. We are in the middle of a health-tech secular change. This is for good. This is unstoppable. This is the best part of the story. Yet, healthcare spending is unsustainable in an aging world. Technology, as every tool, brings its risks. Global levels of assistance are outrageously unequal. Mental disorders are exponentially growing. That’s the other part of the story. How will the lab of the future adapt to the entire story? Will it “just” be able to sustain the health-tech revolution, or will it also play a role in making global health systems more sustainable, equal, and safer?
The speaker of this panel will present his view on how “deeptech” (a synergetic combination of leading-edge technology which today assembles Internet of Things, Artificial Intelligence, Digital and Space) coupled with rising individual expectations for highly personalized and accessible healthcare, will redefine the boundaries of healthcare system and the concept itself of lab.
Internet of Things will be the main key to acquire all the right data. Trillions of “sensors” supporting data gathering from multiple sources: humans, plants, things, places, food, animals, environment. Contextualized data gathering to acquire different data in different contexts, “actuators” instructing devices to do the right thing in the right moment, surveillance networks for enhanced threats.
Artificial Intelligence will be non-optional. With health-related knowledge doubling in months, AI will become a mandatory survival kit. Yet, it still will see things that human eye might miss. Or making correlations that are simply too difficult anyway else. Yes, it will be both defensive for professionals and offensive to diseases, if used in the right way, as every tool.
Digital will influence behaviors, create communities, and redefine the patient-professional interaction. With big part of the clinical outcome depending on context and behaviors, Digital will help to “nudge” patients with personal, doable, life-enhancing tips. But Digital is also the “home of increased expectations”. Patients are individuals, parents, children, workers, citizens, consumers, with ever-growing expectations on what and how can be done through a smartphone. Health won’t make any exceptions.
Space and satellite technology will provide the communication background for all above, from remote surgery to distributed expert network, etc. But Space will also bring additional data coming from macroscopic data gathering, earth observation, context-related data and gravity-less phenomenon analysis.
Can the lab of the future stay immune from all above? Hard to believe. While exact predictions are useless, some trends are clearly visible and point to the raise of a next-generation ubiquitous lab. An “always-on” set of competences, data, technologies, uniquely positioned to close the fracture existing between everyday life/health of individuals and the infinitely smaller portion of its current clinical representation.

SS06-CO05

LARGE GENOMIC ALTERATIONS (LGAS) PROFILES IN HBOC PATIENTS USING SHALLOW WGS (SWGS) PIPELINE FOR THE ASSESSMENT OF HOMOLOGOUS RECOMBINATION DEFICIENCY (HRD) SCORE

G.L. Scaglione, C. Nardelli, M. Setaro, E.D. Capoluongo

Lab. Genomica Oncologica, CEINGE Biotecnologie Avanzate S.C.R.L., Napoli

Background: The homologous recombination (HR) pathway is essential for DNA double strand break (DSB)

repair and involves several genes. HR deficiency (HRD) arises upon inactivation of BRCA1/2, RAD51C or PALB2. The impairment of this pathway is a common characteristic of many tumors and it is frequently observed in breast and ovarian cancer. Consequently, accurate detection of HRD is of clinical relevance as it is indicative of sensitivity to targeted therapy with poly ADPribose polymerase inhibitors (PARPi) as well as to DNA damaging reagents.
Methods: Shallow Whole Genome Sequencing (sWGS) was performed on sixteen ovarian cancer (OC) samples. The training set included 13 samples (6 somatic, 7 germline) carrying both BRCA+ve (n=9) and BRCA-ve (n=4) status. The germline and somatic samples were prepared according to SeqCap EZ HyperCap protocol (Roche). Briefly, 50 ng of input DNA was used for libraries preparation using the KAPA HyperPlus Library Preparation Kit (Roche). Samples were then pooled and sequenced on NextSeq550 Dx platform (Illumina). Sequencing files were quality checked, analyzed and processed using our dedicated bioinformatics pipeline. In this workflow, LGAs profiles were calculated using whole genome sequencing data at low coverage (0.4-1.0X) using different sliding window size spanning 5 to 1000 Kbases. The HRD score was then estimated by measuring the level of agreement in the segmentation profiles of each samples.
Results: The BRCA status was assessed in 13 samples (training set) and the HRD score was estimated as follow: 2 of the 3 somatic BRCA positive (sBRCA+ve) samples were classified as HRD positive while 2 out of 3 sBRCAve samples were scored as negative. As expected, both positive (+ve) and negative (-ve) germline BRCA (gBRCA) samples were classified as HRD negative. Lastly, Among the remaining samples (n=3) for which the germline status of BRCA genes was not available, the algorithm classified 1 sample carrying PALB2 CNV as HRD positive.
Conclusions: Germline BRCA1/2 mutation status is currently the main genetic biomarker of HRD but it has its drawbacks: among others HRD can be driven purely by somatic events. In this scenario we have customized a simple and robust bioinformatics workflow to infer the HR status of breast and ovarian tumor based on sWGS to support patients' treatments and follow up strategies.

SS06-CO06

THE BIG DATA ANALYSIS APPLICATION TO EVALUATE THE CLINICAL UTILITY OF IGM ANTI SARS-COV-2 DETERMINATION: THE EXPERIENCE OF MODENA

V. Pecoraro[1], T. Pirotti[1], T. Trenti[1], M. Plebani[2]

[1]Department of Laboratory Medicine and Pathology, Azienda USL of Modena, Italy
[2]Department of Laboratory Medicine, University-Hospital of Padova, Italy

Background: Serological tests identifying SARS-CoV-2 IgG and IgM in serum play an important role in understanding the disease epidemiology. However, their immunological significance are currently undefined. There are many methods available for the detection of specific Abs whit suboptimal diagnostic accuracy and relatively high throughput capacity and less stringent specimen requirements compared to RNA-based assays. We aimed to assess the clinical utility of IgM detection in SARSCoV- 2 using the big data analysis. Methods: We conduct a retrospective study analyzing with a big data analysis all samples collected between 11 March and 30 September 2020. All serum samples received at the laboratory were processed using qualitative and commercially available rapid lateral flow immunoassay tests for 2019-nCoV IgG and IgM. Positive results were confirmed using a chemiluminescent method. Subjects with a positive result were contacted from the Department of Public Health for further tests (viral RNA research or subsequent serological tests) for definitive diagnosis.Results: A total of 69,343 serological tests (in 42,911 subjects) and 140,065 oropharyngeal or nasopharyngeal swabs (in 88,771 subjects) were performed. 94.5% of subjects screened (n=40,559) had negative results for both IgG and IgM. Of the 640 subjects with both IgG and IgM positive results, viral RNA research confirmed positivity in 16%. Of the subjects with IgG negative and IgM positive results, a positivity was confirmed in 1.4% (n=7/478) subjects. Subsequent serological testing confirmed IgG positivity in 8 subjects (1.6%). Conversely, in subjects with IgG positive and IgM negative results, a positivity was confirmed in 7.9%. Therefore, analysis suggests that up to 94% of serological test results of IgM positivity and IgG negativity are false positive whereas, serological test results of IgG positive and IgM negative are confirmed true positives in around 7.9% of subjects. Discussion: Our study, based on big data analysis application, confirms the scarce clinical utility of IgM anti SARS-CoV-2 detection in COVID-19 management, and underlines the responsibility of laboratory medicine professionals to highlight limitations of the SARS-CoV-2 serological tests due to uncertainty in their interpretation.

SS07 - La diagnostica molecolare decentrata: "rischi e opportunità"

SS07-01

IL RUOLO DEI PRESCRITTORI: IL PDTA NEI TUMORI OVARICI

P. Scollo

Dipartimento Materno Infantile e dell'U.O.C. di Ginecologia ed Ostetricia
Azienda Ospedaliera per l'Emergenza Cannizzaro (A.O.E.C.) di Catania.

Il carcinoma ovarico rappresenta la settima neoplasia più frequente nella popolazione femminile mondiale e la principale causa di morte tra i tumori ginecologici. In Italia, sono stati registrati circa 5300 nuovi casi nel 2019, ed è la quinta causa di morte per cancro nelle donne di età compresa tra 50 e 69 anni, con una sopravvivenza netta a 5 anni dalla diagnosi stimata intorno al 40%.
Ad oggi mancano delle strategie di screening efficaci per l'identificazione precoce della malattia, e l'assenza di sintomi predittivi ed il ritardo nella diagnosi che ne consegue fa si che oltre il 75% delle donne al momento della diagnosi presenti un tumore in stadio avanzato necessitando di una gestione multidisciplinare da parte di professionisti con specifiche competenze. Solo centri di riferimento possono offrire un'assistenza così complessa

ed è ormai documentato come le curve di sopravvivenza delle pazienti migliorino (> 40%) in centri con esperienza specifica per il trattamento del carcinoma ovarico. La disponibilità di una guida nei percorsi diagnostici è una esigenza per medici di medicina generale e specialisti di altre discipline che scaturisce dalla necessità di dover garantire al paziente il percorso diagnostico più appropriato, meno impegnativo, più utile e meno dispendioso. Lo scopo dei PDTA è quello di incrementare la qualità dell'assistenza percepita ed effettivamente erogata, migliorando gli outcomes e promuovendo la sicurezza del paziente attraverso l'utilizzo delle giuste risorse necessarie. L'approccio chirurgico riveste un ruolo fondamentale ed imprescindibile nel programma di cura ed il residuo tumorale assente è stato definito come l'unico residuo tumorale che si associ a curve di sopravvivenza ottimali e anche la chemioterapia antiblastica che segue sempre la chirurgia nel cancro ovarico avanzato, ottiene risultati migliori dopo una chirurgia ottimale. Esperienza, cultura e preparazione tecnica dell'operatore hanno un impatto sulla curva di sopravvivenza della paziente motivo per cui è fondamentale che tale procedura sia eseguita in centri di riferimento specializzati, con una esperienza del chirurgo ginecologo oncologo certificata da percorso formativo o casistica adeguata ed una organizzazione strutturale complessiva multidisciplinare, in modo da garantire la migliore sequenza terapeutica per la paziente. La prima rete di riferimento e diffusione dei PDTA è costituita dalla medicina generale. Non si può infatti prescindere dalla condivisione con tutta la rete regionale dei medici curanti che sapranno così a chi inviare la paziente con sospetto o diagnosi già accertata di cancro ovarico, per un percorso diagnostico e terapeutico adeguato anche nei tempi permettendo un feedback sulle condizioni ed i percorsi clinico-terapeutici delle singole pazienti. Inoltre vanno incentivati i rapporti informativi e di riferimento con le associazioni di volontariato e le associazioni di pazienti che costituiscono patrimonio fondamentale nella realizzazione e sviluppo di informazione, cultura sanitaria di prevenzione primaria e secondaria e di affiancamento nelle cure domiciliari o in strutture di assistenza anche ai malati terminali.

SS08 - Young Scientists - Evoluzione della Medicina di Laboratorio: strategie e nuove sfide

SS08-CO09

MELANOQ WEB-APP: AN INNOVATIVE TOOL FOR COLLECTING, HARMONIZING, AND MANAGING CLINICAL AND GENETIC DATA OF MELANOMA PATIENTS USING A WEB-BASED PLATFORM

C. Pellegrini, L. Cardelli, M. Mastrangelo, M. Bressan, M.C. Fargnoli

Dermatologia, Dipartimento di Scienze Cliniche Applicate e Biotecnologiche, Università degli Studi dell'Aquila

With more than 320,000 new cases and 57,000 deaths worldwide in 2020, melanoma remains one of most aggressive cancer globally. The incidence varies widely among white-skinned populations and has significantly increased in recent years in Australia, US and in Europe, including in the Mediterranean area. Given the heterogeneous recording of epidemiological, clinical and genetic variables across melanoma studies, the MelaNostrum Consortium was confronted with the difficult task of harmonizing all information from different international centers to perform association studies. Therefore, MelaNostrum investigators developed a questionnaire for data collection that could be used as a full or itemized template for the standardization. On the base of that questionnaire, we built a web-based tool that allow to collect data taking advantage of a telemedicine approach. The App allows to complete online the MelanoQ questionnaire using a tablet, and automatically generates the database with all the collected data of each patient. Similar to the MelanoQ questionnaire, the MelanoQ Web-App is organized in 4 main sections (A-D) and includes a total number of 64 items related to: general and demographic information (section A); phenotypic, UV exposure risk factors and lifestyle habits (section B); clinical examination, medical history and family history (section C); tumor characteristics, including histology, staging and molecular profile (section D). Different subsections are designed for self-administration, patient/ control interviews performed by a physician or study nurse, and data collection from medical records. A specific attention has been paid to the anonymization of data, to solve privacy concerns and to collect different signed consensus format by international patients.In conclusion, we sought that a web-tool to generate a comprehensive database allowing to pool data on melanoma from different centers world-wide is of great opportunity for clinicians and for patients.

SP01 - Il ruolo del POCT nella diagnosi delle sindromi coronariche acute

SP01-01

hs-cTnI: RETI POINT OF CARE E RISCHIO CLINICO

M. Mele

Giomi
Unità operativa: Laboratorio Analisi Cliniche Struttura:ICOT Città: Latina Mansione: Responsabile del Servizio Laboratorio Analisi Cliniche

La ISO 15189 e la ISO 22870 ci impongono l'adozione di cruscotti gestionali, indicatori e analisi del rischio, nell'ottica di un miglioramento continuo della qualità del dato diagnostico e riduzione del TAT. Nella gestione delle SCA-NSTEMI dobbiamo determinare il peso che, la caratteristica della Troponinal- HS (CV contenuto entro il 10%) e il valore delta del 20% come variazione significativa nel monitoraggio, potrebbero avere nell'inficiare il valore predittivo negativo di un dato rilasciato in assenza di un controllo di qualità interno che non restringa l'imprecisione analitica, soprattutto per i valori di concentrazione vicini ai limiti decisionali. Nel nostro laboratorio è stato reclutato un campione di pazienti con età > 65 anni, degenti nella struttura e sottoposti ad interventi di sintesi o protesizzazione di fratture di femore entro le 48 ore dal ricovero e aventi sintomatologia riconducibile a cardiopatia ischemica (dispnea, dolore toracico, aritmie ed ipotensione). Per tali pazienti è stato misurato il dosaggio

delle troponine su POCT sottoposti a controlli di qualità parte terza; tali controlli sono stati validati da software gestionale al fine di ridurre la variabilità analitica e consentire il monitoraggio dei pazienti a rischio, con protocollo 0-1h, direttamente nelle TIPO ad opera dei cardiologi; questo ha inoltre permesso al laboratorio di misurare, valutare e ridurre il rischio di causare danni al paziente attraverso il sistema IQCP (Individualized Quality Control Plan) e la supervisione dei sistemi integrati come da linee guida a garanzia e tutela del medico e soprattutto del paziente.

SP01-CO10

EVALUATION OF A HIGH-SENSITIVITY CARDIAC TROPONIN POINT-OF-CARE ASSAY: A COMPARISON WITH A LABORATORY PLATFORM

R. Aloe[1], P. Avanzini[1], R. Musa[1], P. Cerati[1], M. Di Pietro[2]

[1]SSD Biochimica ad Elevata Automazione, AOU Parma
[2]Laboratorio Unico Metropolitano (LUM), AUSL Bologna

Background: Until recently, high sensitivity in cardiac troponin testing was achieved exclusively by laboratory platforms, whereas point-of-care systems lacked the required analytical performance. In 2020, however, the first high sensitivity cTn POC assay (TriageTrue, Quidel) was successfully evaluated (1) and assay-specific cutoffs for the 0/1-h algorithm were included in the ESC guidelines for the management of NSTEMI (2). In this work, the performance of TriageTrue in comparison with the hs-cTn assay currently in use in our institution (Access Hs TnI, Beckman Coulter) was evaluated.
Methods: Linearity of TriageTrue was assessed on 11 plasma samples, each in 5 replicates (cTn = 3.13–800 ng/L). Precision was evaluated on 3 pools with different concentrations (5 aliquots/pool × 5 days). For the comparison study, 146 plasma T0/T1 samples coming from 73 ED patients with chest pain were tested using both methods. Agreement was assessed with Spearman's correlation, Passing-Bablok regression and Bland-Altman analysis (MedCalc 18.6).
Results. Linearity: Spearman's correlation coefficient = 1.000; p <0.001. Total precision (CV): 4.5% (low), 9.2% (medium), 9.0% (high). Within-run precision: 4.82%– 9.25%. Passing-Bablok regression: intercept = 0.6674 (95% CI: -0.6031–1.2862), slope = 0.886 (95% CI: 0.8301–0.9792) for T0; intercept = 0.01802 (95% CI: -1.0898–0.07439), slope = 0.9463 (95% CI: 0.8777–1.0347) for T1. Bland-Altman analysis: mean bias = -0.4 ng/L (T0); 1.4 ng/L (T1). Spearman's correlation: 0.914 (95% CI: 0.865–0.946; p <0.001) for T0; 0.924 (95% CI: 0.880–0.952; p <0.001) for T1.
Conclusion: CV was <10% for all the analyzed pools. Precision was particularly high (CV = 4.5%) in the low pool, which is relevant for the reduction of analytical noise and the reporting of small cTn variations around the 99th percentile URL. The tested methods show great concordance, with high correlation for T0/T1 samples and a slight proportional difference at T0. Overall, TriageTrue is a valid alternative to the tested laboratory assay and its adoption by EDs may considerably reduce turnaround times and streamline clinical decision-making at the onset of myocardial infarction.

REFERENCES

1. Boeddinghaus et al., JACC 2020, 75, 1111–1124.
2. https://doi.org/10.1093/eurheartj/ehaa575.

SP02 - Alleanza per la qualità e miglioramento degli outcome clinici

SP02-01

RISK MANAGEMENT FOR POINT-OF-CARE TESTING

J.H. Nicholas

Vanderbilt University Medical Center, Nashville, Tennessee, USA

Introduction: Point-of-care testing (POCT) is laboratory testing conducted close to the site of patient care. POCT is growing in popularity with manufacturers offering a wide menu of tests and devices where the operator can obtain a rapid test result with the potential to initiate faster patient care decisions. But POCT is not fool-proof, and any test can and will fail if operated under the wrong conditions.
Methods: Risk management is a process where laboratories can assess their weaknesses, implement a control plan to detect and prevent erroneous results, and monitor the effectiveness of their plans.
Results: The Clinical and Laboratory Standards Institute (CLSI) EP23-A: Quality Control Based on Risk Management provides guidance based on risk management for laboratories to develop quality control plans tailored to the particular combination of measuring system, laboratory setting, and clinical application of the test.
Discussion: This presentation will describe how laboratories can partner with manufacturers to conduct risk assessments and implement quality control plans in their laboratory and at the point-of-care. The advantages of utilizing a risk management approach to controlling laboratory errors will be emphasized along with the efficiencies gained from conducting a risk assessment and implementing a quality control plan. A revision of CLSI EP23-A is currently being drafted, and this presentation will preview a few of the updates that can be expected in the next version of the guidance document.

SP03 - Diagnostica decentrata di COVID-19: rischi e opportunità

SP03-01

TESTING FOR SARS-COV-2: SELF-SAMPLING AND PRE-ANALYTICAL ISSUES

A. Padoan

Department of Medicine, DIMED, University of Padova and Department of Laboratory Medicine, University Hospital of Padova, Italy

Although reverse real-time PCR (rRT-PCR) remains the gold standard for detecting SARS-CoV-2, high tests demanding has overwhelmed molecular laboratory capacities in all countries around the world, especially during early pandemics. During the second wave, the validation of SARS-CoV-2 antigen rapid diagnostic tests (RDT) has substantially changed testing strategies globally, since results were available within 30 min, reducing turnaround time and therefore exposure risk. Recently, validated self-tests for SARS-CoV-2 based on the

nasopharyngeal swab (NPS) or saliva have prompted for the empowerment of the general population in the fight against the spread of infectious.
Swabbing is a complex task requiring training and competency assessment, and thus they are performed by trained nurses or physicians. The complexity of NPS, coupled with a lack of a standard swabbing practice may contribute to a high number of false-negative results for SARS-CoV-2. SARS-CoV-2 rRT-PCR false-negative results have been reported to be as high as 41% and several reports exist of patients negative to NPS, who are subsequently positive on repeat testing [1]. Differently, the false-positive ratio for the SARS-CoV-2 molecular test is expected to be very low, since PCR design is mostly unaffected by false-positive results. Recently, Tsang et al. compared the diagnostic performance of different clinical specimens, including nasopharyngeal, nasal, throat, and oropharyngeal swabs and saliva and they found that using NPS as the gold standard, moderate sensitivities were achieved by saliva (85%, 75–93) and nasal swabs (86%, 77–93) and a much lower sensitivity by throat swabs (68%, 35–94). The Authors concluded that saliva and nasal swabs are clinically acceptable alternatives to commonly used nasopharyngeal swabs. Saliva is a matrix elective for self-collection, and molecular testing is reliable but require laboratory instrumentation to be performed. Indeed, antigen determination on salivary samples is still under debate [2].
Most of the errors occur in the preanalytical phase, with relatively few analytical and post-analytical errors. Some issues arising during the pre-analytical phase of SARS-CoV-2 diagnostics regards: the time of swab, swabbing practice, sample handling and conservation and RNA extraction. NPS should be taken at the time of symptom onset when the highest viral load occurs in COVID-19, thus not the day immediately before (and not too far from) possible close contact with positive subjects. Sample handling and storage were only partially a limiting factor when samples are kept a 4 °C and processed within 5 days [2]. Differently, sample preparation is a crucial factor for antigen testing, and centrifuged vs non-centrifuged samples give discordant results.
In conclusion, self-testing could be of aid in the screening programs for reducing viral spread, but other alternatives are possible, such as self-collection of samples with analytical tests performed in clinical laboratories. These required the optimization of pre-analytical steps to reduce the impact on results.

REFERENCES

1. Woloshin, S.; Patel, N.; Kesselheim, A.S. False Negative Tests for SARS-CoV-2 Infection — Challenges and Implications. N. Engl. J. Med. 2020, 383, e38.
2. Basso, D.; Aita, A.; Navaglia, F.; Franchin, E.; Fioretto, P.; Moz, S.; Bozzato, D.; Zambon, C.-F.; Martin, B.; Dal Prà, C.; et al. SARS-CoV-2 RNA identification in nasopharyngeal swabs: issues in pre-analytics. Clin. Chem. Lab. Med. 2020, 58, 1579–1586.

SP03-02

DECENTRALIZED COVID-19 DIAGNOSTICS: RISKS AND OPPORTUNITIES. RAPID SEROLOGICAL TESTING

G. Lippi

Section of Clinical Biochemistry, University of Verona, Verona, Italy

The knowledge that has been garnered so far on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is that humoral immunity encompasses the generation of immunoglobulins of most classes against surface viral antigens, which mostly involve the spike protein, the nucleocapsid protein, but also envelope and membrane proteins. Since the spike protein is the anchor that the virus uses for penetrating the host cells through biding with its natural host cells receptors, it can be assumed that antibodies binding to spike protein of SARS-CoV-2, and especially to its receptor binding domain, would retain stronger neutralizing potency against the virus. Serological testing has been conventionally defined as a diagnostic procedure used for detecting an immune response against an infectious agent. This definition shall dissipate any residual doubt about the fact that this type of testing is not intended to replace the identification of viral RNA for diagnosing SARS-CoV-2 infection, but must be rather used for establishing if individuals have been infected by the virus and/or have developed an immune response. The diagnostic sensitivity stratified according to the assay methodology is highly variable. Recent evidence confirms better performance for chemiluminescent and ELISA anti-SARS-CoV-2 IgG immunoassays compared to lateral flow immunoassays, whose sensitivity remains far below 80%. Additionally important drawbacks of rapid serological tests include the facts that the information provided by the companies is concerning because often lacks details, its quality is considerably variegated among different devices, several claims are vague, there is a lack of transparency along with the fact that human aspects are not been adequately addressed for purpose of alleviating the risk of inappropriately using the device. The risk of misinterpreting tests results by patients when rapid kits are used for self-diagnosis is another aspect that must be considered. Recent evidence was provided that over 2% of patients may misdiagnose themselves as being negative while the device generates positive results. This was mostly due to objective difficulties encountered by the patients in reading and interpreting the results of the strips. Important considerations for implementing point-of-care serology testing thus include (i) usage of well-validated tests, evaluated against a gold standard; (ii) performance characteristics - thus encompassing sensitivity, specificity, positive and negative predictive values or cross-reaction with other coronaviruses - shall be tested using serum samples collected from patients infected with SARS-CoV-2, with other respiratory viruses (including seasonal coronaviruses) and also from healthy controls; (iii) adequate training of healthcare workers is needed (iv) and, finally, (IV) provisions must be in place, encompassing the capture of testing data for individual patient records and surveillance purposes, and the participation to external quality assessment schemes, to systematically monitor the quality of this type of testing.

SP03-CO07

DETERMINATION OF SARS-COV-2 ANTIBODIES IN SALIVARY SAMPLES FROM VACCINATED INDIVIDUALS AND COVID-19 PATIENTS

C. Cosma[1], A. Padoan[1], C. Di Chiara[2], D. Rinaldi[1], D. Donà[2], A. Gastaldi[3], D. Basso[1], C. Giaquinto[2], M. Plebani[1]

[1]Department of Laboratory Medicine, University-Hospital of Padua, Italy
[2]Division of Pediatric Infectious Diseases, Department for Women's and Children's Health, University of Padua, Italy
[3]Department of Pediatrics, Woman and Child Hospital, University of Verona, Verona, Italy

Background and Aim: Salivary SARS-CoV-2 Ab determination could be suitable for monitoring the viral spread and vaccination efficacy, especially in pediatric patients. We investigated N/S1-RBD IgG antibody levels in salivary samples of infectious-naïve vaccinated subjects and of COVID-19 patients, further comparing levels with serum anti-SARS-CoV-2 S-RBD IgG.
Methods: A total of 72 subjects were enrolled at the Padova University Hospital: 36 COVID-19 patients and 36 health care workers (HCW), who underwent a complete vaccination campaign with BNT162b2 (BioNTech/Pfizer). All collected a salivary sample, using Salivette (Sarstedt, Nümbrecht Germany). For 9 HCW, salivary samples were collected at three different times within the same day (before breakfast, at 10 am, and after lunch). A serum sample was also collected for all individuals. Time post symptoms onset or time from the first vaccine were also recorded. Salivary COVID-19 N/S1 RBD (sal-IgG) ELISA (RayBiotech, GA, USA) and anti-SARS-CoV-2 S-RBD IgG Ab (ser-IgG) (Snibe Diagnostics, Shenzhen, China) were used for determining IgG Ab.
Results: Subjects' mean age (±sd) was 35.8±18.2 yrs. Age significantly differed ($p<0.001$) from COVID-19 patients [29.7±17.3 yrs] and HCW [47.1±12.9 yrs]. Positive sal-IgG were found in 70/72 (97.2%) samples; in sera, 71/72 (98.6%) samples were positive to ser-IgG. The sal-IgG median levels differed from COVID-19 to vaccinated HCW, being in salivary samples 0.21 kAU/L and 0.8 kAU/L ($p =0.030$), respectively; median levels for ser-IgG in COVID-19 and vaccinated HCW were 135 kBAU/L and 940 kBAU/L, respectively ($p<0.001$). Salivary IgG levels were not influenced by time post-symptom onset or time post-vaccination, both on vaccinated HCW (rho= -0.147, $p=0.402$) and COVID-19 subjects (rho=0.0267, $p=0.986$). Ser-IgG levels was not influenced by the time post-symptom onset for COVID-19 subjects (rho=0.102, $p=0.419$), while a strong significant correlation was found with time post-vaccination in HCW (rho=-0.6292, $p<0.001$). Sal-IgG levels were not-influenced by the daytime of collection (rho=0.148, $p=0.373$). Passing-Bablok regressions showed that sar-IgG and ser-IgG comparability was assessable only when ser-IgG values were divided by 1000, being slope and intercept 0.068 (95%CI: 0.069-0.341) and 0.221 (95%CI:-0.097 to 0.786), respectively. Conclusions: Salivary IgG is efficiently detectable both in COVID-19 and in vaccinated individuals and analyses appeared to be not influenced by the daytime of collection. The analyses performed showed that, overall, sal-IgG were lower than ser-IgG, and thus comparability with serum levels needs to be better explored.

SP03-CO08

MDW IS A NOVEL INFLAMMATORY BIOMARKER WITH PROGNOSTIC RELEVANCE IN COVID-19 PATIENTS

G. Riva[1], V. Nasillo[1], A.M. Ottomano[1], G. Bergonzini[1], A. Paolini[2], B. Lusenti[1], S. Castellano[1], R. Rizkallah[1], P. Ferrari[1], M. Varani[1], M. Luppi[2], E. Tagliafico[1], T. Trenti[1]

[1]Dip. Int. Interaz. Medicina di Laboratorio e Anatomia Patologica, AOU/AUSL di Modena, Italy
[2]UO Ematologia, Univ. Modena e Reggio Emilia, AOU di Modena, Italy

Monocyte Distribution Width (MDW), a new hematologic parameter correlating with cytomorphologic changes occurring during monocyte activation, has recently been described as promising early biomarker of sepsis. Similar to sepsis, in SARS-CoV-2-associated disease (COVID-19), monocyte/macrophage subsets are considered key mediators of the life-threatening hyperinflammatory disorder –commonly defined as 'cytokine storm'– which is part of the complex infectionassociated immune dysregulation observed in severe COVID-19 cases (possibly, representing a new kind of viral sepsis). Thus, we aimed at investigating possible prognostic roles of MDW testing during monitoring of COVID-19 patients.In this work, we longitudinally measured MDW values in a cohort of 87 patients with molecularly-proven COVID-19 diagnosis, consecutively admitted to our intensive/subintensive clinics in early 2020. Statistical analyses were applied to correlate MDW values with common inflammatory markers, disease severity, clinical trajectories and final outcome.We found significant direct correlations between MDW and different inflammatory markers routinely assessed during hospitalization, namely CRP ($p<0.001$), fibrinogen ($p<0.001$) and ferritin ($p<0.01$). Moreover, high MDW values were remarkably associated with fatal outcome (AUC=0.76, sensitivity 0.75, specificity 0.70, MDW threshold 26.4; RR=4.91, OR=7.14). Furthermore, evaluating MDW dynamics in cases with longer followup, we frequently observed progressive MDW increments in patients with worsening inflammatory conditions, while clinical recoveries were consistently associated with MDW decreases.Our study shows, for the first time, that MDW can be useful in the prognostic monitoring of hospitalized COVID-19 patients, as it is: (i) easy and rapid to obtain, (ii) directly related to the activation state of a fundamental inflammatory cell subset (i.e. monocytes, pivotal both in cytokine storm and in sepsis immunopathogenesis), (iii) strongly correlated with clinical severity of COVID-19- associated inflammatory disorder, and, in turn, (iv) endowed with relevant prognostic significance. Additional studies are needed to define the role of MDW monitoring in other clinical settings, including COVID-19 outpatients.

SS08 - Young Scientists - Evoluzione della Medicina di Laboratorio: strategie e nuove sfide

CJ01

LABORATORY MEDICINE WOULD MERIT A FURTHER SMALL REVOLUTION

F. Salvatore

Professor of Human Biochemistry, Emeritus
Dipartimento di Medicina Molecolare e Biotecnologie Mediche
Università di Napoli Federico II
CEINGE-Biotecnologie Avanzate
Founder and Principal Investigator
Past (president and scientific coordinator)
Member of Accademia Nazionale delle Scienze (detta dei XL) - Roma

In the last couple of decades, Laboratory Medicine has made giant steps forward in terms of innovative technology and has made major scientific breakthroughs in the medical field as a whole. Indeed, a plethora of both in vitro and in vivo assays and tests in biological fluids of the human hydrodynamic system are now available.
The importance, for clinical purposes, of novel metabolic processes and protein cross-talk mechanisms is being increasingly recognized. The increased survival period of sick, elderly people, plus the therapeutic aspects of precision medicine, in which the drugs selected resulted in a series of direct approaches to altered target molecules, have made it difficult to identify the most effective molecules to use as biomarkers in most of this population scenario.
In this optics, the clinical value of Laboratory Medicine has now reached about 70% of the most important measures used to diagnose diseases, but at a cost to the National Health System of many countries that reaches a maximum of 5% of the global health costs. Therefore, it seems that Laboratory Medicine does not need to increase further value in the contribution to the care of fragile individuals, and in people affected by chronic degenerative diseases.
Notwithstanding all these premises, and the increase in Clinical Laboratory testing, which is, and will continue in the future to be an indispensable ally of medical care, the correct diagnosis of a single or of multiple diseases occurring in a single individual will benefit enormously from this Discipline, if some steps forward will be made. I believe that the enormous amount of knowledge now accumulating in the field of Laboratory Medicine will revolutionize, not only the medical care of people, but, in the various areas of the medical scenario, also the field of Laboratory Medicine Science itself and the practice deriving from it.
However, there is a grey, largely neglected area in humankind: although life-span has almost doubled in the last 150 years, I believe the time has come to look at the wellbeing of each individual during his/her lifespan, particularly because multimorbidity occurs during the lifetime of each individual. In other words, we should all begin to be mindful of our state of health as early as about 20-25 years of age, when most auxological aspects have been reached, and sexual maturity completed. Consequently, I believe that each person should start a “health diary” at that age. Therefore, also healthy people should be monitored as well as patients , which should be one of the tenets of preventive medicine Having said that, I must now say that chronological age is practically meaningless in calculating health status. In fact, only a very careful analysis of an individual’s personal functional and morphological aspects will reveal early signs of disease, and enable physicians to prevent its progression. This, of course, applies much more to multimorbidity; in fact, once identified them, measures can be made to eradicate or to delay the start or the progression of each illness, therefore determining a better state of health during the progression of chronological age.
All this would revolutionize today’s medicine where the physician looks for early signs and symptoms of each possible alteration/disease, before deciding on treatment. The revolution I am talking about is to look at each individual when they are enjoying still normal health, as mentioned above. Indeed, current practice will become to measure a large series of functional laboratory parameters relating to all the functional assets of single tissues and/or organs, and also by imaging assessment, namely by looking at the morphological aspects of all the organs and tissues of the human body, mainly using echography approaches, and also by making a total body scan of different parts of the organism, and this, as said before, starting very early, i.e ,at 20-25 years old.
This approach may be considered too costly, but in effect it is much less costly than waiting for the appearance of an overt disease, which must then be treated for decades, frequently with very expensive drugs and tests (laboratory and imaging).
In this short presentation, I’ll also discuss theoretical and practical methods that can be used in the practice of predictive medicine at genomic level, as well as secondary preventive aspects aimed at improving the health of each individual, taking into consideration the primary prevention of diseases, also by decreasing environmental nocive factors, and tailor beneficial personal life style approaches for each person.
The inversion of the “paradigm” between aging and disease (1), the misnomers of the use of the term “age-associated disease”, and other considerations related to ideological aspects of so-called oxymoron term, physiological aging, are discussed to illustrate the need for this expected revolution in medical care, including Laboratory Medicine approaches. This will also support the joining of Preventive Medicine to effective Individualized Medicine.

REFERENCES

1. Salvatore F. The shift of the paradigm between ageing and diseases. Clin Chem.Lab Med. 2020 Sep 25;58(10):1635-1644. doi: 10.1515/cclm-2020-0125. PMID:32286241.

CJ02

DRIVE THROUGH DIFESA: L’ITALIA DIVENTA AREA DI CRISI

C. Renzi

Policlinico Militare - Roma

Con l'avvio dell'Operazione IGEA, la Difesa ha fornito il suo supporto al Servizio Sanitario Nazionale per l'attività

di screening del Coronavirus mettendo a disposizione della Nazione circa 200 postazioni distribuite su tutto il territorio nazionale. Ad oggi sono operativi circa 72 Drive-Through-Difesa. I contributi, forniti da ciascuna Forza Armata, sono diretti e coordinati fin dalla prima ora dal Comando Operativo di vertice Interforze (COI) per mezzo di una Sala Operativa dedicata, composta da personale interforze. In aggiunta al Policlinico Militare di Roma "Celio" sono stati inoltre messi a disposizione anche ulteriori 11 laboratori in tutta Italia e sono già stati eseguiti ben oltre 2,9 milioni di tamponi. Il concorso della Difesa nel contrasto al COVID 19 ha visto impegnati dal 23 ottobre 2020 circa 1.895 militari al giorno, per un totale di 434.107 giornate/uomo complessive. Con l'arrivo delle prime dosi di Vaccino, su richiesta della Struttura Commissariale, il Comando di Vertice Interforze della Difesa ha avviato l'Operazione EOS e, facendo tesoro dell'esperienza e delle specifiche competenze logistiche acquisite in questi anni di impegno nelle varie missioni nei diversi Teatri Internazionali, ha approntato e implementato un piano di distribuzione delle dosi mettendo a disposizione un dispositivo, prontamente adattabile in base alle esigenze, composto da 11 aerei, 73 elicotteri e 322 mezzi. Nel valutare alcune richieste pervenute dalle ASL, la Difesa ha successivamente convertito i Drive Through in "presidi per la somministrazione dei vaccini"(PVD), attività questa che si è aggiunta a quella già in atto, di stoccaggio dei vaccini presso l'aeroporto di Pratica di Mare e il successivo trasporto in tutte le regioni italiane. Ad oggi sono stati attivati sul territorio nazionale 30 Presidi Vaccinali della Difesa che supportano la Sanità Nazionale con le vaccinazioni a favore della popolazione civile, i quali hanno eseguito già oltre 473 mila vaccinazioni. Ad essi si aggiungono i 35 Presidi Vaccinali Mobili della Difesa con il compito di supportare la campagna vaccinale soprattutto in quelle località più difficili da raggiungere, che hanno eseguito oltre 53 mila vaccinazioni. È stata avviata inoltre la campagna vaccinale a favore delle isole minori tra cui le Tremiti, le Eolie, Pantelleria, Ustica, le isole della regione Sardegna, l'isola di Capraia e del Giglio, per le quali sono state somministrate oltre 25 mila vaccinazioni. Preziosissimo il contributo del Policlinico Militare "Celio", uno dei tre Ospedali Militari di riferimento, che è stato riconvertito in tempi rapidissimi in Covid Hospital ed inserito nella rete nazionale anti Covid quale riferimento delle strutture sanitarie del Centro Italia, mettendo a disposizione 152 posti letto di cui 100 di degenza ordinaria e 52 di sub-intensiva/intensiva.

CJ03

TRATTAMENTO DEI DATI SANITARI: PROGRESSO SCIENTIFICO E PRIVACY

V. Notarangelo

Data Protection Officer & Legal Counsel nei settori banking e sanità".

Al centro della società 4.0 ci sono i dati, che sono il motore di tutto e, per mezzo di essi, si muovono il progresso, l'economia e, oggi più che mai, la sanità e la ricerca scientifica.
È in tali settori che, ultimamente, si sono registrati i maggiori investimenti nella digital transformation finalizzata a sfruttare -attraverso i dati- tutte le nuove tecnologie presenti ed emergenti, dall'Internet of Things (IoT) all'Artificial Intelligence (IA). Lo sfruttamento dei Big Data, di fatto, costituisce il punto di partenza e la risorsa indispensabile per lo sviluppo della medicina innovativa e di precisione, fornendo supporto scientifico, organizzativo e infrastrutturale per promuovere la ricerca ed accelerare gli studi preclinici e clinici.
Tale sviluppo, tuttavia, avendo aumentato il numero dei soggetti che detengono dati relativi alla salute, la velocità di trasmissione di tali dati e la quantità delle informazioni elettronicamente archiviate (spesso non in territorio nazionale), ha determinato un aumento esponenziale della pericolosità dei trattamenti di dati dal punto di vista della riservatezza e un'accresciuta possibilità di ledere la dignità e le libertà fondamentali della persona. Ne è conseguita un'accentuata sensibilità del legislatore europeo e, a seguire, di quello nazionale, nei confronti della tutela di tali dati e tutele connesse. Oltre al Regolamento Europeo sulla Protezione dei Dati, che ha rivoluzionato il modo di concepire la data economy, è, infatti, in corso di valutazione -da parte delle istituzioni europee- la prima bozza di Regolamento sull'Intelligenza Artificiale, che costituirà il vero trampolino di lancio all'utilizzo massivo e regolamentato degli algoritmi, soprattutto in ambito healthcare.
Per la verità, tale ultima normativa citata, non farà altro che definire gli argini all'utilizzo di sistemi algoritmici già ampiamente in uso. Ne è una prova il recente intervento[1] dell'Agenzia Italiana del Farmaco (AIFA), che ha inteso richiamare e sottolineare tutte le normative da rispettare ai fini della presentazione e della conduzione di sperimentazioni attraverso algoritmi e tecniche di Machine Learning. L'AIFA, mediante tale guida, ha descritto alcuni case study, mostrando alcuni workflow che rappresentano le norme impattate a seconda della tipologia di sistema utilizzato e ponendo particolare attenzione alla compliance legata al trattamento dei dati e ai profili connessi di cybersecurity.
La sfida nell'utilizzo dei dati sanitari con applicazione di sistemi di AI in sanità e nella ricerca scientifica è, dunque, quella di trasformare la "consueta" classificazione di una malattia in un migliore processo decisionale clinico, definendo più precisamente le caratteristiche e i contorni che la singola patologia evidenzia nel caso di specie, per poi ottenere – grazie ad algoritmi e processi di machine learning – protocolli di cura specifici, personalizzati, eventualmente predittivi.

1. AGENZIA ITALIANA DEL FARMACO (AIFA), Guida alla presentazione della domanda di autorizzazione alla Sperimentazione Clinica che preveda l'utilizzo di sistemi di Intelligenza Artificiale (AI) o di Machine Learning (ML), 24.05.2021 https://www.aifa.gov.it/

SS09 - CASI CLINICI selezionati da abstract

CC001

Utilità dell'analisi genetica nel tumore del testicolo

M. Nunziato[1,2], F. Di Maggio[1,2], G. Damaggio[3], F. Crocetto[4], S.D. Pandolfo[4], A. Aveta [4], A. Calabrese[5], C. Imbimbo[4], V. Colonna [3], F. Salvatore[1,2]

[1]Ceinge-Biotecnologie Avanzate,Naples, Italy

[2]Department of Molecular Medicine and Medical Biotechnologies, University of Naples Federico II, Naples, Italy

[3]Institute of Genetics and Biophysics "A. Buzzati-Traverso", National Research Council (CNR), Naples, Italy

[4]Department of Neurosciences, Human Reproduction and Odontostomatology, University of Naples Federico II, Naples 80131, Italy

[5]Istituto Nazionale Tumori IRCCS "Fondazione G. Pascale", Via Mariano Semmola, 53, 80131 Napoli NA, Italy

Testicular cancer (TC) is a rather common neoplasia in men; the incidence in 2020 worldwide was 74.458, and the mortality accounts for about 12%. In this context, the early diagnosis and the comprehension of genetic pathogenesis gain an important role. A 18 years old patient was enrolled for a neoformation of the left didymus; he underwent an orchifunicolectomy of the left testicle. The histological diagnosis indicated mixed germ cell tumor consisting mainly of embryonic carcinoma (~80%) with a share of teratoma (~15%) and a share of Yolk-Sak tumor (~5%). Due to the early-onset and familiarity for seminoma (father, onset at 40 ys), we performed, firstly, a genetic analysis on the family-trio using our customized 48 multi-gene panel, which accounts for 883 target regions and includes all coding regions/gene and some adjacent noncoding regions. The 48 genes selected are associated to various typology of cancer as colon, prostate, breast and ovarian ones. The libraries have been obtained using the HaloPlex Target Enrichment System (Agilent Technologies). Sequencing run was performed using MiSeq platform (Illumina) and data analysis for variants identification was performed using Alissa Software. The average read depth obtained, was 511X, 515X and 605X for the proband, his father and his mother, respectively. We identified, two interesting variants: one shared with his mother in CHEK2, c.721+3A>T, reported as conflicting for pathogenicity in ClinVar database, and one de novo in ELAC2, c.2389G>T (p.Glu797Ter), predicted as Likely Pathogenic with in silico analysis.Then, we extended the analyses to Whole Genome Sequencing (WGS) using the Oxford Nanopore Technology (ONT) that allows both, (i) genome comparison between the trio and (ii) whole methylome analysis. The libraries have been prepared without fragmentation step (Ligation Sequencing Kit, Nanopore Oxford). Three different sequencing runs, for each patient, were performed on Promethion-24. The bioinformatics analysis was performed using Deep Variant for variant calling, Nanopolish and PycoMeth for methylome analysis.Comparison of the methylation profile, among the members of the trio, in 1.8M windows of the genome (size 500bp), revealed 260 windows with significant differences in methylation (p-value<0.01). Overall, the father and the child genome are less methylated compared to the mother. Interestingly, a 607kb region on Chromosome 20 (GRChg38) is significantly unmethylated in the father and the child compared to the mother. In conclusion, with the Nanopore approach, in a single step strategy we can obtain all the information for a complete first glance characterization of the genomic sequencing profile.This work is supported by CIRO project (to FS) from Campania Region (Italy), SATIN “Neoplasia studies” from Campania Region (to FS) and “Predictive Medicine in neoplasia” (to FS) from Campania Region (Italy).

CC002

Tre casi di possibile tossicità da farmaci

R. Simeoli[1], S. Cairoli[1], D. Cocciadiferro[2], E. Agolini[2], M. Marano[3], L. Lancella[4], L. Cursi[4], A. Vitale[1], V. Ventura[1], G. Antonetti[1], B.M. Goffredo[1]

[1] 1Department of Pediatric Specialties, Division of Metabolic Biochemistry, Children's Hospital Bambino Gesù, IRCCS, Rome, Italy;

[2] 2Laboratory of Medical Genetics, IRCCS, Children's Hospital Bambino Gesù, IRCCS, Rome, Italy;

[3] 3Pediatric Poison Control Center, Children's Hospital Bambino Gesù, IRCCS, Rome, Italy;

[4] 4Pediatrics and Infectious Diseases Unit, Children's Hospital Bambino Gesù, IRCCS, Rome, Italy;

Introduction: Therapeutic Drug Monitoring (TDM) is defined as the measurement of drug plasma concentrations at a specific time in a dosing interval. This application is particularly useful in pediatric patients where drug metabolism is often affected by developmental changes resulting in more variable plasmatic levels compared to adults. Variability in drug response may be caused by genetic polymorphisms, therefore in order to obtain a personalized medicine approach, TDM can be combined to Pharmacogenetics (PGx) tests especially when cases of altered drug responses or toxicity are reported.

Methods: Using Liquid Chromatography associated to Mass Spectrometry (LC-MS/MS), we routinely analyze plasmatic concentration of several drugs. In addition, Laboratory of Medical Genetics is able to run a Next Generation Sequencing (NGS) gene panel in order to evaluate presence of genetic variations or single nucleotide polymorphisms (SNPs) in genes encoding for proteins involved in drug metabolism and transport.

Results: During TDM routine, when analysis of plasma concentrations are above the laboratory alert values and patients presented signs of toxicity, PGx tests were performed. We described three clinical cases of patients with clinical signs of toxicity due to Phenytoin (PHT), Isoniazid (INH) and Voriconazole (VO) accumulation, respectively. Analysis of plasma concentrations confirmed levels above the laboratory alert values for Phenytoin, Isoniazid and Voriconazole. PGx tests were performed for these patients revealing for PHT patient, the homozygous variant c.1075A>C (p.Ile359Leu) in the CYP2C9, corresponding to the CYP2C9*3 haplotype, while the NAT2*5C/6B haplotype was identified in the INH patient. Therefore, patients were classified as poor metabolizer for PHT and INH, respectively. Similarly, patient treated with VO showed the variant NM_000769.2 (CYP2C19): c.681G>A; p.Pro227Pro, corresponding to the CYP2C19*1/*2 haplotype classifying this patient as intermediate metabolizer.

Conclusions: These cases show that TDM combined with PGx could help clinicians during the diagnostic process. In particular, this approach can be used not only for monitoring drug concentrations but also for predicting different pharmacogenetic variants accountable of altered responses to different pharmacological treatments. Therefore, combination of TDM and PGx represents a valid tool for a personalized medicine approach reducing risks of drug toxicity and/or therapeutic failures during the routine clinical practice.

CC003
Preziose informazioni dall'esame del liquido pericardico

A. Sharif Rouhani[1], F. Spanu[1], C. Ferraris Fusarini[1], M. Ammirabile[1], A.C. Migliorini[1], M. Vidali[1], G.A. Croci[2], F. Ceriotti[1]

[1]U.O.C. Lab. Analisi, Osp. Maggiore Policlinico, Milano
[2]U.O.C. Anatomia patologica, Osp. Maggiore Policlinico, Milano

Il pericardio è una membrana sottile che ricopre il cuore e la radice dei principali vasi sanguigni. È formato da due strati, separati dalla cavità pericardica, e contiene il liquido pericardico (LP, volume 20-50 ml). Si parla di versamento pericardico (VP) se il volume di LP nella cavità supera il valore normale. Il tamponamento cardiaco (TC) si ha quando un abbondante VP ostacola il riempimento delle cavità cardiache durante la diastole, con alterazioni del sistema cardiovascolare. Nell'aprile 2021, accede in pronto soccorso (PS), per dispnea ingravescente, un uomo di 63 anni fumatore, iperteso e in terapia con Rivaroxaban. Mediante ecocardiogramma si evidenzia un TC: il paziente è sottoposto a pericardiocentesi. In laboratorio arriva un campione di LP per la conta dei globuli bianchi (GB) e gli esami morfologico e chimico-fisico. Esso presenta 6800 GB/µL, con LDH, albumina e proteine rispettivamente di 3601 U/L, 2.5 g/dL e 4.80 g/dL. L'esame morfologico mostra un tappeto di emazie, granulociti neutrofili (11%), linfociti (70%) ed elementi monocito-macrofagici (19%). Si trovano, inoltre, numerose cellule di natura non ematopoietica (CDNNE) con aspetto dismorfico, risultato comunicato al PS. La TAC eseguita rileva un versamento pleurico: si preleva un campione di liquido (LPL) per le analisi. Anche il LPL mostra numerose emazie, granulociti neutrofili (3%), linfociti (45%), elementi monocito-macrofagici (52%) e CDNNE, simili a quelle di LP. Queste ultime si trovano anche nel liquido ascitico, prelevato per ulteriore presenza di versamento addominale. Alla TAC si vede una trombosi a livello dell'arteria polmonare comune, adenomegalie multiple, in particolare adenopatia in sede paratracheale e sovraclaveare. Si effettua pertanto un'agobiopsia del linfonodo sovraclaveare che consente di porre diagnosi di carcinoma polmonare scarsamente differenziato, a fenotipo citocheratina 7+/-, citocheratine 5/6+, p63+/-, compatibile con un istotipo squamocellulare. In conclusione, l'analisi di laboratorio del LP ha permesso, in tempi brevi, di dare un'indicazione ipotetica della presenza di neoplasia. Solo in tempi successivi al ricovero del paziente, e mediante altri esami, è stata confermata la presenza di una forma tumorale ormai metastatizzata.

CC004
Una diagnosi difficile in un paziente dializzato peritoneale

G. Canu[1], G. Alfano[2], S. Tagliavini[1], C. Canali[1], L. Giampaolo[1], M. Tagliazucchi[1], A.M. Ottomano[1], E. Tagliafico[1], T. Trenti[1], M. Varani[1]

[1]Department of Laboratory Medicine and Pathology AUSL-AOU Modena
[2]Nephrology, Dialysis and Transplant Unit, University Hospital of Modena

Introduction: Interleukin-6 (IL-6) is a pro-inflammatory cytokine secreted by lymphocytes, fibroblasts and macrophages involved in B-cell differentiation and stimulation of acute-phase proteins. IL-6 is associated with high serum levels in viral human infections such as hepatitis B and C virus, influenza virus, herpes simplex virus, HIV and also in coronavirus disease 2019. This cytokine is also produced by a wide array of intraperitoneal cells after the exposition to a local noxa patogena.Icodextrin has been associate with sterile peritonitis in patients on peritoneal dialysis (PD): this type of peritonitis is a cause of cloudy effluent and mild abdominal discomfort that resolved with the discontinuation of icodextrin. The diagnosis of icodextrin-associated peritonitis is critical to avoid unnecessary antibiotic prescription.Here, we described a case report of steril icodextrin-associated peritonitis coupled with elevation of IL-6 on peritoneal dialysate occurred in a male 76 years old undergoing PD.
Methods: IL-6 (DXI 800 Beckman Coulter) and leucocyte count were performed on peripheral blood sample and peritoneal dialysate before and after icodextrin rechallenge. Serum reactive C protein (PCR), serum procalcitonin (PCT) and peritoneal effluent colture were performed before and after icodextrin rechallenge.
Results: After 48 hours from the start of icodextrin rechallenge, peritoneal effluent became cloudy with a slight increase in leucocyte count (178 cells/microlitro with 11% neutrophil granulocytes, vs 62). Dyalisate colture, serum PCR and PCT resulted negative; leucocyte count in peripheral blood resulted normal (7300/µL). IL-6 level increased steeply in peritoneal effluent when compared to baseline (1124 vs 114 pg/mL) and subsided to baseline levels with the withdrawal of the icodextrin solution without increase in serum IL-6 (15.7 vs 12 pg/mL); likewise leucocyte count on peritoneal dialysate decreased (63 cells/µL).
Discussion: IL-6 can be used, along with peritoneal leukocyte count, as a precocious and sensitive marker of local inflammation and, in these cases, to discriminate from non-inflammatory peritonitis.
Reference: Yang X, Tong Y, Yan H, et al. High Intraperitoneal Interleukin-6 Levels Predict Peritonitis in Peritoneal Dialysis Patients: A Prospective Cohort Study. Am J Nephrol. 2018;47(5):317-324

CC005
Due pazienti fragili in Pronto Soccorso

M. Casini[1,2], T. Pavia[1], V. Guerri [1,2], M. Martelloni [3], L. Macchia[3,2], B. Fedi[1], C. Giusti[1], E. Gaudio[1], P. Chiarugi[1,2], G. Pellegrini[1,2]

[1]Lab. Analisi Chimico Cliniche Azienda Ospedaliero Universitaria Pisana
[2]Centro FCSA n. 281 Azienda Ospedaliero Universitaria Pisana
[3]SD Patologia Clinica Azienda Ospedaliero Universitaria Pisana

Two cases of dabigatran intoxication are suitable for our observation in recent months: they consist in two frail women both treated with dabigatran for atrial fibrillation (AF).
First case. A 68-years-old woman, accessed to the first aid for an accidental fall, non-commotional head trauma. Potus, general conditions expired, melena and rectorrhagia, anemia. Coagulation tests (done probably three days after the suspension of dabigatran) were found: Prothrombin Time (PT) 3,02, activated Partial Thromboplastin Time (aPTT) 2,45. Since dabigatran therapy is not declared, clinicians required the study of coagulation factors. All factors were moderately reduced, excepted the FVIII which was normal; the FII was significantly reduced (6%). There was a transient decrease in liver function. Suspecting previous assumption of dabigatran, laboratory doctors performed the Thrombin Time test, which was not clottable, and therefore the dabigatran resulted 658 ng/mL. Second case. A 94-years-old woman, accessed to the first aid for presyncope, anuria, asthenia and soporous state, in therapy with verapamil. Laboratory tests revealed severe anemia (hemoglobin 5,6 g/dL), creatinine 2,03 mg/dL, PT 4,97, aPTT 3,27, dabigatran 1,224 ng/mL.
Conclusions - In accordance with the guidelines of the European Heart Rhythm Association (2021 EHRA), stroke prevention in older AF patients is of great importance as stroke risk rises greatly with age. These patients have more favourable outcomes on directed oral anticoagulant (DOAC) than without it, and on DOAC than on vitamin K antagonist (VKA). Frailty, cognitive decline and risk of falling should not generally be a reason not to anticoagulate patients. However care needs to taken to minimize the risk of complications, it is crucial to ensure a structured follow-up with periodic visits, to reassess stroke and bleeding risk, to evaluate kidney and liver function, to check blood count and to measure the concentration of DOAC. Excessive DOAC plasma concentrations expose the patients to an increased risk of bleeding, This may occur when the patient has taken an overdose; also intercurrent events such as acute renal failure (especially with dabigatran) or administration of drugs with known drug-drug interaction, such as dabigatran/verapamil.

CC006
Le malattie rare richiedono approcci multidisciplinari

M. ZAGAGLIA[1,2], L. MARINELLI[1], R. MANISCALCO[4], B. BRUSCHI[3], P. COCCIA[3], D. LIGI[4], E. DAMIANI[2]

[1]Lab. Patologia Clinica, Osp. Riuniti Ancona, Ancona
[2]Dip. Scienze della Vita e dell'Ambiente, Univ. Politecnica delle Marche, Ancona
[3]U.O. Oncoematologia Pediatrica, Osp. Riuniti Ancona, Ancona
[4]Dip. Scienze Biomolecolari, Univ. Urbino Carlo Bo, Urbino

Afibrinogenemia is a rare bleeding disorder with an estimated prevalence of 1-2 per 1,000,000 people; in countries where the inbreeding rate is very high (such as in South India) the number of cases of congenital Afibrinogenemia is significantly higher.The Afibrinogenemia is an inherited disease with an autosomal recessive trait; while homozygous individuals are clinically symptomatic, heterozygotes (simple and/or compounds) usually do not show significant symptoms.In the present study, the homozygous variant IVS4 + 1G> A (c.718 + 1G> A) of Intron 4-¤ chain has been described for the first time from a genetic-molecular point of view, as the cause of Afibrinogenemia. Moreover, an in-depth biochemical study was carried out both on the proband and parents to determine whether the pro-inflammatory status was related to genetic-molecular disorders of fibrinogen.The increased levels of C-reactive protein (an acute phase protein) correlate with an increased concentration of several pro-inflammatory cytokines and a consistent lympho-monocytosis in the afibrinogenemic proband.In our knowledge, this is the first evidence describing in an afibrinogenemic proband a significant increased plasma values of the platelet-derived growth factor-beta (PDGF-¤), a growth factor involved in platelet genesis.This novel panel of cytokines could help to explain the increased thrombotic risk that patients suffering from Afibrinogenemia are subjected to, providing possible interpretations of both inflammatory and proteolytic pathways involved in the genetic anomaly of Afibrinogenemia.Although the present novel set of biochemical-molecular laboratory data should be carefully evaluated, it may open new frontiers for future clinical-medical use adding innovative biochemical-molecular biomarkers for the diagnosis of this rare genetic disease.
References:
Pieters M et al., Res Pract Thromb Haemost. 2019;3:161–172
Asselta et al., J Thromb Haemost 2006; 4: 2115–2129

CC007

Una anemia in gravidanza da indagare attentamente

G. Barberio, F. Bassetto, E. Cover, E. Gorrasi, F. Martignago, C. Mattiuzzo, A. Pegoraro, S. Zago

U.O.C. Medicina di Laboratorio, Azienda Ulss 2 "Marca Trevigiana", Treviso (TV)

L'emoglobina (Hb) Torino è una variante instabile delle catene alfa globiniche descritta la prima volta nel 1968 in una famiglia veneta abitante a Torino e successivamente segnalata in altre tre famiglie originarie del Veneto ed in un soggetto libanese nel quale è stata ipotizzata un'origine "de novo". In letteratura la Hb Torino è stata descritta talvolta associata ad anemia emolitica e splenomegalia, mentre in altri casi risultava meno sintomatica, senza essere mai stata provata tale eterogeneità fenotipica. Una donna di 29 anni, splenectomizzata, alla seconda gravidanza, è pervenuta in laboratorio per il controllo dell'anemia con una diagnosi generica fatta nel passato di anemia emolitica. Alcuni membri della sua famiglia erano stati esaminati per controllo e non mostravano particolari segni di anemia.
L'esame dell'assetto emoglobinico della probanda, mediante cromatografia ad alta risoluzione (HPLC) e successivo controllo in elettroforesi capillare (CE), ha fornito profili "normali" con indici eritrocitari variabilmente e moderatamente diminuiti. La quantificazione della Hb A1c (12 mmoli/moli) e della Hb A2 (1,5-2,3%), nonché la storia clinica della paziente suggerivano l'esame "in vitro" della stabilità della Hb con risultati positivi sia nella probanda che nella figlia. Lo studio strutturale mediante spettrometria di massa, successivamente confermata con l'analisi dei geni alfa, previa ricerca di difetti alfa talassemici, indicavano nella probanda la presenza di alfa talassemia (-3.7kb del) allo stato eterozigote e una mutazione T>G al nt130 del gene Alfa2, associato alla sostituzione amminoacidica Phe>Val al codone 43, corrispondente alla Hb Torino. La presenza dell'alfa talassemia associata ad una variante instabile come la Hb Torino correla con il fenotipo emolitico osservato nella probanda. L'utilizzo di metodi separativi ha rilevato valori significativamente ridotti di Hb A1c e di Hb A2 e ha quindi permesso di utilizzare risorse diagnostiche che hanno consentito di risalire alla causa della condizione emolitica. Questo caso contribuisce a fornire esempi di interazione tra difetti globinici fenotipicamente rilevanti, consente di capire come pervenire a diagnosi differenziali più corrette e come risulta utile poter disporre comunque di metodi dedicati che consentano di risalire alle caratteristiche globiniche anche mediante la quantificazione della Hb A1c.

CC008

Importanti informazioni dall'esame dello striscio periferico

M. Lorubbio, P. Anedotti, C. Artini, F. Cinci, S. Di Mario , S. Fabbroni, C. Gatteschi, L. Giannotti, F. Pulcinelli, E. Tripodo, A. Ognibene

Clinical Chemical Analysis Laboratory, San Donato Arezzo Hospital

Introduction: Rarely, platelets can interact with other blood elements, forming platelet aggregates. This paper presents an isolated case of platelet satellitism around neutrophils, lymphocytes and monocytes with platelet phagocytosis by both neutrophils and monocytes.
Case presentation: The subject was an 89-year-old woman with breast cancer on anti-estrogenic hormone cancer therapy. Whole blood sample collected in a tube with K2EDTA (Ethylenediaminetetra-acetic acid) anticoagulant was analysed within 4 hours, using an XN haematology analyser (Sysmex). The CBC (complete blood count) presented the following results: WBC (White blood cell) 4.0 x 10^9/L, RBC (Red blood cell) 3.58 x 10^12/L, haemoglobin 116 g/L, haematocrit 34.9%, MCV (Mean corpuscular volume) 97.5 fL, MCH (Mean corpuscular haemoglobin) 32.5 pg, MCHC (Mean corpuscular haemoglobin concentration) 33.2 g/dL, RDW (Red blood cell distribution width) 14.6% and PLT (Platelet) 136 x 10^9/L.
Conclusion: This case report describes the platelet satellitism around neutrophils, lymphocytes and monocytes and the interesting, very rare and singular phenomenon of platelet phagocytosis by not only neutrophils but also monocytes.

53° Congresso Nazionale della Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica (SIBioC - Medicina di Laboratorio)

Virtual Edition, 11-13 ottobre 2021

Riassunti Poster

Codice Poster	Argomento
• **P134**	Big Data ed Intelligenza Artificiale
• **P005, P023, P143, P122, P123**	Biochimica clinica dei liquidi biologici non ematici e malattie neurodegenerative
• **P003, P008, P010, P015, P017, P018, P032, P033, P052, P070, P075, P077, P090, P107, P115, P130, P132, P136, P162**	Casi Clinici
• **P002, P007, P014, P016, P028, P029, P030, P035, P040, P046, P047, P051, P063, P064, P067, P071 P081, P089, P091, P094, P095, P097, P111, P128, P139, P146, P147, P151, P157, P158, P159, P165, P178**	Diasgnostica del COVID-19
• **P056, P058, P069, P106, P110, P114, P117, P140, P145, P155, P166, P170, P171, P173**	Diagnostica decentrata (POCT)
• **P006, P037, P038, P057, P062, P066, P068, P074, P088, P100, P150, P164, P169**	Diagnostica ematologica integrata
• **P031, P096, P103, P105, P112, P113, P124, P126, P156, P163, P167**	Diagnostica dell'emostasi e trombosi
• **P025, P148, P176**	Diagnostica infettivologica (non COVID-19)
• **P0138**	Diagnostica delle malatie autoimmunitarie ed allergologiche
• **P013, P101, P102, P109**	Diagnostica cardiovascolare
• **P012, P099, P119, P135, P141**	Diagnostica della malattia diabetica e sindrome metabolica
• **P022, P121, P152**	Diagnostica delle malattie metaboliche ereditarie e screening neonatale
• **P011**	Diagnostica delle malattie epatiche
• **P019, P039, P054, P055, P085, P086, P092, P108, P120, P127, P129, P133, P160, P177**	Diagnostca oncologica
• **P079**	Diagnostica delle alterazioni delle proteine
• **P009, P024, P048, P082**	Diagnostica delle malattie renali e urologiche
• **P154**	Farmacogenetica e patologie genetiche
• **P045, P172**	Farmacotossicologia clinica, forense e doping

- **P044, P084** Gestione d'organizzazione del laboratorio
- **P050, P087, P116, P131, P168** Qualità analitica
- **P027, P042** Standardizzazione, armonizzazione e tracciabilità dei dati e delle informazioni
- **P002, P020, P072** Sostanze d'abuso e farmaci
- **P001, P004, P021, P026, P034, P036, P041, P049, P059, P060, P061, P065, P073, P076, P078, P080, P083, P093, P098, P125, P142, P149, 174** Varie

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

PO001

Use of Rheology and portable Low-Field NMR for the monitoring of lung functions in cystic fibrosis patients

G. Grassi[1], M. Abrami[2], M. Maschio[3], M. Conese[4], M. Confalonieri[5], F. Gerin[1], B. Dapas[1], R. Farra[5], A. Adrover[6], L. Torelli[5], B. Ruaro[5], M. Grassi[2]

[1]*Università di Trieste, DSV*
[2]*Università di Trieste, DIA*
[3]*IRCCS Burlo Garofolo, Trieste*
[4]*Università di Foggia*
[5]*Università di Trieste, DSMC*
[6]*Università la Sapienza, Roma*

Background. Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the gene encoding of the cystic fibrosis transmembrane conductance regulator (CFTR) responsible for chloride and sodium ion exchange across epithelial membranes. Dysfunctional CFTR induces the production of thick/viscous mucoid secretions in multiple organs, in particular the airways, where an augmented mucus viscosity is determined by the pathological increase in proteins, mucin and biological polymers. This process impairs mucociliary clearance, promoting chronic inflammation and bacterial infection leading to airway remodeling. This, in turn, may progress to respiratory failure, the most common cause of death for CF patients. Therefore, as sputum is a rich and noninvasive source of biomarkers of inflammation/infection, the determination of mucus properties is a relevant parameter able to indirectly monitor lung disease.We previously (Abrami et al Magn Res Med, p.427, 2020; Abrami et al Magn Res Med, p. 2323, 2018;) employed Low Field Nuclear Magnetic Resonance (LF-NMR) to measure the spin-spin relaxation time (T2m) of the water hydrogens present in the CF sputum. These data showed that the T2m had an indirect correlation with circulating/local inflammation markers and a direct correlation with FEV1 (forced expiratory volume in the first second, i.e. the amount that is exhaled in the first second purposefully trying to breath out as much air as possible). Thus, the assessment of the physical properties of sputum by T2m, may well provide a useful tool for the indirect monitoring of lung disease in CF patients. Here the T2m significance in the monitoring of CF lung disease was further investigated by studying the correlation of T2m with: 1) sputum viscoelasticity, 2) mucociliary clearability index(MCI)/cough clearability index(CCI) and 3) sputum average mesh-size.

Methods. Sputum samples from 25 consenting CF subjects were analyzed by rheology tests (elastic modulus G and zero shear viscosity ▫0) and LF-NMR (T2m). MCI/CCI were calculated from the rheological parameters. The average mesh-size (▫) of the sputum structure was then evaluated by rheology/LF-NMR, together with FEV1 for each patient.

Results. There was an inverse correlation between G/▫0 and T2m, indicating that a worsening of the lung condition (T2m-FEV1 drop) is paralleled by an increase in sputum viscoelasticity (G and ▫0) favoring mucus stasis/inflammation. A direct correlation was also observed between T2m and MCI/CCI, showing that T2m provides information as to airway mucus clearing. Moreover, there was a direct correlation between T2m and the average sputum mesh size (▫).

Conclusions. We demonstrated a correlation between T2m and the sputum viscoelasticity/average mesh-size and with MCI/CCI, parameters related to airway mucus clearing. This correlation was assessed, for the first time, by a combined analysis based on rheology and LF-NMR. This novel approach provided information as to the macro-(viscosity, elasticity, average magnetic relaxation time) and micro-scale (mesh size distribution) characteristics of CF sputum. In conclusion, the present data strengthen the potential of our test (T2M) to provide indirect monitoring of airway disease course in CF patients.

PO002

Valutazione del valore prognostico del biomarcatore MR-pro-ADM in pazienti COVID-19

T. Callegari[1], M. Cassinari[1], R. Guaschino[1], C.L. Magnano[1], D. Iodice[1], P. Iodice[1], E.C. Lauritano[2]

[1]S.C. Lab. Analisi, A.O. SS.Antonio e Biagio e C.Arrigo, Alessandria

[2]S.C. Medicina d'Urgenza, A.O. SS.Antonio e Biagio e C.Arrigo, Alessandria

INTRODUZIONEQuesto studio si è svolto presso il laboratorio analisi dell'A.O. SS. Antonio e Biagio e C. Arrigo di Alessandria nel periodo marzo-giugno 2020. Trattasi di uno studio di tipo osservazionale, prospettico e monocentrico effettuato su una coorte di pazienti con diagnosi di ARDS secondaria ad infezione da SARS-CoV2 ricoverati nella prima ondata della pandemia presso il reparto di Terapia sub-Intensiva.OBIETTIVOLo scopo di questo studio è stato di valutare le concentrazioni plasmatiche del biomarcatore pro-Adrenomedullina medio regionale (MR-proADM) al momento dell'accesso del paziente in PS (T0) e successivamente dopo 1, 3 e 5 giorni dal ricovero, per verificare il suo valore prognostico in termini di mortalità a 30 giorni e di efficacia nella stratificazione del rischio.PAZIENTI E METODISono stati arruolati 21 pazienti ricoverati in IMCU con diagnosi di infezione da SARS-CoV2. Il dosaggio di pro-ADM è stato effettuato su campioni di plasma mediante strumento B.R.A.H.M.S. KRYPTOR® compact PLUS. L'analisi statistica è stata effettuata con Stata version 16.RISULTATITra i biomarkers dosati, solo le concentrazioni di MR-proADM e LDH sono risultate significativamente elevate nei pazienti deceduti rispetto ai pazienti sopravvissuti, mentre nessuna differenza statisticamente significativa è stata osservata per le concentrazioni di PCR e PCT. Al T0 la media dei valori di MR-pro-ADM è risultata più alta nei pazienti non-survivors rispetto ai pazienti survivors (3.5 vs. 1.1 nmol/L, $p<0.05$). Livelli crescenti di MR-proADM tra il terzo e il quinto giorno di ricovero nel reparto di terapia intensiva, rappresentano la chiave per la definizione del rischio di mortalità. Il cut-off ottimale per MR-pro-ADM sembra essere di 1.07 nmol/L che risponde a una sensibilità del 91% e a una specificità del 71%.CONCLUSIONI Mr-proADM è risultato un biomarcatore più efficace di altri convenzionali (PCR, WBC, Procalcitonina) nell'identificare precocemente i pazienti Covid-19 con prognosi infausta. Questo studio ha permesso ai clinici di inserire in routine questo dosaggio già nella seconda ondata dell'epidemia, in quanto sembra essere utile, in aggiunta ai comuni scores clinici, nella stratificazione di tali pazienti e nel predire un più o meno elevato rischio di mortalità.

PO003

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

A.M. DI FABIO, G. DI MICHELE

U.O.C. Patologia Clinica Ospedale Civile San Salvatore L'Aquila ASL 1

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

Anna Maria DI FABIO , Giulio Di Michele.Laboratorio Analisi – Ospedale San Salvatore, L'Aquila ASL 1 – Avezzano, Sulmona, l'Aquila

CLINICAL PICTURE AND LABORATORY INVESTIGATION IN A COVID-19 PATIENT.

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

PO004

Analisi delle urine: nefelometria e turbidimetria a confronto.

B. Bernardi, N. Jordaney, M. Di Benedetto

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

Introduzione: La ricerca e misurazione di alcune proteine urinarie risulta utile nella diagnosi di un eventuale danno renale. La differenziazione tra proteine ad alto e basso peso molecolare permette di distinguere tra proteinuria glomerulare e tubulare, la presenza di catene leggere libere kappa (K) e lambda (L) urinarie consente di individuare una proteinuria di Bence Jones (BJ) in un contesto di discrasia plasmacellulare. Abbiamo confrontato la determinazione dell'alfa-1 microglobulina, dell'alfa-2 macroglobulina, dell'albumina, delle immunoglobuline G e della BJ urinarie ottenuta su strumentazione nefelometrica Bn ProSpec (Siemens) e su strumentazione turbidimetrica Optilite (The Binding Site). Metodi: Lo studio di comparazione è stato condotto analizzando campioni di urina (raccolta 24 ore) con il kit K.BNA.FRK.FRL (New Scientific Company) su Bn proSpec e con il kit Freelite Mx su Optilite (33 campioni per le catene leggere libere lambda e 41 campioni per le catene leggere libere kappa). 28 campioni di urina (raccolta estemporanea) sono stati analizzati con kit Siemens (Bn proSpec) e kit The Binding Site (Optilite) per la ricerca degli analiti urinari alfa-1 microglobulina, alfa-2 macroglobulina, albumina e immunoglobuline G. La concordanza tra i valori ottenuti in nefelometria ed in turbidimetria è stata valutata mediante analisi statistica (Passing-Bablok e Bland-Altman). Risultati: E' stata riscontrata una buona correlazione per alfa-1 microglobulina (Correlation R=0.997), albumina (R=0.993) e immunoglobuline G (R=0.998); per alfa-2 macroglobulina R non è calcolabile. L'analisi dei dati delle catene leggere libere ha evidenziato per L una buona correlazione con un Agreement rate=93%, mentre per K un Agreement rate=71%. Il risultato ottenuto per K dovrà essere approfondito per valutare se la discordanza può essere attribuibile ai diversi controlli automatici dell'eccesso di antigene. Bergon E., et al. Classification of Renal Proteinuria: A Simple Algorithm. Clin Chem Lab Med 2002; 40(11):1143–1150.Caldini A. el al. New patterns of relapse in multiple myeloma: a case of "light chain escape" in which FLC predicted relapse earlier than urine and serum immunofixatio. Clin Chem Lab Med 2016; 54(6): 991–995.

PO005

A convenient method for extraction and analysis with High-Pressure Liquid Chromatography of catecholamine neurotransmitters

S. De Francia[1], F. Chiara[1], S. Allegra [1], F. Montarolo[2]

[1]Clinical and Biological Sciences Department, University of Turin

[2]Neuroscience Institute Cavalieri Ottolenghi (NICO), Orbassano (Turin)

The extraction and analysis of catecholamine neurotransmitters in biological fluids is of great importance in assessing nervous system function and related diseases, but its precise measurement is still a challenge. Many protocols have been described for neurotransmitters measurement by a variety of instruments, including high-pressure liquid chromatography (HPLC). However, there are shortcomings, such as complicated operation or hard-to-detect multiple targets, that can no longer be avoided. Presently, the dominant analysis technique is still HPLC, due to its high sensitivity and good selectivity. Here, a detailed protocol is described for the pretreatment and detection of catecholamines with high pressure liquid chromatography coupled with ultraviolet detection (HPLC-UV) in plasma and brain samples of mice. The calibration curve of catecholamines was established in the concentration range of 0.01 – 5.00 ug/mL. 100 µl of plasma or brain samples were extracted by protein precipitation using 300 ul of freeze solution of formic acid 0.5%v/v in acetonitrile. Each sample was vortexed for at least 15 s and then stocked in freezer at -20°C for 15' and later centrifuged at 4,000 rpm for 10 min. The 250ul of surnatant was transferred to an injection vial. Chromatographic separation was performed at 35°C, using a column oven, on a RP column (Atlantis T3 4.6 x 50 mm, 5 µm, Waters, USA). A gradient chromatographic elution was executed by mixing three solutions: water; acetonitrile; 100mM Ammonium Formate, pH = 3.00 (with formic acid). The flow rate was set at 1 mL/min. Catecholamines plasma concentrations were reported as ug/mL, instead brain amount were converted in ng/mg of tissue weight. Previously, prior to start extraction procedure, weighted brain samples were frozen in liquid nitrogen, sonicated for 1 min, reconstituted in 1 mL of water and sonicated for another min. The established protocol was applied to assess the differences of plasma and brain levels of catecholamines between genetically different mice. Applications of our methods are very broad as wide is the field of neurodegerative diseases.

PO006

Ruolo del laboratorio di Patologia clinica nella diagnostica di I livello delle Emoglobinopatie: esperienza della ASL Roma 1.

F.G. Martino, D. Frattolillo, G. Raccosta, M. Vitillo

UOC Patologia Clinica HUB, Dipartimento dei Laboratori, PO S. Filippo Neri, ASL Roma 1

SCOPO: Le Emoglobinopatie (EP) sono patologie autosomiche recessive caratterizzate da anomalie della struttura, funzione o produzione di emoglobina. Al fine di garantire all'utenza della ASL Roma 1 una corretta diagnosi in un'ottica di appropriatezza, efficacia e sostenibilità, a partire dal 1/7/2019 è stato messo a punto un percorso diagnostico multidisciplinare offerto alle coppie afferenti ai Centri di Procreazione Medicalmente Assistita (PMA) della ASL e alle donne in gravidanza afferenti ai Centri Prelievo. Questo percorso garantisce l'accesso sul territorio alla diagnostica di I e II livello e alla consulenza genetica; inoltre offre alle coppie afferenti ai percorsi di PMA programmi di diagnosi prenatale, prevenzione, presa in carico e follow-up. MATERIALI E METODI: Dal 1° luglio 2019 al 31 maggio 2021 abbiamo esaminato 4007 Pazienti con range di età 11-90 anni, 932 maschi (23%) e 3075 femmine (77%) che hanno effettuato un prelievo presso i centri della ASL Roma 1 per la ricerca di EP. Gli esami specifici di I livello sono l'assetto emoglobinico (elettroforesi capillare - SEBIA), l'esame emocromocitometrico e l'assetto marziale. Nel referto viene fornito un commento interpretativo al fine di giungere ad una diagnosi definitiva o presuntiva, quando possibile. RISULTATI: In 2406 Pazienti (60%) i risultati erano compatibili con lo stato di normalità; in 478 casi (11,9%) erano presenti anomalie riconducibili ad una EP da confermare con esami di secondo livello; in 1123 (28%) la mancanza dei parametri emocromocitometrici e/ o dell'assetto marziale non hanno permesso di produrre un referto conclusivo. DISCUSSIONE: La valutazione integrata dei risultati della ricerca delle EP con gli indici eritrocitari e con l'assetto marziale ha consentito, nella nostra esperienza, una diagnosi di esclusione di EP nell'83,4% dei casi. Nel restante 16,6% è stato necessario un approfondimento con metodiche di 2° livello. Il commento interpretativo nel referto si è rivelato molto utile nella comprensione dei risultati. Sebbene sia ancora presente un numero significativo di richieste non complete per arrivare a una definizione diagnostica, il percorso si è rivelato utile sia per i pazienti che per i clinici.

PO007

LA TERAPIA CON PENTAGLOBIN NELL'INFEZIONE DA SARS-COV2: INNESCO DELLA IMMUNITA' UMORALE.

S. Leonardi, V. Santocchio, N. Carannante[2], N. Schiavone[2], F. Garziano, C. De Falco, B. Maione, S. Sarpa, L. Atripaldi

[1]UOC Biochimica Clinica, Osp. Monaldi, Napoli
[2]UOC Malattie Infettive, Osp. Cotugno, Napoli

BACKGROUND: La patogenesi da SARS-CoV-2 sembrerebbe essere mediata da risposte immunitarie sproporzionate, nonchè dalla capacità del virus di aggirare l'immunità innata. È stato anche osservato che l'infezione COVID-19 induce la produzione e la secrezione di citochine pro-infiammatorie, come l'IL-6, che disregola le risposte infiammatorie locali, parzialmente responsabili della devastante sindrome da distress respiratorio acuto. Pertanto l'IL-6 potrebbe essere considerata come potenziale marker prognostico della gravità della malattia COVID-19.MATERIALI E METODI: I pazienti sono stati reclutati presso la III Divisione Covid-19 dell'Ospedale Cotugno; ad essi è stato somministrato Pentaglobin® in infusione lenta 5 mL/Kg al T0, T7 e T10 per valutarne gli effetti. I criteri di inclusione sono stati maggior età, polmonite da 7 gg non responsiva ad altri trattamenti, e IL-6 < 20 pg/mL, condizione per cui i pazienti non erano arruolabili per il protocollo che prevede l'utilizzo del Tocilizumab. Sono stati valutati, oltre agli esami di routine previsti dal protocollo, l'andamento pre e post-trattamento con Pentaglobin dei seguenti marcatori: IgM, IgG, IgA e 2019-nCoV IgM e IgG, Proteina C Reattiva (PCR), Procalcitonina (PCT), Adrenomedullina (MR-proADM), Copeptina (COP), Globuli bianchi (WBC).RISULTATI: Dopo 6,5 giorni da T0, abbiamo osservato un incremento del valore di quasi il doppio delle IgG, IgM, IgA, oltre che delle Immunoglobuline specifiche per la diagnosi di COVID-19. Contestualmente, i livelli di IL-6, PCT, PCR, ADM e COP mostravano un significativo decremento del valore iniziale, coerentemente al miglioramento dello stato clinico del paziente.CONCLUSIONI: Nei pazienti trattati con Pentaglobin si è evidenziata una netta correlazione tra l'aumento delle Immunoglobuline aspecifiche e l'incremento delle Immunoglobuline anti-COVID-19, con un significativo miglioramento dello stato clinico del paziente, confermando dunque che l'incremento del titolo anticorpale specifico è associato ad un outcome migliore.

PO008

Discordanza fra sesso cromosomico e sesso fenotipico: case report

D.D. Dell'Edera, A. Allegretti, A.A. Epifania, F. Mangia, F. La Rocca, B. Persia, F. Paolicelli, F. Simone, R. Ambrosecchia, G. Schiuma

U.O. Lab. Genetica Medica, Ospedale Madonna delle Grazie, Matera

È giunto alla nostra osservazione il signore V.Q. per sottoporsi allo studio del cariotipo da sangue venoso. Dagli esami di laboratorio visionati si osserva che il suddetto presenta azoospermia, LH 7,1 IU/L (v.n. 1,24-7,8), FSH 15,6 IU/L (v.n. 1,5 a 12,4), AMH 5,02 ng/ml, Testosterone 3,94 µg/L (1,75-7,81). Da una visita uroandrologica si è evidenziato quanto segue: ipotrofia testicolare bilaterale, deferenti presenti, didimi ad ecostruttura e morfologia normale ma nettamente ridotti di dimensioni (1,88ml il sinistro, 2,63ml il destro), prostata di dimensioni ed aspetto normale, vescicole seminali bilateralmente in sede e nella norma.Dall'esame del cariotipo si è evidenziato che il signor V.Q. presenta un cariotipo femminile (46,XX). Per comprendere la discrepanza fra sesso cromosomico e sesso fenotipico osservato abbiamo eseguito la ricerca del gene SRY. L'indagine molecolare ha confermato la presenza del gene SRY che mediante FISH è risultato localizzato nella regione terminale del braccio corto del cromosoma X.Il riscontro di un cariotipo 46,XX in un soggetto fenotipicamente maschile è una condizione rara, con prevalenza stimata intorno a 1:20.0001. La diagnosi è molto spesso tardiva: i soggetti affetti presentano genitali esterni maschili alla nascita, raramente ambigui (<20% dei casi), e solo durante lo sviluppo il riscontro di severa ipotrofia testicolare bilaterale associata ad azoospermia consente di ipotizzare un disturbo dello sviluppo sessuale. Generalmente tale quadro è correlato ad ipogonadismo ipergonadotropo. In questi soggetti l'ecografia addominale è utile per escludere strutture mulleriane residue. Dai dati presenti in letteratura la biopsia testicolare, per verificare la presenza di spermatozoi nei testicoli, non è raccomandata e l'adozione o la fecondazione in vitro (IVF) con un donatore di sperma sono le uniche possibilità2.

Riferimenti Bibliografici

1 Amaral RC et al. Quality of life of patients with 46,XX and 46,XY disorders of sex development. Clin Endocrinol (Oxf). 2015 Feb;82(2):159-64.

2.Terribile M et al. 46,XX Testicular Disorder of Sex Development (DSD): A Case Report and Systematic Review. Medicina (Kaunas). 2019 Jul 12;55(7):371.

PO009

La refertazione dello studio metabolico per il rischio nefrolitiasico: il commento biochimico

G. Cangiano[1], A. Latte[2], V. Capobianco[1], P. Iardino[1], F. Fedele[1], M. Boscaglia[1], F. Gaeta[1], L. Paragliola[1], M. D'Amora[3]

[1]UOC Patologia Clinica - P.O. Pellegrini - ASL NA 1 Centro

[2]UOC Farmacia - Ospedale Santa Chiara - Trento - APSS Trento

[3]UOC Patologia Clinica - Ospedale San Paolo - Napoli - ASL NA 1 Centro

La valutazione dello studio metabolico per il rischio nefrolitiasico e l'inquadramento clinico del Paziente, possono aiutare il Nefrologo, l'Urologo o il MMG esperto, ad indirizzare il Paziente verso gli stili di vita (correttivi dietetici ed eventualmente farmacologici) atti a prevenire la formazione calcoli renali. Le determinazioni degli analiti urinari delle 24 ore si effettuano utilizzando clorexidina al 20% (dosaggi di sodio, potassio, cloro, proteine urinarie, urea, creatinina ed acido urico e determinazione del pH) ed acido cloridrico 37% (determinazioni di calcio, fosforo, magnesio, ossalato, citrati, solfati e cistina). Inoltre, su urina estemporanea, prelevata al termine della raccolta delle 24 ore, si praticano i dosaggi del calcio e della creatinina. Il referto è elaborato da un software dedicato, costruito in Access, che oltre a raccogliere i parametri sopra citati, include anche il volume urinario e le supersaturazioni renali di calcio ossalato, calcio fosfato, acido urico e cistina. La lettura del referto è facilitata dalla presenza di un commento biochimico finale che sintetizza le più importanti variazioni dei parametri laboratoristici. L'aumento (o la diminuzione) patologica di ognuno di questi ultimi viene infatti confrontata con dei range presenti all'interno del programma, generando così un opportuno commento parziale. La sommatoria di questi ultimi viene a costituire il commento finale biochimico che può riassumere in maniera diretta al clinico la carenza di inibitori, l'aumento di promotori, l'acidità urinaria, la eventuale presenza di ipercalciuria metabolica, la diuresi e la tendenza a formare un certo tipo di calcolo.

Cangiano G, Buccino G, et Al. La calcolosi renale in un laboratorio di patologia clinica: modello organizzativo e nuove tecniche analitiche. Giornale di Tecniche Nefrologiche e Dialitiche, 2019,1,31,22-29

PO010

The importance of capillary electrophoresis for HbA1C analysis in haemoglobinopathies: a case report

L. Agnello[1], E.M. Pappalardo[2], S. Maestri[2], R.V. Giglio[1], P. Altavilla[2], C.M. Gambino[1], A.M. Ciaccio[3], B. Lo Sasso[1], M. Ciaccio[1]

[1]*Institute of Clinical Biochemistry, Clinical Molecular Medicine and Laboratory Medicine, Department of Biomedicine, Neurosciences and Advanced Diagnostics, University of Palermo, Palermo, Italy.*

[2]*Department of Laboratory Medicine, University Hospital "P. Giaccone", Palermo, Italy.*

[3]*University of Palermo, Palermo, Italy.*

Background. Haemoglobinopathies, inherited disorders of haemoglobin, are the most common monogenic diseases worldwide (1). They can be classified as qualitative (haemoglobin variants) and/or quantitative (thalassemias) disorders. The clinical manifestations are highly variable, ranging from asymptomatic to severe haematological diseases, with multiple end-organ damages. Among structural haemoglobin variants, HbS, HbE and HbC are the most common. The diagnosis of haemoglobinopathies mainly relies on Complete Blood Count (CBC) and on the analysis of haemoglobin fractions, according to guidelines (2). Sometimes the diagnosis is casual during routine laboratory analysis. Case report: We report a case of a 51-year-old Caucasian man who had to undergo an odontostomatological surgery. Before the operation, he underwent routine pre-operative evaluation, including also laboratory testing, such as CBC and glycated haemoglobin (HbA1c). The HbA1c measured by high performance liquid chromatography (HPLC) resulted undetectable. In order to confirm such unexpected laboratory finding, we measured HbA1C by capillary electrophoresis on CAPILLARYS 3 TERA instrument (Sebia Labordiagnostische Systeme GmbH, Fulda, Germany), turning out the same result. The capillary electrophoresis of HbA was performed to identify the possible cause of the absence of HbA1C. The analysis revealed the total absence of HbA0 and the presence of the HbC and HbE variants. Then, the sample was further analysed by capillarys haemoglobin (E) kit (Sebia Labordiagnostische Systeme GmbH, Fulda, Germany), which provides a complete haemoglobin profile for the quantitative analysis of the normal haemoglobin fractions A, A2, and F and allows the detection of haemoglobin variants, using capillary electrophoresis. The analysis confirmed that the absence of HbA1C was due to haemoglobinopathy and it was not related to potential analytical or pre-analytical errors. Discussion. In this case report, the capillary electrophoresis has been fundamental to confirm the absence of HbA1C and to detect the total absence of HbA0 as well as the presence of two structural variants of haemoglobin, leading to the diagnosis of a haemoglobinopathy. The optimal analytical performances make capillary electrophoresis a solution of choice for identifying haemoglobinopathies at a high degree of confidence. Indeed, it allows an accurate separation and quantification of the haemoglobin fractions.

References:

1. Ghosh K, Ghosh K, Agrawal R, Nadkarni AH. Recent advances in screening and diagnosis of hemoglobinopathy. Expert Rev Hematol. 2020;13:13-21.
2. Ghosh K, Colah R, Manglani M, Choudhry VP, Verma I, Madan N, Saxena R, Jain D, Marwaha N, Das R, Mohanty D, Choudhary R, Agarwal S, Ghosh M, Ross C. Guidelines for screening, diagnosis and management of hemoglobinopathies. Indian J Hum Genet. 2014;20:101-19.

PO011

Hepatocellular carcinoma cell line-secreted extracellular vesicles: miRNA and long non coding RNA transcriptional expression

M. Cabiati[1], C. Salvadori[1], G. Basta[1], S. Del Turco[1], A. Cecchettini[2], S. Del Ry[1]

[1]Institute of Clinical Physiology, CNR, Pisa, Italy
[2]University of Pisa, Dept. Experimental and Clinical Medicine, Pisa, Italy

Extracellular vesicles (EVs) are membrane-released vesicles vesicles acting as transporters of proteins, lipids and short/long non-coding RNA (miRNAs and lncRNAs). They are released by normal and pathological cells, including hepatocellular carcinoma (HCC). To date, studies focused on miRNAs and lncRNAs contained in EVs derived from HCC are limited. Our aim was to analyze the transcriptional profile of potential regulating miRNAs and lncRNAs in EVs secreted by HCC tumor cell line (HepG2), and from a non-tumorigenic hepatocyte cell line (WRL68), to compare their differential expression profile and to identify novel molecular diagnostic markers of HCC. EVs were isolated from the conditioned medium, through differential centrifugations. The expression profile of miRNAs (miR-23a, miR-16-2, miR-181a, miR-373, miR-205, miR-27a, miR-1323, miR-532) and lncRNAs (HULC, HOTAI, XIST, MALAT-1, GAS-5, H19) was performed in Real-Time PCR and their transcript were found both in HepG2 and WRL68 EVs. Lower miR-181a, miR-205 and miR-1323 expression was detected in EVs secreted by HepG2 compared to WRL68, while an opposite trend was observed for miR-23a, miR-16-2, miR-373, miR-27a, and miR-532. Several significant correlations were found between miRNA and lncRNA.The results obtained could identified them as new potential diagnostic and prognostic biomarkers of HCC.

PO012

Putative Long non-coding RNA in childhood obesity: Screening and identification of their transcriptional levels

M. Cabiati[1], M. Fontanini[1], M. Giacomarra[1], G. Politano[2], E. Randazzo[3], D. Peroni[3], G. Federico[3], S. Del Ry [1]

[1]Bichemistry and Molecular Biology Laboratory, CNR, Institute of Clinical Physiology, Pisa Italy
[2]Department of Control and Computer Engineering, Politecnico di Torino, Italy
[3]Unit of Pediatric Endocrinology and Diabetes, Dep. Clinical and Experimental Medicine, University of Pisa, Italy

Long non-coding RNAs (LncRNAs) and microRNAs are involved in the pathogenesis of obesity, a multifactorial disease characterized by inflammation, cardiometabolic complications and increased cancer risk among other co-morbidities. The up/down regulation of LncRNAs and microRNAs may play an important role in this condition to identify new diagnostic/prognostic targets. Aim of the study was to identify circulating inflammatory LncRNAs in obese adolescents (n=54) and to evaluate whether their expression behaved differently compared to normal-weight adolescents (N, n=26). To have a more complete insight, the expression of some circulating miRNAs linked to obesity (miR-33a, miR-223, miR-142, miR-199a, miR-181a, miR-4454) were also analysed. LncRNA and miRNAs were extracted simultaneously from plasma samples and Real-Time PCR was performed. Among the 86 LncRNAs analysed with custom pre-designed plates only four (RP11-347E10.1, RP11-10K16.1, LINC00657, SNHG12) were amplified in both normal-weight and obese adolescents and only SNHG12 showed significantly lower expression compared to normal-weight adolescents (p=0.026). Circulating miRNAs showed a tendency to increase in obese subjects, except for miR-181a expression. We observed significant correlations among LncRNAs themselves as well as among miRNAs. This is the first study analyzing LncRNAs in metabolic processes, such as obesity, and, although further investigations are needed to better recognize the mechanisms underlying the metabolic disorder and propose them as potential therapeutic targets for the treatment of childhood obesity, the result obtained can be considered encouraging as they suggest that, subject studied had a basal level of inflammation without an active carcinogenesis.

PO013

BMP4 system expression in human coronary artery endothelial and smooth muscle cells under dynamic flow: effect of medicated Bioresorbable Vascular Sca#olds at low and normal shear stress.

M. Cabiati [1], M. Giacomarra[1], M. Fontanini [1], A. Cecchettini [2], G. Pelosi [3], F. Vozzi [3], S. Del Ry [1]

[1]Laboratory of Biochemistry and molecular biology, Institute of Clinical Physiology CNR, Pisa, Italy
[2]Laboratory of Proteomics, Institute of Clinical Physiology IFC-CNR / c University of Pisa, Dept. Experimental and Clinical Medicine, Pisa, Italy
[3]Laboratory of Biomimetic Materials and Tissue Engineering, Institute of Clinical Physiology CNR, Pisa, Italy

Endothelial and smooth muscle cell dysfunction is an early event at the onset of atherosclerosis, a heterogeneous and multifactorial pathology of the vascular wall. Bone morphogenetic protein (BMP)-4, a mechanosensitive autocrine cytokine, and BMPR-1a, BMPR-1b, BMPR2 specific receptors play a key role in atherosclerotic plaque formation and vascular calcification and BMP4 is regarded as a biomarker of endothelial cell activation. The study aimed to examine the BMP4 system expression by Real-Time PCR in Human Coronary Artery Endothelial (HCAECs) and Smooth Muscle Cells (HCASMCs) under different flow rates determining low or physiological shear stress in presence/absence of medicated Bioresorbable Vascular Scaffold (BVS). The HCAEC and HCASMC were subjected to 1-10-20 dyne/cm2 shear stress in a laminar-flow bioreactor system, with/without BVS+Everolimus (600 nM). In HCAECs without BVS the BMP4 expression was similar at 1-20 dyne/cm2 decreasing at 10 dyne/cm2, while adding BVS+Everolimus, it decreased both at 1-10 compared to 20 dyne/cm2. In HCASMCs without BVS+Everolimus, the BMP4 system mRNA expression was significantly reduced at 1-10dyne/cm2 compared to 20 dyne/cm2 while in presence of BVS+Everolimus, higher BMP4 mRNA levels were observed at 10 compared to 1-20 dyne/cm2. In HCAECs and HCASMCs BMPRs were expressed in all experimental conditions except for BMPR-1a at 1 dyne/cm2 in HCAEC. Significant correlations were found between BMP4 and BMPRs.The less effect on BMP4 expression due to low shear stress in HCAEC compared to HCASMC as well as its reduction in presence of BVS+Everolimus at low shear stress highlighted a protection BMP4-mediated against endothelial dysfunction and neoaterogenesis.

PO014

Rilevazione dell'antigene del virus SARS-CoV-2 in tamponi nasali nello screening di soggetti asintomatici

T. Ciarma, F. Capanna, A. Arcangeli, L. Pianese, A. Fortunato

U.O.C. Patologia Clinica ASUR Marche – Area Vasta 5, Ascoli Piceno

SCOPO: il tampone nasale si è dimostrato una valida alternativa al prelievo nasofaringeo quando si utilizza un metodo rapido per la rilevazione dell'antigene del virus SARS-CoV-2. Questo metodo può essere eseguito più agevolmente e può facilitare una rapida estensione delle strategie di analisi dell'antigene. È stato condotto uno studio prospettico con l'obiettivo di valutare un metodo per la ricerca dell'antigene, automatizzato e ad elevata produttività, per rilevare le persone infette da COVID-19 asintomatiche, utilizzando un tampone nasale eluito direttamente in una soluzione inattivante il virus.
METODO: nel mese di febbraio 2021, nel Laboratorio ASUR di Ascoli Piceno, è stata effettuata la ricerca dell'antigene di SARS-CoV-2 in 6241 soggetti asintomatici utilizzando un nuovo metodo quantitativo su un analizzatore, ad elevata produttività e con tecnica immunochemiluminescente (CLIA), che rileva la proteina del nucleocapside del virus. Dopo la raccolta, il tampone nasale è stato eluito nel terreno inattivante e, trascorso il tempo previsto per la completa inattivazione del virus (30 minuti a T.A.), è stata eseguita la determinazione. Tutti i campioni positivi all'antigene sono stati analizzati con tecnica molecolare (reverse RT-PCR).
RISULTATI: 290 campioni su 6241 sono risultati positivi per SARS-CoV-2 con il metodo antigenico e 235 sono stati confermati dalla RT-PCR. La prevalenza dei soggetti positivi per la ricerca dell'antigene è stata del 3,7% con il 96,3% di campioni negativi. La concordanza percentuale con RT-PCR è stata dell'89,52% per i positivi e del 99,54% per i negativi, considerando un valore soglia di 100 TCID50/mL.
CONCLUSIONI: la ricerca dell'antigene con metodo CLIA risulta adeguato per lo screening di un numero elevato di soggetti asintomatici, al fine di identificare i portatori del virus che sono la fonte primaria di trasmissione.
BIBLIOGRAFIA: Pieter Vermeersch, Confronto tra il test antigenico quantitativo DiaSorin Liaison e la RT-PCR per la diagnosi di COVID-19 in pazienti sintomatici e asintomatici. J Clin Microbiol doi:10.1128/JCM.00374-21

PO015
CASO CLINICO: UNA FALSA EQUIVALENZA

C. Bellini[1], I. Baldi [2], P. Calzoni[3], R. Leoncini[4], G.P. Caldarelli[1], B. Morelli[5]

[1]UOC Analisi Chimico Cliniche, PO Misericordia Grosseto, AUSL Toscana Sud Est
[2]UOC Chirurgia Vascolare, PO Misericordia Grosseto, AUSL Toscana Sud Est
[3]UOS Coagulazione, UOC Laboratorio Patologia Clinica, AOU Senese
[4]Dip. Biotecnologie Mediche, UNISI - UOC Laboratorio Patologia Clinica, AOU Senese
[5]Synlab Castanedolo (BS)

PC giunge al PS di Grosseto per anemizzazione acuta e ematoma al muscolo psoas da caduta. Alla TC emerge un aneurisma dell'arteria femorale profonda operato in Chirurgia Vascolare. Il paziente era in terapia domiciliare con Edoxaban 60mg per Fibrillazione Atriale. PT e aPTT post-intervento risultano 1,11 e 0,99 ratio. Per ripristinare l'anticoagulazione viene scelta la dose minima (30mg) poiché il paziente non ha documentazione. Alla somministrazione però Edoxaban non è disponibile, ma è presente Apixaban 5mg. Nel consulto rapido tra infermiere e chirurgo avviene un fraintendimento. Poco dopo il chirurgo intuisce l'errore e corre in reparto, ma il paziente ha già ricevuto 6 dosi di Apixaban, perciò viene trasportato in PS per la gastrolusi. A 30 minuti dall'overdose si evidenzia un allungamento più marcato per il PT (2,01) rispetto all'aPTT (1,23): infatti la sensibilità dei reagenti per PT e aPTT agli xabani è scarsa e particolarmente bassa per aPTT e soprattutto per Apixaban rispetto agli altri. La normalità di PT e aPTT non consente di escludere livelli terapeutici o in eccesso di farmaco. Tuttavia oltre una certa soglia, specifica per reagente, si ha un allungamento più evidente sul PT [1]. Il centro antiveleni indica di determinare l'attività anti-Xa per valutare il rischio emorragico. Il Laboratorio invia un campione per la consulenza del Laboratorio dell'Azienda Ospedaliera Universitaria Senese, allegando le informazioni utili per l'interpretazione: tempo tra assunzione e prelievo (3h), principio attivo, dose (30mg) età (75), altezza (175cm) e peso (100kg). L'attività anti-Xa risulta 557 ng/mL che, vista l'ampia variabilità delle concentrazioni attese al picco, corrisponde a circa 2-3 dosi di Apixaban 5mg: la gastrolusi ha contenuto l'assorbimento. Infatti per il reverse in caso di sovradosaggio non è raccomandato l'antidoto se non c'è sanguinamento maggiore attivo o non è urgente una procedura a rischio [2]. Segue monitoraggio con emocromo, PT e aPTT che ritornano nei limiti nelle 48h seguenti. Dunque si evidenzia l'importanza dei test per i DOAC in urgenza per misurare l'attività anticoagulante, come nei casi di overdose. Inoltre una buona rete tra Laboratorio e Clinica e tra Laboratori ha consentito l'immediata risposta alle necessità diagnostiche.

Bibliografia

1) Gosselin R, et al. Comparison of the effect of the anti-Xa direct oral anticoagulants apixaban, edoxaban, and rivaroxaban on coagulation assays. Int Jnl Lab Hem. 2016;38:505–13. DOI: 10.1111/ijlh.12528

2) Cuker A, et al. Reversal of direct oral anticoagulants: Guidance from the Anticoagulation Forum. Am J Hematol. 2019 Jun;94(6):697-709. DOI: 10.1002/ajh.25475.

PO016

Early Prediction of In-Hospital Death of COVID-19 Patients: A Machine-Learning Model Based on Age, Blood Analyses, and Chest X-Ray Score

E. Garrafa, M. Vezzoli, M. Ravanelli, S. Calza, R. Maroldi

Lab Biochimica Clinica Spedali Civili di Brescia

Background: To develop and validate an early-warning model to predict in-hospital mortality on admission of COVID-19 patients at an emergency department (ED).Methods: In total, 2782 patients were enrolled between March 2020 and December 2020, including 2106 patients (first wave) and 676 patients (second wave) in the COVID-19 outbreak in Italy. The first-wave patients were divided into two groups with 1474 patients used to train the model, and 632 to validate it. The 676 patients in the second wave were used to test the model. Age, 17 blood analytes and Brescia chest X-ray score were the variables processed using a Random Forests classification algorithm to build and validate the model. ROC analysis was used to assess the model performances. A web-based death-risk calculator was implemented and integrated within the Laboratory Information System of the hospital.Results: The final score was constructed by age (the most powerful predictor), blood analytes (the strongest predictors were lactate dehydrogenase, D-dimer, Neutrophil/Lymphocyte ratio, C-reactive protein, Lymphocyte %, Ferritin std and Monocyte %), and Brescia chest X-ray score. The areas under the receiver operating characteristic curve obtained for the three groups (training, validating and testing) were 0.98, 0.83 and 0.78, respectively.Conclusions: The model predicts in-hospital mortality on the basis of data that can be obtained in a short time, directly at the ED on admission. It functions as a web-based calculator, providing a risk score which is easy to interpret. It can be used in the triage process to support the decision on patient allocation

PO017

IgD □ Multiple Myeloma and post-treatment isotype switching: a case report

L. Agnello[1], E.M. Pappalardo[2], S. Maestri[2], M.N. Alongi[2], P. Altavilla[2], R.V. Giglio[1], A.M. Ciaccio[3], B. Lo Sasso[1], M. Ciaccio[1]

[1]*Institute of Clinical Biochemistry, Clinical Molecular Medicine and Laboratory Medicine, Department of Biomedicine, Neurosciences and Advanced Diagnostics, University of Palermo, Palermo, Italy.*

[2]*Department of Laboratory Medicine, University Hospital "P. Giaccone", Palermo, Italy.*

[3]*University of Palermo, Palermo, Italy.*

Background: Multiple myeloma (MM) is a malignancy of B cells characterized by an atypical proliferation of plasma cells. MM can be classified according to the type of light and heavy chains produced. The most common form of MM is IgG, followed by IgA. Case report: We report the case of a 57-year old woman admitted to the Haematology Unit of University Hospital “P. Giaccone” of Palermo, Italy, for suspect of MM. She underwent all routine laboratory tests for the initial clinical evaluation. The serum protein electrophoresis showed a monoclonal component in the □-□ region. In order to characterise it, the immunotyping of the monoclonal component was performed by capillary electrophoresis on Capillarys 3 TERA (Sebia) using antisera against □,□,□,□ and □ chains. The analysis revealed the presence of □ light chains not bound to □, □ and □ heavy chains. The serum immunofixation performed by zonal electrophoresis on Hydrasys (Sebia) confirmed such finding. Next, we further investigate if □ light chains were free. Accordingly, we performed the serum immunofixation using anti-sera against free light chains (□ and □), which showed a negative result. Then, in order to test the hypothesis that the nature of monoclonal component could be D or E, we performed the serum immunofixation using antisera against □,□,□ and free □, finding the presence of IgD □, which was successively confirmed by turbidimetric immunoassay. Thus, the diagnosis of IgD □ MM was made. The patient promptly started the treatment with VTD. Interestingly, after 1 month, she showed an isotype switching from IgD □ to IgG k. Whether the isotype switching is intrinsically transient, or instead reflects differing selection by the treatment, remains to be determined.Discussion: IgD MM has a very low incidence (2% of total MM cases) and it is characterized by a worse prognosis. Here, we described a case of IgD □ MM characterised, additionally, by an isotype switching. The recognition of IgD monoclonal component can be sometimes difficult and requires expertise. Indeed, the commonly used immunotyping methods are based on the use of antisera against □,□,□,□, and □, and not include antisera against □ and □, leading to the potential misclassification of the monoclonal component as free light chains. High variability in cancer evolution among patients with MM, and an alternating dominance of cancer sub-clones during treatment responses has been previously described (1). However, further studies are mandatory to elucidate the

contribution of different drugs, depth of response, clinical variables, to the isotype switching.References: 1. Keats JJ, Chesi M, Egan JB, Garbitt VM, Palmer SE, Braggio E, Van Wier S, Blackburn PR, Baker AS, Dispenzieri A, Kumar S, Rajkumar SV, Carpten JD, Barrett M, Fonseca R, Stewart AK, Bergsagel PL. Clonal competition with alternating dominance in multiple myeloma. Blood. 2012;120:1067-76.

PO018

UN CASO DI NEOPLASIA A CELLULE DENDRITICHE PLASMACITOIDI BLASTICHE SENZA LESIONI CUTANEE ALL'ESORDIO

M. Seghezzi, M.d.C. Baigorria Vaca, G. Previtali, G. Guerra, P. Dominoni

Laboratorio Analisi Chimico Cliniche, ASST Papa Giovanni XXIII, Bergamo

Riportiamo il caso clinico di una donna di 38 anni originaria della Repubblica Dominicana arrivata al pronto soccorso dell'ASST Papa Giovanni XXIII di Bergamo con sospetto di leucemia acuta.L'esame emocromocitometrico (effettuato con lo strumento Sysmex XN-module-[Sysmex, Kobe , Japan]) ha mostrato anemia con emoglobina 89 g/L (i.r 120-160), lieve neutropenia (1,43x10^9/L ; i.r 2,00-9,00), piastrinopenia (42x10^9L;i.r 150-400) e presenza di eritroblasti . L'osservazione del citogramma WDF(White Blood Cell Differential) evidenzia la presenza di un cluster di cellule ad alta fluorescenza che viene colorato metà con il codice colore dei monociti e metà con quello dei granulociti immaturi, che potrebbe indicare la presenza di blasti. E'stato eseguita la revisione dello striscio di sangue periferico che mostra la presenza di granulociti immaturi e di blasti di grandi dimensioni, con citoplasma basofilo, alterato rapporto nucleo /citoplasma e vacuoli evidenti . L'analisi citofluorimetrica del sangue periferico ha mostrato la presenza di blasti mieloidi CD34-,CD117-, CD33+,CD4+,CD14+CD64+,CD56+,CD123+. La biologia molecolare è risultata positiva per alterazione nel gene NPM1. È stata così posta la diagnosi di neoplasia a cellule dendritiche plasmacitoidi blastiche (BPDCN). Nonostante il protocollo chemioterapico, la paziente è deceduta dopo pochi mesi dalla diagnosi. La BPDCN è una patologia a decorso aggressivo, classificata dalla World Health Organization nel 2008 nell'ambito delle "acute myeloid leukemia and related precursor neoplasms". Nelle fasi iniziali di malattia, il primo organo coinvolto risulta essere quasi sempre la cute. L'esame istopatologico e l'immunofenotipizzazione sono necessari per una corretta diagnosi; in particolare, la diagnosi immunofenotipica si basa sulla contemporanea positività per i marker CD123, CD4 e CD56. In questo caso clinico, l'osservazione dello striscio di sangue periferico è stata dirimente per la diagnosi e l'esecuzione di ulteriori approfondimenti; infatti le caratteristiche morfologiche peculiari dei blasti sono risultate fondamentali per confermare il sospetto di leucemia a cellule dendritiche plasmacitoidi blastiche.

PO019

Plasma integrity index is a novel predictor of pathological complete response (pCR) in breast cancer patients after neoadjuvant chemotherapy.

M. Dameri[1], G. Cirmena[1], F. Ravera[1], A. Garuti[1], V. Barbero[3], L. Ferrando[3], P. Fregatti[2,3], L. Del Mastro[1,3], A. Garlaschi[3], D. Friedman[2,3], A. Ballestrero[1,3], G. Zoppoli[1,3]

[1]Dep. of Internal Medicine, University of Genoa
[2]Dep. of Surgical Sciences and Integrated Diagnostic DISC
[3]Osp. Policlinico San Martino, Genoa

Background: Locally advanced breast cancer (BC) is currently treated with neoadjuvant chemotherapy (NACT) followed by surgery. NACT is able to achieve about a 20% rate of pathological complete response (pCR). To date the pre-surgical assessment of clinical complete response (cCR), which guides the surgical management of post-NACT BC, is based on magnetic resonance imaging (MRI) with suboptimal accuracy. Plasma cell-free DNA integrity (DI) can provide useful information in regard to early diagnosis, recurrence, and response to therapy. The aim of this study is to estimate the accuracy of an electrophoresis-based method for DI assessment in the evaluation of the response to the systemic treatment in BC patients undergoing NACT.
Methods: 62 BC patients undergoing anthracycline/ taxane based NACT followed by surgery were recruited. Plasma samples were collected at diagnosis, after anthracycline administration, and after NACT completion before surgery. After cfDNA extraction, cfDNA fragmentation profiling (DFP) was performed by automated electrophoresis on an Agilent Tapestation 2200 device. The concentration of differently sized cfDNA fragments was assessed and specific cfDNA fragments size ranges were selected to calculate a normalized measure of DI, namely cfDNA integrity index (DII), expressed as the ratio of 321-1000 to 150-220 bp sized fragments. DII was used to build an explorative classifier for BC response to NACT, comparing its performance with MRI.
Results: DFP was performed on 38 plasma samples collected from post-surgery patients, with a 30/70 ratio between pCR and non pCR patients. DII showed an overall accuracy in correctly predicting the achievement of pCR of 81.6, with a cutoff above 2.71 having sensitivity = 81.8 and specificity = 81.5. MRI overall accuracy in the same cohort amounted to 77.1, with a sensitivity and a specificity of 72.7 and 81.5. The performance of the two techniques combined, achieved an overall accuracy of 92.6 with a predictive value of pCR of 87.5 and a predictive value of absence of pCR of 94.7.
Conclusions: DII measured before surgery after NACT completion shows great potential to correctly predict the achievement of pCR in BC patients. The evaluation of its use in combination with MRI is warranted in prospective studies.

PO020

EVALUATION OF SENSITIVITY AND SPECIFICITY PERFORMANCE OF TWENTY DRUG SCREENING IMMUNOASSAYS

R. Spataro[1], M. Stornaiuolo[1], M. Marinova[1], C. Artusi[1], M. Zaninotto[1], M. Plebani[1,2]

[1] U.O.C. Medicina di Laboratorio, Azienda Ospedale-Università Padova
[2]Dipartimento Didattico-Scientifico-Assistenziale (DIDAS) Servizi di Diagnostica Integrata

Background: A urine drug screen is quick and painless. It tests urine for the presence of illicit drugs but it exhibits some limitations. It is well known that false-positive results (FP) can lead to serious medical or social consequences if results are not confirmed by a reference method, while reporting a false-negative results (FN) is a severe oversight from clinical point of view. Aim of the study was to evaluate, in according to CLSI EP12-A2 guideline, the sensitivity (SE) and specificity (SP) performance of twenty toxicology immunoassays in urine samples using two different analytical principles: Cloned Enzyme Donor Immunoassay (CEDIA®) and homogeneous enzyme immunoassay (DRI®) applied on ILab Taurus analyser. Materials and Methods: Diagnostic accuracy evaluation was performed using a total of 286 urine samples and 61 EQA materials to obtain at least 50 positive and 50 negative samples for each one of the following tested immunoassays: DRI®Amphetamine, Ecstasy, Methadone, Cannabinoid, Cocaine, Opiate, Barbiturate, Oxycodon, Benzodiazepine, Ketamine and CEDIA®Amphetamine, Methadone, EDDP, Cannabinoid, Cocaine, LSD, Benzodiazepine, Opiate, 6-MAM and Buprenorphine. For each analytical run (n=150), analytical session acceptability was assessed by controls material provided by the manufacturer, being acceptable within CV<±15%. All results were confirmed by HPLC-MS/ MS method using Chromsystems® MassTox® Drugs of Abuse Testing kit. Results: SE and SP were 100% for most assays except SP for DRI®Amphetamine (88.5%), DRI®Ecstasy (99.1%), CEDIA®Opiate (98.9%), CEDIA®Benzodiazepine (96%) and DRI®Ketamina (77.9%). SE for DRI®Cannabinoid (96.7%) and DRI®Benzodiazepine (90.4%). Discussion: 13 FP for DRI®Amphetamine were due to the presence of MDMA >700µg/L (cross-reactivity declared from manufacturer, 800µg/L); for DRI®Ecstasy 1 FP was due to the presence of “Baby Jhonson Head to Toe” shampoo added to the EQA; 1 FP for CEDIA®Opiate was due to the presence of Metadone and EDDP >1500µg/L; 2 FN for DRI®Cannabinoid were around the cutoff and 8 FN for DRI®Benzodiazepine due to the presence of Lorazepam at 175-799 µg/L (cross-reactivity declared from manufacturer, Lorazepam, 1000 µg/L).

PO021

Evaluation of multiple polymorphisms in vitamin D-related genes, Vitamin D status and Multiple Sclerosis

L. Agnello[1], C. Scazzone[1], M. Vidali[2], M. Casisa[3], R. Valveri[3], G. BIvona[1], A.M. Ciaccio[4], C.M. Gambino[1], M. Ciaccio[1]

[1]*Institute of Clinical Biochemistry, Clinical Molecular Medicine and Laboratory Medicine, Department of Biomedicine, Neurosciences and Advanced Diagnostics, University of Palermo, Palermo, Italy*

[2]*Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy*.

[3]*Department of Laboratory Medicine, University Hospital "P. Giaccone", Palermo, Italy.*

[4]*University of Palermo, Palermo, Italy.*

Background. Multiple Sclerosis (MS) is a multifactorial disease whose pathogenesis results from the interaction among genetic, epigenetic and environmental factors. Among these, a role for hypovitaminosis D has emerged in the last decades. Vitamin D status is influenced by both environmental and genetic factors. Accordingly, single nucleotide polymorphisms (SNPs) in genes codifying for molecules involved in vitamin D metabolism have been associated with an increased risk of developing MS (1). However, few studies assessed the association of such SNPs with the severity of the disease. The aim of this study was to evaluate the potential association among vitamin D status, MS severity and vitamin D-related SNPs, alone or in combination. Methods. In this observational study, we enrolled one hundred MS patients: 82% had relapsing remitting-MS form and 14% secondary progressive-MS form. Serum 25(OH)D3 levels and genotyping of SNPs in vitamin D-related genes were evaluated in all participants by high-performance liquid chromatography and real-time polymerase chain reaction, respectively. Specifically, we genotyped 18 SNPs in the following genes: NAD synthetase 1, CYP2R1, vitamin D binding protein, vitamin D receptor, Retinoid X Receptor-□, KLOTHO, CYP24A1, and CYP27A1. MS severity was assessed by Multiple Sclerosis Severity Score (MSSS). Results. Univariate and multivariate analysis showed that form of the disease but none of the investigated SNPs was an independent predictor of MSSS. A score summing up all polymorphisms (using 0 as wildtype and 1 as the presence of polymorphism) was calculated. The median (IQR, min-max) number of polymorphisms was 11 (10-13, 7-16). Number of polymorphisms did not correlate with MSSS (rho=0.051, p=0.676) or with 25(OH)D3 levels (rho=-0.046, p=0.681). Accordingly, patients with more polymorphisms (n□14) did not show significantly higher MSSS or 25(OH)D3 levels than patients with fewer polymorphisms (n□9), respectively p=0.526 and p=0.917.Conclusion. In this study, we assessed the hypothesis that the presence of SNPs in vitamin D-related genes could influence MS severity. We found that all investigated SNPs, alone or in combination, were not associated with the severity of the disease. To the best of our knowledge, this is the first study that evaluates the cumulating effect of vitamin D-related SNPs on MS severity. References. 1. Scazzone C, Agnello L, Bivona G, Lo Sasso B, Ciaccio M. Vitamin D and Genetic Susceptibility to Multiple Sclerosis. Biochem Genet. 2021;59:1-30. doi: 10.1007/s10528-020-10010-1.

PO022

Mass Spectrometry approach to quantifying orotic acid and its metabolites in urine and plasma

C. Francesco [1], S. Allegra[1], S. De Francia [1], M.P. Puccinelli[2], G. Mengozzi[2]

[1]*Clinical and Biological Sciences Department, University of Turin*

[2]*Laboratory of Clinical Biochemistry "Baldi e Riberi", Metabolic Diseases Unit, AOU Città della Salute e della Scienza di Torino*

The orotic acid (2,4-dioxo-1H-pyrimidine-6-carboxylic acid; Vitamin B13) is an intermediate metabolite of pyrimidine nucleotides biosynthesis and represents a minor diet constituent. The precursors of orotic acid in human metabolism are the cytosolic carbamoyl phosphate and aspartate via dihydrorotate: this biosynthesis is catalyzed by CAD gene encoding multifunctional enzyme. The multimeric protein called uridine 5'-monophosphate synthase is constitued by two domains that catalyze uridine monophosphate synthesis: orotatephosphoribosyltransferase (OPRTase; EC 2.4.2.10) and orotidine 5'-phosphate decarboxylase (OMPdecase; EC:4.1.1.23). The orotic acid biosynthesis is directly involved in metabolism of 5-Fluorouracil (5-FU), because this anticancer drug is competitive substrate of OPRTase. In particular, the transferase activity of OPRTase multicomplex enzyme is inhibited by 5-FU at 59% level of control. The other end OPRTase is involved in metabolic disorders as congenital orotic aciduria and consequently the urinary orotic acid is quantified in clinical routine analysis for differential diagnosis of hereditary metabolic disease. Therefore, we aimed to develop a high performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) assay that allows the simultaneous and sensitive detection of an orotic acid and orotidine 5'-monophosphate as metabolic and toxicological biomarkers in plasma and urine.The implementation and validation of chromatography and spectrometric method are developed in accordance with UNI EN ISO/IEC 17025:2018 and Eurachem Guide Lines (Method validation in clinical chemistry follows the established standards and procedures accepted by all disciplines of chemical metrology). The clinical aspects are tested by analyzing diagnostic proficiency testing (external quality assessment - EQA) and other samples from patients with metabolic and malignant disorders. The analytes, orotic acid and orotidine-5'-monophosphate are identified and quantified with high performance parameters of repeatability, reproducibility, robustness, precision and accuracy. The quantification method is based on internal standard approach for signal and matrix effect suppression. Whatever analytes is identified and by two MRM transition with S/N>50 in LOD range. The analytical method clearly distinguishes between urine and plasma specimens from the normal and pathological patients at 97.5% of level of confidence. The HPLC-MS/MS method to be suitable for differential diagnosis of hereditary metabolic disease and metabolic monitoring of toxicity induced by anticancer drug. The analytical protocol is rapid and ideal to be used in routine analysis of clinical chemistry laboratory.

PO023

Establishing reference intervals of Neurogranin in the cerebrospinal fluid

L. Agnello[1], R.V. Giglio[1], M. VIdali[2], F. Coraci[3], R. Monteleone[3], C.M. Gambino[1], C. Scazzone[1], G. Bivona[1], A.M. Ciaccio[4], B. Lo Sasso[1], M. CIaccio[1]

[1]*Institute of Clinical Biochemistry, Clinical Molecular Medicine and Laboratory Medicine, Department of Biomedicine, Neurosciences and Advanced Diagnostics, University of Palermo, Palermo, Italy.*

[2]*Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy.*

[3]*Department of Laboratory Medicine, University Hospital "P. Giaccone", Palermo, Italy.*

[4]*University of Palermo, Palermo, Italy.*

Background: Neurogranin is a post-synaptic protein expressed in the neurons of the hippocampus and cerebral cortex. It has emerged as a promising biomarker of synaptic dysfunction in Alzheimer's Disease (1). However, there are still some limitations in transferring neurogranin from bench to bedside, including the lack of reference interval (RI). Establishing RI is mandatory before introducing a biomarker in clinical practice in order to ensure its appropriate interpretation. The aim of the study was to establish RI of cerebrospinal fluid (CSF) neurogranin levels in individuals with non-neurodegenerative diseases.Methods: We performed a retrospective observational study at the University Hospital "P. Giaccone" of Palermo on 113 controls, including patients with non-neurodegenerative disorders, which are not characterised by synaptic degeneration, who underwent lumbar puncture for CSF analysis as part of their diagnostic evaluation. The CSF samples were collected between 8:00 a.m. and 10:00 a.m. from fasted patients. Specifically, the CSF was obtained by a lumbar puncture at the L3/4 or L4/5 interspace using a 21-gauge needle, collected in polypropylene tubes, centrifuged at 500 g for 20 min, aliquoted in propylene tubes and stored at #80°C until analysis. CSF neurogranin levels were measured by a commercial ELISA kit (Euroimmun, Lübeck, Germany), according to manufacturer instructions. CSF RI of neurogranin was calculated by the robust method.Results: A total of 113 individuals, 44 women and 69 men, were included in the study. Specifically, the study population consisted of patients with non-neurodegenerative diseases, including vascular encephalopathy, epilepsy, leukoencephalopathy, cervical myelopathy, normotensive hydrocephalus, idiopathic polyneuropathy, and conversion disorder. The median (IQR) age of the whole population was 63 (47-73) years. Lower and upper reference limits of the RI were 28 pg/mL (90%CI 21-39) and 1362 pg/mL (90%CI 1062-1820), respectively. No sex and age influence on CSF neurogranin levels was observed. Conclusion: This is the first study establishing the RI of CSF neurogranin. The next step will be to establish the clinical decision limits for diagnosing AD, identifying AD patients with accelerated cognitive decline, and monitoring therapies. References: Agnello L, Gambino CM, Lo Sasso B, Bivona G, Milano S, Ciaccio AM, Piccoli T, La Bella V, Ciaccio M. Neurogranin

as a Novel Biomarker in Alzheimer's Disease. Lab Med. 2021;52:188-196. doi: 10.1093/labmed/lmaa062.

PO024

La refertazione della cistinuria in Pediatria

G. Cangiano[1], V. Capobianco[1], P. Iardino[1], F. Fedele[1], M. Boscaglia[1], F. Gaeta[1], L. Paragliola[1], C. Taranto[1], A. Contina[2], M. D'Amora[2]

[1]UOC Patologia Clinica Ospedale dei Pellegrini, ASL Napoli 1 Centro, Napoli

[2]UOC Patologia Clinica Ospedale San Paolo, ASL Napoli 1 Centro, Napoli

Nel nostro laboratorio si effettua lo studio metabolico per il rischio nefrolitiasico e la determinazione della cistinuria su campioni di Pazienti pediatrici provenienti dalle Nefrologie dell'Azienda Santobono-Pausilipon e dell'Azienda Universitaria Vanvitelli di Napoli. Quest'indagine si effettua generalmente su campioni di urine raccolti nelle 24 ore usando sia acido cloridrico che clorexidina; nei bambini, per la difficoltà nel praticare una raccolta urinaria articolata, si usano le urine estemporanee oppure le urine delle 24 ore trattate almeno, con 2 ml di clorexidina digluconato 20% . Il dosaggio della cistinuria è richiesto anche per verificare le risposte alle strategie terapeutiche adottate per i pazienti cistinurici come: introito di almeno 3 – 3,5 litri di liquidi al giorno, alcalinizzazione con bicarbonato di sodio o citrato di potassio, dieta iposodica a ridotto contenuto animale, eventuale terapia farmacologica con D-penicillamina o tiopronina. Nel referto elaborato in Access (specifico programma che gestisce la calcolosi renale), oltre ad esprimere la concentrazione di cistinuria in mg/dL (cys), determinata col metodo fotometrico all'acido fosfotungstico, ed il rapporto con la creatinina (espresso in mmol/mmol), si determina il pH in potenziometria e si referta la relativa supersaturazione renale (SSR cys) ricavata con il seguente algoritmo: SSR cys = cys/25 . [(- 0,0172 . pH3) + (0,2617 . pH2) - (1,3044 . pH) + 2,8661]. Questa nuova modalità di refertazione consente di evidenziare sia la positività in campioni urinari diluiti (rapporto cistina/creatinina) ma anche la tendenza alla precipitazione (SSR cys) in urine non opportunamente corrette con dieta, integratori, alcalinizzazione ed eventualmente farmaci. Cangiano G, Buccino G, et Al. La calcolosi renale in un laboratorio di patologia clinica: modello organizzativo e nuove tecniche analitiche. Giornale di Tecniche Nefrologiche e Dialitiche, 2019,1,31,22-29

PO025

Definition and independent validation of a new tool for sepsis screening based on CBC parameters

L. Agnello[1], M. Vidali[2], C. Scazzone[1], S. Maestri[3], A. Iacona[3], P. Tortorici [3], I. Sciortino[3], S. Mancuso[1], A.M. Ciaccio[4], G. Bivona[1], M. Ciaccio[1]

[1]Institute of Clinical Biochemistry, Clinical Molecular Medicine and Laboratory Medicine, Department of Biomedicine, Neurosciences and Advanced Diagnostics, University of Palermo, Palermo, Italy

[2]Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy.

[3]Department of Laboratory Medicine, University Hospital "P. Giaccone", Palermo, Italy.

[4]University of Palermo, Palermo, Italy.

Background. The early diagnosis of sepsis is still challenging today. In this study, we sought to develop and validate an index, the Sepsis Index (SI), for early detecting patients at high risk of sepsis. SI is based on the relationship between two parameters of complete blood count (CBC): monocyte distribution width (MDW) and mean monocyte volume (MMV).Methods. The derivation and validation cohorts consisted, respectively, of 2215 and 703 patients consecutively admitted to the emergency department (ED) of the University Hospital “P. Giaccone”, Palermo. According to Sepsis-2 criteria, patients were classified into 4 groups: controls; SIRS (patients with at least 2 SIRS criteria); infection (patients without sepsis and with 0 or 1 SIRS criterion); sepsis (patients with confirmed or suspected infection and SIRS). SI is calculated as the ratio between the estimated MDW and a theoretical MDW (MDWth), which is obtained by a polynomial function based on MDW and MMV. A SI value□1 was selected to define sepsis. Results. The derivation cohort consisted of 2215 patients: 1855 controls, 100 SIRS, 172 infection and 88 sepsis. The validation cohort consisted of 703 patients: 498 controls, 52 SIRS, 105 infection, and 48 sepsis. In the validation cohort, the sepsis subgroup displayed the highest MDW (median 27.5, IQR 24.6-32.9) and SI (median 1.15, IQR 1.05-1.29) values. At the ROC curve analysis for the prediction of sepsis, the AUC of MDW and SI were, respectively, 0.876 (95%CI 0.812–0.942) and 0.877 (95%CI 0.810-0.944). Using the cut-off of 23 and 1 for MDW (1,2) and SI, respectively, we found out that sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio and negative likelihood ratio for sepsis were respectively 0.79 (MDW) vs 0.79 (SI), 0.84 vs 0.91, 0.27 vs 0.38, 0.98 vs 0.98, 5.08 vs 8.52 and 0.25 vs 0.23. Discussion. SI showed good diagnostic accuracy for sepsis detection and displayed better diagnostic performance than MDW alone. In particular, SI displayed an increased specificity, PPV and LR+ than MDW. Overall, SI improves the diagnostic accuracy of MDW alone for the early identification of patients at high risk of sepsis in the ED by reducing the rate of false-positive. Conclusion. SI represents a promising tool for sepsis screening. Considering that MDW and MMV are both included in a routine CBC, the estimation of the SI could represent a rapid tool for supporting the early detection of sepsis in ED.

References.

1. Agnello L, Sasso BL, Giglio RV, Bivona G, Gambino CM, Cortegiani A, Ciaccio AM, Vidali M, Ciaccio M. Monocyte distribution width as a biomarker of sepsis in the intensive care unit: A pilot study. Ann Clin Biochem. 2021;58:70-73. doi: 10.1177/0004563220970447.

2. Agnello L, Bivona G, Vidali M, Scazzone C, Giglio RV, Iacolino G, Iacona A, Mancuso S, Ciaccio AM, Lo Sasso B, Ciaccio M. Monocyte distribution width (MDW) as a screening tool for sepsis in the Emergency Department. Clin Chem Lab Med. 2020;58:1951-1957. doi: 10.1515/cclm-2020-0417.

PO026

SPE and HPLC-UV method for the quantification of venetoclax in human plasma

S. Allegra, F. Chiara, I. Federico, S. De Francia

Clinical and Biological Sciences Department, University of Turin

Venetoclax (ABT-199) is an orally bioavailable BH3 mimetic drugs; it selectively blocks BCL-2 signaling within the cell and inducing the TP53-independent apoptotic pathway. Venetoclax has been introduced in treatment of chronic lymphocytic leukemia (CLL), indolent Non-Hodgkin lymphoma (iNHL), acute myeloid leukaemia (AML) and multiple myeloma (MM). Drug maximum plasma concentration is reached 5–8 hours and the elimination half-life (t 1/2) ranges between 14 and 18 hours. Venetoclax is metabolised by the CYP3A pathway and through the hepatic-faecal system. Moreover, the drug is a P-glycoproteins (p-gp) substrate. Our aim was to obtain a simple, fast and reliable method for the quantification of venetoclax for clinical routine use. We here describe the development and validation of an analytical method for drug determination in human plasma by high pressure liquid chromatography coupled with ultraviolet detection (HPLC-UV). Solid phase extraction (SPE) was performed whit Oasis MAX 30um 100mg, conditioned with 1ml of ACN / water, 1/10. 1ml of plasma with was diluted with 2.0ml of deionized water. 10ul of the internal standard solution was added to the sample (1:10 of the stock solution in ACN + 0.1% v / v TFA). The pretreated sample was dispensed in the SPE column, washed with 500ul of NH4OH 5% v / v and with 750ul of slz ACN / water, 2/10 and then eluted with 250ul of ACN 0.1% TFA. 150 ul of the eluate was transferred into vials by adding 50ul of diluent solution (ACN / water, 60/40 + 0.1% TFA) and inject into HPLC. The calibration curve is performed on four points above zero: 0 - 1.0 - 2.5 - 5.0 - 10.0 ug/ml.The method was then applied on real samples from AML patients treated with venetoclax, assessing its eligibility for the routine use. Observed data suggest the usefulness of investigating intracellular and cerebrospinal fluid concentration of the drug. Moreover, further studies are necessary to test the correlation of venetoclax pharmacokinetics with treatment outcome and toxicity.

PO027

LIPOPROTEIN(a): COMPARATIVE EVALUATION OF TWO AUTOMATED ASSAYS

F. Dragone[1], N. Montenegro[1], M. Scalise[1], M. Sgro[1], F. Lucia[2], L. Martino[2], G.C. Antico[2], A. Cerra[3], F. Galato[2], L. Giaquinto[2], P. Iania[3], S. Mancuso[2], M. Martucci[2], C. Teti[2], F.F. Fabiano[3], E. Angotti[2]

[1]Department of Experimental and Clinical Medicine, Magna Græcia University, Catanzaro, Italy
[2]O.U. of Clinical Biochemistry – AOU Materdomini, Catanzaro, Italy
[3]O.U. of Clinical Pathology - AOU Materdomini, Catanzaro, Italy

Aim The different analytical methods for the measurement of serum Lipoprotein(a) (Lp(a)) are not yet fully harmonized and no consensus exists on a measurement unit. In this study we compared the results obtained from the assays of Lp(a) concentrations performed with two different methods to evaluate i) the presence of a potential bias and ii) how much this bias may influence the assignment of patients to the upper or lower cut-off categoryMethods Tina-quant Lipoprotein (a) Gen.2 Roche and N Reagent Latex Lp(a) Siemens were used for the comparison study. The first is an immunoturbidimetric assay calibrated with the reference material SRM2B and the results are expressed in nmol/L, while the second is an immunonephelometric assay, the reference material is not specified and the measurement unit is mg/dL. A total of 42 samples assayed with Roche method and ranging of serum Lp(a) concentrations from 25 to 224 nmol/L, were selected from the daily work routine and immediately re-assayed with the Siemens method. For comparison purposes, the Siemens results were converted in nmol/L. Comparability of methods was established performing the Passing-Bablok regression and the Bland-Altman analysis to prove whether the bias founded was less than the preliminarily calculated maximum acceptable biasResults Precision was verified and the maximum acceptable bias was calculated as 14.7%. Passing Bablok regression exhibited the presence of both a proportional and constant systematic error. Bland Altman analysis showed a bias=9.7%, (95% IC 2.3-17.1) so that the results were not comparable. To evaluate whether the two methods had the same ability to classify patients, we categorized the results in two reference classes one below and one upper the cut off. Siemens categorized most patients into the class below the cut off (<75 nmol/L) whereas Roche categorizes the lowest number of patients. The strength of the agreement calculated by the Cohen's kappa statistical coefficient, was 88% with a coefficient of 0,76Conclusions The compared assays have shown good reliability in measuring Lp(a) levels in the routine laboratory. We showed that the assays are not interchangeable due to the lack of the results comparison although there was a substantial agreement in the patient's classification

PO028

Evaluation of detection efficiency by SARS-CoV-2 Assay performed on the VitaPCRTM Instrument in samples collected in UTM

V. Sorbello, S. Carbonari, M. Costantini, M. Ferri, V. Formisano, A. Leone, D. Vandini, S. Barocci

U.O.C. Patologia Clinica – Laboratorio Analisi, Ospedale Santa Maria della Misericordia di Urbino, ASUR Marche AV1, Urbino (PU), Italy

Background The diagnosis of SARS-CoV-2 infection is essential for the control of the epidemic, the establishment of protecting measures and the therapeutic management of patients. Among diagnostic tests utilizing a real-time reverse transcription polymerase chain reaction (RT-PCR) amplification technology, VitaPCRTM SARS-CoV-2 Assay was used in our Laboratory for the rapid qualitative detection and discrimination of SARS-CoV-2 viral RNAs in direct nasopharyngeal (NP) or oropharyngeal (OP) swabs.
Aim of the study To test SARS-CoV-2 Assay on NP or OP swabs collected in UTM instead of directly melted in the provided buffer of the kit in order to assess the detection efficiency of the system in relation to the potential interference of UTM in the PCR reaction and to viral RNA concentration.
Methods A pool of ten SARS-CoV-2 positive samples collected in UTM, previously tested by quantitative real-time RT-PCR, was assayed on the VitaPCRTM Instrument at several dilutions (from 1:10 to 1:100000) in the provided buffer of the kit, immediately and after a cycle of freezing and thawing at -20°C.
Results VitaPCRTM Instrument was able to detect SARS-CoV-2 viral RNA at four out of five tested dilutions, with a good efficiency especially at 1:10 and 1:100 dilutions, while at 1:1000000 the result was doubtful. After freezing and thawing the detection efficiency sensibly decreased and the Instrument was able to detect the viral RNA only at 1:10, 1:100 and 1:1000 dilutions, while at 1:10000 and 1:1000000 the result was doubtful.
Conclusions These preliminary results are promising suggesting the potential use of SARS-CoV-2 Assay on the VitaPCRTM Instrument even with swabs collected in UTM, increasing the versatility of this system. Moreover, the reduced performance of VitaPCRTM Instrument after freezing and thawing the sample suggests a consistent degradation of viral RNA, advising against the use of this type of frozen samples with this assay. Further investigations are recommended.

PO029

Development of qPCR tests for monitoring SARS-CoV-2 variants through high resolution melting analysis

S. BAROCCI[1], A. DIOTALLEVI[3], G. BUFFI[3], M. CECCARELLI[1], C. ORLANDI[3], A. CASABIANCA[3], M. MAGNANI[3], E. CARLOTTI[4], D. VANDINI[1], M.P. CANCELLIERI[4], G. CAPPELLI[4], L. GALLUZZI[2]

[1]UOC Patologia Clinica ASUR Marche AV1
[2]Dipartimento di Scienze Biomolecolari (DISB) Università degli Studi di Urbino
[3]Servizio Prevenzione e Sicurezza Ambienti di Lavoro (SPSAL) ASUR Marche AV1

The current global pandemic (COVID-19) caused by the new Betacoronavirus SARS-CoV-2 is characterized by successive waves of infection due to new variants that include mutations in the gene encoding the Spike protein, the main target of the nucleic acid-based vaccines. In fact, as of autumn 2020, several countries have reported the detection of SARS-CoV-2 variants that have spread more efficiently (referred to as "variants of concern" by WHO). Such variants include the Alpha variant (English variant, B.1.1.7), the Beta variant (South African variant, B.1.351), the Gamma variant (Brazilian variant, P.1), and the more recent Delta variant (Indian variant, B.1.617. 2). Therefore, it is pivotal to monitor the virus and the onset of SARS-CoV-2 variants characterized by high transmissibility or reduced susceptibility to neutralizing antibodies induced by vaccination.Surveillance of genomic variants is currently based on sequencing of viral genomes performed on a random fraction of samples positive by molecular test. The sequencing of 228 SARS-CoV-2 positive samples by ASUR Marche Area Vasta 1 (Fano-Pesaro-Urbino) from February to June 2021 highlighted the progressive increase of variants (mainly B.1.1.7 and to a lesser extent P.1) from early February until March 18th. From March 18th onwards, only variants B.1.1.7 and P.1 were detected. DNA sequencing involves high costs and extended analysis time compared to a PCR-based diagnostic test. To rapidly identify the samples containing virus variants to be sequenced for complete characterization, in synergy with the University of Urbino, five rapid tests based on real-time PCR and high-resolution melting (HRM) were designed on the gene encoding the Spike protein. Preliminary results indicated that the sensitivity of the assays was not significantly different from that of commercial molecular tests. Furthermore, through HRM analysis, it was possible to discriminate amplicons with mutation 1709 C > A causing the amino acid substitution A570D, specific for the alpha variant.

PO030

MOLECULAR DIAGNOSIS FOR THE NOVEL CORONAVIRUS SARS-COV-2: THE EXPERIENCE OF THE SAN GIULIANO HOSPITAL (ASL NAPOLI 2 NORD)

I. Piccirillo, V. Federigo, L.A. Piccolo, C. Romano, E. Mauriello, A. Sorrentino

P.O. San Giuliano ASL Napoli 2 Nord Lab.Patologia Clinica, Giugliano, Italy

The current COVID-19 pandemic, caused by SARS-CoV-2 virus, has made it necessary to identify positive, symptomatic and asymptomatic patients, in a timely manner to activate appropriate containment measures, in order to limiting the spread of infection. The standard test for the diagnosis of COVID-19 is based on the detection of the viral RNA in respiratory specimens using the Real Time reverse-transcription PCR (rtRT-qPCR). In our Molecular Medicine Laboratory, from November 2020 to May 2021, we tested 98.540 respiratory samples collected in UTM swabs (Copan Diagnostics). Viral RNA were extracted with Nextractor magnetic automated system NX48S (Genolution) and they were tested for the detection of SARS-CoV-2 with two different rtRT-qPCR kits: i) NeoPlex kit COVID-19 Detection Kit (GeneMatrix, Eurospital), whose gene targets are RdRp and N (N=68.951), and ii) KHB Diagnostic kit for Sars-CoV-2 (KHB), whose gene targets are ORF1ab region, N and E (N=29.589). About 30% of the total analyzed samples, in the mentioned period, were found to be positive to SARS-CoV-2 RNA. However, 1.538 samples found to be positive to only ORF1ab region with KHB Diagnostic kit for Sars-CoV-2, resulting then negative to both N and RdRP genes after being re-tested, within the same working day, with NeoPlex kit COVID-19 Detection Kit, as confirmed by further clinical/diagnostic and follow-up insights. This indicated a false positive rate of about 5% by using ORF1ab region as target gene in SARS-CoV-2 detection. In conclusion, our preliminary data strongly suggest to use a kit for RdRp gene, whose detection has a greater clinical specificity, particularly, in the late phase of infection. From May 2021, we introduced in the diagnosis of SARS-CoV-2 routine a new kit, 1copy COVID-19 qPCR 4plex (1drop, Eurospital), which not only amplifies three genes in a single tube (i.e., RdRp, N and E), but allows to obtain results after 50 minutes from the extraction, thereby improving the TAT. These results suggest the need of use more than one diagnostic kit, with different targets, in order to obtain more reliable diagnostic data.

PO031

Evaluation of the correlation between anticoagulant therapy adherence and knowledge and INR values in a population of patients on chronic warfarin therapy

M. Stella[1,2], D. Mirata[2], L. Robbiano[2], G. Antonucci[3], L. Innocenti[4], F. Mattioli[1,2], F. Sacco[1,2]

[1]Unità di Farmacologia Clinica, E.O. Osp. Galliera, Genova

[2]Dip. di Medicina Interna, Unità di Farmacologia e Tossicologia, Università di Genova, Genova

[3]Dip. di Medicina, E.O. Osp. Galliera, Genova

[4]Lab. di Analisi, E.O. Osp. Galliera, Genova

Oral anticoagulant drugs are one of the most frequently prescribed pharmacological classes, proving to be effective in many clinical situations, but they are accompanied by an increased risk of bleeding, which implies a radical change in the patient's lifestyle. The prevention of this side effect necessarily requires from Patients a precise adherence to the therapy. For this purpose, the therapeutic education, allowing the Patient to acquire the knowledge and skills necessary for an accurate management of his disease, has proved to be a valid strategy to improve the adherence of Patients to a specific medical treatment, pharmacological or not. The intent of this work is to evaluate whether better therapeutic education can promote adherence and optimise therapy management. Materials and methods: two questionnaires, the Anticoagulation Knowledge Tool and the Morisky Questionnaire, were administered to Patients attending the thrombosis outpatient clinic and the TAO centre of the E.O. Ospedali Galliera in Genoa, to assess knowledge and adherence to anticoagulation therapy, respectively. Based on the results of the questionnaire, Patients were offered a short educational session in which the centre's clinical pharmacologist informed them about the risks and benefits of anticoagulation therapy. Subsequently, for Patients treated with warfarin, possible correlations between the results of the questionnaires and their INR values (assessed at the time of the session and after the treatment education interview) were evaluated. Preliminary results: 53 Patients have been enrolled to date, 21 of whom are on warfarin therapy and 32 on direct-acting anticoagulants. Among Patients, 64% demonstrated excellent adherence (8/8), 28% intermediate adherence (7/8 or 6/8) and 8% poor adherence (5/8 or less); 18 patients were highly acquainted, 21 Patients were moderately acquainted and 14 Patients were poorly acquainted. Among Patients on warfarin therapy, 6 improved their INR target range as a result of the interview. As adherence was already highest in most patients, only 6 out of 21 patients showed an improvement in INR. Conclusions: As already supported by other studies, effective therapy education can help to achieve a better clinical outcome and adherence to therapy.

PO032

IL LABORATORIO NELLA DIAGNOSI DELLA PORPORA TROMBOTICA TROMBOCITOPENICA: UN CASO CLINICO

M. Castriota, C. Linari, A.M. Massobrio, G. Mengozzi

Lab. di Biochimica Clinica, AOU Città della Salute e della Scienza, Torino

Donna di 48 anni, accede al Centro Prelievi per l'esecuzione di esami ematochimici per la comparsa di ittero, astenia, dolore all'ipocondrio destro. All'esame emocromocitometrico si evidenziano anemia normocitica, normocromica (Hb= 7,7g/dl) e grave piastrinopenia (piastrine=2 10^9/L). L'analisi dei grafici strumentali mostra una anomala distribuzione dei globuli rossi e delle piastrine in impedenziometria, che pone il sospetto della presenza di schistociti. All'esame microscopico dello striscio di sangue periferico si evidenzia la presenza di un elevato numero di schistociti (10%). Sono inoltre presenti: aumento della bilirubina indiretta (1,7 mg/dL), marcato aumento della lattato deidrogenasi (LDH=4223 UI/L). In presenza di valori critici e nel sospetto di porpora trombotica trombocitopenica (TTP), la paziente viene contattata prontamente ed indirizzata con urgenza in Pronto Soccorso. In base al quadro clinico e all'esito degli esami ematochimici viene confermato il sospetto di TTP (PLASMIC score=7), e avviata la terapia con plasmaferesi e steroide e successivamente con caplacizumab. La paziente mostra una buona risposta alla terapia con progressivo miglioramento del conteggio piastrinico. Prima dell'avvio della terapia viene richiesto il dosaggio dell'attività dell'ADAMT13 (<0.01 IU/mL) e degli Ab anti ADAMTS13 (51 U/mL), che confermano la diagnosi di TTP.La porpora trombotica trombocitopenica (TTP) è una emergenza ematologica con elevata mortalità in assenza di appropriata terapia messa in atto tempestivamente. E' una forma di microangiopatia trombotica (TMA); si distingue dalle altre TMA per il deficit di ADAMTS13. La diagnosi è impegnativa per la presentazione clinica variabile e la possibile sovrapposizione con altre TMA. Nella conferma della diagnosi un importante ruolo è svolto dal dosaggio dell'attività dell'ADAMTS13 e degli anticorpi anti ADAMTS, che, in un appropriato contesto clinico, consentono di distinguere la TTP da altre TMA e differenziare la TTP immunomediata dalla TTP congenita. Abbiamo presentato questo caso di TTP, data l'importanza di un corretto e precoce inquadramento ai fini terapeutici e prognostici, in cui il laboratorio di diagnostica ematologica può fornire un importante contributo nell'attivazione di un rapido iter diagnostico.

PO033

INTERFERENZA DELLE CRIOGLOBULINE NELL'ESAME EMOCROMOCITOMETRICO E NEL QUADRO PROTEICO: UN CASO CLINICO

M.P. Monaco, V. Santocchio, F. Garziano, A. Lombardo, R. Capuano, I. Piccirillo

Dipartimento di Medicina di Laboratorio di Patologia Clinica, Ospedale "San Giuliano" - Giugliano

BACKGROUND: Le crioglobuline sono un gruppo eterogeneo di proteine che precipitano a 4°C, ma ritornano in soluzione a 37°C. La ricerca delle crioglobuline non richiede strumentazioni analitiche particolarmente sofisticate e non presenta particolari difficoltà dal punto di vista esecutivo, ma è fortemente condizionata dal rispetto rigoroso delle condizioni operative e questo vale in particolare per le interferenze che esse determinano nei campioni analizzati.
CASE REPORT: Il paziente è un uomo di 65 anni, ricoverato nel reparto di Ematologia nel mese di marzo 2021. Gli esami ematochimici di routine all'ingresso non hanno evidenziato particolari alterazioni ad eccezione dell'esame emocromocitometrico. Infatti, l'emocromo eseguito su ADVIA 2120 e ripetuto, per conferma, su DxH1 Beckman Coulter mostrava grandi incongruità nei parametri analizzati: RBC: 1,36 x 10^6/¤L; Hb: 10,9 gr/dL; Hct: 11,7%; MCH: 80,2 pg; MCHC: 93,2 gr/dL; WBC: 1,52 x 10^3/¤L. Ad approfondimento diagnostico è stato eseguito, successivamente, il quadro sieroproteico elettroforetico (QPE) su Capillarys (SEBIA) rilevando la presenza di un doppio picco in zona Beta 2 probabilmente da riferisi all'evoluzione della malattia e alla terapia. Tuttavia, non è da escludere la possibilità che la crioglobulina possa aver interferito nella giusta e corretta valutazione della componente monoclonale nel momento in cui il campione non è stato processato in modo corretto. Pertanto, riscontrando una serie di incongruità nella refertazione dei risultati, si è proceduto contattando il medico di reparto per un confronto sui dati rilevati e, appurata la patologia di cui il paziente era affetto, Macroglobulinemia di Waldenstrom, tutti gli esami anormali, emocromo e QPE, sono stati ripetuti dopo incubazione a 37°C.
DISCUSSIONE E CONCLUSIONI: Abbiamo descritto il caso clinico di cui sopra per sottolineare quanto, in talune circostanze, sia indispensabile l'osservanza di regole scrupolose nella fase pre-analitica affinchè il dato laboratoristico avvalori il sospetto clinico-diagnostico.

PO034

Molecular biomarkers correlate with brain grey and white matter changes in patients with mitochondrial m.3243A>G mutation

S. Evangelisti[1], L.L. Gramegna[1,2], C. La Morgia[3,4], L. Di Vito[3], A. Maresca[4], L. Talozzi[1], C. Bianchini[1], M. Mitolo[2], D.N. Manners[1], V. Carelli[1,4], R. Lodi[1,5], C. Testa[2,6], C. Tonon[1,2]

[1]*Dep. of Biomedical and NeuroMotor Sciences, University of Bologna, Bologna, Italy*

[2]*IRCCS Istituto delle Scienze Neurologiche di Bologna, Functional and Molecular Neuroimaging Unit, Bologna, Italy*

[3]*IRCCS Istituto delle Scienze Neurologiche di Bologna, UOC Clinica Neurologica, Bologna, Italy*

[4]*IRCCS Istituto delle Scienze Neurologiche di Bologna, Programma di Neurogenetica, Bologna, Italy*

[5]*IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy*

[6]*Dep. of Physics and Astronomy "Augusto Righi", University of Bologna, Bologna, Italy*

INTRODUCTION - The mitochondrial DNA (mtDNA) m.3243A>G mutation in the MT-TL1 gene results in a multi-systemic disease, commonly associated with degenerative brain pathology. The aim was to explore correlations between circulating and peripheral biomarkers and brain grey matter density and white matter microstructure alterations to gain new insights into the pathophysiology of neurodegeneration related to the m.3243A>G mutation.

METHODS - Seventeen patients harboring the m3243A>G mutation were enrolled (age 43.1±11.4 yrs, 7F). A panel of blood biomarkers including lactate acid, alanine, L-arginine, fibroblast growth factor 21 (FGF-21), growth/differentiation factor 15 (GDF-15) and circulating cell free mtDNA (ccf-mtDNA), as well as blood, urine and muscle mtDNA heteroplasmy were evaluated. Patients also underwent a standardized Magnetic Resonance (MR) protocol that included volumetric T1-weighted images and diffusion-weighted MRI. Twenty sex- and age-matched healthy controls were included.

RESULTS - A widespread cortical grey matter (GM) loss was observed, more severe ($p<0.001$) in the bilateral calcarine, insular, frontal and parietal cortex, along with infratentorial cerebellar cortex. High urine mtDNA mutation, high levels of plasma lactate and alanine, low levels of plasma arginine and high levels of serum FGF-21 significantly ($p<0.05$) correlated with the reduced brain GM density. Widespread microstructural alterations were highlighted in the white matter, significantly ($p<0.05$) correlated with plasma alanine and arginine levels, mtDNA mutation load in urine, and with high level of serum GDF-15 and high content of plasma ccf-mtDNA.

CONCLUSIONS – Besides the correlation of MR metrics with mutation loads, we also found correlations with plasma levels of lactate and alanine (peripheral markers of altered mitochondrial metabolism), arginine (marker of altered nitric oxide metabolism), ccf-mtDNA (marker of massive cell death), serum FGF-21 and GDF-15 (both documenting a stress response). This suggests that an in vivo interplay between the mtDNA-related mitochondrial respiratory deficiency and defective nitric oxide metabolism, synergizing two distinct pathogenic mechanisms, is involved in the brain neurodegeneration in m.3243A>G patients.

PO035

Comparison of the clinical performance of two SARS-CoV-2 antigen testing with rapid chemiluminescent immunoassays

A. Aita[1,2], D. Basso[1,2], S. Schiavon[3], L. Rossi[4], E. Franchin[4], R. Rigoli[3], A. Crisanti[4], M. Plebani[1,2]

[1]*Department of Medicine-DIMED, University of Padova, Padova, Italy*

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

[3]*Department of Clinical Pathology, AULSS 2, Marca Trevigiana, Treviso, Italy*

[4]*Department of Molecular Medicine, University of Padova, Padua, Italy*

Background. One of the strategies suggested for the containment of SARS-CoV-2 pandemic is testing at risk populations with rapid turn-out of results, contact tracing and isolation of infected individuals, at least until vaccination programs have been completed. Molecular testing of naso-pharyngeal swabs (NPS) is considered gold-standard, but antigenic testing should offer the advantage of being more rapid. Aim. The aim of this study was to evaluate the clinical performance of two chemiluminescent immunoassays on laboratory automated platforms, LIAISON® SARS-CoV-2 Ag Assay (DiaSorin) and Elecsys SARS-CoV-2 Antigen (Roche), to detect SARS-CoV-2 N antigen in NPS. Patients and Methods. A total of 281 subjects were consecutively enrolled (116 M, 165 F) from three different cohorts: 14 were COVID-19 in-patients (Group 1), 149 were patients enrolled at the emergency unit (Group 2) while 118 were healthcare employees under SARS-CoV-2 periodic surveillance (Group 3). All subjects underwent NPS with eSwab Copan. Antigen and molecular testing were performed soon after collection. Results. Thirty subjects were SARS-CoV-2 positive at molecular testing. Liaison antigen was positive (>200 TCID50/ml) in 22/30 (Se=73.3%), equivocal (100-200 TCID50/ml) in 4/30 and negative (<100 TCID50/ml) in 4/30 subjects. Specificity was 61.8% since 60/157 negative samples had equivocal results. With Elecsys sensitivity was 75.9% and specificity 99.5%. ROC curves were performed to compare the two assays and to identify the best cut-off. The areas under the curves were not different (¤2=0.14; Prob>¤2=0.7077). The highest likelihood ratio for Liaison corresponded to 150 TCID50ml cut-off, while for Elecsys to 1 index value. With these thresholds sensitivity of these two assays were 86% and 87% respectively, with 99% specificity. The limitations in sensitivity were due to false negative results for samples with Ct values at molecular analysis higher than 25. No false negative case was recorded among those with Ct lower than 25. Conclusions. In conclusion NPS SARS-CoV-2 antigen testing with chemiluminescent immunoassays allows the rapid detection of positive samples with a sensitivity and specificity that meet the recommendations of the WHO for this type of testing.

PO036

ATP produced by CD4 + T cells: innovative immunological parameter in the monitoring of liver and kidney transplant patients.

M. BASSI[1], P. SELVA[1], E. MAGRINI[1], S. BERGAMINI[1], M. RIGA[3], R. MANCINI[1]

[1]*Laboratorio Unico Metropolitano AUSL-Bologna*

[2]*Università degli Studi di Bologna*

INTRODUCTION

The optimal "target" of immunosuppression in the transplant patient with solid organs (liver, kidney) has been the goal of many researchers and doctors working in the transplant sector for many years. Immunosuppression prevents rejection of the transplanted organ, but exposes the patient at an increased risk of infections. Calcineurin inhibitor drugs, cyclosporine and tacrolimus, therefore constitute the pillars of immunosuppression (2-3).Unfortunately to date, the only tool used to evaluate the type of immunosuppression was the dosage of drugs at the serum level, which moreover does not allow to understand the patient's degree of immunosuppression.The ATP produced by CD4+ T lymphocytes represent a biomarker of global and personalized immunocompetence of the transplant patient.

MATERIALS AND METHODS

From July 2019 to date, heparinized blood samples of 260 transplant patients from the S. Orsola Malpighi Hospital were analyzed with Cylex ImmunoKnow (ImmuKnow, Viracor Eurofins Immune Cell function Assay-ADA) (1).The test measures the ability of TCD4+ to respond to mitogenic stimulation with phytohemagglutinin-L (PHA) in vitro, quantifying the amount of adenosine triphosphate (ATP) produced by CD4+ cells after stimulation.

RESULTS

The analysis of the risk curves for rejection and infection showed that patients maintained at ATP levels between 130-450 ng /mL were at minimal risk of infection or rejection; the value of 280 ng/mL of ATP was found to be a target level for individualizing immunosuppressive therapy, defining the highest probability of maintaining clinical stability with lower rate of immunosuppression (2-3).

BIBLIOGRAPHY

Correlation Between Immunosuppressive Therapy and CD4D T-Cell Intracellular Adenosine Triphosphate Levels in Liver Transplant Qu, Z.-J. Zhu*, L.-Y. Sun, L. Wei, Y. Liu, and Z.-G. Zeng2.Immunosuppression Modifications Based on an Immune Response Assay: Results of a Randomized, Controlled Trial. Ravaioli et al Tranplantation 2015 Immune Function Assay (Immunknow) Drop Over First 6 Months After Renal Transplant: A Predictor of Opportunistic Viral Infections? Quagliaa,*, T. Cenab, R. Fenoglioa, C. Musettia, D. Cagnaa, E. Radina, S. Roggeroc, A. Amorosoc, Magnanib, and P. Strattaa

PO037
DIAGNOSI DI LABORATORIO EMOMETRICA DI LEUCEMIA ACUTA PROMIELOCITICA

V. Latella, M. Caracciolo, S. La Scala, C. Alati, B. Modafferi

G.O.M. Bianchi Melacrino Morelli Reggio Calabria

INTRODUZIONE Paziente donna (52 aa), viene ricoverata presso U.O.C. di Terapia Intensiva Post Operatoria della nostra Azienda a seguito di in un intervento chirurgico. L'esame emocromocitometrico evidenziava anemia, piastrinopenia e monocitosi.. WBC: 7.590 ▫L ; HGB. 7.9 g/dL ; PLT: 32.000 ▫L. MATERIALI E METODI I grafici attenzionati evidenziavano dei cluster di distribuzione cellulare anomala. L'esame morfologico, subito eseguito, pone in evidenza la presenza di cellule blastiche con presenza di numerosi Corpi di Auer. Contestualmente e con i clinici della TIPO si coinvolge la divisione di Ematologia che prende in carico la paziente. A poche ore dall'esecuzione dell'esame emocromocitometrico e dalla valutazione morfologica dello striscio periferico e midollare, accompagnati dalla valutazione clinica, viene formulata la diagnosi definitiva di Leucemia Acuta Promielocitica . I promielociti leucemici sono elementi di grossa taglia con nucleo eccentrico, cromatina meno fine rispetto ai mieloblasti e nucleoli ben evidenti. L'elemento morfologico caratteristico è costituito dalla presenza di numerosissimi granuli primari, che hanno la peculiarità di coprire il nucleo. Si possono osservare elementi simili ai corpi di Auer, riuniti in ammassi. I granuli sono poco evidenti e il nucleo si presenta bilobato con ponti cromatinici tra le sub-unità nucleari, che determinano caratteristiche "figure a occhiali". CONCLUSIONI. La leucemia acuta promielocitica è la forma più aggressiva di leucemia mieloide acuta . L'esordio della malattia è improvviso e, in alcuni casi, può essere caratterizzato dalla comparsa di una grave sintomatologia emorragica dovuta alla presenza di un ridotto numero di piastrine e all'alterazione dei meccanismi della coagulazione . Se la leucemia acuta promielocitica non viene identificata in tempi rapidi in centri di riferimento esperti ed attrezzati per affrontarne la gestione, il rischio di mortalità precoce è molto elevato. L'emocromo e l' esame morfologico del sangue periferico rappresentano la base per un rapido sospetto diagnostico. E' ovvio come, nel caso in esame, la tempestività della diagnosi innescata dall'osservazione dei cluster cellulari e soprattutto dall'esame morfologico del sangue periferico, abbia posto la paziente nella condizione avere una rapida diagnosi e conseguente terapia.

PO038
DIAGNOSI DI LABORATORIO EMOMETRICA DI LAM CON IPEREOSINOFILIA

V. Latella[2], B. Modafferi[2]

[1]

[2]G.O.M. Bianchi Melacrino Morelli Reggio Calabria

Introduzione La Leucemia Mieloide Acuta è una malattia caratterizzata dalla proliferazione di cloni di cellule staminali ematopoietiche che non hanno completato il processo regolare di maturazione. Queste cellule, lentamente, rimpiazzano il normale tessuto ematopoietico nel midollo, alterando la produzione delle altre cellule (globuli rossi, piastrine e granulociti), per poi entrare nel circolo sanguigno ed infiltrarsi in altri organi. Lo scopo di questo lavoro è porre l'attenzione sull'importante ruolo che ad oggi riveste la medicina di laboratorio nella valutazione di patologie di natura ematologica anche di complessa identificazione. Materiali e Metodi . Paziente donna (40 anni) viene ricoverata presso l'U.O.C di Ostetricia della nostra Azienda. Viene eseguito un emocromo, come richiesto dal reparto, ed emerge quanto segue : GB 139.000/mmc PLT 64.000/mmc HGB 7.9 gr/dl MCV 107.0fl. Risultati Un'attenta analisi degli scattergram WDF e WPC pone il sospetto diagnostico di LAM. All'esame morfologico risulta : N 15 L 18 M 15 E 35 PMC 2 BL 15 A questo punto viene allertata l'U.O.C di Ematologia della nostra Azienda che osservati i dati del laboratorio e valutata la clinica pone la diagnosi di Leucemia Acuta Mieloide ipereosinofila. Conclusioni La medicina di laboratorio , ed il linguaggio laboratoristico in particolare, si basano sulla capacità di trasformare semplici dati numerici in informazioni diagnostiche ed interpretative. Il quadro che ne emerge è ricco di informazioni analitiche che saranno fondamentali nella fase post-analitica con commenti di refertazione , che opportunamente valutati offrono la chiave d'apertura alla diagnosi per il clinico.

PO039

Automation of a capture-based NGS workflow: one thousand patients experience in a diagnostic clinical routine framework

E. Tenedini [1,2], F. Celestini[3], P. Iapicca[4], M. Marino[1], S. Castellano[2], L. Artuso[1], F. Biagiarelli[4], L. Cortesi[5], A. Toss[5,6], E. Barbieri[5], L. Roncucci[2], M. Pedroni[2], R. Manfredini[7], M. Luppi[2], T. Trenti[1], E. Tagliafico[1,2,8]

[1]Department of Laboratory Medicine and Pathology, Diagnostic Hematology and Clinical Genomics Unit, Modena University Hospital, Modena, Italy

[2]Department of Medical and Surgical Sciences, University of Modena and Reggio Emilia, Modena, Italy

[3]Hamilton Italia S.r.l., Agrate Brianza (MB), Italy

[4]SOPHiA GENETICS SA HQ, Saint-Sulpice, Switzerland

[5]Department of Oncology and Hematology, Modena University Hospital, Modena, Italy

[6]Department of Surgery, Medicine, Dentistry and Morphological Sciences with Transplant Surgery, Oncology and Regenerative Medicine Relevance, University of Modena and Reggio Emilia, Modena, Italy

[7]Life Sciences Department University of Modena and Reggio Emilia, Centre for Re-generative Medicine, Modena, Italy

[8]Center for Genome Research, University of Modena and Reggio Emilia, Modena, Italy

Clinicians are increasingly using the results of Next Generation Sequencing (NGS) tests to take first-line treatment decisions or choose among surgical options. So, genomics put under progressive pressure the traditional diagnostic laboratory to expand its sequencing ability and to adopt a laboratory medicine model, where a large-scale automation is supporting the testing process ubiquitously. NGS libraries preparation for multigene panels are designed either with amplicon-based or with hybridization capture-based target enrichment methods. Amplicon-based approaches are simpler and ask for a very little DNA input, but it has been demonstrated that hybridization-based procedures are less likely to generate false positives/negative SNVs, and notably they perform better in terms of coverage uniformity, which is essential for correctly predicting CNVs. Nevertheless, this kind of workflow is well known to consist of multiple and hands-on demanding steps, possibly prone to human bias. Automation may definitely help dealing with these limitations, especially in the clinical routine of a public healthcare system, where diagnostics have to work in a cost-effective manner. We therefore directed our interest towards the automation with a liquid handler that can integrate all the devices necessary for the execution of the protocol. With these premises, we implemented the automated SOPHiA Genetics ce-IVD Hereditary Cancer Solution (HCS) library preparation workflow on the Hamilton's STARlet platform and adopted it in the diagnostic routine. After processing more than 1000 genomic DNA samples, we compared the NGS results carried out with this automated protocol with the ones carried out manually on 240 samples and we get some conclusions. We proved that an accurate automated design can minimize the risk of human-introduced errors, standardizing the analytical performances and decreasing sequencing data variability. Variant calling accuracy resulted very good, as far as no false positive likely pathogenic or pathogenic variants were found. In a more complete laboratory picture, automation allowed a more efficient working agenda and an improvement of the samples' flow, scheduling consumes, achieving a better supply and making NGS more affordable. To make the economic investment of automation paid off, we tested the same platform with other commercial or custom SOPHiA panels, to diagnose hereditary rare diseases or hematological cancers. The automated solution showed broad versatility, with easily scaling number of samples and reagents volumes, as well as adjustments to hybridization and amplification programs to adapt to different working protocols.

PO040

Molecular Diagnostic for SARS-CoV-2: an experimental External Quality Assessment Scheme

L. Galla[1], L. Sciacovelli [1], A. Padoan[1,2], M. Plebani[1,2]

[1]Department of Laboratory Medicine, University-Hospital of Padova, Italy.
[2]Department of Medicine-DIMED, University of Padova, Italy

Background: External Quality Assessment (EQA) is a valuable tool to monitor and improve the analytical performances of clinical laboratories. During the COVID-19 pandemic, the number of kits to detect the infection and the number of tested samples intensified to satisfy the test request. To guarantee suitable results, EQA scheme providers have implemented specific schemes assessing different SARS-CoV-2 diagnostic systems. This study aims to describe the results collected in an experimental EQA scheme for molecular diagnostic of SARS-CoV-2 managed by INSTAND e.V with the collaboration of the Centre of Biomedical Research for Quality in Laboratory Medicine for Veneto Region Laboratories.
Methods: The qualitative EQA results collected, two surveys in 2020 and one in 2021, for 18 samples total, have been summarized to identify the percentage of laboratory results per sample. Control samples included SARS-CoV-2 or other seasonal coronaviruses (MERS-CoV, HCoV 229E, HCoV OC43) provided by Nationales Konsiliarlaboratorium fur Coronaviren of Berlin. SARS-CoV-2 Variants of Concern (VOCs) were included only in the 2021 survey.
Results: The average of the participating laboratories strongly decreased between the first survey of 2020 (927) and the last analysed survey, March 2021 (594). The main analytical procedures used, in the first, second and third survey respectively were CEPHAID kits (11.6%, 12% and 11.7%), in-house produced assays (10.4, 6.2 and 5%), SEEGENE kits (8.5%, 8.1% and 7.9%), ROCHE Diagnostics (8.3%, 8.5% and 6.9%) and ALTONA Diagnostics kits (6.1, 6.2 and 4.5%). A good agreement was found among laboratories results, with an overall range from 95% to 99.8%. Furthermore, generally from 0.2% to 2.9% of incorrect results and 0% to 1.1% of indeterminate results were reported.
Conclusions: The EQA programs are a fundamental quality assurance tool to evaluate the laboratory performance and know the State-of-the-Art diagnostic systems used by participating laboratories. The need for an EQA scheme for every test performed in the laboratory is mandatory to guarantee patient safety.

PO041

Polyunsaturated fatty acid biomarkers in nonagenarians: a preliminary study

S. Ali[1], A. Aiello[2], S. Davinelli[1], G. Accardi[2], G. Scapagnini[1], C. Caruso[2], G. Candore[2]

[1]Dept. of Medicine and Health Sciences, University of Molise, Campobasso
[2]Lab. of Immunopathology and Immunosenescence, Dept. of Biomedicine, Neuroscience and Advanced Diagnostic, University of Palermo, Palermo

Polyunsaturated fatty acids (PUFA) are extensively studied in human metabolism. Prospective studies and controlled trials support that the effects of these essential fatty acids are clinically relevant. PUFAs profile in the blood reflects both diet and metabolism, and their levels may be related to disease risk. To date, the n-3 index, n-6/n-3 ratio, and arachidonic acid (AA)/ eicosapentaenoic acid (EPA) ratio are the most promising biomarkers associated with PUFA metabolism. These indices are considered to be markers for several diseases, especially cardiovascular events and brain disorders. Despite widespread interest, there is little evidence regarding the association between PUFA biomarkers and longevity. We conducted the present study to determine whether the n-3 index, AA/EPA ratio, and n-6/n-3 ratio are associated with aging and longevity. We compared the PUFA biomarkers from a cohort of 30 nonagenarians with 74 matched controls from Western Sicily/Italy. We further classified the participants into four age groups, from young adults to nonagenarians. Gas chromatography was employed to determine the blood PUFA profile. The nonagenarian group had a significantly lower AA/EPA ($p = 0.005$) and n-6/n-3 ($p = 0.02$) ratios compared to the controls. When stratified by age groups, a significantly better n-3 index ($p = 0.03$), AA/EPA ($p = 0.07$), and n-6/ n-3 ($p = 0.05$) were observed in the nonagenarians of age-group 65-89, compared to the controls. We concluded that PUFA biomarkers derived from nonagenarians are different from the general population.

PO042

L'analisi delle crioglobuline e del criofibrinogeno nei laboratori italiani: risultati di un sondaggio a cura del Gruppo di Studio Proteine di SIBioC

P. Natali[1], D. Debbia[1], F. Lavatelli [2], U. Basile[3], M. Savoia[4], I. Catalodo[5], F. Turra[6], I. Crespi[7], D. Farci Santarcangeli[8], T. Troiano[9], A. Picanza[10], A. Russo[10], R. Minerba[10], M. Mussap[11], M.S. Graziani[12], G. Palladini[2]

[1]Dip. Interaziendale Integrato di Medicina di Laboratorio e Anatomia Patologica Azienda OU e Azienda USL di Modena, Modena

[2]Fondazione IRCCS, Policlinico San Matteo, Università di Pavia, Pavia

[3]Dip. Medicina di Laboratorio, Policlinico Universitario A. Gemelli, IRCCS Università Cattolica del "Sacro Cuore", Roma

[4]Dip. di Medicina Molecolare e Biotecnologie Mediche, Azienda Ospedaliero Universitaria, Università Federico II, Napoli

[5]Azienda Sanitaria Locale 2, Lanciano Vasto Chieti, Ospedale "Ss Annunziata", Chieti

[6]Laboratorio Unico Metropolitano Ospedale Maggiore - Azienda USL di Bologna, Bologna

[7]Azienda Ospedaliera Universitaria "Maggiore della Carità" di Novara, Novara

[8]Ospedale Policlinico di Milano, Milano

[9]Azienda Ospedaliero Universitaria Consorziale Policlinico di Bari, Bari

[10]Dip. Diagnostico, Azienda Ospedaliero Universitaria di Parma, Parma

[11]Medicina di Laboratorio, Dip. di Scienze Chirurgiche, Università di Cagliari

[12]Sezione di Biochimica Clinica, Università di Verona

INTRODUZIONE: Lo studio delle crioproteine (CP), cioè crioglobuline (CG) e criofibrinogeno (CF), è importante nell'ambito delle patologie reumatologiche ed autoimmuni.Diverse sono le criticità legate all'analisi delle CP: un alto livello di manualità, è operatore dipendente, manca una metodica di riferimento univoca. Il gruppo di Studio Proteine di SIBioC ha elaborato un sondaggio per verificare le procedure analitiche eseguite nei laboratori italiani.

METODI: È stato somministrato un questionario costituito da 40 domande riguardanti la fase pre-, post- e analitica sia della determinazione delle CG che del CF.

RISULTATI: Al questionario hanno risposto 66 laboratori, 4 (6%) non eseguono la ricerca di CG, 2 dei quali si riferiscono ad un altro centro, mentre la ricerca di CF è eseguita in 7 laboratori (11%) di cui 4 (6%) eseguono anche la conferma con antisiero anti-fibrinogeno.Fase preanalitica: sul tipo di provetta, se preriscaldata, come è trasportata al laboratorio, si sono ottenute risposte molto diversificate, mentre c'è concordanza (94%) sul mantenimento del campione a 37°C per la formazione del coagulo, che solo nel 30% dei casi viene però centrifugato a 37°C.Fase analitica: il 71% esprime il criocrito in percentuale rispetto al volume del siero, nell'11% dei casi senza preventiva centrifugazione. L'immunofissazione di conferma è eseguita nel 78% dei casi, nel 14% viene eseguita sempre, mentre gli altri casi solo se il criocrito (CCT) è >1%, se il paziente è di nuova diagnosi o se la tipizzazione è richiesta dal medico.Fase post-analitica: nel 90% dei laboratori le CG sono identificate secondo la classificazione di Brouet. Nel 29% dei casi il CCT non viene indicato, ma la solo presenza assenza di CG. Partecipano ad un programma di controllo esterno di qualità sulle crioglobuline il 18% dei laboratori.

DISCUSSIONE: Dal sondaggio risulta che la ricerca e tipizzazione delle CG presenta una forte eterogeneità inter-laboratorio, spesso condizionata dall'organizzazione locale. Scarsa è la diffusione della ricerca del CF. Emerge la necessità di avviare un processo di armonizzazione delle fasi pre-, post-, e analitica basato su raccomandazioni che il GdS Proteine della SIBioC sta preparando per migliorare la qualità dei risultati e della refertazione.

PO044

Implementation and decentralization of the analytical process in molecular biology: the experience of Modena for COVID-19 diagnosis

V. Pecoraro, T. Pirotti, T. Trenti

Dipartimento Interaziendale ad Attività Integrata di Medicina di Laboratorio ed Anatomia Patologica-AUSL Modena

Background: Real time reverse transcriptase polymerase chain reaction (RT-PCR) on clinical specimens is considered the first line test for the diagnosis of SARS-CoV-2 infection. This test involves different steps: RNA extraction, reverse trancription, PCR setting-up, amplification and analysis of results. To date, several analyzers for extraction and amplification phases are availabe, while few are able to guarantee the automation of the entire workflow. To optimise the allocation of swabs, with the aim of maximising the number of swabs tested and reducing the wait time for results, we applied a decentralysed system.Methods: In Provincia of Modena a network of 5 suburban laboratories referring to central laboratory was developed.The work was focused not only on the implementation of new analyzers, but also on the organization and consolidation of the whole workflow integrating the pre-analytical, analytical and post-analytical phases and including the molecular biology tests report on LIS (Laboratory Information System). Furthermore, all laboratory professionals attended a specific training.Results: Between march 2020 and June 2021 450,000 RT-PCR for RNA of Sars-Cov-2 research were performed. The central laboratory analyzes the majority of swabs (n= 259832, 58%), including those enrolled at drive through (44%). 76% were analyzed with a automated system, and 24% with a manual procedure. The laboratory is open every day including Sunday, from 8:00 a.m. to 8:00 p.m. engaging two biologists and five laboratory technicians per day. Our project allowed to increase significantly the number of swabs tested in a day (from 100 in march 2020 to 4600 in march 2021), guaranteeing their reporting within 3 hours in emergency or 24 hours for routine and drive setting.Conclusions: The employ of automated, user friendly and with a guided interface instrument facilited the entire workflow, reducing the operator work and the reporting time. RT-PCR executed with a manual procedure need of a specific expertise to read the reaction products, but the shorter reporting time makes it useful for swabs made in emergency. However, the presence of the barcode reader and the connectivity with the management LIS facilitated the traceability of the samples for the entire diagnostic pathway and the minimization of human error. The implementation of our workflow has involved an important rationalization and optimization of the staff, while integration of new knowledge about SARS-COV-2 and optimal analytical performance of analyzers has allowed a modern management of the samples maximising the laboratory's test capacity for RT-PCR tests.

PO045

Intossicazione da Amanita phalloides: misura di miRNA circolanti come marcatori precoci di danno epatico

F. Luceri[1], E. Bigagli[2], M. Cirronis[3], L. Cinci[2], N. Cini[1], M. D'Ambrosio[2], V. Petrolini[3], G. Mannaioni[2], A. Fanelli[1], C. Luceri[2]

[1]Laboratorio Generale, Azienda Ospedaliero-Universitaria Careggi, Firenze

[2]Dipartimento di NEUROFARBA, Università di Firenze

[3]Centro Antiveleni e Centro Nazionale di Informazione Tossicologica, Ist. Clin. Scientif. Maugeri IRCCS, Pavia

Non ci sono linee guida condivise per il trattamento di pazienti intossicati da Amanita Phalloides e la loro sopravvivenza è influenzata da vari fattori: la gravità del danno epatico, la capacità di rigenerazione delle cellule epatiche residue e la gestione delle eventuali complicazioni. Nei casi più gravi il trapianto di fegato diventa l'unica opzione clinica possibile. Per la misura dell'a-amanitina urinaria esiste un solo kit ELISA commerciale e, in alternativa, alcuni laboratori utilizzano metodiche LC-MS. Lo scopo di questo studio è di valutare se l'analisi di microRNA (miRNA) possa essere utilizzato per diagnosticare precocemente un'intossicazione da A. Phalloides. In una prima fase, 11 differenti miRNA associati a danno epatico sono stati ricercati nel siero e nelle urine di 6 pazienti intossicati da amanitina e di 6 soggetti di controllo. Sono stati identificati 2 miRNA circolanti (miR-320a e miR-155) poco espressi o assenti nei campioni dei soggetti sani e presenti e/o sovra-espressi in quelli dei pazienti. I 2 miRNA sono stati ricercati in un secondo gruppo di casi, 14 sieri e 10 campioni urinari di pazienti intossicati da A. Phalloides, confermando lo stesso andamento osservato nel training set. Successivamente, cellule di epatocarcinoma umano HepG2, sono state trattate con una concentrazione sub-tossica di a-amanitina valutando la citotossicità, le variazioni di vitalità e il ciclo cellulare dopo 24, 48, 72 e 96h di esposizione. Le cellule HepG2 mostrano una notevole riduzione della vitalità già dopo 24h che rimane costante fino a 96h; dopo 24-48h di esposizione, circa il 30% delle cellule muore e dopo 72-96h, il numero di cellule vive si riduce quasi completamente. I 2 miRNA identificati sono stati misurati nelle cellule e nel terreno di coltura, dopo 24 e 48h di esposizione, associando i risultati ottenuti con i profili di espressione genica delle cellule, per identificare i processi biologici controllati dai 2 miRNA. In conclusione, la misura di miRNA sierici e urinari potrebbe essere un interessante biomarcatore di danno epatico di intossicazione acuta da A. Phalloides. Lo studio del ruolo biologico di questi miRNA potrebbe inoltre consentire di ipotizzare nuove strategie terapeutiche per questa grave intossicazione.

PO046

COVID-19 mRNA BNT162b2 Vaccine: Anti-SARS-Cov-2 S-RBD IgG Antibodies response in a large population.

B. Lo Sasso[1,2], M. Vidali[3], C. Scazzone[1], G. Bivona[1], C.M. Gambino[1,2], R. Tomasino[2], V. Cappa[2], G. Candore[1,2], M. Pantuso[1], R.V. Giglio[1], M. Ciaccio[1,2]

[1]Department of Biomedicine, Neurosciences and Advanced Diagnostics, Institute of Clinical Biochemistry, Clinical Molecular Medicine and Laboratory Medicine, University of Palermo, Palermo, Italy

[2]Department of Laboratory Medicine, University Hospital "P. Giaccone", Palermo, Italy

[3]Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy.

Background: Vaccines have been rapidly developed to control Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2) diffusion among the population. The immune surveillance by assessing anti-spike protein receptor-binding domain (S-RBD) antibodies levels is fundamental to evaluate the individual protection against SARS-CoV-2 infection.
Methods: We evaluated the anti S-RBD IgG levels on a large cohort consisting of recipients of COVID-19 mRNA BNT162b2 (Pfizer-BioNTech) vaccine, without or with previous SARS-CoV-2 infection (2872 and 149 subjects, respectively), and 318 recovered COVID-19 patients that did not receive the vaccination. Among vaccinated, 450 subjects performed a re-dosing after about 72 days from the first measurement. Anti S-RBD IgG levels were evaluated by chemiluminescence immunoassay on Maglumi 800 (SNIBE, China).
Results: Anti S-RBD IgG levels were significantly lower in subjects with the previous infection than vaccinated subjects, both with and without previous infection (Bonferroni's correction, both $p < 0.001$). No difference was observed between vaccinated subjects with and without previous SARS-CoV-2 infection ($p = 0.118$). General linear model analysis revealed that age ($p = 0.012$), development of adverse effects after vaccination ($p = 0.001$), and sex ($p = 0.003$), but not the presence of previous infection ($p = 0.660$), were independent predictors of anti S-RBD IgG levels. Anti S-RBD IgG levels were significantly higher in women than men (2300 vs. 1462 BAU/mL; $p < 0.001$) as well as in subjects with one or more symptoms after vaccination than asymptomatic ones (2150 vs. 1374 BAU/mL; $p = 0.001$). Additionally, anti S-RBD IgG levels decreased with age (rho= -0.190; $p < 0.001$). Finally, a significant decrease in anti-RBD IgG levels was observed within a short period (median -1.1% day) not influenced by age and sex.
Conclusions: This observational study has revealed a robust response to COVID-19 vaccine administration, characterized by a good antibody production with age- and sex-related differences. Additionally, we showed a rapid antibody decay rate within a short period after a completed two-dose vaccine cycle.
Reference: Padoan et al. Clin Chim Acta. 2021 Aug; 519:60-63.

PO047

Monocyte Distribution Width (MDW) as a potential biomarker of sepsis in COVID-19 patients

B. Lo Sasso[1,2], R.V. Giglio[1], G. Bivona[1], C.M. Gambino[1,2], G. Candore[1,2], F. Ferro[2], R. Andolina[2], C. Scazzone[1], M. Ciaccio[1,2]

[1]Department of Biomedicine, Neurosciences and Advanced Diagnostics, Institute of Clinical Biochemistry, Clinical Molecular Medicine and Laboratory Medicine, University of Palermo, Palermo, Italy

[2]Department of Laboratory Medicine, University Hospital "P. Giaccone", Palermo, Italy

Introduction: Monocyte Distribution Width (MDW), a measure of monocyte anisocytosis, has emerged as a reliable biomarker for screening sepsis in the acute setting, including the emergency department (ED) and intensive care unit (ICU). Noteworthy, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection can cause sepsis, representing the most common complication in Coronavirus Disease 19 (COVID-19), associated with high mortality. However, only a few studies examine the MDW in COVID-19. Therefore, this study aimed to investigate the role of MDW as a sepsis biomarker in patients with COVID-19.
Materials and Methods: We enrolled one hundred ten COVID-19 patients hospitalized in the COVID-19 Units at the University Hospital "P. Giaccone" of Palermo, Italy, from September to October 2020. MDW was measured in samples collected in K3 EDTA tubes by a UniCel® DxH 900 hematology analyzer (Beckman Coulter's Inc, Brea, California). Statistical analysis was performed using MedCalc v12.1.4.0 statistical software (MedCalc Software, Mariakerke, Belgium).
Results: COVID-19 patients showed elevated MDW values with a median of 25.3 (IQR 23 – 27). About 70% of patients on admission had MDW values higher than the cut-off of 23.5, which we established in previous studies.
Conclusions: Our results support the clinical utility of the novel biomarker MDW in COVID-19 patients. Further studies are needed to explain better the mechanisms involved in the increase of MDW in COVID-19 patients and, in particular, its relation with the severity of the disease.

References: Agnello L, Bivona G, Vidali M, Scazzone C, Giglio RV, Iacolino G, Iacona A, Mancuso S, Ciaccio AM, Lo Sasso B, Ciaccio M. Monocyte distribution width (MDW) as a screening tool for sepsis in the Emergency Department. Clin Chem Lab Med. 2020 Oct 25;58(11):1951-1957.

PO048

"Albumin-Creatinine Ratio" (ACR) e "Protein-Creatinine Ratio" (PCR): verifica della concordanza di risultati tra Atellica 1500 e Cobas8000.

A. Milano[1], F. Cappellini[2], M. Casati[2], S. Carnicelli[1], L. Fossati[2], G. Sala[2], R. Di Pierri [2], E. Zucchetti[2], F. Basta[2], L. Zullo[2], R. Brivio[2]

[1]Dip. Medicina e Chirurgia, Università degli Studi Milano-Bicocca
[2]U.O. Laboratorio Analisi Chimico Cliniche, ASST Monza

Introduzione e scopo del lavoro
Le linee guida per per l'esecuzione dell'esame chimico-fisico e morfologico delle urine (ECMU) (1) e per la gestione della CKD (2) raccomandano che albuminuria e proteinuria su campione estemporaneo siano refertate come ACR (albumin-creatinine ratio) e PCR (protein-creatinine ratio). I risultati (mg/g) possono essere classificati in 3 categorie di escrezione proteica: fisiologica (ACR <30, PCR <150), moderata (ACR 30-300, PCR 150-500), severa (ACR >300, PCR >500). ACR e PCR sono oggi disponibili su alcune strumentazioni per l'esame chimico-fisico delle urine; se da un lato ciò rappresenta un valore aggiunto per l'ECMU, dall'altro presuppone che il laboratorio valuti il grado di concordanza tra i risultati quantitativi (strumentazioni di chimica-clinica) e quelli semiquantitativi (strisce reattive) prodotti; scopo del lavoro è stato verificare l'accordo tra le due metodologie d'analisi per garantire l'allineamento dei risultati forniti ai pazienti.
Materiali e metodi
100 campioni estemporanei di urina sono stati analizzati su Clinitek Novus, (Siemens) e su Cobas 8000, modulo c702 (Roche). I risultati di ACR e PCR ottenuti sono stati confrontati in termini di accordo tra le categorie di appartenenza mediante il test K pesato di Cohen.
Risultati
ACR: il 73% dei campioni ha mostrato concordanza esatta (<30: n=32; 30-300: n=15; >300: n=26), il 27% discordanza di una categoria (Novus ha sovrastimato e sottostimato rispettivamente il 18 % e il 9% dei casi). k di Cohen = 0,71 (CI 95%: 0.61-0.81), grado di concordanza: buono.PCR: l'81% dei campioni ha mostrato concordanza esatta (<150: n=36; 150-500: n=17; >500: n=28), il 18% discordanza di una categoria (Novus ha sovrastimato e sottostimato rispettivamente il 5% e il 14% dei casi), l'1% discordanza di 2 categorie (1 caso di sottostima per Novus). k di Cohen = 0,79 (CI 95%: 0.70-0.88), grado di concordanza: buono.
Conclusioni
Lo studio ha fornito un risultato di buona concordanza tra le categorie di escrezione proteica ottenute coi metodi confrontati, sia per ACR che per PCR. Considerate le differenze intrinseche tra le performance analitiche dei metodi anche le frequenze di discordanza riscontrate sono da considerarsi adeguate.
Bibliografia
1) PMID: 23732715
2) PMID: 28134409

PO049

"DEVELOPMENT AND VALIDATION OF METHOD TO SEPARATE AND LABELING GRANULOCYTES WITH 99mTc-HMPAO"

A. Sammartano[1], S. Migliari[1], M. Scarlattei[1], G. Baldari[1], S. Pipitone[3], L. Ruffini[1], R. Aloe[2]

[1]Nuclear Medicine Division, Azienda Ospedaliero-Universitaria di Parma, via Gramsci 14, 43126 Parma, Italy
[2]Struttura Semplice Dip. Biochimica ad Elevata Automazione, Azienda Ospedaliero-Universitaria di Parma, via Gramsci 14, 43126 Parma, Italy
[3]U.O. Diagnostica Ematochimica, Dipartimento Diagnostico, Azienda Ospedaliero-Universitaria di Parma, via Gramsci 14, 43126 Parma, Italy

Labeled leukocytes with 99mTc-HMPAO are routinely used for infection imaging. In particular, granulocytes are exploited as the cells are mainly involved in inflammation. They are divided into neutrophils, basophils and eosinophils. Neutrophils involved particularly in the acute inflammatory response in which through different cellular interactions, they can to cross the endothelium and reach inflammation sites where they carry out their phagocytic action. Aim of the study was to validate a method to separate granulocytes from the blood and labelling them with a radiopharmaceutical (Ceretec®).The separation and labelling validation process was executed on three different patients who were taken for a blood sample in a syringe containing ACD and HAES. The supernatant plasma obtained through the sedimentation and centrifugation was subsequently added delicately in a test tube, by means of Butterfly, containing a density gradient solution of 1,077 g/ml "Biocoll". Before labeling, the supernatant plasma sample was analyzed with hematology instrument Siemens ADVIA 2120 (more sensitive and precise) to verify the real presence of granulocytes in the sample whose presence shows that the previous phases of sedimentation and centrifugation were successful. The quality controls were the visual inspection of final product with Trypan Blue test, the granulocytes viability (> 99%), bacterial endotoxin test (LAL Test) and labelling efficiency (LE%). The results of the analysis of our three samples 66,2% 68,7% 68,2% (Fig 12.a-b-c) show a high percentage of neutrophils of the hemogram.We didn't observed presence of macroaggregates during the entire process, until the final sample. Labelling efficiency resulted at very high values in the three consecutive measured aliquotes (mean value 74.56%) In conclusion, the results shows that cell-based infection imaging with 99mTc-HMPAO-labeled granulocytes will be easy implemented in routine clinical practice.

PO050

Development and validation of a HPLC method to determine chemical purity and radiochemical purity of [68Ga]-DOTA-Pentixafor (PET) tracer

A. Sammartano[1], S. Migliari[1], M. Scarlattei[1], G. Baldari[1], L. Ruffini[1], R. Aloe[2]

[1]*Nuclear Medicine Division, Azienda Ospedaliero-Universitaria di Parma, via Gramsci 14, 43126 Parma, Italy*

[2]*Strutture Semplice Dip. Biochimica ad Elevata Automazione, Dip. Diagnostico, Azienda Ospedaliero-Universitaria di Parma, via Gramsci 14, 43126 Parma, Italy*

INTRODUCTION The C-X-C chemokine receptor 4 (CXCR4) and its ligand CXCL12 are overexpressed in in a variety of tumor types, strongly promoting tumor growth, invasiveness and metastasis through multiple signal pathways. Moreover, the CXCR4/CXCL12 axis is a key factor involved in the process of cell migration at sites of infection and/or inflammation. Recently, new imaging probes targeting CXCR4 have been developed for PET imaging of several different hematologic and other neoplasms including leukemia, lymphoma, multiple myeloma, adrenocortical carcinoma or small cell lung cancer and also in other solid tumors and disease conditions, such as splenosis, stroke, atherosclerosis, and myocardial infarction in humans and in animals.Among CXCR4-directed imaging agents, Pentixafor labeled with Gallium-68 (Ga-68) has shown a unique position, because of its elevate affinity and selectivity to CXCR4, its low unspecific binding and adequate distribution profile accompanied by quick renal excretion.Preparation conditions may influence the quality and in vivo behaviour of this tracer and no standard procedure for the quality controls (QCs) is available. During recent years, attention to QC methods has grown up in the development of radiopharmaceuticals, especially in establishing clinical grade compounds. So that, HPLC method has become crucial for identification/ characterization of the final product, demanding higher resolution than standard TLC method. Before their use in routine quality control procedures, analytical methods must be validated. AIM. Aim of this study was to develop a new rapid and simple HPLC method of analysis for the routine QCs of [68Ga]-DOTA-Pentixafor to guarantee the high quality of the finished product before release. The method was validated to fulfill ICH requirements and EDQM guidelines including specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy and precision. MATERIALS AND METHODS. A range of concentration of PENTIXAFOR (4.00, 2.00, 1.50, 1.00, 0.80, 0.50 µg/mL) and [68Ga]-PENTIXAFOR were analyzed. The identification of peaks was achieved on a symmetry C18 column 3µm 120Å (3.0 mm × 150 mm spherical particles) using water +0.1% trifluoroacetic acid (TFA) and B) acetonitrile +0.1% TFA, as the mobile phases at a flow rate of 0.600#mL/min and monitored at 220#nm. The run time was 16#min. The developed analytical test method was validated because a specific monograph in the Pharmacopoeia is not available for [68Ga]-DOTA-Pentixafor. RESULTS. The purity and quality of the radiopharmaceutical obtained according to the proposed method resulted high enough to safely administrate it to patients. Excellent linearity was found between 0.5 and 4 ▫g/ml, with a correlation coefficient (R2) for calibration curves equal to 0.999, the average coefficient of variation (CV%) resulted <2% (0.10%) and the average bias% value was 1.44%. The limits of detection and quantification (LOQ) for DOTATATE were 0.5 and 0.1 µg/mL respectively.
CONCLUSION. Our QCs validation protocol include not only typical analytical characteristics (radiochemical purity, chemical purity, pH, integrity filter, radionuclidic purity) but also precision, accuracy, specificity, limit of detection and quantification, linearity and range of HPLC method in order to guarantee simple and safe implementation of the produced radiopharmaceutical in the diagnostic activity.The developed method to assess the radiochemical and chemical purity of [68Ga]-DOTA-Pentixafor is rapid, accurate and reproducible allowing routinely use of this PET tracer as diagnostic tool for imaging CXCR4 expression in vivo, also assuring patient safety.

PO051

THE ANALYSIS OF THE SPREAD OF COVID-19 AND FLU IN THE SAN GIULIANO HOSPITAL (ASL NAPOLI 2)

V. FEDERIGO, A. SORRENTINO, L.A. PICCOLO, C. ROMANO, E. MAURIELLO, R. RICCIO, I. PICCIRILLO

P.O. San Giuliano ASL Napoli 2 Nord Lab. di Patologia Clinica, Giugliano, Italy

INTRODUCTION: Influenza (the flu) and CoViD-19 are contagious respiratory illnesses, both affecting lungs and breathing that can be easily spread between people. Although Flu and CoViD-19 share similar symptoms (including fever, cough, body aches, and sometimes vomiting and diarrhoea), these diseases are caused by different viruses. The aim of this study is to compared the spread of Flu and CoViD-19 by means of Real time PCR test.

METHODS: From January 2020 to March 2021 a total of 200 respiratory samples from both male and female symptomatic patients in the age group of 15-70 years were tested for the detection of the Flu A (Matrix Protein gene), Flu B (Non Structural Protein gene) and SARS-CoV-2 (RdRp and N genes) viruses with Neoplex FluCovid kit (GeneMatrix, distributed by Eurospital). Samples were divided into three groups: i) collected from patients hospitalized in the E.R. (N=30); ii) collected from patients with respiratory distress 7 days after the first respiratory swab, which resulted negative at the molecular testing for SARS-CoV-2 (N=40); iii) collected from patients with typical symptoms of both Flu and CoViD diseases.

RESULTS AND CONCLUSIONS: Real time PCR test on samples of the first group revealed that 30% of patients were positive to Flu A, 67% were positive to SARS-CoV-2 and the remaining 23% negative for both Flu and CoViD-19 etiological agents. In the second group, only the 5% of patients were positive to Flu A, 25% positive to SARS-CoV-2 and 70% negative to both Flu and CoViD-19. In the third group, only <1% of patients revealed to be co-infected with both Flu A and SARS-CoV-2 viruses, whereas the 67% were found positive to only SARS-CoV-2 and the remaining 32% negative to both Flu and SARS-COV-2 target genes. Overall, our data suggest the importance of using an appropriate diagnostic testing to get an accurate diagnosis in order to define the most right and effective therapy. Moreover, our analysis revealed a low incidence of Flu, which can be related to the extensive use of masks.

PO052

Il ruolo del Laboratorio nell'analisi di componenti monoclonali: un caso clinico particolare

M. Cilento, A. Contina, I. Campanile, G. Giordano, L. Ferraro, M. D'Amora

UOC Medicina di Laboratorio P.O. San Paolo Asl Napoli 1 Centro

L'elettroforesi delle sieroproteine rileva l'omogeneità molecolare delle Ig e, ove presente, una componente monoclonale. L'importanza della metodologia nella ricerca delle CM nel siero è descritta nel documento del GdS "Proteine"della SIBioC.La quantificazione delle CM correla con le dimensioni del clone cellulare in proliferazione nel midollo purché secernente. La quantificazione delle CM rappresenta un biomarcatore necessario per: a) porre diagnosi differenziale tra componente monoclonale di incerto significato (MGUS) (CM <30 g/L) e mieloma multiplo (MM) (CM ¤30 g/L); b) definire alla diagnosi il rischio di progressione di una MGUS vs malattia linfoproliferativa maligna come MM o macroglobulinemia di Waldenström, stimato ~1% /anno, che non si stabilizza nel tempo; c) definire il rischio di progressione del MM asintomatico "smoldering" verso il MM clinicamente conclamato, rischio definito ~10%/ anno per i primi 5 anni che poi scende a ~3%/anno nei successivi 5 anni e a 1-2%/anno nei successivi 10 ;d)valutare la risposta al trattamento in corso di MM e disordini correlati.Perciò la quantificazione della CM occorre all'inizio del sospetto clinico di MM ed anche nel monitoraggio della malattia. Si sottolineano altri due aspetti relativi all'impiego della quantificazione delle CM nella pratica clinica: a) la linea guida dell'"International Myeloma Working Group" ha introdotto il concetto di "malattia misurabile" basato sulla quantificazione della CM, eventualmente anche urinaria (proteina di Bence Jones) o la determinazione delle catene leggere libere nel siero, prerequisito per l'applicazione dei criteri descritti; b) la concentrazione della CM è solo uno dei criteri che consente di valutare il suo impatto clinico. Nelle patologie linfoproliferative più rare manifestazioni cliniche e prognosi sono determinate più dalle caratteristiche fisico-chimiche e/o anticorpali della proteina monoclonale che non dall'espansione del clone cellulare. Caso clinico:nel 2017 analizziamo un paziente di 54a con infezione da varicella zoster evidenziando al QPE un picco monoclonale quantizzato (28,4%.-2,4 g/dl) e tipizzato con metodica immunotyping quale IgG-kappa. L'immunofissazione urinaria evidenzia due componenti: la prima costituita da catene leggere di tipo kappa (Bence Jones) e la seconda di tipo Ig-kappa. Il paziente acquisita conoscenza del problema clinico si ricovera in struttura universitaria lombarda. Nel 2018 ritorna al ns ambulatorio e si riscontra aumento del picco monoclonale (30,8%.-2,8 g/dl).Nel 2021 registriamo un ulteriore aumento del picco monoclonale (34,5% - 2,9 g/dl). L'elettroforesi capillare delle urine delle 24hh evidenzia n.3 componenti monoclonali. L'immunotyping delle urine evidenzia due componenti monoclonali costituite da catene leggere kappa ed una IgG-kappa.Si è proceduto al dosaggio semiquantitativo delle componenti monoclonali ritenuto utile per il monitoraggio del paziente che ad oggi è ancora in osservazione.

PO054

C-reactive protein monitoring and clinical presentation of fever as predictive factors of prolonged febrile neutropenia and blood culture positivity after autologous hematopoietic stem cell transplantation. Single center real-life experience.

P. Castorina[2,1], D. Carcò [3,2], P. Guardo[3,2], V. Iachelli[3,2], T. Pace[3], u. Markovic[3], G. Moschetti[3]

[1]Faculty of Mathematics and Physics, Charles University, V Holešovi#kach 2, 18000 Prague
[2]Istituto Oncologico del Mediterraneo, Viagrande,

Background: Febrile neutropenia is a medical emergency that requires urgent evaluation, timely administration of empiric broad-spectrum antimicrobials especially in the setting of both allogeneic and autologous hematopoietic stem cell transplant. Methods: In this retrospective study, a total of 49 consecutive episodes of FN was evaluated in 40 adult patients affected by either multiple myeloma or lymphoma, following ASCT, with nine patients having fever in both transplantations. Results: We evaluated 40 patients with FN following ASCT, twenty-nine patients were affected by multiple myeloma, while eleven had lymphoma. Extremely drug-resistant (XDR) germs, Pseudomonas aeruginosa and Klebsiella pneumoniae were isolated in a total of 14 patients. Febrile neutropenia occurred at a median of 7 days from PBSC reinfusion with twelve patients that suffered early fever onset within the first 5 days from ASCT. Median duration of FN was 2 days. Ten patients had at least one fever spike superior to 39°C. Twenty patients had positive blood cultures with XDR germs present in 7 cases, respectively Pseudomonas aeruginosa and KPC in five and two. Daily values of WBC, CRP and body temperature were recorded following ASCT. The combined study of sensitivity and specificity gave a cut-off of less than 300 WBC for the onset of fever the day after, with area under curve (AUC) around 92%. Analogous ROC curve for CRP measured the day before fever had weak impact on fever onset, given that AUC resulted around 60%. ROC analysis of peak CRP values was done based on blood culture positivity and a value of 12 mg/dL resulted significant. Subsequently Odds ratio evaluated the predictivity for fever duration greater than 3 days that was associated with the presence of both peak number of 3 and body temperature greater than 39°C. Blood culture positivity and peak CRP values greater than 12 mg/dL were also associated with prolonged fever duration. Conclusions: In our study both clinical characteristics of fever and peak CRP levels were associated with higher probability of both prolonged fever duration and positive blood culture, needing extended antibiotic therapy. Further studies are needed thus improving the outcome of patients affected by febrile neutropenia following ASCT.

PO055

Ricerca Proteinuria di Bence Jones: studio comparativo tra metodo immunonefelometrico ed immunofissazione

C. De Falco, A. Petti, L.A. Catapane, R. Sabatino, M. Tenga, A. Petruzziello

U.O.C. Patologia Clinica, Dipartimento dei Servizi Sanitari, A.O.R.N. "Sant'Anna e San Sebastiano", Caserta, Italia

La ricerca della Proteina di Bence Jones (PBJ) riveste un importante ruolo nella diagnosi, nella prognosi e nel monitoraggio delle malattie immunoproliferative e delle discrasie plasmacellulari. Ad oggi il gold standard per evidenziare e caratterizzare la presenza delle catene leggere libere nelle urine è l'immunofissazione urinaria (IFU). Il dosaggio quantitativo delle catene leggere totali urinarie può essere effettuato anche con tecniche immunochimiche (turbidimetriche o nefelometriche), impiegando anticorpi specifici. Tale metodo, però, non fornisce un dato adeguato al monitoraggio della malattia, in quanto l'uso degli antisieri anti-catene leggere totali non permette di distinguere le catene leggere legate da quelle libere.
Scopo di questo studio è stato comparare la metodica nefelometrica (BN ProSpec®, Siemens Healthineers) con l'immunofissazione (Hydragel 4 Bence Jones®, Sebia).
53 campioni di cui 38 Maschi (72%) e 15 Femmine (28%) con età media di 67,27, afferenti, nel periodo Febbraio - Giugno 2021, all'A.O.R.N. ''Sant'Anna e San Sebastiano'' di Caserta, sono stati sottoposti, mediante nefelometria, alla ricerca delle catene ◘ e ◘ urinarie. Di questi, 40 campioni sono risultati PBJ positivi (75%) e 13 PBJ negativi (25%). L'IFU ha mostrato che dei 40 campioni PBJ positivi in nefelometria, solo 14 (40%) si confermano positivi, mentre 24 (60%) erano negativi. Dei 13 campioni PBJ negativi in nefelometria, 12 (92,3%) sono stati confermati negativi all'IFU, mentre 1 (7,7%) è risultato positivo.
Se ne conclude che il test nefelometrico mostra una sensibilità del 94,12%
(CI 95%, 71,31% - 99,85%) e una specificità del 33,33% (CI 95%, 18,56% - 50,97%) con PPV del 40% (CI 95%, 33,96% - 46,36%) e NPV del 92,31% (CI 95%, 62,90% - 98,84%)
I dati ottenuti mostrano come l'immunofissazione rappresenti il metodo più valido per la determinazione della PBJ rispetto alla quantificazione immunochimica che, dosando catene leggere totali, può determinare dei risultati falsi positivi in caso di danno renale.

PO056

Qualificazione del contaglobuli Horiba Microsemi CRP come strumento point of care (POCT) in un Servizio Trasfusionale.

S. D'Antico, V. Granero, S.G. Foddai, D. Ongaro, S. Irelli, R. Roncato, F. Azzalin, F. Fila, G. Giustetto, F. Castagno

S.C. Banca del Sangue e Immunoematologia, A.O.U. Città della Salute e della Scienza, Torino

Introduzione e scopo dello studio.
Una pronta disponibilità dei parametri dell'emocromo sono essenziali per le decisioni mediche in ambito ambulatoriale, ad esempio l'Hct per stabilire la necessità di un salasso e il conteggio Plts per valutare l'idoneità alla donazione di piastrine in aferesi. Lo strumento in uso è un contaglobuli di laboratorio convenzionale (Sysmex XN-1000), ma la logistica del trasporto dei campioni, il TAT e la refertazione di ritorno comportano un notevole impatto sulle tempistiche. Uno strumento POCT potrebbe migliorare l'efficienza delle prestazioni ambulatoriali. In questo lavoro è stato analizzato l'impatto sulla decisione clinica secondo i risultati ottenuti da due differenti analizzatori.
Materiali e metodi.
Abbiamo analizzato 100 campioni, prelevati in 5 giorni consecutivi da 100 diversi soggetti, con due strumenti: Sysmex XN-1000 Dasit (A-1), quale strumento in uso, e Microsemi CRP Horiba ABX (A-2) come possibile POCT. Test di regressione di Passing-Bablok per comparazione di A-1 vs A-2. Test di McNemar per valutazione di correlazione clinica: salasso per Hct¤45%; piastrinoferesi per Plts¤180 x10e3/¤l. Valutazione dei tempi per il trasporto dei campioni al laboratorio, il TAT e la refertazione.
Risultati.
L'analisi di regressione di Passing-Bablok evidenzia una correlazione analitica per i parametri esaminati (Hct: y=-1,3956+1,0294x; Plts: y=17,4606+0,9199x). L'analisi dei tempi rileva l'impiego di 25 minuti per la disponibilità del referto con A-1 contro 5 minuti con l'analizzatore A-2. L'analisi di McNemar eseguita per il valore di Hct dimostra una differenza non statisticamente significativa tra le decisioni cliniche prese sulla scorta dei risultati di A-1 rispetto a quelle che sarebbero state prese sulla scorta dei risultati di A-2 (¤2=0,083; p=0,7728). Per il valore di Plts, McNemar dimostra una differenza non statisticamente significativa tra le decisioni cliniche (¤2=0,0001; p=1,000).
Conclusioni.
I parametri misurati con A-2 sono comparabili a quelli di A-1 e possono essere usati per le medesime decisioni cliniche. Lo strumento Microsemi CRP Horiba ABX come POCT consente di ottimizzare i tempi dell'analisi migliorando l'efficienza di un ambulatorio Trasfusionale riducendo i tempi necessari ad erogare la prestazione, salasso terapia o donazione.

PO057

DIAGNOSI DI LABORATORIO EMOMETRICA DI LABORATORIO IN UN CASO DI MIELOMA MULTIPLO

V. Latella, B. Oliva, C. Garreffa, B. Modafferi

G.O.M. Bianchi Melacrino Morelli Reggio Calabria

Introduzione. Il mieloma multiplo è una neoplasia sostenuta dalla proliferazione di un clone neoplastico di natura plasmacellulare. Materiali e Metodi. Paziente uomo (75 anni) viene ricoverato presso l'U.O.C di Chirurgia Generale della nostra Azienda per un intervento programmato. Viene eseguito un emocromo di routine che esprime i seguenti dati: GB 4.330/mmc PLT 31.000/mL HGB 7.3 gr/dl MCV 88.0 fl. Risultati. Un'attenta analisi degli scattergram WDF e WPC pone il sospetto diagnostico di Mieloma Multiplo. E' ben evidente sullo scattergram WDF un cluster di distribuzione cellulare anomalao. Lungo l'asse della fluorescenza si estende una popolazione cellulare ad alta fluorescenza (HFLC, linfociti ad alta fluorescenza, verosimilmente plasmacellule). L'esame morfologico del sangue periferico esprime una quota di plasmacellule in periferia pari al 18%.
Si condividono i dati con i clinici dell' U.O.C. di Ematologia che sulla base di ulteriori esami (puntato midollare, citofluorimetria, valutazione clinica) concludono per una diagnosi di Mieloma Multiplo. Conclusioni Il mieloma multiplo rappresenta circa l'1,2% di tutti i tipi di cancro, e si stima che colpisca ogni anno in Italia circa 4.500 persone. È più frequente negli anziani (il 38% dei pazienti ha più di 70 anni), mentre solo il 2% dei pazienti ha meno di 40 anni al momento della diagnosi. Il MM è una malattia dall'andamento croncio-recidivante che non guarisce. Grazie all'introduzione di farmaci di nuova generazione, nei pazienti giovani si è assistito negli ultimi anni ad un progressivo allungamento della sopravvivenza mediana, che attualmente si attesta intorno ai 6-7 anni.

PO058

''Studio valutativo della performance analitica di due dispositivi POCT per il dosaggio glicemico in pazienti ospedalizzati''

V. Lombari, L.A. Catapane, R. Sabatino, A. Petruzziello

U.O.C. Patologia Clinica, Dipartimento dei Servizi Sanitari, A.O.R.N. "Sant'Anna e San Sebastiano", Caserta, Italia

Il monitoraggio dei livelli di glucosio nel sangue nei pazienti ospedalizzati è importante per la gestione ed il mantenimento delle concentrazioni normalizzate del glucosio ematico.Scopo di questo studio è stato valutare le performance analitiche di StatStrip® Glu/Ket (Nova Biomedical, Italia) con il sistema Contour®CARE (Ascensia Diabetes Care, Italia), utilizzando come riferimento il dosaggio del glucosio eseguito su analizzatore COBAS® 6000 (Roche Diagnostics, Svizzera) presso l'A.O.R.N. ''Sant'Anna e San Sebastiano'' di Caserta, Italia.Sono stati analizzati 25 pazienti, di cui 10 maschi (40%) e 15 femmine (60%) con età media di 46 anni, afferenti agli ambulatori dell'A.O.R.N. ''Sant'Anna e San Sebastiano'', al fine di valutare l'affidabilità e l'accuratezza di StatStrip® Glu/Ket anche in presenza di sostanze che notoriamente causano interferenze.Abbiamo valutato l'interferenza simulata in vitro, utilizzando diverse concentrazioni di Acido Ascorbico su campioni di glucosio a concentrazioni note (49,05 mg/dL; 243,70 mg/dL; 376,00 mg/dL). I risultati hanno mostrato che, addizionando il sangue di pazienti con differenti concentrazioni di Acido Ascorbico clinicamente rilevanti (0 mg/dL; 5 mg/dL; 10 mg/dL), StatStrip non mostra interferenze sul valore di glucosio riportato, al contrario di quanto osservato per il lettore Contour®CARE.

È stato poi valutato il valore del glucosio in diversi campioni a concentrazione nota (34,40 mg/dL; 242,20 mg/dL; 376,00 mg/dL) utilizzando campioni con differenti livelli di ematocrito clinicamente rilevanti (28%, 45%, 65%); i risultati ottenuti non hanno evidenziato interferenze. In particolare si evidenzia come, in corrispondenza di valori criticamente bassi ed alti di Ematocrito, il meter comparativo mostri una chiara tendenza alla sottostima del valore del glucosio misurato per un target glicemico medio ed alto.

Abbiamo, inoltre, valutato l'interferenza da Acido Ascorbico in riferimento all'Ematocrito. Con tale prova si ricreano le condizioni tipiche riscontrabili nel paziente critico, cioè campioni con livelli bassi di ematocrito (tipici dei pazienti in terapia intensiva), addizionati con concentrazioni terapeutiche di Acido Ascorbico. Il sistema StatStrip non ha mostrato interferenze dell'Ematocrito, neppure quando sono state addizionate concentrazioni terapeutiche di Acido Ascorbico (0 mg/dL; 5 mg/dL; 10 mg/dL), al contrario di quanto osservato per Contour®CARE.

Abbiamo infine valutato l'interferenza dello Xilosio sul valore di glucosio rilevato nei campioni di riferimento (53,15 mg/dL; 234,35 mg/dL; 339,10 mg/dL), tramite l'aggiunta di concentrazioni crescenti di Xilosio (0 mg/dL; 100 mg/dL; 200 mg/dL).

Il sistema StatStrip non mostra interferenze sul valore di glucosio riportato, a differenza del lettore Contour®CARE che mostra una chiara tendenza alla sovrastima della glicemia nei campioni trattati.

In conclusione, i risultati prodotti hanno permesso di dimostrare come una tecnologia certificata per l'analisi della glicemia POCT in ambito ospedaliero garantisca performance di livello effettivamente elevato in termini di accuratezza e precisione del dato glicemico, anche in condizioni cliniche alterate da molteplici interferenti.

PO059

Hidrox ® and Chronic Cystitis: Biochemical Evaluation of Inflammation, Oxidative Stress, and Pain

R. Siracusa, R. D'Amico, R. Di Paola

Università degli Studi di Messina, Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali

Interstitial cystitis/painful bladder syndrome (IC/PBS) is a chronic bladder condition characterized by frequent urination, inflammation, oxidative stress, and pain. The aim of the study was to evaluate the anti-inflammatory and antioxidant effects of an oral administration of Hidrox® (10 mg/kg) in the bladder and spinal cord in a rodent model of IC/BPS. The chronic animal model of cystitis was induced by repeated intraperitoneal injections of cyclophosphamide (CYP) for five consecutive days. Treatment with Hidrox ® began on the third day of the CYP injection and continued until the 10th day. CYP administration caused macroscopic and histological bladder changes, inflammatory infiltrates, increased mast cell numbers, oxidative stress, decreased expression of the tight endothelial junction (e.g., zonula occludens-1 (ZO-1) and occludin), and bladder pain. Treatment with Hidrox®was able to improve CYP-induced inflammation and oxidative stress via the nuclear factor erythroid 2-related factor 2 (Nrf2)/heme oxygenase 1 (HO-1) pathway. It was also able to reduce bladder pain which was aggravated by the activation of neuroinflammation in the central nervous system. In particular, Hidrox® reduced the brain-derived neurotrophic factor (BDNF), as well as the activation of astrocytes and microglia, consequently reducing mechanical allodynia. These results indicate that nutritional consumption of Hidrox® can be considered as a new therapeutic approach for human cystitis, increasing the conceivable potential of a significant improvement in the quality of life associated with a lowering of symptom intensity in patients with IC/BPS.

PO060

Regulation of Inflammatory and Proliferative Pathways by Fotemustine and Dexamethasone in Endometriosis

R. Fusco

Università degli Studi di Messina

Endometriosis is a common disease. Its pathogenesis still remains uncertain, but it is clear that cell proliferation, apoptosis and chronic inflammation play an important role in its development. This paper aimed to investigate the anti-proliferative and anti-inflammatory effects of a combined therapy with fotemustine and dexamethasone. Endometriosis was induced by intraperitoneal injections of uterine fragments from donor animals to recipient animals. Next, the pathology was allowed to develop for 7 days. On the seventh day, fotemustine was administered once and dexamethasone was administered daily for the next 7 days. On Day 14, the animals were sacrificed, and peritoneal fluids and lesions were explanted. In order to evaluate the gastrointestinal side effects of the drugs, stomachs were harvested as well. The combined therapy of fotemustine and dexamethasone reduced the proinflammatory mediator levels in the peritoneal fluid and reduced the lesions' area and diameter. In particular, fotemustine and dexamethasone administration reduced the heterogeneous development of endometrial stroma and glands (histological analysis of lesions) and hyperproliferation of endometriotic cells (immunohistochemical analysis of Ki67 and Western blot analysis of PCNA) through the mitogen-activated protein kinase (MAPK) signaling pathway. Combined fotemustine and dexamethasone therapy showed anti-inflammatory effects by inducing the synthesis of anti-inflammatory mediators at the transcriptional and post-transcriptional levels (Western blot analysis of NF¤B, COX-2 and PGE2 expression). Fotemustine and dexamethasone administration had anti-apoptotic activity, restoring the impaired mechanism (TUNEL assay and Western blot analysis of Bax and Bcl-2). Moreover, no gastric disfunction was detected (histological analysis of stomachs). Thus, our data showed that the combined therapy of fotemustine and dexamethasone reduced endometriosis-induced inflammation, hyperproliferation and apoptosis resistance.

PO061

Hericium erinaceus and Coriolus versicolor Modulate Molecular and Biochemical Changes after Traumatic Brain Injury

D. Impellizzeri , M. Cordaro, R. Di Paola

Università degli Studi di Messina

Traumatic brain injury (TBI) is a major health and socioeconomic problem affecting the world. This condition results from the application of external physical force to the brain which leads to transient or permanent structural and functional impairments. TBI has been shown to be a risk factor for neurodegeneration which can lead to Parkinson's disease (PD) for example. In this study, we wanted to explore the development of PD-related pathology in the context of an experimental model of TBI and the potential ability of Coriolus versicolor and Hericium erinaceus to prevent neurodegenerative processes. Traumatic brain injury was induced in mice by controlled cortical impact. Behavioral tests were performed at various times: the animals were sacrificed 30 days after the impact and the brain was processed for Western blot and immunohistochemical analyzes. After the head injury, a significant decrease in the expression of tyrosine hydroxylase and the dopamine transporter in the substantia nigra was observed, as well as significant behavioral alterations that were instead restored following daily oral treatment with Hericium erinaceus and Coriolus versicolor. Furthermore, a strong increase in neuroinflammation and oxidative stress emerged in the vehicle groups. Treatment with Hericium erinaceus and Coriolus versicolor was able to prevent both the neuroinflammatory and oxidative processes typical of PD. This study suggests that PD-related molecular events may be triggered on TBI and that nutritional fungi such as Hericium erinaceus and Coriolus versicolor may be important in redox stress response mechanisms and neuroprotection, preventing the progression of neurodegenerative diseases such as PD.

PO062

IMPORTANZA E RUOLO DEI METODI SEPARATIVI NELLA VALUTAZIONE DELL'HbA1c IN PRESENZA DI COMPOSTI INSTABILI DELL'EMOGLOBINA

M. MAFFEI[1], M. MOGNI[1], S. QUINTINO[2], G. FORNI[2], G. IVALDI[3], D. COVIELLO[1]

[1]di Genetica Umana, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Giannina Gaslini, Genova, Italia

[2]Centro Microcitemia e Anemie Congenite, Ospedali Galliera, Genova, Italia

[3]Già Laboratorio di Genetica Umana, Ospedali Galliera, Genova, Italia

I difetti dell'emoglobina (Hb) che possono compromettere la corretta quantificazione dell'emoglobina glicata (Hb A1c) di solito sono giudicati eventi rari. Tuttavia, in una popolazione come quella italiana con una particolare prevalenza di difetti talassemici e strutturali dell'Hb e con una eterogenea presenza di composti Hb o di associazioni di difetti, tali eventi risultano senz'altro più frequenti e in gran parte sottovalutati. Senza poter conoscere l'assetto Hb, nelle sue componenti fisiologiche e patologiche, il valore dell'Hb A1c potrebbe essere sottostimato, sovrastimato o erroneamente considerato normale. Una donna con una storia di emoglobinopatie familiari, è stata recentemente esaminata (assetto Hb mediante HPLC dedicato, emocromo e assetto marziale) all'inizio della gravidanza. Un significativo aumento dell'Hb A2 (8,6%) e una marcata microcitosi deponevano per la presenza di un tratto beta talassemico eterozigote confermato con l'analisi molecolare (Cod39). La successiva valutazione dell'Hb glicata mediante elettroforesi capillare (CE) mostrava un valore marcatamente ridotto di Hb A1c (13 mmol/mom), si confermava il valore elevato dell'Hb A2 e ritenuto che la sola presenza della beta talassemia non potesse giustificare il marcato decremento dell'Hb A1c. Pertanto, sono stati eseguiti ulteriori esami molecolari sospettando la presenza di fattori che potessero ridurre la sopravvivenza eritrocitaria e produrre la condizione di moderata anemia con ferro nella norma. Si sono così riscontrate ben due cause emolitiche: una emoglobinosi H (tetramero comunque non visibile per mancanza di catene beta globiniche normali) e una variante instabile delle catene beta globiniche (Hb Duarte in quantità >80% per la presenza della beta talassemia). Il caso che riportiamo costituisce senz'altro una particolare combinazione rappresentativa di alcune situazioni che potrebbero compromettere una corretta valutazione dell'Hb A1c. L'approfondimento molecolare prodotto, avvalorato anche dai risultati ottenuti con il metodo separativo per l'Hb glicata, potrà suggerire nel futuro il monitoraggio adeguato di possibili condizioni anemiche e l'utilizzo di adeguati indicatori dello stato glicemico della paziente.

PO063

B-Type and NT-proBNP natriuretic peptides as a prognostic marker of COVID-19 disease severity and outcome: which one is the best performer?

D. Palma[1,2], R. De Falco[1], M. Vano[1], G. Cardiero[1,2], C. Anastasio[1], C. Pagano[1], P. Autiero[1], M. Savoia[1], G. Fortunato[1,2]

[1]Dip. di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli Federico II, Via Sergio Pansini, 5 – 80131,Napoli, Italia

[2]CEINGE S.C.a r.l. Biotecnologie Avanzate, Via Gaetano Salvatore 486, 80145, Napoli, Italia

Introduction: B-type natriuretic peptide (BNP) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) are usually considered as equal diagnostic tools for heart failure. Increased concentrations of BNP and NT-proBNP in COVID-19 patients have already been reported. The aim of this study was to evaluate the usefulness of these markers and any potential difference between them in predicting COVID-19 prognosis.Materials and Methods: We retrospectively collected and analyzed data about 174 consecutive adult patients affected with COVID-19. The clinical course of COVID-19 before hospitalization and its related complications were also acquired. In particular, the presence of pre-existing diseases related to cardiac and pulmonary functions was recorded, alongside with diabetes and hypertension. BNP and NT-proBNP of each patients were collected at admission in hospital. BNP plasma concentrations were measured by chemiluminescent microparticle immunoassay on the ARCHITECT i2000SR system (Abbott Laboratories, Wiesbaden, Germany). NT-proBNP was also measured on the ARCHITECT i2000SR system by using the Alere assay (Roche Diagnostics GmbH, Mannheim, Germany). Results: BNP and NT-proBNP values were higher in in-hospital non-surviving patients ($p<0.001$). Despite a high correlation obtained by Spearman's rank correlation coefficient between these two variables (rho =0.716, $p<0.001$), receiver operating characteristics (ROC) curve analysis showed that NT-proBNP (AUC =0.951) performed better ($p=0.01$) than BNP (AUC =0.777). Kaplan–Meier analysis was performed by dividing the population into groups, based on whether NT-proBNP and BNP concentrations at admission were higher than the cut-offs resulting from ROC curves. Both log rank tests resulted significant ($p<0.001$), in the group of patients with NT-proBNP admission values lower than the cut-off showing an absence of fatal outcome, whereas the subgroup of patients with BNP admission values lower than cut-off included 53.84% of all non-survivors of this study.
Conclusion: NT-proBNP proved to be a better prognostic tool than BNP for fatal outcome in COVID-19 patients. In particular, our study highlighted that a value of NT-proBNP below the cut-off of 511 ng/L at admission led to no in-hospital mortality in our population.

PO064

''Comparison between SARS-CoV-2 Antigen Immunoassay and real-time RT-PCR in 392 asymptomatic COVID-19 positive patients.''

R. Sabatino[1], L.A. Catapane[1], C. De Falco[1], A. Petti[1], E. Tripaldelli[1], A. Annecchiarico[2], A. Petruzziello[1]

[1]U.O.C. Patologia Clinica, Dipartimento dei Servizi Sanitari, A.O.R.N. "Sant'Anna e San Sebastiano", Caserta, Italia.

[2]Direttore Sanitario, A.O.R.N. "Sant'Anna e San Sebastiano", Caserta, Italia.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is one of the most current concern to public health. The rapid identification of infected individuals is one of the main strategies adopted by governments to limit its diffusion. At present, the analytical methods used for the identification of SARS-CoV-2 infection are molecular, antigenic and serological. Although real-time RT-PCR represents the gold standard, one of the most important challenge is the adoption of an easy and rapid tool to identify SARS-CoV-2 infection. Aim of this study was to evaluate the performance of the Lumipulse® SARS-CoV-2 antigen assay. 392 oro-nasopharyngeal swabs from asymptomatic patients (208 males, 184 females, mean age:59.6) were collected at the Emergency Department of AORN Sant'Anna e San Sebastiano, Caserta, Italy to evaluate the performance of the Lumipulse® SARS-CoV-2 antigen assay based on chemiluminescence enzyme immunoassay in comparison with qualitative real-time RT-PCR test (TianLong Biotechnology Co, Suzhou, P.R.China). Of the 392 samples tested by Lumipulse® SARS-CoV-2 antigen test, 36.7% (n=144) were positive, while 59.2% (n=232) were negative. Following the Manufacturer's instructions, 16 (4.1%) samples were found to be in grey zone (range 1.67–9.19 pg/mL). Using real-time RT-PCR as reference, the antigen test allowed to detect the presence of the SARS-CoV-2 antigen, showing 96% sensitivity (95% CI, 93-99%) and 98% specificity (95% CI, 97-100%) with 97% PPV (95% CI, 93-99%) and 97% NPV (95% CI, 94-99%), +LR 55.14 (20.86, 145.74) and -LR 0.04 (0.02, 0.09) with an overall agreement rate of 97% (366/376). The median antigen concentration among positive samples was 688.3 pg/mL (range 11.74–5000 pg/mL) and 0.14 pg/mL (range 0.01–1.38 pg/mL) among the negative ones. Among the 10 discordant results, 6 were false negative (3 males and 3 females), and 4 false positive (2 males and 2 females). No one of them showed positivity for ORF1ab gene, whereas the mean cycle thresholds (Ct)-value of false negative samples was 33.5±1.35 (min 31.6–max 34.8) for N genes, with a median Ct-values of 34.1. No one of them showed positivity for ORF1ab gene. The antigen mean value of four false positives was 29.45±12.22 pg/mL with a median value of 25.63 pg/mL. Of the 16 samples found to be in grey zone, the real time RT-PCR assay revealed that 10 (68.7%) were negative and 6 (31.3%) were positive only for N gene with a mean Ct of 27.13±5.29. Furthermore, we divided our population into three groups based on Ct-values: in the group with higher Ct (¤25) the concordance with SARS-CoV-2 antigen positivity was 86.2% (95% CI, 73.7-98.7%), whereas in the second (15<Ct<25) and in

the third group (Ct□15) concordance was to be 100%. In addition, in the first group (Ct□25) the median antigen concentration was 46.84 pg/mL (range 0.06–811.94 pg/mL), while in the second group (15<Ct<25) was 688.3 pg/mL (range 19.90–5.000 pg/mL) and in the third group (Ct□15) was 4891.45 pg/mL (range 39.6–5.000 pg/mL). The data obtained show that the Lumipulse® SARS-CoV-2 antigen test may represent a valid support for the identification of SARS-CoV-2 infection even if in a scenario of higher prevalence (>10%), RT-PCR still remains mandatory to confirm any antigen results.The results showed that Lumipulse® has a high sensitivity and specificity (96% and 98%, respectively) even if sensitivity seems to be Ct-value dependent. Indeed, if we recalculate it in a Ct-dependent manner, we observed that sensitivity decrease from 100% to 86%, as the Ct-value increases.

PO065

VALUTAZIONE DELLE VARIAZIONI INDOTTE SULL'OSMOLALITÀ DALLA FORTIFICAZIONE IN CAMPIONI DI LATTE UMANO DI BANCA DONATO PASTORIZZATO E DI LATTE MATERNO PRETERMINE FRESCO

M.L. Mattei[1], N. Cini[1], F. Luceri[1], S. Rapi[1], S. Elia[2], M. Ciarcià[2], F. Miselli[2], C. Dani[2], A. Fanelli[1]

[1]SOD Laboratorio Generale, Azienda Ospedaliero Universitaria Careggi, Firenze, Italia

[2]SOD Neonatologia e Terapia Intensiva Neonatale DAI Materno-Infantile, Azienda Ospedaliero Universitaria Careggi, Firenze, Italia

Con la definizione nascite pretermine si identificano i bambini nati vivi prima del completamento della 37ma settimana di gravidanza con un'incidenza di 15 milioni l'anno in tutto il mondo. Una non adeguata alimentazione, soprattutto nei primi mesi di vita, è associata a ritardo di crescita e deficit neurocognitivi con ripercussioni nella vita adulta. L'American Academy of Pediatrics (AAP) raccomanda la fortificazione del latte materno e del latte umano di banca per la nutrizione dei neonati con peso <1500g per aumentare l'apporto di nutrienti. L'osmolalità del latte umano è di circa 300 mOsm/Kg, ma aumenta con la fortificazione. Valori troppo elevati (>500 mOsm/Kg) possono essere associati ad effetti potenzialmente avversi sulla tolleranza enterale e sul benessere del bambino prematuro. Per tale motivo l'AAP raccomanda un valore di osmolalità massimo di 450 mOsm/Kg, mentre Ellis et al (Arch Dis Child Fetal Neonatal 2019, 104, F333–F340) indicano un range di sicurezza di valori compresi tra 300-500 mOsm/Kg. Un altro fattore che può influire sull'osmolalità è la conservazione del campione. Attualmente il latte fortificato viene preparato poco prima della somministrazione direttamente nel reparto, dato che le raccomandazioni dell'OMS limitano a 24h il tempo massimo di conservazione. Ciò determina un'elevata variabilità nella preparazione, dovuta alla turnazione del personale, e un possibile aumento del rischio di contaminazione microbiologia. Il presente studio ha lo scopo di valutare le variazioni di osmolalità in base al tipo di latte, al tipo ed alla concentrazione di fortificante ed al tempo di conservazione. La stabilità dell'osmolalità per un tempo maggiore delle 24h costituisce un indice di buona conservazione e consentirebbe di allungare i tempi di utilizzo, permettendo di razionalizzare maggiormente i processi e gli ambienti di preparazione. I campioni di latte sono stati fortificati con 2 tipi di fortificanti (PreNAN FM85 e APTAMIL BMF) a due diverse concentrazioni (2% e 4%), aliquotati e conservati a +4°C. Per ogni campione è stata misurata l'osmolalità ai tempi 0, 6, 24, 48, 72h dopo la fortificazione. I risultati non hanno evidenziato una differenza nei valori dell'osmolalità rispetto al tipo di fortificante ed al tempo trascorso dalla preparazione.

PO066

IL LABORATORIO DI LIVELLO INTERMEDIO PER LA DIAGNOSTICA DELL'EMOGLOBINOPATIE ALLA LUCE DEI RECENTI FLUSSI MIGRATORI

M. Caldora[1], A. Di Domenico[1], M.A. Fernando Kurukulasuriya[1], M. Acunzo[1], F. Balzamo[1], G. Cardiero[2], G. Cangiano[1]

[1]U.O.C. Patologia Clinica P.O. Pellegrini, A.S.L. Napoli 1, Napoli
[2]Institute of Genetics and Biophysics "Adriano Buzzati Traverso", (I.G.B.-A.B.T. , C.N.R.)

INTRODUZIONE: I recenti flussi migratori da Paesi in via di sviluppo stanno determinando un cambiamento nella distribuzione delle emoglobinopatie anche nei Paesi industrializzati. Ciò rappresenta un problema emergente per la sanità pubblica e comporta l'esigenza di adeguare i metodi di laboratorio alla necessità di diagnosticare varianti emoglobiniche finora poco conosciute.
OBIETTIVO: Gli Autori adottano un algoritmo di laboratorio diagnostico – operativo che prevede test per la ricerca di eventuali emoglobine instabili (inclusioni endoeritrocitarie e test in tampone tris - isopropanolo a 37°C) al primo livello di indagine.
RISULTATI: Grazie a questo algoritmo molti campioni di sangue si sono rivelati di interesse perché appartenenti a portatori di nuove mutazioni come l'Hb Policoro, o di alfa talassemia, di Hb Neapolis, di Hb Sun Prairie, etc. Il corretto orientamento delle indagini di laboratorio di primo livello è stato di fondamentale importanza per i biologi molecolari che hanno caratterizzato queste varianti emoglobiniche.
CONCLUSIONI: Per i cambiamenti di tipologia e di distribuzione delle emoglobinopatie anche nei paesi indusrtializzati, gli Autori ritengono utile ampliare lo screening di base con metodi in grado di rilevare varianti emoglobiniche oggi meno rare che in passato, a causa dei flussi migratori.

PO067

''Valutazione della sieroprevalenza da SARS-CoV-2 nella popolazione di dipendenti vaccinati dell'A.O.R.N. Sant'Anna e San Sebastiano, Caserta''

R. Sabatino, G. Loquercio, L.A. Catapane , C. De Falco, E. Tripaldelli, A. Petruzziello

U.O.C. Patologia Clinica, Dipartimento dei Servizi Sanitari, A.O.R.N. "Sant'Anna e San Sebastiano", Caserta, Italia.

La vaccinazione anti-SARS-CoV-2/COVID-19 rappresenta la principale arma a disposizione della comunità scientifica per contrastare la diffusione del coronavirus SARS-CoV-2 e ridurre l'impatto della pandemia da COVID-19. L'obiettivo dei vaccini è quello di produrre una risposta immunitaria rapida e specifica al fine di neutralizzare il virus ed impedire l'infezione cellulare. I vaccini forniscono una protezione diretta contro le evoluzioni gravi della malattia COVID-19 e contribuiscono alla riduzione e alla prevenzione dell'ospedalizzazione e dei decessi associati all'infezione da SARS-CoV-2. In tale contesto, lo scopo del nostro studio è stato quello di valutare la sieroprevalenza IgG anti-spike SARS-CoV-2 nella popolazione dei dipendenti afferenti all'U.O.C. Patologia Clinica dell'A.O.R.N. ''Sant'Anna e San Sebastiano'' di Caserta sottoposta a vaccinazione COVID-19 con il vaccino Comirnaty di Pfizer/BioNTech (2 dosi a distanza di 21 giorni). A tal fine si è provveduto a monitorare l'andamento del titolo anticorpale IgG anti-spike a 15, 30, 45, 60 e 75 giorni dopo la somministrazione della II dose vaccinale. La determinazione qualitativa e quantitativa degli anticorpi IgG anti-spike è stata eseguita tramite dosaggio chemiluminescente a cattura di microparticelle (CMIA) utilizzando il sistema Architect della Abbott Laboratories in campioni di siero ed un cut off <50 AU/mL per campioni considerati negativi ed un cut off >50 AU/mL per campioni considerati positivi. L'azienda produttrice ha dichiarato un valore >3550 AU/mL corrispondente a una probabilità del 95% di essere pari o superiore alla diluzione 1:160 del test PRNT (test di neutralizzazione con riduzione delle placche). In linea con il primo Standard Internazionale WHO (IS) per l'attività di legame delle immunoglobuline anti SARS-CoV-2, i risultati sono riportati anche in Binding Antibody Unit (BAU) per mL, al fine di consentire di confrontare risultati ottenuti con test diversi. La popolazione studiata è costituita da 21 femmine (55.3%) e 17 maschi (44.7%) con un'età mediana di 52.4 anni. I risultati ottenuti, dimostrano in primo luogo l'efficacia della vaccinazione, avendo la totalità dei dipendenti sviluppato anticorpi ◘3550 AU/mL dopo 15 giorni dalla seconda dose vaccinale. In nessuno dei 38 dipendenti si è osservato un decremento del titolo anticorpale <50 AU/mL dopo 75 giorni dalla seconda dose vaccinale; 14 pazienti hanno mostrato un decremento del titolo anticorpale che li collocava nell'intervallo compreso tra 3550 e 50 AU/mL (504.1-7.1 BAU). Inoltre, l'andamento del titolo anticorpale IgG anti-spike mostra un decremento medio del 74.9% a 75 giorni dalla somministrazione della seconda dose. Quanto tale decremento abbia rilevanza sulla durata dell'immunità è uno dei quesiti più discussi a livello scientifico. L'ECDC ha dichiarato che sebbene le risposte immunitarie siano evidenti nel periodo che segue l'infezione o la

vaccinazione, esse tendono a diminuire nel tempo, con un numero decrescente sia di cellule linfocitarie che di anticorpi sierici. Tuttavia, la valutazione dell'impatto di tale declino immunitario richiederà un'attenta valutazione sia in termini di riduzione quantitativa, che del cambiamento qualitativo o funzionale.

PO068

DIAGNOSI DI HBE ETEROZIGOTE NEL LABORATORIO DI LIVELLO INTERMEDIO

M. Caldora[1], A. Di Domenico[1], M.A. Fernando Kurukulasuriya[1], M. Acunzo[1], F. Balzamo[1], G. Cardiero[2], G. Cangiano[1]

[1]U.O.C. Patologia Clinica P.O. Pellegrini, A.S.L. Napoli 1, Napoli
[2]Institute of Genetics and Biophysics "Adriano Buzzati Traverso", (I.G.B.-A.B.T. , C.N.R.)

INTRODUZIONE: L'emoglobina E (¤2 ¤2 26Glu # Lys), molto diffusa nei vari Paesi dell'Asia, è la seconda emoglobina anomala nel mondo. È dovuta ad una mutazione della catena beta che la rende instabile quando è esposta ad agenti ossidanti, per labilizzazione dei legami tra i monomeri che costituiscono il tetrametro dell'emoglobina. L'HbE nella forma eterozigote è clinicamente asintomatica, mentre genera quadri clinici più o meno gravi quando è trasmessa in associazione con l'alfa talassemia, con la beta talassemia e/o con varianti emoglobiniche comuni. I recenti flussi migratori hanno determinato un incremento di questa variante anche in Italia ove in associazione con altre emoglobinopatie si possono generare eterozigosi composte.
OBIETTIVO: Da ciò l'esigenza di un iter diagnostico di laboratorio che ottimizzi la diagnosi preventiva e quindi l'orientamento all'eventuale studio molecolare successivo nei casi in cui si rilevi una frazione emoglobinica a comportamento cromatografico tipo HbE.
L'iter diagnostico prevede:
Esame emocromocitometrico
Cromatografia liquida ad alta prestazione
Elettroforesi alcalina
Test di stabilità
Elettroforesi capillare
CONCLUSIONI: Gli Autori hanno elaborato un algoritmo di laboratorio che permetta di caratterizzare l'HbE nel laboratorio intermedio e/o di orientare in modo più mirato l'eventuale studio molecolare successivo.

PO069

D-DIMER POINT-OF-CARE TEST (POCT): COMPARISON WITH LABORATORY GOLD STANDARD TEST AND ITS SCREENING VALUE IN PATIENTS WITH SUSPECTED VENOUS THROMBOEMBOLISM

A. Biasotto[1], M. Cipriani[2], D. Nigris[2], E. Fontanini[2], R. Giacomello[1,2]

[1]Dep. of Medicine (DAME), University of Udine, Udine
[2]Inst. of Clinical Pathology, Dep. of Laboratory Medicine, Azienda Sanitaria Universitaria Friuli Centrale (ASUFC), Udine

The term "D-dimer" identifies the soluble peptides produced after the systematic degradation of cross-linked fibrin through the fibrinolytic mechanism, representing a valuable marker of activation of coagulation and fibrinolysis. Due to the low specificity of the test for thrombotic disorders (~40-45%), D-dimer measurement alone is not employed for the effective diagnosis of venous thromboembolism (VTE) and must be used in combination with predictive scores and imaging techniques. The absence of a rise in D-dimer values, instead, may help excluding the diagnosis of VTE; in the emergency setting it is necessary to employ a very sensitive test (~98%) with a high negative predictive value (NPV).
The aim of the study is to compare a quantitative POCT D-Dimer test with the laboratory gold standard test in patients with suspected VTE presenting to the emergency department (ED) of ASUFC.
Twenty consecutive adult subjects with clinical suspicion of TVE were enrolled in the study. The concentrations of D-Dimer in sodium citrated plasma specimens were measured using both the fluorescence immunoassay POCT LumiraDx™ D-Dimer Test (LumiraDx Ltd, Alloa, UK) and the Enzyme Linked Fluorescent Assay (ELFA) VIDAS® D-Dimer Exclusion™ II (bioMérieux SA, Marcy l'Etoile, France).The upper limits of normal (ULN) declared by the manufacturers, respectively 533 FEU ng/ml for LumiraDx™ and 500 FEU ng/ml for VIDAS®, were adopted as the cut-off for diagnosis exclusion.
Median values obtained with the two assays were, respectively, 972 FEU ng/ml for LumiraDx™ e 563 FEU ng/ml for VIDAS®; no significant statistical difference between the two methods was found with Mann-Whitney test (p=0,1167). Considering VIDAS® as the reference method, Spearman rank correlation was very good (r= 0,943; 95% CI 0,859-0,978, p<0,0001) as well as the Passing-Bablok regression (y = 79,300 + 1,188 x); Bland-Altman agreement showed a mean difference of +363,6 with all the values comprised between ±2SD. The overall concordance rate in excluding (<ULN) or considering a possible VTE diagnosis (¤ULN) between the two tests was 85%.
In conclusion, LumiraDx™ test was comparable with the laboratory VIDAS® assay and was sufficiently accurate to be employed as a screening tool in patients with suspected VTE in ED.

PO070

Un utile riscontro occasionale di variante emoglobinica

M. Carta[1], G. Barberio[2], L. Forner[1], G. Marzotto[1], M.G. Meneghini[1], L. Pressi[1], A. Rodolfi[1], D. Giavarina[1]

[1]Medicina di laboratorio, AULSS 8 Berica, Vicenza
[2]Medicina di laboratorio, AULSS 2 Marca Trevigiana, Treviso

L'elettroforesi capillare consente di visualizzare la presenza di varianti emoglobiniche durante la determinazione della concentrazione di emoglobina glicata (HbA1c).Anche se la maggior parte delle varianti (HbC, HbS, HbD, HbE) non interferisce con la determinazione di HbA1c, in altri casi può essere presente una sotto o sovrastima del dato ottenuto. Inoltre la segnalazione di una variante emoglobinica può essere utile per un corretto inquadramento clinico-diagnostico.Caso clinico: DS è un uomo di 38 anni proveniente dalla Serbia. Dopo un riscontro di glicemia (121 mg/dL) compatibile con IFG (Impaired Fasting Glucose) torna per ripetere glicemia e dosare HbA1c. Capillarys Sebia evidenzia un tracciato definito "atipico" con la presenza di uno sdoppiamento della frazione HbA0 che impedisce una corretta integrazione delle frazioni: viene evidenziato un picco relativo ad HbA1c ma non ne viene fornito il dosaggio.L'assetto emoglobinico su Capillarys Sebia evidenzia la presenza di una variante pari al 43% in zona Z8.Presso il laboratorio di Treviso vengono eseguiti i test per valutare l'eventuale presenza di una variante instabile: il test di Carrell (termolabilità a 37°C) ed il test al BCB (Blu Brillante di Cresile) per la ricerca di inclusi endoeritrocitari. Il test di Carrell risulta positivo mentre il test al BCB non è dirimente.Il campione viene inviato presso il Laboratorio di genetica medica dell'Ospedale Maggiore di Milano per l'analisi molecolare dove viene riscontrata la presenza della mutazione c.304 G>C del gene beta globinico, compatibile con lo stato di portatore di Hb Rush.Hb Rush è una emoglobina lievemente instabile che in combinazione con HbE o con trait beta talassemico può dare un quadro clinico simile a quello di una talassemia intermedia.La valutazione del cromatogramma ha permesso di segnalare al medico curante l'impossibilità di fornire un dato accurato di HbA1c in questo paziente, sia per i limiti tecnici dovuti alla presenza di una variante emoglobinica che migrando in zona molto vicina ad HbA non è da essa distinguibile, ma anche perché trattandosi di una variante instabile potrebbe accompagnarsi ad un accorciamento dell'emivita eritrocitaria. Inoltre il paziente e la sua partner sono stati inseriti un appropriato percorso di consulenza genetica.

PO071

Evaluation of SARS-CoV-2 S-RBD IgG and NAb antibody levels in vaccinated subjects.

S. Sarubbi[1], M. Pelagalli [1], M. Nuccetelli[2], E. Nicolai[1], M. Pieri [1,2], S. Bernardini[1,2]

[1]Department of Experimental Medicine, University of Tor Vergata, Via Montpellier 1, 00133 Rome, Italy
[2]Department of Laboratory Medicine, "Tor Vergata" University Hospital, Viale Oxford 81, 00133 Rome, Italy

BACKGROUND: Covid 19 disease represents the largest public health emergency.Following the spread of Covid vaccines, it has become of central importance for laboratories to assess immunity, protection against SARS-CoV-2 and whether booster shots will be needed.Aims of the study are:Detection of antibodies following SARS-CoV-2 vaccination;Monitoring of anti-Spike SARS-CoV-2 antibody levels (S-RBD) induced by vaccination;Monitoring of neutralizing SARS-CoV-2 antibody levels (NAb) induced by vaccination;Identifying antibody levels of previously infected subjects;Correlation between antibody levels and type of vaccine used;METHODS: A total of 70 workers from the University 'Tor Vergata' in Rome and from the University hospital, were monitored during their vaccination program (Astra Zeneca and Pfizer-BioNTech vaccines). Serum samples collected at different time points 10, 20, 35, 50, 80, 120, 150 days after the first and second dose of vaccine. A chemiluminescent assay for the quantitative determination of SARS-CoV-2 S-RBD IgG and NAb was used, performed on the fully automated Mindray CL 1200i analytical system.RESULTS: The antibody concentrations detected in the two groups of workers after the first dose made us able to distinguish them into three different groups: subjects previously naturally infected, with higher antibody production; uninfected with the lowest antibody concentration values and a group with antibody values in the middle between the two, probably workers with asymptomatic infection. The amount of S-RBD and NAb antibodies in vaccinated subjects with pre-existing immunity is almost as twice as high than in naive vaccinated subjects at the same time points. Both vaccinations, although with differentiated antibody concentrations, reach a peak about 30 days after the first dose and then decrease up to 150 days, stopping at a steady state of around 150 - 200 BAU/ml. CONCLUSIONS: These data highlights: the importance of serologic testing before vaccination in order to distinguish previously infected asymptomatic persons thus avoiding possible side effects such as the development of antibody-dependent enhancement (ADE); the importance of completing the two doses recommended for non-infected subjects in order to achieve strong levels of immunity.

PO072

Carbamazepine and carbamazepine-10,11-epoxide assessment with a reference and a routine assay

D. Negrini, W. Magon, D. Peserico, G. Lippi, E. Danese

Section of Clinical Biochemistry, University of Verona, Verona, Italy

Introduction
Carbamazepine is a common antiepileptic drug used to block fast voltage-dependent sodium channels for treatment of epilepsy and other neurologic conditions such as trigeminal neuralgia.
Carbamazepine-10,11-epoxide, one of its metabolites, is pharmacologically active and its values increase with concomitant use of other anticonvulsants.
This study was aimed to compare the method used in our routine laboratory to a reference method (LC-MS/MS) for carbamazepine assay, and then to compare the concentrations of the drug and one of its main metabolites.
Methods
Plasma samples were collected from patients needing carbamazepine assessment. For each plasma sample, carbamazepine was assayed on a Roche Cobas c702 with proprietary kit (immunoassay) on the same day of collection, whilst residual sample volume was anonymized, aliquoted and kept at -20°C until LC-MS/MS analysis.
LC-MS/MS analysis was performed with Shimadzu Nexera X2 UHPLC coupled to AB Sciex 4500 MD MS/MS. ClinMass Antiepileptic Drugs kit (Recipe GmbH) was used for measuring Carbamazepine and Carbamazepine,10-11,epoxide, after method verification in agreement with local certification procedures.
Results
The study population included 22 subjects (6 females and 16 males; median age and range: 42 and 0-80 years). The difference between immunoassay and LC-MS/MS was non-significant (paired t-test, p=0.14), with Pearson's correlation (r) of 0.978.
LC-MS/MS concentrations of carbamazepine and its epoxide values were instead non significantly correlated (r=0.579), displaying significant differences (paired t-test, p<0.05). The ratio calculated between carbamazepine and its epoxide displayed a broad range of values, between 3.37 and 12.55 (mean, 6.62±2.27).
Conclusions
Considering the clinical significance of carbamazepine assessment as part of the therapeutic drug monitoring, we confirm the validity of the immunoassay tested as an easier and faster alternative for routine quantification of plasma carbamazepine concentration.
Nonetheless, when patients have uncontrolled symptoms or are challenged by dose adjustment, a more comprehensive assessment of carbamazepine metabolites should be considered.

PO073
ANEMIA E FERRITINA NEI PAZIENTI COVID-19

M.P. Monaco, L. Frascogna, A. Lombardo, I. Piccirillo

Lab.Patologia Clinica,Osp.San Giuliano,Giugliano

Introduzione
L'infezione da SARS-COV-2 si può presentare con manifestazioni cliniche diverse, che vanno dal portatore asintomatico alla grave compromissione respiratoria e\o multiorgano. (1)
Numerosi biomarcatori legati all'infiammazione (PCR, IL-6, D-DIMERO) sono stati studiati e valutati per il loro probabile ruolo prognostico nell'evoluzione della patologia, soprattutto se associati a comorbidità e all'età del paziente.
Scopo del lavoro
Nel nostro studio abbiamo posto particolare attenzione alla valutazione dell'anemia e dell'assetto del ferro nei pazienti COVID.
Materiali e metodi
Abbiamo monitorato circa 160 pazienti (M/F 2:1) età media 55 anni, ricoverati presso il reparto COVID, dopo esito positivo del tampone molecolare in RT-PCR per SARS-COV-2.
Per ogni paziente è stato eseguito esame emocromocitometrico con ADVIA 2120 Siemens, markers bioumorali volti a valutare l'assetto del ferro e gli indici di infiammazione con ALINITY Abbott, test coagulativi di base con ACL TOP. (2)
Conclusioni
Dal nostro studio è emerso che i pazienti COVID-19 con il progredire della malattia mostrano livelli più bassi di emoglobina e sideremia, livelli più elevati di PCR e ferritina con differenze significative tra casi moderati e gravi e sopravvissuti e non sopravvissuti.
Infatti, l'aumento della ferritina e la riduzione della sideremia sono importanti indici di gravità clinica legati sia allo sviluppo di anemia che di ipossia nei pazienti COVID-19 che necessitano di ossigenoterapia.
Bibliografia
1) Ruan Q. et al. Clinical predictors of mortality due to COVID 19 based on an analisys of data of 150 patients from Wuhan, China. Intensive Care Med. 2020; published online March 3
2) Ganz T. Anemia of inflammation. N. Eng. J Med. 2019; 381:1148-57

PO074
HBC E VARIANTI SIMILI: DIAGNOSI DIFFERENZIALE PER LA PREVENZIONE DELLE FORME GRAVI

M. Caldora[1], A. Di Domenico[1], M.A. Fernando Kurukulasuriya[1], M. Acunzo[1], F. Balzamo[1], G. Cardiero[2], G. Cangiano[1]

[1]U.O.C. Patologia Clinica P.O. Pellegrini, A.S.L. Napoli 1, Napoli
[2]Institute of Genetics and Biophysics "Adriano Buzzati Traverso", (I.G.B.-A.B.T. , C.N.R.)

INTRODUZIONE: L'HbC è un'emoglobina variante dovuta ad una mutazione del gene codificante per la beta globina che determina la sostituzione di un residuo amminoacidico di acido glutammico con uno di lisina in posizione 6. Molto diffusa in Africa Occidentale (Senegal, Nigeria, Nuova Guinea, ecc) è clinicamente asintomatica nella forma eterozigote ed in quella omozigote ma in associazione con l'HbS genera la Sickle Cell Disease (SCD), forma clinicamente grave. L'intensificarsi dei flussi migratori , ha determinato una maggiore diffusione di questa variante emoglobinica anche in Italia, facendo nascere l'esigenza di adeguare metodi di laboratorio alla necessità di identificare e caratterizzare l'HbC.
OBIETTIVO: L'accurata diagnosi differenziale tra l'emoglobinopatia da HbC e quella da altre varianti Hb O-Arab, HbC Harlem e HbC Ziguinchor, è importante perché le loro rispettive interazioni con HbS sono associate a quadri clinici di diversa gravità.
RISULTATI: Le tre varianti (Hb O-Arab, HbC Harlem e HbC Ziguinchor) presentano la stessa migrazione elettroforetica (come l'Hb A2), ma Hb O-Arab in HPLC ha un picco cromatografico minore nella D window, l'Hb C Harlem ha test di solubilità e falciformazione positivi, la HbC Ziguinchor è falcizzante ed elettroforeticamente migra in posizione più catodica rispetto all'Hb A2.
CONCLUSIONI: La diversa gravità del quadro clinico di queste varianti in associazione con l' HbS ha spinto gli Autori a descrivere un iter diagnostico differenziale mediante cromatografia a scambio cationico ed elettroforesi dell'emoglobina.

PO075

Urinary protein electrophoresis in children with renal disease

A. Vasco[1], E. Scopetta[1], M. Ciarlo[1], F. Caprio[2], E. Zulli[3], C. Pecoraro[4], M. Savoia[1,5]

[1]*Dep. of Molecular Medicine and Medical Biotechnology, University Federico II, Naples*

[2]*Dep. of Medical Translational Sciences, University of Campania, Luigi Vanvitelli, Naples*

[3]*Dep. of Public Health, University Federico II, Naples*

[4]*Unit of Pediatric Renal Transplantation, Nephrology and Dialysis, Santobono Children Hosp., Naples*

[5]*Dep. of Laboratory and Transfusion Medicine, AOU Federico II, Naples*

The presence of protein in urine is a common laboratory finding in children. Although proteinuria is usually benign in the form of transient or orthostatic, the persistent type can be a marker of a serious underlying renal disease.
Orthostatic proteinuria is a condition without clinical significance and is the most common type observed in children, especially in adolescent males. Persistent proteinuria can be classified as glomerular or tubular in origin.
Glomerular type is due to increased permeability of the glomerular basement membrane. Two forms are distinguished: the selective one, characterized by an early and reversible glomerular lesion with loss mainly of albumin (ALB) and transferrin (Tf); the non-selective one, indicative of an advanced glomerular lesion with loss of high molecular weight protein as polyclonal immunoglobulins (Ig).
Tubular proteinuria is due to an increased excretion of normally filtered low molecular weight proteins, especially alpha-1-microglobulin (A1M), beta-2-microglobulin (B2M), retinol binding protein and cystatin C (CysC), because of impaired reabsorption by the proximal tubules.
This preliminary study has involved 14 patients from Pediatric Nephrology and Dialysis of Santobono Children Hospital of Naples: M/F=9/5; age 1-18 y; proteinuria ranging from 162 to 4330 mg/die.
Urinary protein electrophoresis performed in agarose electrophoresis (Hydrasys2, Sebia, Italy) has shown that: glomerular proteinuria is detected in all patients for the concurrent presence of ALB and Tf; in 7 patients Ig are observed too, as demonstration of an advanced glomerular lesion in these cases; in 4 patients the presence of low molecular weight proteins is detected, particularly B2M and CysC, an accurate biomarker for the detection of tubular dysfunction.
Urinary protein analysis and other investigations performed have allowed us to confirm the following diagnoses: 3 patients with orthostatic proteinuria; 1 with cystinosis; 3 with nephrotic syndrome; 1 with renal hypodysplasia; 1 with Dent syndrome; 1 with Gitelman syndrome; 4 with undefined diagnosis proteinuria, awaiting genetic analysis.
Urinary protein electrophoresis has led to identify the type of kidney damage and its extent and was fundamental in the diagnostic and therapeutic process.

PO076

Cannabidiol determination on peripheral blood using volumetric absorptive microsampling (VAMS) and LC-MS/MS: a useful tool for TDM of epilepsy patients

F. Pigliasco[1], M. Marcenaro[2], P. Striano[2], A. Riva[2], A. Cafaro[1], T. Lomonaco[3], G. Tripodi[1], G. Cangemi[1]

[1]*Chromatography and Mass Spectrometry Section, Central Laboratory of Analysis, IRCCS Istituto Giannina Gaslini, Genoa*

[2]*Pediatric Neurology and Muscular Diseases Unit, , IRCCS Istituto Giannina Gaslini, Genoa, Italy*

[3]*Department of Chemistry and Industrial Chemistry, University of Pisa, Italy*

Interest in cannabis-based therapies has recently increased, due to the availability of cannabidiol (CBD) for the treatment of epilepsy without psychoactive effects. Therapeutic drug monitoring can prevent drug interactions and minimize drug toxicity. The aim of this work is to evaluate volumetric absorptive microsampling (VAMS) from capillary blood as an alternative strategy for therapeutic drug monitoring (TDM) in patients treated with the newly available GW-purified form of cannabidiol (Epidiolex®). A fast ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) coupled to an online sample preparation system analysis was carried out on a Thermo Scientific Ultimate 3000 LC system coupled to a TSQ Quantiva triple quadrupole for the quantification of cannabidiol (CBD) and, in addition, delta-9-tetrahydrocannabinol (#9-THC). After validation using European Medicine Agency (EMA) guidelines the method was applied to samples obtained by finger prick of five pediatric patients treated with Epidiolex® for Dravet syndrome and the results were compared to those obtained from venous blood and plasma. The method is linear in the range of 1–800 #g/L for both CBD and THC with intra- and inter-day precisions ranging from 5% to 14% and accuracies from -13% to +14% starting from 30 #L of sample. Stability in VAMS is ensured for up to 4 weeks at 25 0C thus allowing simple delivery. There was no difference (p = 0.69) between concentrations of CBD measured from VAMS sampled from capillary or venous blood (range: 52.19–330.14 or 72.15–383.45 #g/L) and those obtained from plasma (range: 64.3–374.09 #g/L). This proof-of-concept study suggests that VAMS allows monitoring of CBD plasma levels and can offer valuable support
for personalized therapy in refractory epilepsy.

PO077

Effects of Hemoglobin Osu-Christiansborg on HbA1c measurement.

A.R. Cussigh[2], S. Cmet[1], E. Fontanini[1]

[1]*Dip. Medicina (DAME), Università di Udine, Udine*
[2]*Ist. Patologia Clinica, ASUFC, Udine*

Many clinical situations like thalassemia and structural hemoglobin (Hb) variants make accurate estimation of glycated hemoglobin (HbA1C) difficult. An unusually low or high HbA1C results should alert about possibilities of Hb variants. Awareness about such interferences in different assay methods is essential while reporting or interpreting HbA1C results.We report a case of a 68 year old woman who had an elevated HbA1c of 8.8% (72 mmol/mol) performed on cation exchange HPLC (Tosoh HLC-723 G11 variant mode); this method has high processivity cause of a short run time, but makes the instrument vulnerable to interference from Hb variants and other substances. The chromatogram also revealed the presence of Hb variant: the sample was analyzed on Tosoh HLC-723 G11 in the B-Thalassemia mode and it result in a peak of 42% in D window retention time.Glucose and fructosamine were also tested spectrophotometrically (Roche Diagnostics Cobas8000) to verify correlation and accuracy of the HbA1c value. The fructosamine was 306 uMol/L (205-285) and blood glucose 116 mg/dL (74-109).Even if it is well known that there isn't always good correlation between HbA1c, glucose and fructosamine values, we decided to verify HbA1c accuracy with to two other methods: in capillary electrophoresis (CE Sebia Capillarys Flex Piercing) HbA1c was 6.0% (42 mmol/mol) and latex agglutination immunoassay (Nihon Kodhen CHM-4100 Celltac chemi HbA1c) revealed a 5.8% (40.0 mmol/mol) value.CE hemoglobin mode confirm the variant in HbD zone. DNA analysis was performed for the final diagnosis and confirmation of the Hb variant. Advanced technologies as Next Generation Sequencing (Thalassemia Devyser) and Sanger sequencing were used.The variant was identified as Hemoglobin Osu-Christiansborg CD52 GAT>AAT Asp>Asn (HBB:c.157 G>A) in heterozygosis.Hb Osu-Christiansborg is rare non pathological beta chain globin variant that is generally only recognized as a part of routine screening. In 2005 Kapoor et al. described a case of falsely high HbA1c because of Hb Osu-Christiansborg variant using HPLC method.Through this case we want to highlight the importance of capillary electrophoresis for accurated HbA1c measurement in presence of hemoglobinic variant and its simultaneously signaling also in routine laboratory screening.

PO078

Development and validation of UHPLC–MS/MS methods for the quantification of aciclovir and ganciclovir in plasma and dried plasma spot

F. Pigliasco[1], S. Barco[1], A. Cafaro [1], R. Simeoli[2], A. Magnasco[3], M. Faraci[4], B.M. Goffredo[2], G. Cangemi[1]

[1]*Chromatography and Mass Spectrometry Section, Central Laboratory of Analysis, IRCCS Istituto Giannina Gaslini, Genoa*
[2]*Metabolic Pathology Laboratory, IRCCS Ospedale Pediatrico Bambino Gesù, Rome, Italy*
[3]*Pediatric Nephrology, IRCCS Istituto Giannina Gaslini, Genoa, Italy*
[4]*Department of Pediatric Hematology and Oncology, IRCCS Istituto Giannina Gaslini, Genoa, Italy*

The role of TDM of aciclovir (A) and valganciclovir/ganciclovir (V/G) in critically ill patients is still a matter of debate. More data on dose-concentration relationship might therefore be useful, especially in pediatrics where clinical practice is not adequately supported by robust PK studies. We have developed and validated a new LC-MS/MS micromethod to simultaneously quantify A and G from plasma and dried plasma spot (DPS).
The method is based on a rapid organic extraction from DPS and separation on a reversed-phase C-18 UHPLC column after addition of deuterated internal standards. Accurate analytes quantification using SRM detection is then obtained using a Thermofisher Quantiva triple quadruole MS coupled to a Ultimate 3000 UHPLC. It has been validated following international (EMA) guidelines for bioanalytical method validation and has been tested on samples from pediatric patients treated with A, V or G for cytomegalovirus infection following solid organ or bone marrow transplantation. Concentrations obtained on plasma and DPS were compared using Passing Bablok and Bland Altman statistical tests.
The assay is linear over wide concentration ranges (0.033-40 mg/L) in both plasma and DPS for A and G, suitable for the expected therapeutic ranges for both Cmin and Cmax, accurate and reproducible in the absence of matrix effects. Results obtained from plasma and DPS were interchangeable. The application of the LC-MS/MS method allowed us to obtain a very specific, sensitive, and rapid quantification of the antiviral drugs starting from very low volumes (50 #L) of plasma samples and DPS. The stability of analytes for at least 30 days allows cost effective shipment and storage at room temperature.
Our method is suitable for PK studies and TDM allowing to study TDM-guided dosing of the antivirals in critically ill pediatric patients.

PO079

La complessa determinazione della Malattia da Catene Pesanti

D. Debbia, M.R. Cucinelli, T. Trenti, P. Natali

Medicina di Laboratorio-Dip. Interaziendale integrato di Med. di Laboratorio e Anatomia Patologica AOU- AUSL Modena

Introduzione: la malattia da catene pesanti (HCD) è definita come un raro disordine a carico dei linfociti B (plasmacellule; linfociti plasmacitoidi) caratterizzata dalla produzione di una proteina a catena pesante gamma troncata, incapace di collaborare con le catene leggere nella formazione di una molecola di immunoglobulina completa. La catena pesante anormale viene rilevata nelle urine e/o nel siero senza una catena leggera associata. A seconda dell'isotipo della catena pesante alterata, queste condizioni possono essere sotto classificate come malattia della catena pesante alfa, gamma o mu. A causa del difettoso assemblaggio della immunoglobulina, il tracciato elettroforetico può apparire normale o caratterizzato da ipogammaglobulinemia. E' necessario eseguire un approfondimento con immufissazione su gel d'agarosio (IFE) (Sebia, Firenze) utilizzando una specifica combinazione di antisieri. Metodi: in un tubo si miscela 10 µl di siero con 100 µl di anti-□ e in un secondo tubo 10 µl di siero+100 µl di anti-□ e vengono incubati over night a 4°C. Si centrifuga a 10000 rpm e si recupera il surnatante su cui si esegue IFE. Nelle prime 3 posizioni si semina il contenuto del primo tubo e nelle restanti tre quello del secondo tubo, aggiungendo nella prima e nella terza posizione del gel l'antisiero della catena pesante coinvolta; nelle restanti posizioni gli antisieri anti-□ e anti-□. Risultati: si osserva su IFE una banda monoclonale nelle sole posizioni con antisiero anti catena pesante, e l'assenza di bande di catene leggere □-□. Questo conferma la presenza di una sola catena pesante non legata ad alcuna catena leggera e la diagnosi di malattia da catene pesanti. Conclusioni: la diagnosi avviene in laboratorio tramite la dimostrazione che la immunoglobulina coinvolta è troncata delle catene leggere □ e □. Infatti le manifestazioni cliniche variano a seconda dell'isotipo della catena pesante coinvolta, da assenza di sintomi a sintomatologia simile a un linfoma aggressivo. La maggior parte dei pazienti sono asintomatici e quindi si possono considerare delle MGUS ma con un elevato rischio di progressione verso linfoma o mieloma, proprio per questo la corretta e preventiva diagnosi è fondamentale per trattare adeguatamente il paziente.

PO080

UHPLC-MS/MS Analysis of Cannabidiol and its Metabolites in Serum of Patients with Resistant Epilepsy Treated with CBD Formulations

S. Malaca[1], M. Gottardi[2], F. Pigliasco[3], S. Barco[3], A. Cafaro[3], E. Amadori[4,5], A. Riva[4,5], M. Marcenaro [5], P. Striano[4,5], G. Cangemi[3], R. Pacifici[6], S. Pichini[6], F.P. Busardò[1]

[1]Department of Excellence-Biomedical Sciences and Public Health, Università Politecnica delle Marche, Ancona

[2]Comedical S.Rl, 38123 Trento

[3]Chromatography and Mass Spectrometry Section, Central Laboratory of Analyses, IRCCS Istituto Giannina Gaslini, 16147 Genoa

[4]Pediatric Neurology and Muscolar Diseases Unit, IRCCS Istituto Giannina Gaslini,16147 Genoa, Italy

[5]Department of Neurosciences, Rehabilitation, Ophtalmology, Genetics, Maternal and Child Health, University of Genoa, 16126 Genoa, Italy

[6]National Centre on Addiction and Doping, Istituto Superiore di Sanità, 00161 Rome

Cannabidiol (CBD) is a promising therapeutic agent with analgesic, myorelaxant, and anti-epileptic actions. Recently, a purified form of CBD (Epidiolex®) has been approved by the European Medicines Agency (EMA) for the treatment of two highly-refractory childhood-onset epilepsies (Dravet and Lennox-Gastaut syndrome). Given the interindividual response and the relationship between the dose administered and CBD blood levels, therapeutic drug monitoring (TDM) is a valuable support in the clinical management of patients. We herein report for the first time a newly developed and validated method using ultra-high-performance liquid chromatog-raphy coupled with tandem mass spectrometry (UHPLC–MS/MS) to evaluate CBD and its metab-olites (i.e., cannabidiol-7-oic acid (7-COOH-CBD), 7- hydroxycannabidiol (7-OH-CBD), 6-□-hydroxycannabidiol (6-□–OH–CBD) and 6-□-hydroxycannabidiol (6-□–OH–CBD)) in serum samples. The method reached the sensitivity needed to detect minimal amounts of analytes under investigation with limits of quantification ranging from 0.5 to 20 ng/mL. The validation results in-dicated in this method were accurate (average inter/intra-day error, <15%), precise (inter/intra-day imprecision, <15%), and fast (8 min run time). The method resulted to be linear in the range of 1–10,000 ng/mL for CBD-COOH, 1–500 ng/mL for 7-OH-CBD and CBD and 1–25 ng/mL for 6-□–OH–CBD and 6-□–OH–CBD. Serum levels of CBD (88.20–396.31 and 13.19–170.63 ng/mL) as well as of 7-OH-CBD (27.11–313.63 and 14.01–77.52 ng/mL) and 7-COOH-CBD (380.32–10112.23 and 300.57–2851.82 ng/mL) were significantly higher ($p < 0.05$) in patients treated with GW pharma CBD compared to those of patients treated with galenic preparations. 6-□–OH–CBD and 6-□–OH–CBD were detected in the first group and were undetectable in the second group. 7-COOH-CBD was confirmed as the most abundant metabolite in serum (5–10 fold higher than CBD) followed by 7-OH-CBD. A significant correlation ($p < 0.05$) between the dose

administrated and a higher bioavailability was confirmed in patients treated with a GW pharma CBD prepara-tion.

PO081

Evidence of autoantibodies in hospitalized COVID-19 patients

M.C. Sacchi[1], S. Tamiazzo[1], T. Bensi [1], L. Cellerino[1], R. Doglio[1], M. Sciglitano[1], P. Stobbione[2], E. Lauritano [3], A. Maconi [4], M.M. Ciriello[1]

[1]Lab. Analisi, Dip. Servizi, AON S.S. Antonio e Biagio e C. Arrigo, Alessandria

[2]SSD Reumatologia, Dip Internistico, AON S.S. Antonio e Biagio e C. Arrigo, Alessandria

[3]UO Medicina d'urgenza, Dip Internistico, AON S.S. Antonio e Biagio e C. Arrigo, Alessandria

[4]IRFI (Infrastruttura Ricerca Formazione Innovazione), AON S.S. Antonio e Biagio e C. Arrigo, Alessandria

Recent studies highlight the evidence of autoantibodies in patients affected by Corona Virus Disease-2019 (COVID-19). We evaluated whether severe acute respiratory syndrome (SARS-CoV-2) stimulates autoantibody production and contributes to autoimmunity activation. We enrolled 40 adult patients (66.8 years mean age) admitted to Alessandria hospital between March and April 2020 with a confirmed COVID-19 diagnosis by real-time polymerase chain reaction (RT-PCR) and no previously clinical record of autoimmune disease. 40 blood donors were analyzed for the same markers and considered as healthy controls. All hospitalized patients had high levels of common inflammatory markers, such as C Reactive Protein, Lactate Dehydrogenase, ferritin and creatinine. Interleukin-6 concentrations were also increased, supporting the major role of this interleukin during COVID-19 infection. Lymphocytes number was generally lower compared to healthy individuals. All the patients were also screened for the most common autoantibodies. We found a significant prevalence of ANA (57,5%), ANCA (25%), and ASCA IgA (25%) antibodies in COVID-19 patients compared to healthy controls. We observed that patients having a de novo autoimmune response had the worst acute viral disease prognosis and outcome. Our results sustain the hypothesis that COVID-19 virus might break the body tolerance to itself and stimulate autoimmune responses, suggesting they were directly related to viral infection, instead of being a preexisting condition. The observed increase of autoantibodies remained stable in six-month follow-up of COVID-19 patients. Moreover, preliminary data indicate in a few patients the apparence of clinical manifestations suggestive of autoimmune disease onset. More study will be needed to find out whether these autoimmune profiles persist in COVID-19 affected patients.

PO082

VALUTAZIONE DELLA RATIO ALBUMINA/ CREATININA SU POCT (ALLEGRO-NOVA BIOMEDICAL): RISULTATI PRELIMINARI

M.G. Alessio, M. Seghezzi, G. Previtali, R. Ravasio, R. Marozzi, G. Guerra

Laboratorio Analisi Chimico Cliniche, ASST Papa Giovanni XXIII, Bergamo

La ratio albumina/creatinina (ACR)determinata su primo campione del mattino rappresenta il marker più sensibile di danno renale; la sua valutazione è di grande importanza in patologie quali ipertensione, diabete, cardiopatie. Abbiamo valutato le performance analitiche per ACR di un POCT confrontandole con la determinazione di ACR in chimica liquida, con proteine di un dipstick tradizionale con sensibilità prevalente per albumina e ACR di un dipstick di ultima generazione.

Materiali e Metodi

Sono stati elaborati i dati relativi a 191 campioni, raccolti random tra le urine per esame completo pervenute al Laboratorio Analisi Chimico Cliniche dell'ASST Papa Giovanni XXIII di Bergamo. I campioni appartenevano a 99 M e 92 F; range di età 2 settimane-94 anni (mediana 63 anni), 88 ambulatoriali esterni e 103 ricoverati. I campioni sono stati analizzati in routine con: dipstick Meditape UC-11A (Sysmex) processato su UC3500 (Sysmex), che dispone di un pad tradizionale per proteine in valore assoluto con sensibilità prevalente per albumina e due pad specifici per albumina e creatinina, con calcolo di ACR; ADVIA 1800 (Siemens) per la determinazione di creatinina enzimatica (ECRE2,Siemens), albumina (µALB2, Siemens) e ACR, e successivamente valutati per albumina, creatinina e ACR sul POCT Allegro (UACR-Nova Biomedical). E' stata inoltre valutata la ripetibilità intra e tra le serie su campioni a diversa concentrazione di ACR.

Risultati

La regressione di Passing Bablock ha mostrato una elevata correlazione tra ACR-Allegro e ACR-ADVIA (r=0.989; slope 0.9800; intercetta 0.3000); l'agreement tra i due strumenti risultava ottimo utilizzando due differenti cut off (agreement 0.953 sia per 30 mg/g che per 10 mg/g). La correlazione di Allegro e ADVIA vs dipstick con pad tradizionale per le proteine e ACR calcolato con pad specifici è risultata scarsa per entrambi gli strumenti (agreement < 0.600). Il CV% intraserie variava da 0.8 a 13.2, quella tra le serie tra 1.5 e 3.3.

Conclusioni

Allegro fornisce ottime prestazioni per ACR, praticamente sovrapponibili a quelle della strumentazione analitica di routine, e pertanto può esserne proposto l'utilizzo in particolari contesti clinici (PS, ambulatori specialistici, laboratori).

PO083

Influence of physical activity on interleukins in childhood obesity.

M. Brancaccio[1], C. Mennitti [1], A. Ranieri[2], M.G. Pascale[2], G. D'Alicandro[3], M.R. Licenziati[4], G. Frisso[1,2], O. Scudiero[1,2,5], B. Lombardo[1,2]

[1]Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, 80131 Naples, Italy.

[2]Ceinge Biotecnologie Avanzate S. C. a R. L., 80131 Naples, Italy

[3]Department of Neuroscience and Rehabilitation, Center of Sports Medicine and Disability, AORN, Santobono-Pausillipon, 80122 Naples, Italy.

[4]UOSD Centro Obesità e Patologie Endocrine correlate, AORN, Santobono-Pausillipon, 80122 Naples, Italy.

[5]Task Force on Microbiome Studies, University of Naples Federico II, 80100 Naples, Italy.

Childhood obesity (CO) is a problem of considerable social importance worldwide. In Italy it affects 1/4 children. CO has a multifactorial genesis; in fact, it is the result of different causes, more or less evident, which interact with each other. Main causes are an inadequate diet, linked to a reduced physical activity and a genetic predisposition. During CO, there is an increase in adipose tissue that occurs with weight-gain; consequently, persistent inflammatory state is created. The most commonly inflammatory markers associated with obesity are the cytokines, chemokines and interleukins. The release and the increase of these inflammatory markers affect insulin sensitivity, glucose metabolism and atherosclerosis, eventually leading to health problems. The aim of our study was to understand the predicted role of interleukins concentration in relation to childhood obesity. We evaluated by ELISA assay the following parameters: platelet-derived growth factor ▫ (PDGF-▫), the granulocyte-macrophage colony stimulating factor (GM-CSF), interferon-▫ inducible protein 10 (IP-10), eotaxin and interleukins (IL-1ra, IL-9, IL-17, IL-6 and IL-8) on two population: 45 obese children (O), age=11±3.3, weight=70±23.3 kg, height=1±0.27 m, BMI=31±6.9 and 31 obese children who practice sports (SO), age=10±2.5, weight=61±17.3 kg, height=1±0.13 m, BMI=28±4.4 . Our results showed an increase in serum level of PDGF-▫, IL-9, IL-6, IL-8 IP-10, eotaxin and GM-CSF in O population in comparison to OS. At the same time, we did not observed any significant variation in serum level of IL-1ra, IL-9, IL-17 in both population. Probably, the increase in serum level in O of PDGF-▫, IP-10, IL-9, IL-6, IL-8 and eotaxin induces the formation of adipose tissue; on the other hand a simultaneously increase of GM-CSF, pro-inflammatory cytokine implicated in reducing food intake and body weight, promotes onset of obesity. In conclusion, an early evaluation of these parameters could represent a predicted tool to monitor the health status of children population and allows medical team to evaluate the importance of physical activity in the remission/monitoring of childhood obesity.

PO084

Telegenetics during Covid-19 pandemic: advantage and critical issue

A.P. Capra, S. Briguori, M.A. La Rosa, S. Briuglia

Department of Biomedical, Dental, Morphological and Functional Imaging Sciences, University of Messina

COVID-19 pandemic has radically altered how medical practitioners provide care to patients, in our case interrupting the outpatient activity of clinical genetics.In March 2020 the University Hospital of Messina communicated: “in order to avoid overcrowding within the hospital and in order to further implement the level of public health protection, a service for the delivery by email of laboratory tests must be activated”. To this end, a specific informed consent has been prepared to receive by email medical reports, such as biochemical analysis and pathological anatomy. This form compiled and accompanied by copy of the identity document of the patient has to be send to the hospital email address.In accordance with this initiative, we decided to offer genetic counseling in remote mode in order to guarantee the ongoing clinical genetics service.Genetic reports require special attention because contain sensitive data and because the clinical management need a pre-test and post-test genetic counseling.The counseling requested with SSN prescription was performed by video call and then genetic reports were send by email, protected by a password communicated to the patient during the teleconsultation.Between March and May 2020, the number of clinical consultations was 147, of which 119 in remote mode: 38 pre-test, 80 post-test and 1 prenatal.During the same period in 2019, 558 genetic counseling were carried out.Certainly COVID-19 emergency forced us to change our working habits. We have experienced benefits and drawbacks of telemedicine: we preserve patients privacy and minimize the risk of hospital infection. At the same time, unappealing characteristics included that genetic counseling face-to-face has an average duration of 30-40 minutes, while the time of tele consultation is very variable.This innovative approach also remained active in the following months and in the next future it can be improve using new real-time videoconferencing tool, even if the mixed mode face to face vs. telegenetics has a more complex organizational management.

PO085

ESAME URINE E CELLULE ATIPICHE: NUOVE FRONTIERE PER L’ANALISI AUTOMATIZZATA DEL SEDIMENTO URINARIO

G. Previtali, M. Seghezzi, E. Tirloni, V. Moioli, G. Guerra, M.G. Alessio

SMeL 2 analisi chimico-cliniche, ASST Papa Giovanni XXIII, Bergamo

Introduzione: L’analizzatore automatizzato UF-5000 (Sysmex; Kobe, Japan), oltre al conteggio e alla classificazione dei comuni elementi del sedimento urinario, permette di identificare le cellule atipiche nei campioni di urina rappresentando potenzialmente un interessante strumento di screening per il tumore della vescica. Scopo dello studio è valutare le performance analitiche ed il cut-off del parametro Atypical Cells (Atyp.C) su UF-5000, mettere a punto un pannello di anticorpi monoclonali in citofluorimetria (FCM) per la conferma dei campioni positivi e valutare le prestazioni diagnostiche ottenute da UF-5000 e dalla combinazione i due sistemi.

Materiali e metodi: 322 campioni (236 M, 91 F, età media e mediana 51/60, intervallo 5-92), di cui 104 positivi (prima diagnosi o recidiva di tumore alla vescica confermato istologicamente), 104 controlli negativi e 114 controlli patologici (patologie urologiche non neoplastiche), sono stati analizzati su UF-5000 e in FCM con gli anticorpi Ki67 e CK7.

Risultati: Le equazioni di linearità del parametro Atyp.C sono: $y=-0,01x^2+1,23x-0,18$; $R^2 = 1,00$ (regressione polinomiale) e $y=1,00x+0,65$; $R^2 = 0,999$ (regressione lineare). I CV% intraserie sono compresi fra 36,9% e 49,0% (1 cellula/µL) e tra 20,2% e 37,8% (2 cellule/µL). Limite del bianco, limite di rilevabilità e limite di quantificazione sono rispettivamente 0,3 0,5 e 0,9 cellule/µL; non è stato rilevato carry-over (<1%). Il cut-off calcolato sulla curva ROC è 0,9 cellule/µL. CK7 e Ki67 in FCM mostrano sensibilità del 78,4% e 30,8% e specificità del 48,6% e 92,5%, rispettivamente. UF-5000 permette di identificare il 22,8% dei campioni positivi mentre il 4,3% con l’algoritmo combinato UF-5000 e FCM (UF-5000+Ki67).

Conclusioni: nonostante la bassa sensibilità rilevata, questo primo studio pone le basi per il concreto utilizzo di tecnologie automatizzate come UF-5000 per lo screening del tumore alla vescica applicato alla valutazione del sedimento urinario di routine. Serviranno ulteriori studi per capire definitivamente le effettive potenzialità di questo nuovo approccio.

PO086

LONGITUDINAL TEAR PROTEINS CHANGES ARE RELATED TO OCULAR CHRONIC GVHD DEVELOPMENT IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT PATIENTS

C. Ciavarella[1], G. Astolfi[1], C. Coslovi[1], M. Arpinati[2], F. Bonifazi[2], P. Versura[1]

[1]*Laboratory for Ocular Surface Analysis and Translational Research - DIMES – Alma Mater Studiorum University of Bologna, Bologna, ITALY*

[2]*IRCCS Azienda Ospedaliero Universitaria di Bologna, Policlinico di Sant'Orsola*

Aim: Graft-versus-host disease (GVHD) is complication occurring after allogeneic Hematopoietic stem cell transplant (HSCT), carrying a high risk of morbidity and mortality. Ocular (o)GVHD is a frequent manifestation of chronic GVHD, affecting a large proportion of patients postoperatively. In this study, we analyzed individual tear proteins before and after allogeneic (allo)-HSCT, and correlated their levels with the oGVHD development.
Methods: Data were retrieved from charts of 102 patients subjected to allo-HSCT at the IRCCS Azienda Ospedaliero Universitaria di Bologna in the period april 2010-2020 (40 females and 62 males, aged 42.8 and 46.2 at the time of transplant, respectively). oGVHD was diagnosed according to the International Chronic Ocular GVHD Consensus Group criteria. Data were recorded during the ophthalmological visits performed before the conditioning treatment, and after 3 to 6 months postoperatively. Tears sampled individually were analysed with the Chip-based Agilent 2100 Bioanalyzer system. Total protein (TP), Lysozyme-C (LYS-C), Lactoferrin (LACTO), Lipocalin-1 (LIPOC-1), Transferrin (TRANSF), Albumin (ALB), and Zinc-alpha-2-glycoprotein (ZAG-2) content (ug/mL) were collected and statistically analyzed.
Results: Forty-two patients developed oGVHD after HSCT. TP, LACTO, ZAG-2 levels were significantly lower post HSCT as compared to pre HSCT levels (p=0.01, 0.03, 0.02 respectively). In univariate analysis, TP and ZAG-2 decrease was associated with an increased occurrence of oGVHD (Odd Ratio = 4.49; 95% CI, 1,9 to 10,5; P <0.001; OR=6; 95% CI 2,1 to 16,8; P<0.01, respectively). TRANSF post HSCT levels significantly increased (p=0.02, univariate analysis OR 5.7; 95% CI, 0,8 to 40,7; P<0.01). LYS-C, ALB, LIPOC-1 levels were not statistically signficant altered pre-post HSCT changes.
Conclusion: Our results suggest that total tear protein content, ZAG-2, and TRANSF levels might be significant predictors of oGVHD development, giving Ophthalmologists the chance to manage these patients at an earlier stage.

PO087

Uncertainty of faecal immunochemical methods for haemoglobin used in Colorectal Cancer screening.

S. Rapi[1], L. Lanzilao[1], A. Bonari[1], T. Rubeca[2]

[1]*Laboratorio Generale, AOU Careggi. Firenze*

[2] *Istituto per lo Studio, la Prevenzione e la Rete Oncologica (ISPRO). Firenze*

Introduction. ISO-15189 requires to investigate all components of variability. In estimating the measurement uncertainty the requirement is to include, all the significant sources of variability linked to sampling or to each phase of measurements. Faecal sampling represent a significant source of errors in the immunochemical tests for haemoglobin (FIT-Hb), used in colorectal cancer screening. In the paper we evaluate the uncertainty of the used method including pre analytical variability. The "top-down" method proposed by the SIBioC Study Group on Analytical Quality in Laboratory Medicine was used to calculate the uncertainty on analytic phase. Data on sampling uncertainty, retrieved from a previous investigation on the performance of commercial dipsticks was used to calculate sampling uncertainty treating the trueness on sampling like the analytic bias from external quality assessment (EQA) schemes. To evaluate the 'state of the art' of FIT-Hb an Allowable Operating Limit (AOL) was defined by using the iQC limits 'commonly' accepted by laboratories and maximum bias indicated by EQA schemes supplier. Overall uncertainty of FIT-Hb was obtained by adding sampling and analytic components. Methods. Data from iQC and EQA schemes from routine working flow of 3 instruments were used to calculate the uncertainty for the analytic phase. AOL was calculated using 10% for precision and 24% for bias (indicated by EQA provider) as allowable limits. Sampling-related uncertainty was retrieved from data reported in literature on faecal sampling by commercial dipsticks. Results. Expanded uncertainty of analytic phase, on the 3 instruments ranges 21.1÷28.5 (ng Hb/mL buffer) whereas expanded uncertainty of AOL results 63.9. Pre-analytic component of uncertainty on commercial dipsticks ranges from 16.8÷31.1 (mg stool). Expanded uncertainty including analytic and pre analytic on the monitored instruments range 43.3÷47.3 (µg Hb /g stool). While expanded uncertainty of AOL for commercial methods ranges 72.2÷89.2. Discussion. Expanded uncertainty of FIT-Hb including analytic and pre-analytic components can be estimated over 70%. Analytic component related expanded uncertainty can be expected up to 64% mainly for bias between different brands. Sampling-related uncertainty account up to 50% on the variability of monitored instruments underlining the importance to address pre-analytical on non-conventional materials

PO088

Utilizzo dei marcatori biochimici ed ematochimici nella diagnosi delle anemie da disturbi del metabolismo del ferro

G. Cammarota, L. Gentile , C. Anastasio, F. Conte , E. Scopetta , M. Carucci, A. Miscioscia, M. Sindona

DAIMEDLAB Università Federico II, Napoli

In ambito laboratoristico ematologico vi è la necessità di individuare percorsi diagnostici di precisione e personalizzati per le esigenze di singoli individui e di utilizzare un referto chiaro e completo da fornire ai clinici per l'adeguata terapia. La diagnosi e cura delle Anemie da disturbo del metabolismo del ferro fino ad oggi si è basata su uno studio "statico", che poco rivela le alterazioni comuni a molti tipi di anemie. Abbiamo scelto per il nostro scopo di individuare quattro gruppi di pazienti con anemia lieve/moderata, costantemente presente dalla diagnosi alla dimissione, in cui si escludeva la possibilità di anemie legate a disturbi midollari primitivi o perdite acute di sangue: i pazienti di sesso maschile reclutati nello studio presentavano livelli inferiori a 13 g/dL e superiore a 9 g/dL di emoglobina, mentre le donne inferiore a 12g/dL e superiore a 9 g/dL.I pazienti afferenti alle varie cliniche erano divisi in quattro gruppi di patologie: 1) pazienti oncologici, 2) pazienti affetti da malattie infiammatorie croniche ed acute, 3) pazienti con patologie renali e 4) pazienti anziani con età superiore ai 65 anni. I nostri risultati ribadiscono che il metabolismo del ferro va indagato con esami che riescano ad offrire un quadro "dinamico" ed evolutivo del processo patogenetico e della sua correzione con adeguata terapia. Il primum movens in fase diagnostica deve essere la determinazione e la quantizzazione di un'eventuale patogenesi infiammatoria con la determinazione di analiti quali la ferritinemia e la PCR. La ferritinemia, indagine cardine nella diagnosi e cura delle anemie sideropeniche "pure", diventa non utilizzabile in corso di infiammazione. La diagnosi in tali circostanze va completata con gli indici eritrocitari (MCH, Ret, CHr, %HIPO) che vengono forniti automaticamente dall'esame emocromocitometrico effettuato sulle principali apparecchiature in uso, che sono quindi un mezzo economico e rapido, e con il dosaggio del sTfR. L'insieme di questi dati, unitamente all'utilizzo del Plot di Thomas riesce ad offrire indicazioni terapeutiche precise e personalizzate per patologie ed individuo. Durante il monitoraggio l'esame emocromocitometrico con valutazione di CHr, a breve termine, e di HIPO dopo 4 settimane riesce a valutare il successo terapeutico.

PO089

Risposta anticorpale del personale sanitario vaccinato con BioNTech/Pfizer

S. Masotti[1], V. Musetti[1], V. Casieri[1], A. Spiro[2], M. Maltinti[2], P. Di Cecco[2], D. Battaglia[2], C. Prontera[2], A. Papa[2]

[1]*Istituto Scienze della Vita. Scuola Superiore Sant'Anna, Pisa*
[2]*Fondazione Toscana G. Monasterio, Pisa*

Introduzione: Scopo di questo studio è stato verificare la risposta anticorpale del personale sanitario afferente alla Fondazione Toscana G. Monasterio di Pisa dopo aver ricevuto le due dosi del vaccino mRNA-LNP BTNT162b2 (Comirnaty, Pfizer Inc, NY, USA).
Materiali e Metodi: Campioni di siero sono stati raccolti da 200 soggetti adulti apparentemente sani prima e dopo un mese dal ciclo vaccinale che si è verificato nel gennaio 2021. Su ogni campione sono stati misurati i livelli di IgG diretti contro la proteina del nucleocapside N (CoV-2 IgG) e quelli contro il dominio legante il recettore (receptor binding domain, RBD) della subunità S1 della proteina Spike di SARS-CoV-2 (CoV-2 IgG II Quant). I metodi di misura sono basati sulla tecnologia in chemiluminescenza a cattura di microparticelle (CMIA), utilizzando la piattaforma automatizzata ARCHITECT i1000 (Abbott Diagnostics).
Risultati: I soggetti studiati avevano età media 46±11 anni e comprendevano 125 femmine e 75 maschi. Il personale sanitario vaccinato era senza pregressa infezione da SARS-CoV-2, come dimostrano i valori di IgG anti-N (mediana index 0,03; IQR index 0,02-0,05). Dopo un mese dalla vaccinazione tutti i soggetti hanno sviluppato gli anticorpi attesi, mostrando titoli molto elevati in alcuni casi oltre i limiti misurabili dal metodo (>40000 Au/mL). I livelli di IgG RBD anti SARS-CoV-2 hanno una distribuzione non normale con una mediana di 9808,0 Au/ml (IQR 5486,4-13492,9). È stata riscontrata una differenza significativa tra maschi e femmine [M mediana 8390,6 Au/ml (IQR 4305,3-11295,2) vs F mediana 10377,3 Au/ml (IQR 6609,2-15401,7); p=0,004]. Inoltre, l'analisi bivariata ha evidenziato un trend negativo tra risposta anticorpale ed età.
Conclusioni: I risultati di questo studio confermano i dati presenti in letteratura (Salvagno et al 2021), ovvero una associazione inversa tra età, sesso maschile e risposta anticorpale. Studi futuri saranno necessari per monitorare la permanenza della risposta anticorpale nel lungo periodo (prelievi a 6/12 mesi) e stabilire se anche altre variabili demografiche, che non sono state incluse nel nostro studio, come la presenza di comorbidità, etnicità, BMI e attività fisica possano influenzare lo sviluppo di anticorpi dopo la vaccinazione con mRNA-LNP BTNT162b.

PO090

Use of Fourier Transform Infrared spectroscopy (FT-IR) to improve the analysis of the Urinary Sediment.

S. Rapi[1], A. Bonari [1], S. Dugheri[2], G. Cappelli[3], T. Lucia[3], M. Eva[1], M. Nicola[3], A. Giulio[3], A. Morettini[4], F. Alessandra[1]

[1]*Laboratorio Generale. AOU Careggi. Firenze. Italia*
[2]*Laboratorio Tossicologia Industriale. AOU Careggi. Firenze. Italia*
[3]*Dipartimento di Medicina Sperimentale e Clinica. Università degli Studi Firenze. Italia*
[4]*Medicina Interna 2. AOU Careggi. Firenze. Italia*

Background: Colovesical fistula (CVF) is an abnormal connection between the colon and the urinary bladder. Fecaluria, reported in 40 to 70 percent of cases, is virtually pathognomonic for CVF. Case presentation: During the 5th day of recovery in an 84 years old subject, the passage of cloudy, malodorous urine with visible debris was observed. According to the pathognomonic character of faecaluria, the sample was signed to the laboratory for biochemical and microbiological investigation, able to define the type and origin of materials. Following clinical requirements, both biochemical pathways and instrumental procedures able to confirm or exclude the presence of faecal components in urine were considered. No biochemical compound or component addressing faecal compounds in urine results available between laboratory tests. Our attempt was to characterize urinary pellet investigating the brown powder by Fourier Transform- Infrared spectroscopy coupled to Attenuated Total Reflectance (FTIR-ATR), normally used for renal stone analysis.Method. Urinalysis was performed on IRICELL system (Beckman Coulter, Pasadena. USA). Microscopic evaluation on IRIS system, verified by optical microscopy, report the presence of RBC (1609 / uL); WBC (13900 /uL) and numerous bacteria within the presence of no well-defined images of brown powder. The pellet, obtained by centrifugation, was washed, dried in oven (60 °C for 2h), and analyzed on FTIR-ATR (IRAffinity-1S, Shimadzu, Japan) in the range 4000÷400 cm -1. The resulting spectrum was compared with the instrument library (Spectra Nicodom IR Library')to identify the compound.Results. The brown powder component of the pellet was identified as Keratin, with 90% overlapping with the reference spectrum. Obtained spectra confirm the hypothesis of faecal provenience of collected materials.Discussion. Many imaging and laboratory techniques were proposed to confirm the diagnosis of an entero-urinary tract fistula. The laboratory tests used to confirm the presence of a fistula are based on the recovery in the urine samples of different markers (like barium or poppy seed) after oral or rectal administration. The presence of vegetable matter, leukocytes, and undigested muscle fibers due to rhabdomyocytes was previously reported in literature on urinalysis from CVF subjects. To our knowledge, this is the first case confirming the presence of CVF directly on the urinary pellet analysis on FT-IR spectroscopy. Obtained results show that FT-IR analysis could represent a simple, non-invasive, and fast method to improve the diagnostic course of CVF.

PO091

Anti-SARS-CoV-2 immunoresponse evaluation after vaccination program in healthcare workers of the INT - IRCCS "Fondazione Pascale" Cancer Center: is it time for a third dose?

M.A. Isgrò[1], L. Russo[1], D. Rea[1], L. Di Capua[1], G. Trillò[1], G. Botti[2], L. Miscio[2], A.A.M. Bianchi[2], F.M. Buonaguro[2], E. Cavalcanti[1]

[1]*Laboratory Medicine Unit, Istituto Nazionale Tumori - IRCCS "Fondazione G. Pascale", Napoli, Italia*
[2]*Istituto Nazionale Tumori - IRCCS "Fondazione G. Pascale", Napoli, Italia*

Background: Coronavirus disease 2019 has been characterized by the rapidity of global transmission and the development of diagnostic reagents and vaccines. Aim: the aim of the study was to evaluate SARS-CoV-2 humoral immunoresponse after mRNA anti-spike vaccine (BNT162b2 / Pfizer) administration in two cohorts of healthcare professionals at the INT Cancer Center - IRCCS "Fondazione Pascale": previously exposed and not exposed to SARS-CoV-2 subjects. Materials and methods: 35 healthcare workers with a previous documented history of SARS-CoV-2 infection and 158 healthcare workers without, were enrolled after a written informed consent. Specific anti-RBD (receptor-binding domain) titers against trimeric spike glycoprotein (S) of SARS-CoV-2 were determined by Roche Elecsys Anti-SARS-CoV-2 S immunoassay in serum samples after 1 dose of vaccine in previously exposed subjects and after the first and the second dose in not previously exposed individuals. Geometric mean titers and relative fold changes (FC) were calculated. Results: both previously exposed and not exposed subjects developed significant immune responses to SARS-CoV-2 after the administration of 1 and 2 doses of vaccine, respectively. Anti-S antibody responses to the first dose of vaccine were significantly higher in previously SARS-CoV-2-exposed subjects in comparison to titers of not exposed subjects after the first dose ($p < 0.001$), as well as the second dose of vaccine ($p < 0.001$). FC for workers previously exposed to SARS-CoV-2 were very modest, given the high basal antibody titer, as well as the upper limit imposed by the method. Conversely, for naïve subjects, mean FC following the first dose was low (1.6), reaching 3.8 FC only in 72 (45.6%) subjects following the second dose (▫3.1 in 19.0%).Conclusions: the results showed that, as early as the first dose, SARS-CoV-2-exposed individuals developed a remarkable immune response in comparison to those not exposed, justifying the administration of only one dose in previously exposed subjects. Nevertheless, in 19.0% of not previously exposed subjects, FC after the second dose showed a potential susceptibility to further SARS-CoV-2 infection, suggesting the possibility of administration of a third dose of vaccine in selected less responsive cases.

PO092

Diagnostic accuracy evaluation of urinary para-HydroxyPhenylAcetic Acid (pHPAA) in NeuroEndocrine Neoplasms' (NENs) patients

B. Grilli, L. Russo, G. Trillò, A. Minopoli, P.C. Fiorillo, M.A. Isgrò, E. Cavalcanti

Laboratory Medicine Unit, Istituto Nazionale Tumori - IRCCS "Fondazione G. Pascale", Napoli, Italia

Background: para-HydroxyPhenylAcetic Acid (pHPAA) is an organic acid normally found in the urine of healthy people. Urinary pHPAA is an important metabolite of tyrosine and its increase could be correlated to the altered metabolism of tyrosine and liver diseases. Tyrosine is an amino acid that originates from the hydroxylation of phenylalanine and presents a relationship with some types of cancer such as hepatocellular carcinoma, breast cancer and NeuroEndocrine Neoplasms (NENs).Aim: the aim of the study was to evaluate diagnostic accuracy of pHPAA in NENs' patients.Materials and methods: 17 healthy subjects and 37 NENs' patients were enrolled after a written informed consent and underwent for two days a diet free of potential interfering substances (chocolate, vanilla, banana, kiwi, coffee, tea, pineapple, nuts). Their 24-hour urine levels of pHPAA were determined, after collection, by means of high pressure liquid chromatography (HPLC) with an electrochemical detector (Agilent 1260 Infinity). Diagnostic accuracy of urinary pHPAA was evaluated by drawing the Receiver Operating Characteristic (ROC) curve and calculating the Area under the Curve (AUC). Statistical analysis was performed by using the Statistical Package for Social Science (SPSS Inc., Chicago, IL, USA), version 27.0.Results: urinary pHPAA levels showed significantly higher levels in NENs' patients (median 28.6 mg/24h, Interquartile Range - IR 12.6-86.3 mg/24h, min-max 6.1-794.2 mg/24h) in respect to healthy subjects (median 10.8 mg/24h, IR 6.4-12.1 mg/24h, min-max 3.3-13.7 mg/24h) (Mann-Whitney U test, $p < 0.001$).ROC curve revealed urinary pHPAA as a test with a very good diagnostic accuracy in NENs' diagnosis, with an AUC of 0.851 (95% Confidence Interval: 0.751-0.950).Conclusions: recent studies have shown the importance of pHPAA in some cancers; our study revealed a very good diagnostic accuracy of urinary pHPAA in NENs' patients, suggesting the possibility of performing pHPAA determinations alone or in combination with other laboratory tests in NENs' diagnosis and/or follow-up. Nevertheless, further studies are mandatory in order to evaluate and validate the test in larger cohorts of patients and healthy subjects.

PO093

Urinary para-HydroxyPhenylAcetic Acid (pHPAA) in NeuroEndocrine Neoplasms (NENs): choosing a strategy for detecting a reliable discriminant cut-off

M.A. Isgrò, B. Grilli, L. Russo, G. Trillò, A. Minopoli, P.C. Fiorillo, E. Cavalcanti

Laboratory Medicine Unit, Istituto Nazionale Tumori - IRCCS "Fondazione G. Pascale", Napoli, Italia

Background: para-HydroxyPhenylAcetic Acid (pHPAA) has been demonstrated to be related with some types of cancer. To date, only few works reporting reference values in NeuroEndocrine Neoplasms (NENs) have been published.Aim: the aim of the study was to choose a correct strategy for detecting a reliable discriminant cut-off for pHPAA levels in NENs' diagnosis.Materials and methods: after a two-days diet free of potential interfering substances, 17 healthy volunteers and 37 NENs' patients were enrolled, releasing a written informed consent. Their 24-hour urine levels of pHPAA were determined by means of high pressure liquid chromatography. Different strategies used to calculate a reliable cut-off included: Youden's Index extrapolated from Receiver Operating Characteristic (ROC) Curve, Discriminant Analysis performed by Canonical Equation, ROC Curve's Confusion Matrix Analysis. Statistical analysis was performed by using SPSS software, version 27.0.Results: urinary pHPAA levels showed significantly higher levels in NENs' patients (median 28.6 mg/24h, Interquartile Range - IR 12.6-86.3 mg/24h, min-max 6.1-794.2 mg/24h) in respect to healthy subjects (median 10.8 mg/24h, IR 6.4-12.1 mg/24h, min-max 3.3-13.7 mg/24h) (Mann-Whitney U test, $p < 0.001$). By Youden's index approach, a cut-off of 15.7 mg/24h was detected with a Sensibility (Se) of 70.3%, a Specificity (Sp) of 100.0%, a Negative Predictive Value (NPV) of 60.7% and a Positive Predictive Value (PPV) of 100.0%. By Discriminant Analysis, the following Canonical Discriminant Equation was calculated: $x = 0.007*y - 0.453$, detecting a cut-off of 49.8 mg/24h (Se 35.1%, Sp 100.0%, NPV 41.5%, PPV 100.0%). By means of Analysis of ROC Curve's Confusion Matrix, a cut-off of 12.0 mg/24h was extrapolated, with an Area Under the Curve of 0.851, showing a very good diagnostic accuracy (Se 78.4%, Sp 76.5%, NPV 61.9%, PPV 87.9%, Negative Likelihood Ratio of 0.283, Positive Likelihood Ratio of 3.331, Diagnostic Odds Ratio of 11.8).Conclusions: only Analysis of ROC Curve's Confusion Matrix approach allowed us to detect a reliable discriminant cut-off, finding the right compromise between Sensibility and Specificity percentages and ensuring a very good diagnostic accuracy with a good capability of ruling out NENs' diagnosis.

PO094

Evaluation of ECLIA antigen detection tests as screening methods for COVID-19 in comparison with molecular analysis

F. Tomassetti[1], O. Kolesova[1], P. Cerini[1], D. Finucci[1], G. Turchetti[1], I. Polidori[1], M.L. Frassanito[1], F. Capogreco[1], S. Bernardini[2,3], G. Calugi[1], M. Pieri[2,3]

[1]*Lifebrain Lazio – Guidonia Laboratorio Analisi, Guidonia (RM)*
[2]*Dipartimento di Medicina Sperimentale, Univerisità degli Studi di Roma "Tor Vergata", Roma*
[3]*Lab. di Biochimica Clinica, Policlinico Universitario Tor Vergata, Roma*

In the ongoing COVID-19 pandemic, rapid diagnostic testing for SARS-CoV-2 is necessary to limit virus spread. Different diagnostic rapid tests have been developed as rapid and helpful tools for diagnosis of COVID-19, based on virus proteins detection in respiratory samples.This study aims to evaluate the performances of the Elecsys SARS-CoV-2 Antigen test, in comparison to rRT-PCR, the gold standard.Molecular analysis was carried on 110 swabs from Lifebrain laboratory. According to rRT-PCR, 76 samples were positive, 34 were negative. Initially, the sensitivity and specificity were 85% and 100%, respectively. However, since most of the discordant cases had cycle threshold (Ct) values > 28, it was assumed a new measure to evaluate sensitivity and specificity. At this point, samples with Ct values <28 were selected and a sensitivity of 94% was achieved. The level of agreement between the two tests was 89,1% with ◘ value of 0,77 for total data and 95,9% with ◘ value of 0,95 for samples with <28 Ct.The Antigen test is a well performed tool, timely and effortless, in presence of high viral loads. The comparison data validated the method as a proper approach for rapid screening of patients with high SARS-CoV-2 viral load. Also, a double test for confirmatory analysis on the same swab could increase the overall lab-workflow, but the rate of sensitivity is still highly Ct-dependent.

PO095

Clinical biomarkers and its outcome and complications prediction in Covid-19: an analysis among hospitalized patients.

G. Gallo[1], M.J. Ruiz Álvarez[2], M. Barrionuevo González[2], B. Betré Cubillo[2], K. Sidak[2], E. Coluccia[1], V.F. Alfarano Di Mauro[1]

[1]*Università degli studi della Campania L. Vanvitelli*
[2]*HU Principe de Asturias, Alcalà de Henares*

Introduction: In December 2019 a new pathology emerged in the region of Hubei, China. It is provoked by a virus that was later named 2019-nCov, and then renamed as SARS-CoV-2.Considering previous studies and publications, we decided to evaluate biomarkers to determinate if and how they could be a useful tool to predict complications and lethality.
Goals: we used the clinical data of 147 patients, whom we made a retrospective analysis of. We created two groups: survival and non survivals. We also divided them into two further classes: complications and non complications. We considered the following parameters: age, lymphocyte, neutrophil, platelet, Neutrohpile-to-lymphocyte ratio (NLR), platelets-to-lymphocyte ratio (PLR), CRP and procalcitonine. The Majority of these markers were collected at the emergency room.
Materials and methods: We built a database from 146 Patients that were randomly included, using as criteria of inclusion PCR test positive and admission at the ER, followed by hospitalization. All the data comes from the analytics at the moment of the admission for the big part of them, or, when incompletes, in the next 48/72 hour. We considered as complications the following ones: Trombosis, septic shock, superinfection, acute renal failure and hyponatremia. We collected the data from our Hospital Database, then we performed all the statistical analysis using the IBM SPSS software, version 25. All the findings were considered statistically significative if P value <0.05.
Results: All the patients were included in the study. Among them, 44 were female patiens, 103 were male. We performed Kolgomoroff-Smirnoff test of normality, with the following results: All the parameters showed an asymmetric distribution but CRP. T student and Mann-Whitney tests were performed. Our result showed that the differences in Age, CRP and procalcitonine were significative as p<0,05 between the groups of survaivals and non survivals, while all the rest of parameters showed a non significative result.
Conlcusion: Our results showed a significative difference for the parameters of Age, procalcitonine and CRP when it comes to survival and no survival group. This findigs suggest and somehow confirm the importance of this biomarkers in the evaluation of Covid-19 patients.

PO096

Apulian Regional Network for the integration of Thrombosis Centers and Social Health Districts

G. Dirienzo[1], G. Malcangi[2], E. Adorisio[3], D. Fuzio[1], P. Ranieri[4], L. Dirienzo[4], O. Di Cillo [5]

[1]U.O.S.D .Patologia Clinica Osp.della Murgia - Centro Trombosi Altamura -Asl Bari

[2]U.O.S.D.Centro Emofilia e Trombosi-Azienda Ospedaliera .Universitaria Consorziale Policlinico Bari

[3]U.O.C. Patologia Clinica Osp. di Venere Bari -Asl Bari

[4]U.O.C.Patologia Clinica Universitaria -Azienda Ospedaliera Universitaria Consorziale Policlinico Bari

[5]Area E-Health AReSS Puglia-Centrale Operativa Regionale Cronicità e Reti Cliniche

Background: the use of portable coagulometers and the development of telemedicine have enabled the integration between the territory and Thrombosis Centers, leading to the emergence of Decentralized Specialist Assistance. The clinical advantages of DSA are: reduction of complications, reduction of the workload of the professionals involved, improvement of patients' quality of life and reduction of OAT management costs. In addition, the DSA allows specialists to create a network linking Thrombosis Centers, blood drawing area, and patients. During the SARS-COV-2 pandemic in the ASL BARI and in the A.O.U Policlinico of Bari in collaboration with the E-Health Area of AReSS Puglia and, Werfen, this model was tested.Methods: 200 patients aged 42 to 96 years managed by the Parma-GTS (Global therapy solution) software Instrumentation Laboratory , were enrolled. For each patient, PT was performed using analytical systems in use in the laboratory (ACL TOP 500 CTS with HemosIL RecombiPlastinTin 2G-IL and SYSMEX CS 51000 SYSTEM- SIEMENS with Innovin, Dade) and by digitopuncture with microINR coagulometer (Instrumentation Laboratory). Patients received an electronic report on various devices (PC, tablet, smartphone) ,after expressing informed consent, through homeTAO an optional service integrated in PARMA GTS. Statistical analysis was performed by linear regression and the Bland Altman method. All patients were given an evaluation questionnaire about the reception, the improvement in the quality of life and the simplicity of capillary sampling compared to venous blood sampling as well as the use of homeTAO.Results: The data analysis showed the concordance of the results of the microINR systems with the systems in use in the laboratory in accordance with ISO 17593: 2007. The Pearson's coefficient calculated for all centers is 0.941. In addition, 96% of patients expressed satisfaction with the services offered.Conclusions: the trial demonstrated the possibility of integrating District and thrombosis centres using a single OAT software. The connection of the microINR to the management software allows immediate availability of the analytical data and the homeTAO service allows the domiciliation of the report.

PO097

Immunological profile and diagnostic indices of inflammation as prognostic markers of clinical outcome in patients with SARS-CoV2 infection.

D. Fuzio[1], M. Fasano[2], M. Federico[2], A. Benedetto[1], M. Guida[1], L. Dirienzo[3], G. Dirienzo[1]

[1]U.O.S.D.Patologia Clinica Osp. della Murgia Altamura - Asl.Bari

[2]U.O.C. Malattie Infettive - Covid Ospedale della Murgia - Asl Bari

[3]U.O.C. Patologia Clinica Universitaria - Azienda Ospedaliera Universitaria Consorziale Policlinico Bari

Background The clinical course of pneumonia caused by SARS-CoV-2 is quite peculiar and is characterized by a rapid deterioration of the clinical condition of patients. The aim of this study is to characterize immunological dysfunctions in COVID-19 patients and correlate them with markers of hyperactivation of the inflammatory response, in an attempt to find threshold values indicative of patient outcome. Methods A total of 100 patients were recruited. All patients had a positive PCR test for SARS-COV-2 from nasopharyngeal sample. Patients were grouped into those who did not require mechanical ventilation (n=72) and those who required mechanical ventilation (n=28). Blood samples were collected and analyzed at the point of admission in all patients. Patient immune phenotyping was performed in whole blood samples of patients by flow cytometric analysis. The flow cytometric analysis was performed in an Aquios cytometer (Aquios - Beckman Coulter CA, USA). Antibodies used for cell staining are TETRA-1 Panel (CD45, CD4, CD8, CD3). Data were analyzed using flow cytometric analysis software. Data from the routine biochemical assessment performed in the first 24 hrs after admission to infectious diseases unit will be collected. Results Both the percentage and the absolute number of neutrophils were higher in patients needing ICU care than non-ICU patients, whereas absolute lymphocyte count, and especially the percentage of lymphocytes, presented a deep decline in critical patients. There was no difference between the two groups of patients for CD4 T-lymphocytes, neither in percentage of lymphocyte nor in absolute number, however for CD8 T-cells the differences were significant for both parameters which were in decline in ICU patients. There was a firm correlation between the highest values of inflammation indicators with the decrease in percentage of CD8 T-lymphocytes. This effect was not seen with CD4 cells. Conclusion Our results describe the immune response of severe COVID-19 patients and highlight the value of a novel ratio of CD4/CD8 as a putative marker of poor prognosis. Nevertheless, further research is warranted in order to fully comprehend the transition of the different stages of COVID-19 progression in the context of successful combat of this novel disease.

PO098

R202Q alteration defines anatypical subtype of PFAPA which benefits by colchicine treatment

S. Moz[1], G. Martini[3], M.E. Pinto[3], A. Meneghel[3], F. Zulian[3], M. Plebani[1,2], D. Basso[1,2]

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

[2]*Department of Medicine-DIMED, University of Padova, Padova, Italy*

[3]*Department of Woman and Child Health, University of Padova, Padova, Italy*

Background. PFAPA syndrome is the most common autoinflammatory fever disorder in childhood but its pathophysiology is still unknown. Some studies described the presence of variants of the MEFV gene, including R202Q alteration, in patients with PFAPA. R202Q was first described as a benign polymorphism, but recent studies suggest that R202Q may play as a disease-causing mutation associated with a mild phenotype of FMF.Aims. First objective was to compare clinical features of patients affected byclinical diagnosis of PFAPA but with heterozygous and homozygous R202Q alteration (atypical PFAPA, aPFAPA) to patients affected by typical PFAPA (tPFAPA).Second objective was to compare clinical phenotype of patients with heterozygous R202Q to patients with homozygous R202Q alteration and to evaluate efficacy of colchicine treatment.Patients and Methods. In this study, we reviewed the demographic, clinical characteristics and symptoms of patients with diagnosis of PFAPA. Results. Overall 91 patients were included: 41 with aPFAPA and 50 with tPFAPA.The average age at disease onset was higher in aPFAPA than in tPFAPA (4.5 vs 2.1 years; p = 0.004). aPFAPA had significantly higher ratesof irregular interval between febrile attacks (19.5% vs 2.0%, p = 0.010), abdominal pain (56.1% vs 30.0%, p =0.012), vomiting (22.0% vs 2.0%, p =0.004), diarrhea (19.5% vs 4.0%, p =0.039) and arthralgias (53.7% vs 30.0%, p =0.022). Pharyngitis and apthous stomatitis were significantly less frequent in aPFAPA than in tPFAPA (75.6% vs 100% and 36.6% vs 58.0% respectively). There were no significant differences between the two groups based on family history of recurrent fevers (41.4% vs 48.0%) and cervical adenitis (51.2% vs 68.0%). Colchicine was administered to 48.1% of patients with heterozygous and 63.6% of patients with homozygous R202Q alteration. Both groups had a considerably clinical improvement with colchicine treatment, higher in patients with homozygous R202Q mutation (100% vs 46.2%; p = 0.049). In this group 63.6% of the patients had a complete resolution of symptoms whereas 36.4% had a partial clinical improvement. Colchicine-related side effects lead to withdrawal of therapy in 30.8% of patients (heterozygous R202Q group).Conclusion. R202Q alteration may be associated with an atypical subtype of PFAPA, overlapping some clinical features of FMF and characterized by olderage at onset, less regular interval between febrile attacks, more frequent abdominal pain, vomiting, diarrhea and arthralgias compared to tPFAPA. Patients with homozygous R202Q alteration may benefit by colchicine treatment.

PO099

ACCURATEZZA DIAGNOSTICA DELLA MISURA DELLA HBA1C CON METODICA CAPILLARE IN PRESENZA DI VARIANTE EMOGLOBINICA HB E: CASO CLINICO

F. Martino[1], D. Frattolillo[1], G. Raccosta[1], M. Di Natale[2], C. Codazzo[2], R. Lecce[2], M. Muzi[2], M. Vitillo[1]

[1]*UOC Patologia Clinica HUB, PO San Filippo Neri*

[2]*UOSD Genetica Medica, Centro Sant'Anna, ASL Roma 1*

SCOPO. La misura della emoglobina glicata (HbA1c) è raccomandata per la diagnosi e per il monitoraggio del diabete. La presenza di varianti emoglobiniche può interferire con la determinazione della HbA1c con modalità dipendenti dalla metodologia utilizzata. Vogliamo qui presentare un caso di variante Hb E eterozigote rilevata in un Paziente diabetico originario del Bangladesh che esegue periodicamente la misura della HbA1c. MATERIALI E METODI. La misura della HbA1c è eseguita nel nostro Laboratorio con metodica capillare (HBA1c Sebia Capillarys 3 TERA, Sebia, Francia). Il campione del Paziente in esame è stato analizzato con metodica di primo livello per ricerca di emoglobine patologiche (Hemoglobin(e) Sebia Capillarys 3 TERA, Sebia, Francia). Per il 2° livello è stata eseguita l'estrazione del DNA genomico da leucociti di sangue periferico mediante Kit QIAamp® DSP DNA Blood Qiagen. Inoltre, sono stati effettuati il sequenziamento diretto (strumento 3500 DNA Analyzer Applied Biosystem) dei frammenti corrispondenti all'esone e alle giunzioni introne-esone del gene HBB (NM 000518.5) e la Reverse Dot Blot hybridization (RDB) (a-Globin StripAssay, ViennaLab Diagnostics GmbH CE-IVD). RISULTATI. Il valore della HbA1c è risultato di 57 mmol/mol (7,4%) con glicemia media (eAG) di 165 mg/dL; il picco della HbA1c ha presentato una morfologia normale; il profilo elettroforetico è risultato atipico per la presenza di un extra picco (21,9%) subito prima della HbA2, lievemente aumentata (3,4%) e correttamente risolta. All'analisi con la metodica Hemoglobin(e) il tracciato ha confermato la presenza di una variante emoglobinica migrante nella zona della Hb E in percentuale pari al 24,9% e il lieve aumento di HbA2 (3,7%). Il Paziente ha presentato una Hb di 14,2 g/dL, un MCV di 69,5 fl e un MCH di 22,1 pg/mL; il bilancio marziale è risultato nella norma. L'analisi molecolare ha confermato la presenza della variante beta di tipo Hb E con sensibilità e specificità analitiche dei test utilizzati non inferiori al 99%. DISCUSSIONE. Nella nostra esperienza la metodica capillare consente di misurare la HBA1c permettendone l'uso nella diagnosi e nel monitoraggio del diabete mellito anche in pazienti con varianti emoglobiniche.

PO100

Varianti emoglobiniche G-Philadelphia e S coereditate: valore aggiunto di un metodo separativo ad alta risoluzione per la quantificazione dell'emoglobina glicata

P. Selva, M. Bassi, E. Magrini, R. Alvisi, R. Mancini

LUM (Laboratorio Unico Metropolitano AUSL-Bologna)

L'emoglobina glicata (HbA1c) è un importante marcatore biochimico utilizzato nella diagnosi e nel monitoraggio del diabete, ma la presenza di varianti emoglobiniche può interferire con la sua determinazione rendendo i risultati dell'analisi non attendibili o di difficile interpretazione. Dal momento che la concentrazione di HbA1c dipende dalla vita media eritrocitaria è primariamente importante valutare l'appropriatezza dell'utilizzo del marcatore A1c in pazienti con difetti dei geni globinici e secondariamente, nel caso di vita eritrocitaria normale, valutare l'eventuale interferenza a livello metodologico. Il caso descritto è riferibile ad una paziente di 41 anni originaria della Nigeria che giunge alla nostra attenzione con richiesta di dosaggio di HbA1c. Il sistema diagnostico D-100 di Biorad restituisce un valore di 39 mmol/mol ed il cromatogramma (40 sec) è suggestivo della presenza di frazioni emoglobiniche anomale che non consentono la refertazione del dato. Segue pertanto un approfondimento diagnostico con metodo HPLC Biorad Variant II e Dual kit (6min) che, grazie alla migliore risoluzione, consente di identificare presuntivamente la presenza delle due varianti e restituisce un valore di A1c di 28 mmol/mol. Pertanto si rende necessario un ulteriore approfondimento finalizzato alla verifica dell'accuratezza del valore di A1c e il sangue del paziente viene processato con il metodo capillare Capillarys HbA1c su strumento Capi3 Tera di Sebia. L'elettroferogramma ad alta risoluzione (9min) consente al contempo l'identificazione delle due varianti emoglobiniche con il relativo ibrido e restituisce un valore di A1c di 32 mmol/mol, che viene successivamente confermato anche con metodo immunologico NihonKohden Chemi-4100K. L'insieme dei difetti descritti puo' rappresentare una complicazione diagnostica nella refertazione di A1c nel caso di utilizzo di metodi "veloci" ed a "bassa risoluzione", ottimali per pazienti con normale assetto emoglobinico. In alcuni casi riteniamo che tale complicazione possa essere risolta grazie all'utilizzo di metodi ad alta risoluzione come quello in elettroforesi capillare di Sebia che garantisce al contempo la valutazione della A1c e la risoluzione delle diverse componenti dell'assetto emoglobinico del paziente.

PO101

Identification of Familial Hypercholesterolemia by using data of a clinical-chemistry laboratory: the experience of Reggio Emilia

R. Pullano, E. Bonelli, R. Gnoni, R. Colla, T. Fasano

Laboratorio Analisi Chimico-Cliniche e di Endocrinologia - AUSL IRCCS di Reggio Emilia

Introduction

Cardiovascular diseases (CVD) represent one of the main causes of premature mortality and increase in health care costs around the world. High LDL-C plasmatic concentrations represent a public health concern, being a major metabolic cause of CVD. Familial Hypercholesterolemia (FH) is the archetypal disease for the understanding of the causal relationship between high LDL-C and atherosclerosis. FH is a genetic disorder with a frequency between 1:200 and 1:300 in the general population that is underdiagnosed and undertreated. Clinical diagnosis is performed through the DLCN (Dutch Lipid Clinic Network) criteria but only molecular diagnosis can identify the genetic bases of FH.The purpose of this study was the quantification of putative FH subjects by using laboratory data of the health care system of an Italian province with the aim of designing new diagnostic strategies in a perspective view.

Methods

The study was based on the collection of Electronic Health Records from laboratory database of the AUSL of Reggio Emilia for the years 2014-2015. DLCN score was calculated for clinical diagnosis of FH by using exclusively the biochemical phenotype of subjects. From 2016 onward, clinical laboratory implemented an interpretative comment on lipid profile for LDL-C▫250 mg/dl, suggesting a genetic cause for severe hypercholesterolemia and recommending a specialized clinical evaluation. Effects of the interpretative comment were evaluated.

Results

For the considered timespan, 429967 lipid profiles were extracted from laboratory database for 221644 subjects, corresponding to 41.5% of the relative population.Individual reports with LDL-C▫190 mg/dl or TC▫290 mg/dl (in both cases with TG ▫ 150 mg/dl) were selected. A total of 3243 subjects (1,46%) resulted to have a DLCN score ▫ 3 and classified as at least "possible" FH. A smaller group (178 subjects, 0,08%) had LDL-C▫250 mg/dl and a DLCN score ▫ 5, with the suspicion to be "probable/definite" FH. From 2016 to 2019, 313 subjects received the laboratory alert for LDL-C▫250 mg/dl. Among those, 36 patients (11.5%) underwent a clinical evaluation at the lipid clinic and molecular test for suspicion of FH and 21 subjects were found to carry pathogenic mutations related to FH phenotype.

Conclusion

Clinical laboratory can play a pivotal role for FH identification due to the large amount of lipid profiles performed every year. Data mining of laboratory database, use of specific interpretative comments and the possibility to cross-check Electronic Health Records should improve FH identification and allow therapeutic and preventive strategies in subjects and families.

PO102

Shortening of High-Sensitivity Troponin turnaround time following laboratory technological update

E. Bonelli, R. Gnoni, M. Frattini, R.P. D'Andrea, R. Aleotti, R. Colla, T. Fasano

Laboratorio Analisi Chimico-Cliniche e di Endocrinologia - AUSL IRCCS di Reggio Emilia

Introduction

High-Sensitivity (HS) cardiac troponins have become the preferred biomarker for diagnosis of Myocardial Infarction (MI) and their availability has been one of the major advances in laboratory medicine in the past decades. An efficient troponin testing process is a key to a timely diagnosis of MI in the Emergency Department (ED) and there is a consensus that a turnaround time (TAT) of 1 hour or less should be achieved. In the present study we evaluated the impact of a laboratory technological update on High-Sensitivity Troponin I turnaround time.

Methods

HS Troponin I was performed by Advia Centaur® (Siemens Healthineers) in 2020 and by Atellica® IM analyzer (Siemens Healthineers) in 2021. Laboratory workflow was fully automatized and a FlexLab automation was used for loading, centrifugation and delivery of samples to the analyzers in both years. Precision data were obtained from internal quality controls (Liquichek Cardiac Troponin controls, BioRad, three levels) during the considered periods. Time from samples check-in to clinical validation by laboratory staff was measured from April 1st 2020 to June 30th 2020 and from April 1st 2021 to June 30th 2021. Mean of time to clinical validation and percentage of samples with troponin results available in less than 60 minutes were considered for the whole day and in different time slots (8-14, 14-20 and 20-8).

Results

Precision data in terms of CV%(SD) were 2.5(315.5), 5.5(268.2) and 4(1.6) for concentration of 12377, 1492 and 41 ng/L for Advia Centaur® and 2.8(375.1), 3.3(172.8) and 5.9(2.8) for concentration of 13270, 5249 and 47.5 ng/L for Atellica® IM. Mean of time-to-validation improved from 51.2 mins in 2020 to 42.1 mins in 2021 for the whole day ($P<0.0001$, -17.8%). Better results in terms of percentage reduction were achieved for 8-14 and 14-20 time slots, with a percentage decrease of time-to-validation of 18.1% and 19.1%, respectively (from 55.7 to 45.6 mins for 8-14 slot and from 49.7 to 40.2 mins for 14-20 slot). Mean of percentage of samples with troponin results in less than 60 minutes increased from 87% in 2020 to 95% in 2021 and was always higher than 92%, even at a busy time for samples loading on the automation.

Conclusion

By analyzing daily quality control data, Siemens HS Troponin I was confirmed to be a high-sensitivity assay having an imprecision of < 10% close to the 99th percentile performed both on the Advia Centaur® and Atellica® analyzers. The laboratory technological update with Atellica® IM allowed us to substantially improve the time for troponin reports to clinicians and, ideally, to reduce the time for the diagnosis of MI and the length of stay of patients at the ED.

PO103

ANTI-PLATELET FACTOR 4 PATHOLOGIC ANTIBODIES AFTER ChAdOx1 nCOV-19 VACCINATION MAURIZIANO HOSPITAL DIAGNOSIS AND FINDINGS

B. Montaruli[1], M. Geuna[2], M. Savarese[1], L. Frapiccini[1], S. Bolognese[1], N. Santoro[2], P. Sivera[3], D. Cosseddu[1]

[1]SC Laboratorio Analisi AO Ordine Mauriziano Torino
[2]SS Laboratorio Immunopatologia AO Ordine Mauriziano Torino
[3]SCDU Ematologia AO Ordine Mauriziano Torino

Introduction.Vaccine induced immune thrombocytopenia and thrombosis (VITT) following ChAdOx1 nCOV-19 vaccine has been described, associated with unusual site thrombosis, thrombocytopenia, raised D-dimer and high titre immunoglobulin-G (IgG) class anti-Platelet Factor 4 (PF4) antibodies. Laboratory management of suspected cases begins with a sensitive anti PF4 antibodies binding assay (PF4-ELISA). If the PF4 binding assay is negative, this patient does not have Heparin induced Thrombocytopenia (HIT) or VITT. If the PF4 binding assay is positive, the positivity should be confirmed with one or multiple HIT functional assays as available, such as the serotonin release assay (SRA), heparin-induced platelet activation assay, platelet aggregation (PAT) test, flow cytometry test.Methods.We summarized clinical and laboratory findings of 7 patients in Piedmont who developed thrombosis and thrombocytopenia following AZD1222 vaccination. Plasma from all patients was used to test for anti PF4 antibodies by 2 different ELISA assays (Immucor and Stago) and by 2 different HIT functional assays, PAT and flow cytometry (HIT alert test) both performed in the presence of heparin, PF4 or both.Results.The 7 patients [6 males and 1 female, median age: 38 (range:31-76)] presented with thrombosis 2 to 17 days post vaccination: 5 males had deep vein thrombosis not in unusual sites, 1 male had stroke and the female had cerebral venous thrombosis (CVT). None had received heparin prior symptoms onset. Only 2 out of 7 patients tested positive for anti PF4 ELISA antibodies with both assays: the men with stroke showed low positivity (OD = 0,56 and 0,41) and the female with CVT strong positivity (OD = 3,2 and 3,87). Only the female patient with CVT tested positive with both HIT functional assays, PAT and HIT alert cytometry test in the presence of PF4 independently of heparin. Both assays were inhibited by high concentrations of heparin.Conclusions. In our limited experience VITT demonstrated to be an extremely rare event in the context of AZD1222 COVID-19 vaccination even in the subset of patients with thrombosis and thrombocytopenia.

PO105

THROMBIN GENERATION TIME A HELPFUL TOOL IN PREDICTING BLEEDING RISK

B. Montaruli[1], M. Papandrea[1], F. Bertone[2], P. Sivera[3], S. D'Agnano[4], G. Micca[2], D. Cosseddu[1]

[1]*SC Laboratorio Analisi AO Ordine Mauriziano Torino*
[2]*SC Laboratorio Analisi S. Croce Cuneo*
[3]*SCDU Ematologia AO Ordine Mauriziano Torino*
[4]*Scuola Specializzazione Medicina Interna Università Torino*

The management of patients with acquired or congenital factor deficiencies is challenging because their bleeding risk is highly variable and poorly correlated with routine coagulation tests.We describe the usefulness of Thrombin Generation Time (TGT), a global function test of the clotting system, in predicting bleeding tendency in two patients with coagulation bleeding abnormalities but doubtful clinical haemorrhage tendency. We performed TGT by using STG BleedScreen assay on ST-Genesia STAGO instrument on platelet poor plasma of a 65 year old man setting up for anticoagulant therapy found to be positive for acquired factor V inhibitors (200 UB/ml) without signs of acute haemorrhage and a 15 year old woman with 1% of factor XI activity and a doubtful lupus anticoagulant (LA) antibodies positivity scheduled for tonsil surgery. In the female patient with severe factor XI deficiency and doubtful LA positivity a dramatically impaired TGT was observed suggesting a higher bleeding risk. Low velocity, delayed TGT and reduced Endogenous Thrombin Potential (ETP) were the abnormal TGT parameters observed in this patient. On the contrary in the male patient with acquired factor V inhibitors we did not observed a severely reduced thrombin generation as suggested by coagulation abnormalities diagnosed but we found an increase in thrombin generation ETP suggesting an hypercoagulable state in this patient. Our findings suggest that TGT may be a useful tool in predicting bleeding risk in patients with coagulation bleeding abnormalities but doubtful clinical haemorrhage tendency.

PO106

Riflessioni sull'assetto del POCT intraospedaliero durante la pandemia da Sars-Cov-2: l'esperienza di tre centri italiani.

M. Casati[1], S. Gelsumini[2], M. Savoia[3], E. Rampoldi[4]

[1]*Laboratorio Analisi Chimico Cliniche, Ospedale San Gerardo ASST Monza, Monza*
[2]*UOC SMeL2 Analisi Chimico Cliniche, ASST Papa Giovanni XXIII, Bergamo*
[3]*Dipartimento Medicina di Laboratorio e Trasfusionale , Azienda Ospedaliera Universitaria Federico II, Napoli*
[4]*Coordinatrice Gruppo di Studio POCT per SiBioC*

La pandemia da SARS-CoV-2 ha avuto un notevole impatto sull'organizzazione di molti ospedali italiani: la maggior parte dei reparti è stata convertita repentinamente ad accogliere quasi esclusivamente pazienti Covid. Ciò ha determinato anche una rapida modifica delle attività di laboratorio, in particolare dei POCT, con richiesta, acquisizione, collocazione degli strumenti nella rete POCT e formazione delle nuove unità di personale. Si è garantito così direttamente 'al punto di cura del paziente' il monitoraggio di parametri respiratori, elettroliti e stato glucidico, in relazione allo stadio della malattia e delle relative terapie. Di seguito sono descritti alcuni indicatori di attività in 3 grandi realtà ospedaliere nazionali. Confrontando le richieste complessive pervenute dai reparti interni durante il periodo pandemico Marzo 2020 – Febbraio 2021 vs Marzo 2019 – Febbraio 2020, appare evidente che il numero delle glicemie e delle potassiemie richieste al laboratorio è calato rispettivamente del 12% e del 5%, mentre il numero di glicemie e potassiemie eseguite in POCT è aumentato rispettivamente del 20% e del 30%. Le emogasanalisi in POCT sono aumentate del 30%. Prestazioni essenziali di Biochimica Chimica sono state, quindi, ottenute ricorrendo preferibilmente ai sistemi POCT, preferendoli alla diagnostica tradizionale. Questo, se da un lato ha permesso di minimizzare i rischi legati a ritardi nei tempi di risposta, al trasporto ed alla manipolazione del campione, dall'altro ha potenzialmente aumentato i rischi legati alla non corretta gestione dei campioni da parte di operatori con formazione spesso minimale e/o inadeguata. La mancanza di una normativa in alcune Regioni Italiane rende più difficile la standardizzazione dei Sistemi di gestione ed il confronto tra le prestazioni dei network di POCT: a questa carenza si aggiunge la grave mancanza di connettività che nuoce in numerose realtà. I sistemi di POCT intraospedalieri sono risultati strumenti essenziali per la gestione dei pazienti durante la pandemia: devono perciò essere considerati risorse fondamentali nell'affrontare possibili emergenze sanitarie, ma la loro gestione deve essere implementata in modo completo e tenuta in aggiornamento costante.

PO107

Neurological complication of COVID-19: two different Case Reports

E. De Santis, A. Melegari, T. Trenti

Department of Laboratory Medicine and Pathology, Azienda Ospedaliera Universitaria of Modena, Italy

Here we present two different cases of mild and severe neurological manifestation due to Covid-19 infection. The first case is a woman with anosmia and ageusia still present after 30 days after infection. The autoimmunity tests showed a significant alteration for antibodies against myelin-associated glycoprotein (MAG) and N-methyl-D-aspartate (NMDA) receptor antibodies. The MR revealed a persistent hyperintensity on the olfactory nucleus, a typical sign of encephalitisl with no nasal obstruction or abnormalities. To promote the recovery of both the senses, the patient began an empirical therapy with vitamin C integrators with improvment of symptoms after ninety days. The second case is a woman admitted in ICU for acute severe respiratory sindrome. D-dimer, fibrinogen, prothrombin time (PT), and activated partial thrombopolastine time (aPTT) were no altered, IL-6 level was 123. 50 U/L. After few days patient presented a sudness loss of consciousness with coma due to an acute ischemia with emorragic infartion and after 48 hours she died. We discuss the possible mechanisms of olfactory bulb demage and encephalitis COVID-19 related as well as ischemic stroke, and intracranial hemorrhage. Consequently we try to understand how the laboratory can help physicians in the management of these patients.

PO108

Third generation DNA sequencing for WGS and methylome profile with Oxford Nanopore Technology: a comparison in three genome samples (tumor, paired-healthy tissue and tumoroid colture) from the same patient

F. Di Maggio[1,2], M. Nunziato[1,2], G. Damaggio[3], G. Boccia[4], M. Filotico[4], F. Maione[5], M. Milone[5], G. Luglio[5], G.D. De Palma[5], F. Corcione[4], V. Colonna[3], F. Salvatore[1,2]

[1]Ceinge - Biotecnologie Avanzate, Naples, Italy.
[2]Department of Molecular Medicine and Medical Biotechnologies, University of Naples Federico II, Naples, Italy
[3]Institute of Genetics and Biophysics "A. Buzzati-Traverso", National Research Council (CNR), Naples, Italy
[4]Department of General Surgery and Surgical Specialties, General and Mininvasive Oncological Surgical Unit, University of Naples "Federico II", Via Pansini 5, 80131 Naples, Italy
[5]Department of Clinical Medicine and Surgery, University of Naples "Federico II", Via Pansini 5, 80131 Naples, Italy

Colorectal cancer is the fourth most frequent malignancy and the third leading cause of cancer death worldwide with approximately 1.9 million new cases, and 935,173 deaths estimated in 2020. Tumors are defined as diseases at DNA level due to the accumulation of a number of anomalies, (small to large mutations, chromosomal alterations and epigenetic modifications), mostly in genes that control proliferation, differentiation, death and integrity of the genetic cell heritage. Among common malignancies, colorectal cancer (CRC) bears one of the largest proportion of familial cases; therefore, also the knowledge of the mutational status of predisposing genes in patients may represent an important prevention tool, since it would allow the identification of high-risk subjects to be candidates for targeted prevention and/or therapeutical strategies, also by early finding of cellular signs and symptoms.For many decades, the 2D in vitro cultures have been used, while during the last decade, Patient-derived Organoid (PDO) have emerged as a new tool also in case of their use in preclinical and clinical models. In this scenario, we are stabilizing PDOs (n=10, at the moment) from patients affected by CRC, and following them during the various phases of growth also by advanced microscopy methodology. Moreover, molecular analyses are ongoing, to evaluate the genetic pattern of the three genomes derived from the same patient (tumoroid, tumor-derived tissue and paired healthy tissue). We then performed Whole Genome Sequencing (WGS) analyses by third generation sequencing, with the use of PromethION24- Oxford Nanopore Technologies (ONT). In addition, we designed a multi-gene panel (n=48) to investigate any possible alteration which affects the germinal and the somatic line of the patient's DNA also at tumoroid level. In these experiments, libraries have been obtained using our customized panel by target enrichment system (HaloPlex- Agilent Technologies, Santa Clara, CA, USA). The sequencing run was performed using MiSeq platform (Illumina) and the data analysis, for the

identification of variants, was performed using Alissa Software. With the customized 48 gene panel, in one patient (in the organoid and in tumor-derived tissue) we discovered a pathogenic mutation in TP53 gene, the c.584T>C (p.Ile195Thr). Further experiments are ongoing to extend this comparative analysis of sequences with the aim of analysing the whole mutation spectrum in the framework of precision medicine studies, including library screening of anticancer drugs for specific mutations. This work is supported by CIRO project (to FS) from Campania Region (Italy), SATIN “Neoplasia studies” from Campania Region (to FS) and “Predictive Medicine in neoplasia” (to FS) from Campania Region (Italy).

PO109

Changes in the Biomarkers of Oxidative/Nitrosative Stress and Endothelial Dysfunction Are Associated with Cardiovascular Risk in Periodontitis Patients

M. Currò , M.P. Bertuccio, N. Ferlazzo †, G. Matarese, D. Caccamo, R. Ientile

Dept of Biomedical and Dental Sciences and Morpho-Functional Imaging, University of Messina, Messina, Italy

† In loving memory

A chronic exposure to oral bacteria or their toxins causing periodontitis (PT) can generate pathological alterations affecting the vessel wall [1] and increasing the risk of thrombogenic phenomena [2]. A direct and common thread between PT and cardiovascular disease (CVD) has been suggested since people with PT have a high risk of cardiovascular events. It seems that shared biochemical alterations leading to PT and systemic inflammation are also responsible for CVD development [3]. The aim of this study was to evaluate the possible role of asymmetric dimethylarginine (ADMA), 3-nitrotyrosine (NT), and coenzyme Q10 (CoQ10) as predictive biomarkers in patients affected by both PT and coronary heart disease (CHD) (PT+CHD). In parallel, the relationship between plasma biomarkers associated with endothelial dysfunction and alterations in the inflammatory status in peripheral blood mononuclear cells (PBMC) was examined. Patients with PT, CHD, or both diseases as well as controls were enrolled. Plasma levels of CoQ10, NT, and ADMA were assessed using HPLC method. mRNA levels of caspase-1 (CASP1), NLR family pyrin domain containing 3 (NLRP3), and tumor necrosis factor-α (TNF-α) in PBMC from recruited subjects were quantified using real-time PCR. Patients with PT+CHD showed lower CoQ10 levels and increased concentrations of NT in comparison to healthy subjects. ADMA levels were higher in CHD and PT+CHD patients compared to controls. A negative correlation between CoQ10 and NT concentrations has been observed. Instead, NT concentrations were positively correlated with ADMA levels. Also, ADMA levels were positively correlated with c-reactive protein plasma concentrations. The transcript levels of CASP1, NLRP3, and TNF-α were up-regulated in PBMC from all patient groups when compared to healthy subjects. Our results suggest a possible causal link between oxidative stress, high plasma levels of NT and ADMA, and inflammasome activation, which may be involved in the endothelial inflammatory dysfunction leading to the pathogenesis and progression of CHD in PT patients.

1. Kolltveit KM and Eriksen HM. J. Oral Sci. 2001, 109, 2–7.
2. Young JL et al. Haemost. 2002, 88, 554–567.
3. Paul O et al. Physiol. 2020, 11, 609614.

PO110

Rapid detection of exposure to direct oral anticoagulants: A qualitative urinary dipstick point-of-care assay

M. Marchetti[1], C. Ambaglio[1], E. Sanga[1], L. Barcella[1], F. Schieppati[1], J. Harenberg[3], A. Falanga[1,2]

[1]*UOC Immunoematologia e Medicina Trasfusionale, ASST Papa Giovanni XXIII, Bergamo*

[2]*School of Medicine, University of Milano Bicocca, Milano*

[3]*Ruprecht Karls University of Heidelberg, Heidelberg, Germany*

INTRODUCTION. Knowing whether a patient has been recently exposed to direct oral anticoagulants (DOAC) is a very important issue in the emergency setting for rapid clinical decisions. Chromogenic/coagulation methods for DOAC measurement on plasma samples have several limitations, including limited accuracy, long turnaround time, and are not available in all laboratories. The DOASENSE Dipstick allows to detect the presence of DOACs in urine in one test and discriminate between the presence of a direct factor Xa inhibitor (i.e., rivaroxaban, edoxaban, apixaban) or thrombin inhibitor (i.e., dabigatran).
AIM. To evaluate the operativity of the DOASENSE Dipstick to implement its use in the Emergency Medicine Service at our Hospital.
METHODS. Patients on DOAC referred to our thrombosis and hemostasis outpatient clinic were included into the study, after providing informed consent. Comorbidities and concomitant therapies were recorded. Subjects who were not taking DOACs were also included as negative controls. In all subjects, both venous blood and urine samples were collected. Plasma concentration of DOAC was determined on a Stago STA-R Max® instrumentation. Urine qualitative detection of DOAC was performed using the DOASENSE Dipsticks and the DOASENSE Reader, that reads out specific colors on the DOAC Dipstick pads, designed to measure direct oral factor Xa inhibitors, direct oral thrombin inhibitors, creatinine, and urine color. Specific colors develop on the pads within 10 min depending on whether the DOAC is present or not.
RESULTS. A group of 13 patients (11M/2F) on DOACs with a median age of 69 years (range: 22 - 88 years) were studied: 5 patients were on rivaroxaban, 4 on dabigatran, 6 on edoxaban, and 2 on apixaban. In 3 patients, plasma and urinary tests were performed both at the valley and the peak of drug concentration. In all urine samples we obtained 100% accuracy. In samples of patients collected at valley (n= 10), the drug was clearly detected in urine, despite the low plasma levels (range: 13 – 211 ng/ml). At eye inspection, only 1 urine was abnormal for color (it was particularly clear and transparent), in this case the DOASENSE reader detected the color anomaly as well as the presence of the DOAC. All plasma and urine samples from subjects not on DOACs tested negative.
CONCLUSIONS. Our small study confirmed the high sensitivity and specificity of the urine determination of DOACs by the DOASENSE tool, further supporting its utility at an emergency unit for screening patients with severe bleeding, and/or thrombotic events, or before urgent major surgical interventions.

PO111

First results of an external quality assessment scheme for molecular, serological and antigenic SARS-CoV-2 detection in Lombardy Region

F. Pasotti[1], M. Rizzetto[1], G. Liga[1], A. Scarpignato[1], O.L. Lungu[1], C. Galli[2], L. Bubba[2], E. Pariani[2], M. Corradin[3], S. Buoro[1]

[1]*Centro di Riferimento per la Qualità dei Servizi di Medicina di Laboratorio di Regione Lombardia, Milano, Italia*

[2]*Dipartimento di Scienze biomediche per la salute - Università degli Studi di Milano, Milano, Italia*

[3]*Direzione Generale Welfare Regione Lombardia – U.O. Polo Ospedaliero, Milano, Italia*

La sindrome respiratoria acuta grave (Covid-19) causata dal nuovo coronavirus SARS-CoV-2 scoperto nel 2019 a Wuhan si è rapidamente diffusa determinando una pandemia globale [1]. Il campione di elezione per la conferma della diagnosi di infezione è il tampone naso-faringeo sul quale viene effettuata la ricerca diretta dell'RNA virale per mezzo dell'analisi molecolare [2] oppure, di più recente introduzione, con la ricerca dell'antigene virale [3]. Metodo indiretto per determinare il contatto con il virus è l'analisi sierologica con la ricerca degli anticorpi diretti contro SARS-CoV-2. Nell'arco di breve tempo, per far fronte all'emergenza sanitaria, sono stati resi disponibili numerosi test molecolari, sierologici ed antigenici con caratteristiche molto diverse in termini di sensibilità e specificità. Per l'utilizzo dei test molecolari il Ministero della Salute ha previsto una validazione con un metodo di riferimento [4]. Inoltre per garantire la qualità delle analisi prodotte è fondamentale da parte dei laboratori (SMeL) la partecipazione a programmi di valutazione esterna di qualità (VEQ). Il Centro di Riferimento per la Qualità dei SMeL di Regione Lombardia (CRR_VEQ) ha predisposto dei programmi VEQ ad hoc rivolti a tutti i SMeL di Regione Lombardia che eseguono queste prestazioni. Per i programmi VEQ relativi alla diagnostica molecolare ed antigenica sono stati utilizzati dei campioni stabilizzati da sospensione cellulare contenente il genoma virale. Per la sierologia è stato utilizzato del siero contenente gli anticorpi diretti contro il virus. È stato richiesto ai SMeL un esito per ogni sistema in uso. I risultati dei primi esercizi proposti nel 2020 e 2021 hanno evidenziato buona concordanza verso il risultato atteso. Per la diagnostica molecolare su 1078 determinazioni, 1076 sono risultate concordanti (99,8%). Per la sierologia per 598 determinazioni la concordanza è stata del 95,6% (IgG 98,7%, IgM 83,8%, IgA 100%, IgTotali 100%). Per il test antigenico su un totale di 149 risultati la concordanza è stata del 98%. Da questi primi risultati si evidenzia che, nonostante la rapida implementazione ed evoluzione dei test diagnostici legati alla pandemia da SARS-CoV-2, le performance analitiche sono di adeguata qualità.

PO112

Experimental laboratory protocol as an effective tool to improve the appropriateness of requesting antithrombin in patients receiving direct oral anticoagulants

L. Galasso[1], E. Franceschini[1], D. Fineschi[1], L. Puccetti[3], B. Morelli[4], R. Leoncini[2], P. Calzoni[1]

[1]UOS Coagulazione, U.O.C. Lab. Patologia Clinica, A.O.U.S.
[2]U.O.C. Ematologia, A.O.U.S.
[3]Lab. Analisi Synlab Castenedolo, Brescia
[4]U.O.C. Lab. Patologia Clinica, A.O.U.S.

The new direct oral anticoagulants, DOAC, are comparable to vitamin K antagonists for clinical efficacy and therapeutic target, but have better safety profiles, associated with a general and in particular lower cerebral bleeding risk. In addition, they do not require therapeutic monitoring under routine conditions. However, it is essential for laboratory to establish the drug's plasmatic concentration, in cases of bleeding and/or urgent surgery. For the study of thrombophilia, the patient can undergo further in-depth examinations requiring the dosage of physiological (Antithrombin, Protein C and Protein S) and pathological inhibitors (Lupus Anticoagulant, Anti-Phospholipid antibodies).However, the Guidelines suggest that patients receiving DOAC should not be subjected to such dosages, since the anticoagulant can interfere with the dosing method and alter the test result. In the clinical case presented, it is demonstrated how an experimental protocol for the plasma determination of DOACs can be an effective tool for the appropriateness of the AT dosage request. 65-year-old male patient (M.A.), arrives at the PS in a state of unconsciousness due to a suspected stroke. Laboratory tests show a dosage of AT, performed with the anti FXa method, equal to 101%; however the patient is already known to the laboratory for AT deficiency with a value equal to 40%, previously dosed in the non-acute phase. These results show two possible cases: heparin therapy, supported by the values in the normal range of both the TT ratio (1.13) and the aPTT ratio, or DOAC therapy.The AT dosage with the anti-FIIa method shows the result (42%) confirming the previously diagnosed AT deficiency. The PS is then alerted to the possible ongoing therapy with Xabani, confirmed by the family. Based on the data obtained, a specific protocol was hypothesized for requesting the dosage of AT for patients in DOAC which includes the anti-FXa method for patients receiving Dabigatran and the anti-FIIa method for patients receiving Rivaroxaban, Apixaban and Edoxaban.

PO113

The dosage of anti-phosphatidylserin/prothrombin complex antibodies (aPS / PT) as an additional tool in the approach to patients with suspected Antiphospholipid Antibody Syndrome (APS).

E. Franceschini[1], L. Galasso[1], D. Fineschi[1], B. Morelli[2], L. Puccetti[3], R. Leoncini[4], P. Calzoni[1]

[1] UOS Coagulazione, UOC Lab. Patologia Clinica, A.O.U.S.
[2]Lab. Analisi Synlab Castenedolo, Brescia
[3]UOC Ematologia, A.O.U.S.
[4]UOC Lab. Patologia Clinica, A.O.U.S.

Antiphospholipid Antibody Syndrome (APS) is a clinical condition associated with the presence of antiphospholipid antibodies (aPL) in the circulation. APS correlates with a broad spectrum of clinical manifestations: venous and / or arterial thrombosis and obstetric complications. There are two types of APS: a primary idiopathic, and a secondary often associated with autoimmune diseases such as SLE (Systemic Lupus Erythematosus) and similar (LUPUS-like disease). The aPL term indicates a heterogeneous group of autoantibodies directed against high affinity proteins for phospholipids or phospholipid-protein complexes, both anionic and neutral phospholipids, such as the domain1-beta2GP1 and prothrombin (PT) in particular the phosphatidylserine / prothrombin complex (aPS / PT). In our work we evaluated the role of aPS / PT antibodies as a tool capable of increasing the specificity of the laboratory result for the diagnosis of APS. For this reason were evaluated for classical aPL positivity (anti-Cardiolipin IgG / IgM and anti-Beta2GP1 IgG/IgM antibodies) 460 patients, with clinical criteria for APS and positive for Lupus Anticoagulant (LAC) screening. Of these, 412 patients presented double or triple positivity, therefore perfectly classifiable in APS, while 48 were negative for classic aPL, consequently it was not possible to reach a diagnosis of APS. Therefore negatives were screened for aPS / PT (IgG / IgM) and 45 of them were positive at medium or high titer. The results obtained are in accordance with the diagnostic algorithms developed by Pengo, with Otomo's aPL SCORE and Sciascia's GAPPS SCORE. These results highlight the fundamental role of APS antibody markers, until now considered non-criterion tests, in the diagnosis even in patients previously not classified as positive. In addition, these antibodies can be used both in patients with a single positive ACL, as a test to confirm the actual positivity and in the case of therapy with anticoagulants as they correlate with aPL responsible for ACL and are not affected by the therapy. Finally, they can also be used in patients who are positive for other aPL families for clinical risk stratification.

PO114

VALUTAZIONE DELLA PRECISIONE DEL GLUCOMETRO "MASTER" ACCU-CHEK INFORM II (ROCHE) NEL LABORATORIO SMEL2 DELL'ASST PAPA GIOVANNI XXIII DI BERGAMO

S. Gelsumini, M. Parimbelli, G. Guerra

UOC SMeL2 Analisi Chimico Cliniche - ASST Papa Giovanni XXIII, Bergamo

Il Laboratorio SMeL2 dell'ASST Papa Giovanni XXIII di Bergamo ha implementato una rete di Point Of Care (POC) in cui glucometri (Accu-Chek Inform II, Roche) rivestono un ruolo centrale essendo, oggi, N=124. Il glucometro sfrutta il principio della Glucosio Deidrogenasi mediante aspirazione capillare frontale del campione, ha un intervallo di misura 10–600 mg/dL, supporta due Livelli di Controllo liquidi (CQI), Strisce di misura Plasma calibrate esenti da interferenza da maltosio, compensa gli effetti dell'ematocrito nell'intervallo 10-65%, richiede 0,6 µl di campione (capillare, venoso, arterioso, neonatale) e un tempo di misura di 5 secondi. In Laboratorio è presente un glucometro cosiddetto "Master" di riferimento, che non deve essere assegnato ai Reparti essendo configurato al fine di garantire il corretto passaggio d'informazioni tra i glucometri aziendali ed il software Cobas IT1000 (Roche) espressamente dedicato al POC necessario per la connettività e la gestione remota da parte del POC Manager, identificato dal Direttore del Laboratorio. Il Laboratorio, certificato ISO 9001:2015, anticipando un'ottica di accreditamento ISO 15189, ha verificato la precisione delle prestazioni dichiarate dal produttore seguendo le indicazioni del protocollo CLSI EP 15-A3, usando le soluzioni acquose dei CQI su due Livelli: Low (30-60 mg/dL) e High (261-353 mg/dL). Il produttore definisce accettabile la performance come segue: a concentrazioni di glucosio pari o inferiori a 75 mg/dL, una Deviazione Standard (DS) pari o inferiore a 5 mg/dL; a concentrazioni di glucosio superiori a 75 mg/dL, un Coefficiente di Variazione (CV) pari o inferiore al 5%. Sono stati analizzati 5 replicati di ciascun Livello di CQI per 5 giorni, una volta al giorno e i risultati sono stati elaborati con il software MedComp 1.0. L'assenza di dati estremi è stata verificata tramite il test di Grubbs. La ripetibilità totale è risultata inferiore al valore di verifica ottenuto sulla base della ripetibilità dichiarata dal produttore poiché: per il CQI Low, è risultata essere DS di Laboratorio = 0,867 mg/dL, verso DS = 5 mg/dL; per il CQI High, CV di Laboratorio = 1,046%, verso CV = 5%. I prossimi step includeranno la valutazione della precisione su campioni biologici e la verifica completa del metodo.

PO115

Scoperta del primo caso italiano della variante Hb Broomhill grazie alla quantificazione dell'emoglobina glicata in elettroforesi capillare

L. Persichitti[1], A. Lattanzio[1], M. D'Onofrio[1], M. Basta[1], A.M. Facciolini[1], C. Curcio[2], G. Di Iorio[1]

[1]Unità Operativa Complessa Laboratorio Analisi Cliniche Pescara, Italia

[2]Laboratorio Genetica, Fondazione Ca' Granda, Ospedale Maggiore Policlinico, Milano, Italia

Segnaliamo il riscontro incidentale di una variante rara delle catene alfa globiniche denominata Hb Broomhill in un paziente maschio giunto alla nostra attenzione per la quantificazione di emoglobina glicata(HbA1c).La rilevazione di tale variante,fino ad oggi mai riscontrata in Italia,è stata possibile grazie all'utilizzo del metodo separativo in elettroforesi capillare(Capillarys HbA1c su Capillarys 3 Tera, Sebia).L'elettroferogramma atipico ha evidenziato frazioni emoglobiniche addizionali rispetto a quelle di un campione normale ed in particolare ha mostrato lo sdoppiamento delle frazioni HbA1c, HbA0 e HbA2 tipico della presenza di varianti delle catene alfa globiniche che compongono il tetramero di tutte e tre le frazioni.Il risultato di A1c non è stato conseguentemente riportato.Il sangue del paziente è stato processato anche con metodo cromatografico ad alte prestazioni HPLC (HLC-723G8 A1c e beta thal mode, Tosoh)ma entrambi i cromatogrammi sono risultati normali non mostrando la presenza di frazioni addizionali riconducibili a varianti emoglobiniche.Il valore di A1c col metodo Tosoh è risultato normale(43 mmol/mol).In accordo con le attuali raccomandazioni sulla refertazione dell'emoglobina glicata in presenza di varianti si è proceduto ad ulteriori approfondimenti diagnostici finalizzati alla caratterizzazione molecolare della variante.Il sequenziamento dei geni, avvenuto presso il laboratorio analisi della fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano, ha confermato la presenza in eterozigosi della mutazione c.343C>G del gene alfa2(Hb Broomhill),confermando l'identificazione presuntiva della variante formulata sulla base dei risultati dell'elettroforesi capillare(CE).La ricerca di informazioni su questa variante,sui siti dedicati,non ha consentito di trarre indicazioni utili,universalmente valide,relativamente all'opportunità o meno di refertare il valore misurato di HbA1c.Tale valore non è stato pertanto refertato.Il metodo capillare è risultato fondamentale per la corretta individuazione della variante, informazione chiave per la valutazione dell'appropriatezza di utilizzo di HbA1c quale marcatore per il controllo glicometabolico di questo paziente.

PO116

Microbiome investigation: from research to clinical implementation.

C. Nardelli[1,2,3], I. Granata[4], M. Setaro[2], E. Capoluongo [1,2], L. Sacchetti[2,3]

[1]*Department of Molecular Medicine and Medical Biotechnologies, University of Naples Federico II, Naples*

[2]*CEINGE Biotecnologie Avanzate S.C.a R.L., Naples*

[3]*Task Force on Microbiome Studies, University of Naples Federico II, Naples*

[4]*Istitute for High Perfomance Computing and Networking (ICAR), National Research Council (CNR), Napoli*

The 16S rRNA gene sequencing and metagenomics have revolutionised the study of the microbiome. In fact, the capacity to detect all putative pathogens in a sample has great potential utility in contributing to diagnosis and/or prognosis of several human diseases. However, clinical diagnostic applications of microbiome studies are slow to progress behind research advances owing to a number of factors. In particular, it is mandatory to evaluate the variability in taxonomic profiling of the human microbiome introduced during amplicon data generation, by using reproducible experimental and computational methods. Our group, in the context of gut microbiome research, has recently implemented the following quality control procedural steps. DNA extraction: we used a purified water blank sample as negative control to check for microbial DNA contaminant in DNA extraction kits and laboratory reagents. We used the QIAamp DNA Microbiome kit, that maximizes bacterial DNA by depleting host DNA during the purification process, to overcome decreased NGS sensitivity with high background from the human host (i.e. tissue biopsies). Sensitivity and accuracy of DNA amplification and sequencing. We processed, in parallel to the patient's samples, the standard controls Gut and Oral Microbiome Genomic Mix (ATCC® MSA1006™, MSA1004™), which includes representative gut and oral microbial species with known concentration. Bioinformatics. Sequencing data (Fastq files) were analyzed by several bioinformatics approaches, all identifying the same operational taxonomic units (OTUs). Globally, our results suggested satisfactory absence of DNA contamination, sensitivity and accuracy of microbial taxa identification in the studied gut biopsies. In conclusion, at our knowledge, until now no microbiome-based tests have been clinically validated for the diagnosis or treatment of diseases, in part owing to an incomplete understanding of the complexity of the microbiome and its role in disease pathogenesis, but also for the lack of the adoption of standardized quality control procedures. The latter might contribute to a greater understanding of the role of the microbiome in specific pathological pictures so facilitating its application in the clinical field.

PO117

Efficiency of rapid POC antigen in Frontline Screening for SARS-CoV-2 Infection at Emergency Department Admission

S. Doratiotto, D. Altea, S. Milia, A. Farina, A.M. Mura, T.A. Salis, P. Ragatzu, M. Demontis, D. Masia, M. Atzori, G.A. Porcu, S.A. Congiu, E. Poma, R. Irde

Lab. ASSL Oristano, Osp. San Martino, Oristano

Background: In the emergency department (ED) setting, rapid testing for SARS-CoV-2 enables early but rationale use of limited isolation resources. The use of Antigen Point Of Care tests (POC Antigen) might be an essential tool especially for the Turn Arround Time of response, but it is necessary to clarify whether POC Antigen can be used safely specially for "rule-out" (valid negative testing). Methods: Since September 2020, we have implemented POC antigen evaluation in nasopharyngeal (dNP) dry swab (SD Biosensor Standard F COVID-19 Ag FIA) in the first level evaluation path of the patient at ED admission. In each suspected or not suspected COVID-19 patient two sequential dNP swabs were obtained for RT-PCR viral tests and for POC Antigen. All the results were retrospectively assessed for the performance, in comparison to the RT-PCR (Cepheid Xpert Xpress SARS-CoV-2 and Altona RealStar® SARS-CoV-2 RT-PCR), in terms of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). Results: We included n = 3645 patients from the ED. The sensitivity of the POC Antigen was 73.0 (95%CI: 66.6/78.5)% and the specificity was 97.2 (95%CI: 96.6/97.7)% with a SARS-CoV-2 prevalence of 5.8%; the PPV was 61.8 (95%CI: 55.7/67.7)% and NPV 98.3 (95%CI: 97.8/98.7)%. Thus, n = 57 patients showed false negative POC Antigen results and n = 85 false positive POC Antigen. All positive POC Antigen were quickly confirmed. All the false negative POC Antigen results were tested with high productivity tool. The performance of the POC Antigen was compared with a cut-off threshold cycle (Ct) value of RT-PCR. In patients with Ct values ▫32, the sensitivity was 79.8 (95%CI: 73.6/84.8)% and the (NPV) 98.8 (95%CI: 98.4/99.2)%. In patients with Ct values ▫28, the sensitivity was 88.5 (95%CI: 82.9/92.4)% and the (NPV) 99.4 (95%CI: 99.1/99.6)%. In patients with Ct values ▫25, the sensitivity was 98.1 (95%CI: 94.5/99.3)% and the (NPV) 99.9 (95%CI: 99.7/100)%. Conclusions: We conclude that the use of POC Antigen allowed a rapid classification and an early identification of COVID-19 Infection. In asymptomatic patients, the use of the antigen ensured a low risk of transmission of the infection, being rarely associated to low CT values, index of high viral load.

PO118

Higher serological response to SARS-CoV-2 vaccination in a large unselected population of hospital workers

C. Guiotto[1], I. Casonato [1], M. Daperno[2], G. Pagana[4], G. Pagliaro[3], D. Cosseddu[1]

[1]*S.C. Laboratorio Analisi, A.O. Ordine Mauriziano di Torino*

[2]*S.C. Gastroenterologia, A.O. Ordine Mauriziano di Torino*

[3]*S.S. Medicina del Lavoro, A.O. Ordine Mauriziano di Torino*

[4]*LINKS Foundation, Politecnico di Torino*

INTRODUCTION: At the end of the first pandemic wave in Italy, late in May 2020, all employees of our public hospital in Turin were invited to a SARS-CoV-2 serological observational study. The study enrolled 1,562 subjects and revealed an overall 9.6% positivity for anti-SARS-CoV-2 IgG, with significant differences based on exposure to COVID-19 patients. After the BNT162b2 mRNA vaccine became available in Europe, all hospital workers were invited to vaccination, independently from previous SARS-CoV-2 infection status. AIM and METHODS: All of vaccinated hospital workers were invited to participate to a serologic study focused at exploring short-term serological response (1 month after second vaccine dose), and medium-term serological response (3-months after vaccination), in order to test persistency of the seroconversion, after approval by local Ethical Committee. We used CMIA method for determination of IgG antibodies to SARS-CoV-2, directed against RBD of spike protein, on Alinity platform (Abbott). RESULTS: Interim analysis results (on 1,016 vaccinated workers) showed serological response above the cut-off (50 AU/mL) in all but 1 case (99.9%), and a valid IgG level (>500 AU/ml) in 1,007 subjects (99.1%). Median IgG titre observed was above such limits: 10,197 AU/ml (95%CI 9,705-10,752). Occurrence of adverse events was significantly more common among patients with prior SARS-CoV-2 infection, as confirmed by previous specific PCR swab ($p<0.0001$). Anti SARS-CoV-2 IgG levels were significantly higher in patients with positive swabs (n= 137, median 21,327 AU/mL, 95%CI 19,101-24,063; $p<0.0001$), or seropositivity before vaccination (n=102, median 23,653 AU/mL, 95%CI 20,626-28,292; $p<0.0001$), or with any self reported adverse event after vaccination (n=586, median 12,179, 95%CI 11,087-12,948; $p<0.0001$). A SARS-CoV-2 infection was observed in 11 cases (1%) after full immunization (two vaccine doses); none of the positive cases required hospitalization or presented severe symptoms. Finally, median IgG titre observed 3-months after vaccination was 5,625 AU/mL, as expected. CONCLUSION: According to our preliminary results, early COVID infection among vaccinated subjects is rare. Full results of ongoing study will explore the persistency of seroconversion.

PO119

Next generation sequencing (NGS) for the identification of monogenic diabetes in a cohort of pediatric diabetic patients

F. Iafusco[1], G. Maione[1], C. Mazzaccara[1], F. Di Candia[2], E. Mozzillo[2], A. Zanfardino[3], A. Franzese[2], D. Iafusco[3], N. Tinto[1]

[1]*Department of Molecular Medicine and Medical Biotechnology, University of Naples "Federico II", 80131 Naples, Italy and CEINGE Advanced Biotechnology, 80131 Naples, Italy*

[2]*Center of Pediatric Diabetology, Department Regional of Translational Medical Sciences, Section of Pediatrics, University of Naples "Federico II", 80131 Naples, Italy*

[3]*Department of Pediatrics, University of Campania Luigi Vanvitelli, Naples, Italy*

Introduction: Monogenic diabetes (MD) represents a heterogeneous group of disorders resulting predominantly from defects in single genes. Genetic diagnosis is important to define the specific subtype to choose the most appropriate treatment. The aim of our study is to evidence the importance of Next Generation Sequencing (NGS) analysis in patients with suspected MD in subjects with impaired glycaemia.

Materials and Methods: We analyzed 40 patients. Genomic DNA was extracted from leukocytes and quantified by a NanoDrop ND-1000 spectrophotometer. An NGS analysis, including 42 genes associated with non-autoimmune diabetes, was performed. For each gene, we analyzed the coding regions, 50 bp in each of the intronic boundaries, the promoter, and the 30UTR, for a total target size of about 1 Mb. A total of 50 ng of gDNA was processed through the SureSelectQXT Target Enrichment system for Illumina multiplexed sequencing. Sequencing reactions were carried out on the MiSeq instrument. The sequence reads were aligned to the human reference genome (hg38) using the Alissa Align & Call v1.0.2.10 tool. The evidenced sequence variants were evaluated by Alissa Interpret v5.2.6 CE IVD software and other databases. Finally the variants were classified in according to American College of Medical Genetics and Genomics (ACMG) criteria.

Results: Thirty-three out of forty positive patients (82.5%) were heterozygotes for variants in different genes associated with diabetes and seven out of forty were negative for analyzed genes (17.5%). In many of the positive cases we have found variants in different genes compared to the patient's clinical phenotype that Sanger sequencing wouldn't allow us to identify. Moreover, we also evidenced a case of digenic heterozygous GCK and HNF1A variants in a patient with mild hyperglycemia.

Conclusion:Our study confirms that the clinical phenotype of MD forms is highly variable even within members belonging to the same family, and highlights how in this contest the NGS analyses is very useful both to increase the number of diagnoses and to identify mutations in more than one gene, with a better understanding of the genetic cause and the clinical course of the disease.

PO120

Analisi dei composti organici volatili nell'espirato di pazienti affetti da carcinoma colon retto (CRC) mediante GC-MS.

S. Rapi[2], A. Bonari[2], S. Dugheri[3], G. Cappelli[1], L. Trevisani [1], F. Staderini[4], N. Mucci[1], G. Arcangeli [1], F. Cianchi[4], A. Fanelli[2]

[1]*Università degli Studi di Firenze, Dip. di Medicina Sperimentale e Clinica*

[2]*Azienda Ospedaliero Universitaria Careggi, Lab. Generale*

[3]*Azienda Ospedaliero Universitaria Careggi, Lab. Igiene e Tossicologia Occupazionale ed Ambientale*

[4]*Azienda Ospedaliero Univeristaria Careggi, Chirurgia dell'Apparato Digerente*

Introduzione. L'analisi dei Composti Organici Volatili (COV) rappresenta un'applicazione interessante per la caratterizzazione di numerose patologie. Studi evidenziano l'esistenza in diverse matrici biologiche di un''impronta digitale' di COV soggetta a variazioni in composizione e concentrazione nel corso di diverse patologie trasmissibili e non. Anche se la numerosità dei composti e le variabili pre-analitiche ne complicano la standardizzazione, l'analisi dei COV su campioni di urine e di feci è già in fase applicativa per lo screening del carcinoma colorettale (CRC). Scopo dello studio effettuato dall'AOU Careggi (Comitato etico n. 16770) è la caratterizzazione dei COV nell'espirato in grado di discriminare soggetti con CRC. In questo report sono riportate le informazioni sui COV che caratterizzano i soggetti affetti da CRC. Materiali e metodi. L'espirato di 13 soggetti (età 55-70) affetti da CRC (P) e di 9 soggetti negativi al test del sangue occulto fecale (S) è stato analizzato in gascromatografia -spettrometria di massa (GC-MS) con colonna separativa 5% fenile per l'individuazione e la caratterizzazione dei COV. I picchi risultati dall'analisi sono stati integrati dal software strumentale per quantificare la loro variazione fra i due gruppi di studio. Risultati. L'analisi mostra la presenza di 3 composti con tempo di ritenzione di 12.4, 16.4, 16.8 minuti che risultano variare fra i due gruppi di studio. Gli spettri di massa dei 3 composti sono compatibili con 4 Me-Ottano (C8), 1 I-nonano (C9) e 2,2 di Me-decano (C10). L'espressione media dei 3 composti nei 2 gruppi è: C8-S=120.94±85.39-C8-P=142.76±86.02 (n.s.); C9-S=3371.62±1468.65-C9-P=16060.28± 846.20 ($p<0.01$); C10-S=4186.38±1739.53-C10-P=2016.63±1136.28 ($p<0.01$). Discussione. La caratterizzazione dei COV nell'espirato dei 2 gruppi ha confermato, come già osservata in letteratura, una differenza significativa nella espressione media di C9 e C10, che non si riscontra invece per C8. La forte varianza rilevata può essere dovuta all'esiguo numero di campioni, tuttavia le informazioni sembrano confermare l'associazione fra variazione dell'impronta digitale dei COV e stato di salute/CRC dei pazienti.

PO121

DEVELOPMENT AND PRELIMINARY EVALUATION OF A METHOD FOR THE DETERMINATION OF GROWTH HORMONE IN NEWBORNS ON DRIED BLOOD SPOTS

C. Vantaggiato[1], C. Giavoli[2,3], M. Arosio[2,3], F. Giacchetti[3], A. Sharif Rouhani[1], P. Di Deo[1], C. Varani[1], M. Modica Amore[1], A. Di Modugno[1], F. Napolitano[1], M. Vidali[1], F. Ceriotti[1]

[1]*Clinical Chemistry Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy*

[2]*Endocrinology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy*

[3]*Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy*

Background and aim. Severe deficiency of GH of the newborn is a rare but potentially life-threatening disease. GH can be measured during the first week of life when levels are physiologically higher (neonatal hypersomatotropism). GH evaluation using dried blood spots (DBS) may offer several advantages: easier transportation and storage, reduced costs, allowing centralization and method standardization. Aim of the study was to validate a method for measuring GH in newborns from DBS.

Methods. Whole blood in EDTA and serum was collected from patients attending the Endocrinology Unit. 50 µL of EDTA-blood was spotted onto Guthrie cards (LTA Srl) (diameter disks 13 mm) which were air dried at RT for 4 hours and then processed or stored at -20°C up to 2 months. 3 disks (5.5 mm) were punched out into a 2 mL polypropylene tube and 250 µL of PBS 1X, with or without Tween-20 (0.05%, 0.5% or 1%), were added. Samples were incubated at RT on an orbital shaker for 2 or 16 hours and then centrifuged at 12500 rpm for 1 min. GH in supernatants or undiluted sera was determined by Immulite 2000 (Siemens Healthineers).

Results. Interference by hemolysis present after extraction was evaluated by spiking extracted samples with known concentrations of GH (2 experiments at 3.2 and 9.0 µg/L). No interference was detected (recovery>99%). A calibration curve was built by plotting GH measured in serum vs extracted GH (3 independent replicates, 8 levels from 1 µg/L to 50 µg/L). Recovery at each level was >90%. Linearity was verified ($R2>0.99$) up to a GH serum concentration of 50 µg/L. Mean serum/DBS GH ratio was 14.4. Repeatability at the 8 tested concentrations was 11.1%, 2.4%, 3.5%, 3.5%, 6.1%, 2.9%, 5.2%, 2.7%. Further precision experiments (6 independent replicates at 7.7 µg/L) confirmed previous observations (CV%=3.7%). No appreciable differences were found between samples stored at -20°C up to 2 months or directly processed (similar serum/DBS ratio and recovery>90%), or between samples extracted with Tween or only PBS (at 50 µg/L differences <5%), or between samples incubated for 2 or up to 16 hours (at 2.9, 7.7 and 50 µg/L %differences were 4.2%, 5.9% and 12.6%).

Conclusions. Preliminary evaluation suggests that this method can be used to measured GH in newborns using DBS.

PO122

Monitoraggio terapeutico del Levetiracetam (KEPPRA): confronto e performance analitica di un metodo immunoenzimatico

D. Duranti[1], M. Gambassi[1], B. Sisi[1], F. Mencaroni[1], E. Tavolucci[1], V. Ercolani[1], A. Ognibene[2]

[1]Laboratorio di Tossicologia, Ospedale San Donato Arezzo – AUSL Toscana Sud Est
[2]Direttore UOC Laboratorio Analisi Chimico Cliniche - Arezzo Direttore Dipartimento Medicina di Laboratorio e Trasfusionale Azienda USL Toscana Sud Est - Sede Operativa Arezzo

Introduzione - Il Levetiracetam è un farmaco antiepilettico di seconda generazione, largamente impiegato sia in mono-terapia che in terapia combinata con altri farmaci, nel trattamento delle crisi miocloniche, delle crisi tonico cloniche generalizzate e delle crisi ad esordio parziale con o senza generalizzazione, sia negli adulti che negli adolescenti, e nel trattamento dell'epilessia mioclonica giovanile. L'eliminazione avviene principalmente per via renale. L'elevata efficacia e sicurezza e l'intervallo terapeutico ampio consentono di limitare il monitoraggio terapeutico alla sola fase iniziale per la definizione del dosaggio ottimale. Eventuali aggiustamenti della posologia possono essere richiesti nel caso in cui si manifestino eventi avversi o sia richiesta la contemporanea assunzione di altri farmaci. La metodica di riferimento per il monitoraggio farmacologico è l'HPLC-UV.
In questo contributo si presentano i risultati del confronto di una metodica immunoenzimatica e il gold standard l'HPLC-UV..
Materiali e metodi - Abbiamo confrontato i valori ottenuti dai dosaggi effettuati con la metodica di riferimento su 181 campioni con i valori ottenuti dai dosaggi effettuati con la metodica Siemens.
I risultati sono stati analizzati utilizzando metodi statistici di regressione Passing-Bablock e Bland-Altman.
Sono state effettuate prove di precisione e linearità del metodo secondo le Linee guida e le raccomandazioni CSLI.
Risultati - Dall'analisi condotta sui dati ottenuti con le due metodiche abbiamo ottenuto una retta di regressione con una intercetta di 0,82 e pendenza di 0,96 con r=0,974.
Dal confronto effettuato con l'analisi di Bland-Altman abbiamo ottenuto un BIAS=-0,2622 e un intervallo di confidenza al 95% con limite superiore di 4,9054 e un limite inferiore di -5,4299.
Conclusioni - La metodica immunoenzimatica mostra valori ben correlati rispetto alla metodica di elezione per quanto riguarda valori che cadono all'interno del range terapeutico, in particolare per basse concentrazioni, le maggiori differenze si registrano invece per valori elevati. I profili di precisione per i valori del materiale di controllo utilizzato per le prove risultano lievemente superiori con quanto dichiarato dalla ditta.

PO123

Traumatic Brain Injury: evaluation of potential biochemical markers in vitreous humor

L. Lanzilao[1], M. Da Ros[1], M. Brogi[1], A. Aldinucci[1], S. Raddi[2], B. Defraia[2], M. Focardi[2], A. Fanelli[1]

[1]Laboratorio Generale, AOU-Careggi, Firenze
[2]Sez. Dip. Scienze Medico Forensi, Dip. Scienze della Salute, Università degli Studi di Firenze

The AOU-Careggi General Laboratory is included in the path of organs and tissues donation for therapeutic transplantation purposes, both from beating heart and from a non-beating heart donors. The determination of serum biomarkers to assess the brain damage of the potential donor can be included within an already consolidated process. Proper management of Traumatic Brain Injury (TBI) sequelae can significantly alter their course, especially within 48 h of the injury, and neuroimaging techniques play important roles in the acute therapy. TBI consists of two processes: the initial traumatic impact at the scene causing primary damage to the cerebral parenchyma and blood vessels, followed by the onset of secondary insults. As cells in the Central Nervous System (CNS) are injured, they either secrete, release, or leak proteins, some of which are relatively enriched in the CNS. By measuring these proteins it is possible to assess the extent of cellular injury. The most studied TBI biomarker is S100B, a predominantly intracellular-calcium-binding protein present primarily in mature, perivascular astrocytes. Another brain-specific protein that has been extensively studied in TBI is the glycolytic enzyme Neuron-Specific Enolase. The role and effectiveness of the circulating values of Copeptin in monitoring patients with severe head injury is still uncertain and literature data are still partially discordant. For this reason, the aim of the project will be to look for a correlation between the serum increase of this protein with respect to both a marker whose role is well understood (S100B) and the diagnostic imaging. In addition, we will investigate the potential of C-Reactive Protein, Procalcitonin, Interleukin-6, Ferritin, Lactate Dehydrogenase and Neutrophil Gelatinase-associated Lipocalin, whose biochemical role as acute phase proteins has been already shown in live subjects, to serve as useful post-mortem cerebral biomarkers in forensic setting. Given the assessed mirroring effect binding Vitreous Humor (VH) and plasma, we aim to retrospectively study all the above mentioned biomarkers linked to neuronal damage and to inflammation in VH from TBI patients who died for TBI or other causes, in order to find a correlation with the entity of brain damage.

PO124

L'IMPATTO DELLA SUGGESTIONE PRE-VACCINAZIONE ANTI COVID-19 SUI TEST GENETICI PER LO SCREENING TROMBOFILICO

B. NICCOLETTI[1], M. D'ANZEO[2], N. ONORI[2], B. CINTI[2], M. MORETTI[2]

[1]Dip. Scienze Cliniche e Molecolari, UNIVPM, Ancona
[2]SOD Medicina di Laboratorio, A.O.U. Ospedali Riuniti di Ancona

La trombofilia è una condizione di predisposizione agli eventi trombotici in soggetti che presentano anomalie congenite e/o acquisite dell'emostasi. La ricerca delle mutazioni G1691A del Fattore V e G20210A del Fattore II della coagulazione è considerata da diverse Società scientifiche esame di screening per la trombofilia ereditaria cui sottoporre solo determinate categorie di pazienti. Al fine di migliorare l'appropriatezza delle richieste, abbiamo creato una scheda anamnestica nella quale il medico richiedente l'esame ne specifica la motivazione clinica. Nel periodo 2015-2019 le prescrizioni per i test genetici sono aumentante in maniera costante, mentre nel 2020 l'andamento è stato dicotomico: le richieste pervenute dai reparti della nostra Azienda hanno mantenuto il trend positivo, mentre quelle provenienti da ambulatori e centri prelievi esterni hanno subìto un decremento significativo legato alla pandemia, tendenza che si è invertita nei primi sei mesi del 2021. Analizzando le indicazioni cliniche degli anni 2015-2019, come da Linee Guida la maggior parte delle richieste era riferibile a soggetti con pregressi eventi trombotici o donne con poliabortività, mentre nei primi sei mesi del 2021 circa il 15% delle prescrizioni sono volte a valutare il rischio trombotico pre-vaccinazione anti Covid-19, come conseguenza dell'enfasi mediatica riservata ai rarissimi casi di trombosi post-vaccino. L'analisi dei risultati ottenuti riferiti al 2021 evidenzia l'assenza di mutazioni a carico del Fattore II e del Fattore V di Leiden nel 95% dei casi, dimostrando che queste richieste sono ingiustificate, pertanto tali test non dovrebbero essere eseguiti al di fuori di specifiche condizioni cliniche come da raccomandazioni SISET e SIGU.

PO125

Saliva testing as non-traditional, non-invasive way for monitoring exercise intensity-dependent stress response in teenage elite water polo players

D. Caccamo[1], N. Ferlazzo[1], M. Currò[1], C. Saija[1], A. Trainito[1], F. Naccari[2], D. Di Mauro[1], R. Ientile[1]

[1]Department of Biomedical Sciences, Dental Sciences, and Morpho-Functional Imaging, Polyclinic Hospital University, Messina, Italy
[2]Sport Center CUS UniME, Messina, Italy

A limited number of studies evaluated the correlation between physiological biomarkers and stress in teenage elite athletes. Given their young age, this is crucial for maintaining good health and preventing diseases1. Saliva testing may represent a non-invasive method for tracking individual response to exercise intensity and establishing effective recovery strategies of athletes.
Here we assessed the exercise intensity-dependent variability of stress response biomarkers, at different times of competitive activity, in teenage water polo elite athletes of local team Waterpolo CUS UniMe. The concentrations of cortisol, testosterone, sIgA, and advanced oxidation protein products (AOPP), were measured in saliva samples collected at morning, before match, and after match, on a day of either training match (T1) or competitive match (T2).
Salivary protein content was quantified in order to take into account different salivary flow rate and normalize the concentrations of stress biomarkers. Cortisol/proteins and testosterone/proteins concentrations decreased throughout day T1, while increased throughout day T2. The highest values were measured after match on day T2 (2.5±0.5 vs 14.6±6.3 ng/mg; 0.061±0.024 vs 0.371±0.15 ng/mg, respectively). sIgA/proteins and AOPP/proteins concentrations increased throughout both days, and were higher after T2 match than T1 one (respectively, 1073.0±438.2 vs 71.0±17.3 µg/mg; 78.05±24.2 vs 15.98±3.16 nmol/mg, p=0.0032). Significant differences between concentrations of different biomarkers recorded on T1 and T2 were found only for AOPP, suggesting an increased oxidative stress after match on day T2. T:C ratio after match on day T2 was lower than that at morning (0.053±0.021 vs 0.107±0.031), indicating an increased catabolic response after competitive match. However, T:C ratio values were higher than the set threshold for overreaching.A highly significant positive correlation was found between Cortisol/Proteins and Testosterone as well as IgA/Proteins on day T1, and between Cortisol/Proteins and AOPP on day T2.
Our findings confirm the usefulness of saliva testing as non-invasive way for monitoring the individual response to exercise-induced stress in teenage elite water polo players.
1. Papacosta E, Nassis GP. J Sci Med Sport 2011

PO126

ANALISI DELLE PERFORMANCE STRUMENTALI DELLO STRUMENTO MC4 PLUS PER L'ESECUZIONE DEI TEST DELLA COAGULAZIONE CON METODOLOGIA MECCANICA DI RILEVAZIONE DEL COAGULO

A. Franzoni, C. Bellini, E. Gares, M. Fracchetta, P. Benedetti, V. Volpi, S. Merelli, M. Lombardi, N. Portesi, S. Pontoglio, E. Orlandi, G. Martini, D. Brugnoni

Laboratorio Centrale ASST Spedali Civili di Brescia

INTRODUZIONE. I test di coagulazione eseguiti con strumentazione che utilizza la metodologia foto-ottica per la rilevazione del coagulo possono risultare inaccurati o non eseguibili su campioni che presentano un'elevata torbidità. A causa degli alti volumi di test processati, il Laboratorio Centrale dell'ASST Spedali Civili di Brescia ha in dotazione strumenti automatizzati che utilizzano questa tecnologia (Sysmex CS5100); tuttavia, non di rado, l'analisi su campioni fortemente lipemici risulta di difficile o impossibile esecuzione. Per questo motivo, è stato introdotto in routine un sistema analitico manuale a rilevazione meccanica del coagulo (MC4 Plus), di cui in questo lavoro si riportano i risultati delle prove di verifica.
METODI E RISULTATI. Per PT, APTT e Fibrinogeno sono state effettuate le seguenti prove: 1) Verifica dell'imprecisione di MC4 Plus rispetto alle specifiche dichiarate dalla ditta produttrice e ai traguardi di imprecisione basati sulla Variabilità Biologica (VB): applicando lo schema 3X5 proposto nello Standard CLSI EP15-A3 su 1 pool di plasma umano, tutti gli analiti rispettano i traguardi dichiarati dal fornitore; inoltre PT ha un'imprecisione inferiore al traguardo minimo della VB (3%), mentre APTT e Fibrinogeno rispettano il traguardo desiderabile (rispettivamente 2.03% e 5.35%). 2) Comparazione fra MC4 Plus e Sysmex CS5100 mediante regressione di Passing-Bablok su 50 campioni umani: nessun analita presenta un bias proporzionale e/o costante statisticamente significativo. 3) Esecuzione di Test di Equivalenza per verificare l'accettabilità clinica del bias fra i due strumenti: confrontando l'errore sistematico con i traguardi di massimo bias accettabile basati sulla VB, tutti gli analiti, ad eccezione di Fibrinogeno, rispettano le specifiche di qualità. 4) Determinazione di PT, APTT e Fibrinogeno su 12 campioni lipemici: MC4 Plus è sempre stato in grado di determinare i tre parametri.
CONCLUSIONI: MC4 Plus presenta prestazioni analitiche in linea con quanto dichiarato dal produttore e comparabili con quelle degli strumenti automatizzati che utilizzano una tecnologia foto-ottica di rilevazione del coagulo, consentendo di fornire risultati accurati dei test di base della coagulazione anche in campioni ad elevata torbidità.

PO127

Analisi dei composti organici volatili (COV) nell'espirato di pazienti affetti da carcinoma colon retto (CRC) mediante 'naso elettronico'.

S. Rapi[2], A. Bonari[2], S. Dugheri[3], G. Cappelli[1], L. Trevisani[1], F. Staderini[4], N. Mucci[1], G. Arcangeli[1], F. Cianchi[4], A. Fanelli[2]

[1]Università degli Studi di Firenze, Dip. Medicina Sperimentale e Clinica
[2]Azienda Ospedaliero Universitaria Careggi, Lab. Generale
[3]Azienda Ospedaliero Universitaria Careggi, Lab. Igiene e Tossicologia Occupazionale ed Ambientale
[4]Azienda Ospedaliero Universitaria Careggi, Chirurgia dell'Apparato Digerente

Introduzione. L'analisi dei composti organici volatili (COV) costituisce un interessante applicazione per la caratterizzazione di numerose patologie. Studi recenti riportano una distintiva impronta digitale di COV, che può variare in composizione e concentrazione in base alla matrice biologica ed allo stato di salute o di malattia. L'analisi dei COV presenta alcune criticità, quali le complesse e molteplici variabili pre-analitiche ed analitiche, che ne rendono difficile la standardizzazione; tuttavia, la determinazione dell'impronta digitale dei COV in urine e feci è già in fase applicativa per lo screening del carcinoma colorettale (CRC). Scopo di questo studio, avviato presso l'AOU Careggi (Comitato etico n. 16770), è la messa a punto di un "naso elettronico" capace di rilevare l'impronta dei COV presenti nell'espirato, così da discriminare soggetti con CRC da soggetti sani. In questo report vengono presentati i risultati preliminari dell'addestramento del sistema elettronico.
Materiali e metodi. L'espirato di 13 soggetti affetti da CRC e di 9 soggetti negativi al test del sangue occulto fecale (età 55-70) è stato raccolto in sacche multifoil ed analizzato con naso elettronico a 32 sensori Cyranose 320 (Sensigent, USA). Tale sistema, prima dell'utilizzo, prevede una fase di addestramento per la classificazione dei gruppi, definiti in base alle informazioni cliniche in Negativi, Negativi test FIT-Hb, Positivi e Soggetti CRC.
Risultati. L'addestramento del naso elettronico con questo gruppo di soggetti ha permesso di ottenere una capacità di discriminazione del sistema del 90% fra soggetti sani e malati; tuttavia, il numero ridotto di campioni a disposizione ancora non consente il raggiungimento delle specifiche richieste (capacità di discriminazione 95% e distanza euclidea >5) per l'applicazione clinica dello strumento.
Discussione. Le informazioni raccolte confermano che, avendo un numero di casi maggiori e standardizzando ulteriormente la procedura di raccolta, è possibile addestrare lo strumento elettronico per l'analisi dei COV in espirato per una preliminare e rapida identificazione dei soggetti CRC.

PO128

Evaluation of Loop-mediated isothermal amplification (LAMP) method as an effective molecular point-of-care technique for the rapid diagnosis of SARS-CoV-2 infection.

S. Milia, D. Altea, S. Doratiotto, P. Ragatzu, T. Salis, M. Demontis, A. Mura, A. Farina, D. Masia, G.A. Porcu, M. Atzori, A.S. Congiu, E. Poma, R. Irde

Lab. Analisi, ASSL Oristano

Background: The novel Coronavirus disease-2019 (Covid-19) pandemic emergency is a concrete example of the existing gap between availability of advanced diagnostics and need for cost-effective methodology. The current standard method for Coronavirus detection is the reverse transcription-PCR (RT-PCR), but the recent validation of a new rapid SARS-CoV-2 RT-LAMP assay offers an alternative diagnostic pathway. Unlike PCR tests, LAMP (loop-mediated isothermal amplification) do not require sequential changes of temperature and so can turnaround test results more rapidly. We explored the diagnostic effectiveness of LAMP compared with RT-qPCR traditional assay.Methods: A sample of 1652 UTM NP swabs (collected from suspected or non suspected patient of ED) were tested for SARS Cov2 infection, using IC GENE SARS-CoV-2 POC (Enbiotech) that detect two specific viral targets: S gene and N gene. After a rapid termal RNA extraction protocol, a Real Time amplifier and fluorescence reader allowed us to achieve the result (simultaneously, and up to 12 samples per run) in a time between 30 and 60 minutes. The same samples were further analyzed with a RT-qPCR traditional assay (Cephied Xpert Xpress® SARS cov-2 and Altona RealStar® SARS-cov-2 RT-PCR).Results: The technical performance of assay demonstrated a sensitivity of 72.2 % (95%CI: 61.4/80.8) and specificity of 86.8 % (95%CI: 85.0/88.4), VPP 21.5%, VPN 98.4%, in comparison to current standard of care RT-qPCR testing after RNA extraction, across all samples tested (CT <45 by RT-qPCR), increasing to a sensitivity of 96.6 % for those samples with a higher viral load (CT <25 by RT-qPCR). Our results shows that main limitation of LAMP is the high number of false positive samples (80% of all positive test). Conclusions: in our experience the ICGene RNA RT-LAMP kit only reliably detects very strong positives patients (Ct<25), however, statistically, these are the most infectious cases and so the most urgent to find quickly, particularly in vulnerable settings. Whereby RNA RT-LAMP could replace rRT-PCR where there is need to rapidly identify highly contagious individuals within emergency departments, ensuring results still get laboratory confirmation with highly sensitive nucleic acid amplification testing (NAAT).

PO129

Genes associated to NUP98-rearrangements are involved in primary chemoresistance of pediatric Acute Myeloid Leukemia

V. Di Bella[1], L. Lo Nigro[2,3], N. Andriano [2,3], A.P. Privitera[1], P. Bonaccorso [2,3], M. La Rosa[2,3], V. Iachelli[2,3], G. Spampinato[1], G. Pulvirenti[4], C. Scuderi[1], D.F. Condorelli[1], V. Barresi[1]

[1]Università degli Studi di Catania, Dip. BIOMETEC, Sez. Biochimica Medica

[2]Laboratorio di Biologia Citogenetica-Citofluorimetrica-Molecolare

[3]Centro di Ematologia-Oncologia Pediatrica, Azienda Policlinico - San Marco, Catania

[4]Università degli Studi di Catania

Acute myeloid leukemia (AML) is a haematological disease characterized by the proliferation of haematopoietic precursor cells known as blasts. AML hits commonly adults, whilst in children it represents 15% of acute leukemias. Conventional chemotherapy for AML generally encompasses an intensive induction phase, based on the use of Idarubicin, Cytarabine and Etoposide (ICE scheme), aimed at achieving a morphological remission defined as detection of leukemic blasts less than 5%. Many cases are classified as Primary Induction Response (PIR); unfortunately, 15% of children do not achieve remission [1] and are defined as Primary Induction Failure (PIF). This study aims to characterize the global profile of gene expression in children with PIF-AML, in order to detect molecular pathways disfunctions and identify potential therapeutic targets. As reported in literature [2,3,4] and confirmed in our study, NUP98 gene-fusion products (NUP98 rearrangements) are enriched in PIF-AML pediatric patients: therefore, we decided to investigate the involvement of NUP98-rearrangements, (particularly, NUP98-NSD1 that predict the worse prognosis) in PIF. In order to test such hypothesis, expression data obtained from 85 expression arrays (GEO dataset: GSE75461) and 358 RNAseq samples, from AML-TARGET program, were analysed for “Differentially Expressed Genes” (DEGs) between NUP98+ and NUP98- patients, identifying 110 NUP98/PIF-associated DEGs. Then, we investigated these genes in our local cohort of PIF and PIR patients, analysed by Human Transcriptome Arrays (Affymetrix HTA 2.0): on the basis of diagnostic accuracy, the over-expression of nine DEGs SPINK2, TMA7, SPCS2, CDCP1, CAPZA1, FGFR1OP2, MAN1A2, NT5C3A and SRP54 has been validated by qRT-PCR. Among these transcripts only SPINK2 appears to be also upregulated in adult cohorts, belonging to OHSU study [5] and TCGA database. No association was found between HOX-family genes (HOX-A and HOX-B) and PIF condition. In conclusion, the integrated analysis of NUP98 mutational status and transcriptome profiles allowed us to identify novel putative biomarkers for the prediction of PIF in AML [6].

[1] Pession, A.; Masetti, R.; Rizzari, C.; Putti, M.C.; Casale, F.; Fagioli, F.; Luciani, M.; Lo Nigro, L.; Menna, G.; Micalizzi, C.; et al. Blood 2013, 122, 170–178, doi:10.1182/blood-2013-03-491621.

[2] Bolouri, H.; Farrar, J.E.; Triche, T.; Ries, R.E.; Lim, E.L.; Alonzo, T.A.; Ma, Y.; Moore, R.; Mungall, A.J.; Marra, M.A.; et al. Nat Med 2018, 24, 103–112, doi:10.1038/nm.4439.
[3] Ostronoff, F.; Othus, M.; Gerbing, R.B.; Loken, M.R.; Raimondi, S.C.; Hirsch, B.A.; Lange, B.J.; Petersdorf, S.; Radich, J.; Appelbaum, F.R.; et al. Blood 2014, 124, 2400–2407, doi:10.1182/blood-2014-04-570929.
[4] McNeer, N.A.; Philip, J.; Geiger, H.; Ries, R.E.; Lavallée, V.-P.; Walsh, M.; Shah, M.; Arora, K.; Emde, A.-K.; Robine, N.; et al. Leukemia 2019, 33, 1934–1943, doi:10.1038/s41375-019-0402-3.[5] Tyner, J.W.; Tognon, C.E.; Bottomly, D.; Wilmot, B.; Kurtz, S.E.; Savage, S.L.; Long, N.; Schultz, A.R.; Traer, E.; Abel, M.; et al. Nature 2018, 562, 526–531, doi:10.1038/s41586-018-0623-z.
[6] Barresi, V.; Di Bella, V.; Andriano, N.; Privitera, A.P.; Bonaccorso, P.; La Rosa, M.; Iachelli, V.; Spampinato, G.; Pulvirenti, G.; Scuderi, C.; et al. International Journal of Molecular Sciences 2021, 22, 4575, doi:10.3390/ijms22094575.

PO130

Incidental detection of a rare hemoglobin variant (Hemoglobin Valletta) in a patient with known DM2: a case report

B. Toffoletto, M. Vidus Rosin, C.M. Cheni, E. Ghirotto, E. Albertini, P. Rosa, A. Di Giovanni, F. Zuliani, M. Ruscio

HbA1c (glycated hemoglobin) is a blood marker widely used to routinely monitor long-term glycemic control in diabetic patients. A HbA1c value of below 7% (53 mmol/l) is recommended for adults as it is appropriate to prevent the risk of long chronic complications including cardiovascular diseases. However, many hemoglobin variants interfere with glycated hemoglobin estimation. These variants may lead to either erroneous high or low HbA1c levels. In such cases, HbA1c cannot be used to diagnose diabetes or to assess concordance with glycaemic targets. Here we report a case of a 58-year old male patient affected by diabetes mellitus and ischemic heart disease from 2015. HbA1c estimation was previously done by HPLC (high performance liquid chromatography) with the Tosoh G8 HPLC Analyzer and the last measurement was 7.3% (56 mmol/mol). In none of the previous HbA1c measurements performed with HPLC there was evidence of hemoglobin variant. In may 2021 during a routine check we performed HbA1c analysis by capillary electrophoresis (Capillarys 3 TERA, Sebia) which recently took place of HPLC. Unexpectedly, the HbA1c measurement was undetectable and the value could not be reported. The electrophoretic trace revealed a double peak in the HbA fraction, suggesting the presence of a variant co-migrating with HbA. Therefore the sample was further analyzed performing hemoglobin electrophoresis and molecular studies. Hb electrophoretogram was not conclusive while genotyping detected the presence of the rare hemoglobin variant called Valletta. This variant is clinically silent but as in this case can compromise the HbA1c measurement and interpretation. Our case highlights the importance of rule out any possible interference affecting the HbA1c measure. In the cases no HbA1c value can be reported alternative HbA1c methods that are free of the interference should be evaluated. Otherwise other blood markers instead of HbA1c (i.e. fructosamine) should be considered. Furthermore although this variant is very rare efforts should be made to identify it so that receiving genetic counseling in the case of pregnancy.

PO131

Valutazione del test Bühlmann fCAL® turbo per la determinazione della calprotectina fecale su sistema Alinity ci

S. MATTIOLI, S. SALVADORI, A. BERTOLETTI, M. BOLDINI, A. FANTI, M. MAZZOLI, M. MORANDINI, I. TURETTI, A. MENOLFI, G. BONETTI

Laboratorio Analisi ASST-Valcamonica

Introduzione: La calprotectina, nota anche come proteina L1 o S100A8/A9,è una piccola proteina (36 kDa) che lega principalmente calcio e zinco che si trova all'interno del citoplasma dei leucociti polimorfonucleati. La determinazione della calprotectina fecale (fCal) negli ultimi anni si è consolidata come utile marcatore di supporto nella diagnosi differenziale tra le malattie infiammatorie organiche del tratto gastrointestinale (MICI) come il morbo di Crohn o la colite ulcerosa e quelle funzionali. Scopo di questo studio è valutare il test Bühlmann fCAL® turbo (Bühlmann) [fCAL®] su strumentazione Alinity ci (Abbott) in uso nei laboratori dell'ASST-Valcamonica.Materiali e Metodi: fCAL® è rappresentato da un immunodosaggio turbidimetrico arricchito con particelle (PETIA) che permette la quantificazione della calprotectina in campioni fecali estratti tramite dispositivo B-CALEX® (Bühlmann), con risultati negativi se <80, dubbi tra 80 e 160 e positivi se >160 µg/g di feci. Lo studio di confronto tra metodi è stato effettuato secondo protocollo CLSI EP09c mediante regressione di Deming su n. 40 campioni fecali analizzati con metodo chemiluminometrico su sistema Liaison XL (DiaSorin) con risultati negativi se <50, debolmente positivi tra 50 e 100 e positivi se >100 µg/g di feci. La concordanza tra i due sistemi è valutata tramite il kappa di Cohen pesato.Lo studio di imprecisione è effettuato tramite il protocollo CLSI EP15 A3 impiegando 2 campioni fecali estratti ed un materiale di controllo; obiettivo di Errore Totale (TE): ±10%.Risultati: Alinity fCAL® = 1.58 (95%CI -0.607 - +3.773) Liaison +17.660 (95%CI -122.596 - +157.915), r= 0.9935 (95% CI 0.9877 – 0.9966). Si è riscontrata una buona concordanza con un k di Cohen pesato di 0.725 (95%CI: 0.509-0.942).Nel materiale QC a 82.47 µg/g si è mostrata una DS di 2.396, un CV intra-laboratorio (CVwl) di 2.905% ed un TE di 6.449%.Nei campioni fecali a 78.628 e 195.392 µg/g DS/ CVwl/TE sono stati pari a 1.855/2.359%/4.470 e 2.251/1.152%/2.651, rispettivamente.Conclusioni: il test Bühlmann fCAL® turbo su strumentazione Alinity cI ha mostrato delle buone performance analitiche, rendendolo affidabile quale supporto alla valutazione dell'infiammazione della mucosa intestinale.

PO132

ESPOSIZIONE ACCIDENTALE A BUFOTOSSINA: UN CASO DI INTOSSICAZIONE IN CENTRO ITALIA

A. Bonari[1], M.L. Mattei[1], F. Romano[1], B. Salvadori[1], D. Vitali[1], R. Mannino[1], G. Mannaioni[2], A. Fanelli[1]

[1]Laboratorio Generale, AOU Careggi, Firenze
[2]Università degli Studi di Firenze, Dipartimento di Neuroscienze, Area del Farmaco e Salute del Bambino

Introduzione Un paziente in età pediatrica, successivamente ad un immersione nelle acque di fiume, si presenta al pronto soccorso con nausea, vomito, vertigini, intorpidimento della bocca e debolezza generale. All' ECG vengono riscontrate lievi alterazioni cardiache quali ectopie ventricolari e blocco A-V di modesto grado. I genitori riferiscono di aver rilevato, nel contenuto gastrico del figlio, del materiale organico compatibile con l'ingestione di uova di rospo europeo (Bufo vulgaris), presenti in quantità significativa nelle acque del fiume. Data l'analogia della sintomatologia presentata con l'intossicazione da digitale e le analogie strutturali tra la digitale e le bufotossine presente nelle uova e nella pelle del rospo europeo viene richiesta al Laboratorio Generale della AOU Careggi il dosaggio della digossina ematica. Materiale Metodi. La determinazione della concentrazione della digossina su siero viene effettuata mediante metodica ECLIA su piattaforma Roche Cobas® 8000. Per la ricerca della digossina sul materiale organico recuperato dalle acque del fiume sono stati omogenizzati meccanicamente 0.2 g di uovo con 1 ml di metanolo in agitazione per 20 min. La soluzione è stata poi centrifugata per 10 minuti a 15.000 rpm. Il surnatante è stato analizzato dopo un'ulteriore diluizione 1:3 col solvente TDM diluition buffer (Roche) con la stessa metodica utilizzata per il campione ematico. Risultati. La concentrazione della digossina su siero è risultata di 0.69 ug/L. Il range terapeutico ed il valore critico in uso per adulti presoo il laboratorio sono rispettivamente: 0.9-2.0 ug/L e 2,5 ug/L corrispondenti a 0,012-0,027 ug/Kg e 0,33 ug/Kg per un adulto di 75 Kg. Il dosaggio della digitale sul materiale organico è risultato di circa 4,6 ug/L, corrispondente ad una concentrazione di 23 ng per g di uova. Conclusioni. Le sostanze appartenenti alla famiglia delle bufotossine, in particolare la bufalina, principale tossina del veleno contenuto nelle ghiandole e nella pelle del Bufo vulgaris, sono strutturalmente simili alla digossina. In situazione di emergenza le metodiche di laboratorio utilizzate per la determinazione della digossina possono essere utilizzate per la conferma di intossicazioni da bufalina.

PO133

Identification of cancer driver genes affected by broad copy number aberrations of chromosome 1 and 16 in breast cancer

A.P. Privitera, C. Scuderi, V. Di Bella, D.F. Condorelli, V. Barresi

Department of Biomedical and Biotechnological Sciences, Section of Medical Biochemistry, University of Catania, Via S. Sofia 89-97, 95123 Catania, Italy

Large numerical and structural chromosomal aberrations are a common feature of many solid tumors. Although many arm-level aberrations represent early events in cancer progression and show a remarkable tumor-type specificity, the molecular mechanism linking them to cancerogenesis is still unknown in several cases. In breast cancer, the most recurrent chromosomal aberrations are derivative chromosome der(1;16), deleted 16q and isochromosome 1q. To investigate the role of these aneuploidies and their dysregulated gene expression patterns, we performed a comparative and integrative bioinformatics analysis involving cytogenetic SNP array data (1084 samples), RNA-seq data (1222 samples in total subdivided in 1072 primary tumor and 99 normal breast tissue) and single-point mutation (WES-seq) data from The Cancer Genome Atlas (TGCA). Breast cancer adenocarcinomas were classified in five groups, called 1,16 chromogroups, according to different patterns of arm-level aberrations of chromosome 1 and 16: 1) Group A, bearing 1q-gain and 16q-loss, 2) Group B, bearing 1q-gain/1p-loss, 3) Group C, bearing 1q-gain and normal chr16, 4) Group D, bearing 16q-loss and normal chr1, and 5) Control (CTRL) bearing no aberrations in chr1 and chr16 [1]. Cancer samples were further classified in histological subtype (e.g. Ductal and Lobular) as well as in intrinsic molecular subtypes (e.g. Luminal A, Luminal B, Her2+, Normal-like and Basal-like). The pathway enrichment analysis of the distinctive over-expressed genes in 1,16 chromogroups has highlighted dysregulated pathways involved in processes such as Notch-signaling, formation of the beta-catenin complex and Wnt-signaling. The main candidate driver genes involved in these pathways are BCL9, PYGO2 (Wnt enhanceosome components), APH1A, PSEN2, NCSTN (gamma-secretase subunits), and CDH1 (involved in cell adhesion). On the basis of these data we selected cancer cell models (Cell Model Passports [3]) showing chr1 and chr16 aberrations and characterized the pattern of cell growth, the growth factor dependency and the expression of the candidate cancer driver genes located on those chromosomes. The sensitivity of the selected cancer cell lines to the silencing of the identified overexpressed genes located on chromosome 1q have been evaluated by siRNA transfections.

Bibliography

1) Privitera, A.P.; Barresi, V.; Condorelli, D.F. Aberrations of chromosomes 1 and 16 in breast cancer: A framework for cooperation of transcriptionally dysregulated genes. Cancers (Basel). 2021, 13, doi:10.3390/cancers13071585.

2) McCart Reed AE, Kutasovic JR, Lakhani SR, S.P. Invasive lobular carcinoma of the breast: Morphology, biomarkers and 'omics. Breast Cancer Res. 2015, 17, 12.3.

3) Van Der Meer, D.; Barthorpe, S.; Yang, W.; Lightfoot, H.; Hall, C.; Gilbert, J.; Francies, H.E.; Garnett, M.J. Cell Model Passports - a hub for clinical, genetic and functional datasets of preclinical cancer models. Nucleic Acids Res. 2019, 47, D923–D929, doi:10.1093/nar/gky872.

PO134

A Computational Approach In The Diagnostic Process of COVID-19. Synergy Between The Laboratory And Emergency Department

L. Lanzilao[1], T. Biagioli[1], A. Mariniello[2], B. Polenzani[3], P. Nazerian[4], A. Murri[1], F. Rossi[1], E. Stanghellini[3], A. Fanelli[1]

[1]*Laboratorio Generale, AOU-Careggi, Firenze*
[2]*Dip. di Medicina Sperimentale e Clinica; Università degli Studi di Firenze*
[3]*Dipartimento di Economia, Università di Perugia*
[4]*Dip. Medicina d'Urgenza, AOU-Careggi, Firenze*

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the COVID-19 pandemic.According to the CDC,RT-PCR in respiratory samples is the gold standard for confirming the disease,although it has practical limitations as time-consuming procedures and a high rate of false-negative results. Based on data collected at Careggi Hospital from April 7th-30th 2020,we aim to assess the accuracy of a COVID-19 diagnosis through classification methods based on blood tests and information collected at the ED. 971 pts with pre-specified features of suspected COVID-19 were enrolled;physicians prospectively dichotomized patients in COVID-19 likely/unlikely based on clinical features plus results of bedside imaging.Considering the limits of each method to classify a case COVID-19 positive,further evaluation was performed to form the COVID-19 final diagnosis,established after independent clinical review of 30-day follow-up data. Several classifiers were implemented, both parametric (Logistic Regression, LR;Quadratic Discriminant Analysis,QDA) and non-parametric (Random Forest, RF;Support Vector Machine;Neural Networks;K-nearest neighbour;Naive Bayes). Log transform was applied to some of the covariates and results compared with non transformed data.The dataset was divided in training and validation sets.Results based on validation sample show an AUC>0.8 for all classifiers. Best results are obtained applying RF,LR and QDA to a rebalanced sample using the SMOTE techniques on the log transformed data, showing an AUC of 0.890 (LR),0.896 (QDA) and 0.864 (RF). In parallel,best Sens and Spec are obtained via the above methods, the highest chieved by the LR (Sens 0.696; Spec 0.877). The rather high rate of false negative seems to be a feature inherently characterizing this classification problem.Good discriminatory power was shown for: WBC,Neut,AST,LDH,PCR,Na,IL-6 plus symptoms' information. Parametric models have the additional advantage of allowing a scientific interpretation.The performance of the classifiers with respect to the physician's gestalt and data validation are ongoing. The proposed classifiers show a good level of Sens.To improve Spec,a 3-level classification can be implemented;this tool can help in taking decisions when time and resources are scarce.

PO135

Capillary electrophoresis brings medical added value during HbA1c screening

S. Baglioni, S. Catarzi, B. Di Finizio, F. Veneziani, A. Picchi, P. Casprini

Lab. Patologia Clinica Firenze, Osp. S. Giovanni di Dio, Azienda USL Toscana Centro

Glycated hemoglobin (Hba1c) is an important parameter for the screening and the diagnosis of diabetes mellitus (DM). As HbA1c is part of the hemoglobin fractions, it is subject to clinical variations if patients present pathologies involving Hb. Among them, hemoglobinopathies are important causes. HbP are now common worlwide due to migration. At least 5.2% of the world population carries a hemoglobin (Hb) variant. Thus, it is important for laboratories to be able to diagnose patients carrying HbP during HbA1c measurement and inform the clinicians accordingly in order to adjust DM diagnosis and treatment. We evaluated the ability of capillary electrophoresis (CE) to incidentally discover HbP among patients needing HbA1c measurement and calculated prevalence of HbP. This study was carried out over 2 months. A total of 949 patients samples were received for DM screening. All HbA1c samples were analyzed by CE on Capillarys 3 Tera (Sebia). Profiles showing Hb variants, beta thalassemia (HbA2 >3%) or elevated HbF were counted up. HbA1c by CE allows to highlight Hb abnormalities in clear-cut and precise profiles and brings added value by displacing Hb profiles. A total of 50 atypical profiels were found: prevalence can be considered equal to 5.3%, which is compatible with ones previously calculated. Among them 28 beta-thalassemias were found, 12 profiles with elevated HbF and 10 Hb variants. These Hb disorders have a prevalence of 2.95%, 1.27% and 1.05%, respectively. Presence of these abnormalities has been reported to clinicians with the HbA1c value. Thus, separative method allow the detection of undiagnosed hbP during HbA1c assay, which is not rare in Italy . CE can be used advantageously for this purpose. In our laboratory around 2-5 cases of HbP are found per day, beyond the HbA1c measurement. The incidental observation should be reported to the clinicians and must lead to further investigations (e.g. Hb testing by CE) but also to a better adjustment of DM follow-up and treatment.

PO136

Marcatori di biochimica urinaria nella prognosi dell'ostruzione del tratto urinario inferiore fetale – case report

E. MIlletti[1], A. Mongia[1], L. Lanzilao[1], S. Ciullini Mannurita[1], A. Bonari[1], L. Pasquini[2], A. Fanelli[1]

[1]Laboratorio Generale, AOU Careggi, Firenze
[2]Medicina e Diagnosi Fetale, AOU Careggi, Firenze

L'ostruzione del tratto urinario inferiore fetale (LUTO) è una condizione patologica che determina una marcata morbilità e mortalità perinatale e ha un'incidenza di 2,2 casi su 10000 nascite. È diagnosticata alla fine del primo trimestre o all'inizio del secondo trimestre di gravidanza e le forme lievi provocano sequele cliniche minime, mentre le forme più gravi portano a oligoidramnios, alterazioni displasiche nei reni fetali e ipoplasia polmonare secondaria. È noto che diversi marker biochimici urinari del feto sono correlati alla prognosi di LUTO e, se quest'ultima risulta favorevole, è indicato l'intervento chirurgico come possibile trattamento risolutivo. Il caso riguarda una paziente di 35 anni alla 18a settimana di gravidanza, giunta presso il reparto di Medicina e Diagnosi fetale dell'Azienda Ospedaliero-Universitaria Careggi, al cui feto è stata diagnosticata la LUTO. Presso il Laboratorio Generale è stato eseguito un pannello di analiti biochimici (calcio, sodio, cloro, beta-2 microglobulina e proteine totali) su due prelievi di urina fetale al tempo zero e a distanza di 24 ore (urina neoformata), allo scopo di valutare la funzionalità renale fetale. Sebbene soltanto il dosaggio della beta-2 microglobulina sia risultato associato a prognosi sfavorevole e sebbene sia stata osservata una diminuzione della concentrazione di tutti gli analiti testati alla seconda raccolta di urina fetale, indicativa di prognosi favorevole in base ai dati riportati in letteratura, l'esame ecografico ha mostrato un peggioramento delle condizioni del feto e la gravidanza non è giunta a termine. Sono stati dosati altri marcatori urinari per valutare se potessero contribuire al miglioramento della prognosi, quali creatinina, glucosio, fosfato, urea, ammonio, N-GAL, albumina e cistatina C. Anche per questi analiti è stata osservata una diminuzione della concentrazione alla seconda raccolta. Creatinina e ammonio sono risultati inferiori ai valori di riferimento presenti in letteratura, mentre glucosio e urea sono risultati aumentati. I nostri dati suggeriscono che questi quattro analiti potrebbero essere introdotti come marcatori prognostici più efficaci e la loro determinazione potrebbe essere aggiunta al pannello di analiti urinari comunemente richiesto per questa patologia.

PO138

Riscontro di un nuovo pattern autoanticorpale in pazienti con polmonite da Sars-Cov2

S. Chiappin[2], E. Gnatta[2], A. Romano[1], M. Michelon[2], P. Zavattiero[2], A. Liverani[2], R. Morabito[2], E. Solimbergo[3], A. Battisti[3], A. Filippi[4], S. Barbar[4], N. Simioni[4], L.A. Leone[1], A.M. Leo[2]

[1]Lab.Analisi ULSS 6 – Euganea
[2]Dip. Medicina Generale, Osp. Riuniti Padova Sud ULSS 6-Euganea
[3]Dip. Medicina Trasfusionale, Osp. Riuniti Padova Sud ULSS6-Euganea
[4]Dip. Medicina Generale Osp. Cittadella ULSS6-Euganea

Diversi studi mostrano alterazioni del sistema immunitario nei pazienti affetti da polmonite da Sars-Cov2 suggestivi di una risposta immunitaria maladattativa con un alterato rilascio di citochine e chemochine ad una iperattivazione dei linfociti T.
Tali alterazioni immunitarie possono essere implicate nella perdita di tolleranza immunitaria con attivazione di risposte autoinfiammatorie ed autoimmunitarie sistemiche. Tra le complicanze è stata descritta un'epatopatia Sars-Cov2 correlata, la cui fisiopatogenesi al momento non è nota.
Scopo: Scopo dello studio: indagare il ruolo fisiopatologico dell'autoimmunità nei pazienti affetti da polmonite da Sars-Cov2 descrivendo un nuovo pattern autoanticorpale.
Materiali e metodi: Sono stati reclutati 181 soggetti previo consenso informato, di cui 87 pazienti con diagnosi di polmonite da Sars-Cov2 (, ricoverati presso i reparti di Medicina 1, Pneumologia dell'Ospedale di Cittadella, e di Medicina dell'ospedale di Schiavonia; e 94 donatori afferenti al Centro Trasfusionale non vaccinati per Sars-Cov2 e che all'anamnesi non avessero avuto sintomatologia riferibile a pregressa infezione da Sars-Cov2. Tutti i sieri sono stati analizzati con tecnica IFI su triplo tessuto (fegato, rene e stomaco) di ratto (Mosaic Basic Profile - Euroimmun ® e NOVA Lite Rat Liver, Kidney, Stomach Kit-INOVA Diagnostic Werfen).
Risultati
Nel gruppo dei pazienti il 62% dei sieri analizzati evidenzia una fluorescenza lineare ad aspetto reticolare e ad intensità variabile a livello della membrana cellulare degli epatociti, riferibile a deposito di IgG umane. Nessun siero tra il gruppo dei donatori (0%) ha mostrato un simile pattern fluoroiscopico.
Conclusioni Il riscontro di questo nuovo pattern fluoroscopico nei pazienti con patologia Covid , sembrerebbe confermare il ruolo centrale della risposta immunitaria maladattativa nella fisiopatologia del danno Sars-Cov2 mediato.

PO139

INFEZIONE DA SARS-CoV-2 E RISPOSTA AUTOIMMUNE: QUALE RELAZIONE TRA I DIVERSI BIOMARCATORI?

R. Minerba[1], A. Picanza[1], A. Russo[1], R. Aloe[2], A. Melegari[3], E. De Santis[3], C. Bonaguri[1]

[1]Laboratorio Diagnostica Ematochimica, Azienda Ospedaliero-Universitaria di Parma

[2]S.S.D. Biochimica ad Elevata Automazione, Azienda Ospedaliero-Universitaria di Parma

[3]Laboratorio Patologia Clinica Tossicologia e Diagnostica Avanzata, Ospedale di Baggiovara, Modena

INTRODUZIONE. Studi recenti ipotizzano che i cambiamenti immunologici caratterizzanti la malattia da COVID-19 sono in grado di rompere la tolleranza immunitaria ed attivare una risposta autoimmune in individui geneticamente predisposti.
OBIETTIVO. Si è indagato se la risposta infiammatoria mediata dagli alti livelli di citochine circolanti nei casi gravi di COVID-19 possa stimolare la produzione di autoanticorpi.
METODI. Sono stati analizzati 132 pazienti adulti ricoverati presso i reparti COVID dell'AOU di Parma. Lo stato infiammatorio dei pazienti è stato valutato esaminando IL-6, PCR, D-dimero e procalcitonina (PCT). Per valutare se l'infezione da SARS-CoV-2 può influenzare l'autoimmunità sono stati ricercati gli Ab anti-nucleo(ANA), mentre gli Ab anti-fosfolipidi(aPL) sono stati indagati per studiare la possibile relazione con la coagulopatia indotta da COVID-19.
RISULTATI. La maggior parte dei pazienti aveva alte concentrazioni (▫10 pg/mL) di IL-6 (85%) ed elevati livelli (▫5.0 mg/L) di PCR (95%). Il D-dimero era positivo (▫500 ng/mL) nel 62% dei casi, mentre la PCT (▫0.50 ng/mL) solo nel 3%. Riguardo ai marcatori immunologici, dei 132 pazienti screenati per ANA il 10% era positivo(titolo▫1:160), il 20% debolmente positivo (titolo▫1:80) e il 70% negativo. Il pattern di fluorescenza più frequente era quello granulare. Ab anti-cardiolipina (isotipo IgM più comune) erano presenti nel 20% dei pazienti, mentre Ab anti ▫2-glicoproteina1 costituivano il 2% dei casi. Il 5% dei pazienti aveva entrambi gli Ab.
DISCUSSIONE. I dati inerenti i marker infiammatori sottolineano l'intensità con cui il sistema immunitario reagisce a SARS-CoV-2. Gli ANA sono i marcatori immunologici di varie malattie autoimmuni, ma si possono riscontrare anche in soggetti sani. In assenza di informazioni circa la storia clinica dei pazienti e di conferma ai test di approfondimento (ENA/dsDNA), non siamo in grado al momento di mettere in relazione la positività ANA con l'infezione da SARS-CoV-2. È noto che gli aPL possano essere rilevati transitoriamente in presenza di gravi infezioni. Sarebbe utile indagare i meccanismi alla base della comparsa di Ab anti-cardiolipina da noi osservata nei pazienti COVID-19 per capire se questi autoanticorpi e gli ANA influenzano negativamente l'esito della malattia.

PO140

Evaluation of VivaDiag™ SARS-CoV2 Antigen Rapid Test

G. SAVERIAMPILLAI, A. BETTINARDI, S. BONTEMPI, G. BONETTI, M. MASCHERPA, L. MINOLFI, L. MORANDINI, M. TIGNONSINI, S. SAGGINI, R. VOLPI, G. BONETTI

Laboratory of Clinical Pathology, ASST-Valcamonica, Esine, Brescia, Italy.

Background: The real-time reverse transcription-polymerase chain reaction (RT-PCR) assay represents the gold standard for laboratory diagnosis of SARS-CoV-2 infection but it requires hours and need to be performed by skilled technicians. Therefore, rapid and accurate tests for SARS-CoV-2 screening are essential to expedite disease control and screening. The aim of the present study was to evaluate the VivaDiag™ SARS-CoV2 Antigen Rapid Test (VivaCheck) [VivaDiag] in nasopharyngeal (NP) swab samples. Materials and Methods: VivaDiag uses a lateral flow-based technology and detects the presence of the nucleocapsid protein antigen in 15 min. The manufacturer's instruction for use recommend direct testing from OP or NP swab. The study population consisted of 115 patients (median age, 48 years; 58 men). Diagnostic accuracy was determined in the same NP samples, maintained in 3mL of Universal Transport Medium (UTM™, Copan), type of sample not validated, stored at -80°C until analysis, in comparison to RT-PCR performed on a CFX96™ System (Bio-Rad) using SARS-CoV-2 ELITe MGB amplification Kit (ELITech) detecting the ORF8 and RdRP genetic loci of SARSCoV-2 after RNA extraction). The agreement between VivaDiag and Rt-PCR was analysed using Cohen's weighted kappa. 100 samples had a positive result of molecular testing with cycle threshold (Ct) values of ORF8 ranging from 17.3 to 44.1 and RdRP ranging from 18.2 to 39.0.Results: A fair agreement between VivaDiag and RT-PCR assay with a Cohens's weighted kappa value of 0.242 (95% CI 0.129-0.354) in 115 NP samples.An agreement of 100.0%, 95.7%, 60.9%, 7.7% and 0.0% was found between VivaDiag and rRT-PCR in subgroup of samples with Ct<=25 (n=23),25-30 (n=23),30-35 (n=23),35-40 (n=26) and >40 (n=5), respectively. Considering samples with high viral loads and ORF8 and RdRP Ct value <30 (n=61) the weighted kappa was 0.915 (95% CI 0.800-1.000) showing a very good agreement between VivaDiag and RT-PCR assay was found.Conclusions: The easy-to-perform without need of a reader VivaDiag test evaluated showed a good diagnostic accuracy in NP samples with high viral loads, despite the use of a non-validated sample material and could be used as POCT test in setting with high pre-test probability of SARS-CoV-2 infection.

PO141

Quantificazione dell'emoglobina glicata in elettroforesi capillare in un paziente portatore della variante J- Calabria

T. Biagioli[1], M. Brogi[1], L. Lanzilao[1], A. Aldinucci[1], M. Mogni[2], M. Maffei [2], D. Coviello [2], A. Fanelli[1]

[1]Laboratorio Generale, AOU-Careggi, Firenze
[2]Laboratorio Genetica Umana Ist. G. Gaslini - Istituto Pediatrico di Ricovero e Cura a carattere scientifico

Un paziente M di anni 32 giunge alla nostra attenzione con richiesta di dosaggio di emoglobina glicata (HbA1c) per controlli nell'ambito della medicina del lavoro. Il sangue del paziente viene analizzato mediante metodica HPLC Arkray HA-8180V in uso presso il laboratorio; il relativo cromatogramma non consente la refertazione del dato di HbA1c e si sospetta la presenza di una variante emoglobinica comigrante con HbA0. Si procede pertanto ad ulteriori approfondimenti diagnostici consigliando l'esame della fruttosamina per il controllo glicometabolico del paziente e successivamente processando il campione in elettroforesi capillare (Capillarys HbA1c) su strumento Capillarys 3 Tera di Sebia; l'elettroferogramma delle glicate evidenzia chiaramente la presenza di una variante emoglobinica al 37,8% perfettamente separata e risolta sia da HbA0 che da HbA1c. Stante la formula di calcolo utilizzata dal metodo Sebia [HbA1c = HbA1c/ (HbA1c +HbA0)], si può supporre l'assenza di interferenza a livello metodologico ed il valore risulta di 25 mmol/ mol. La particolarità del caso impone cautela nella refertazione del dato e si procede quindi anche alla determinazione di HbA1c con metodo immunologico Celltac Chemi CHM-4100k di Nihon Kohden (metodo basato sul riconoscimento degli amminoacidi Val-His-Leu terminali della catena beta); il valore di A1c risulta di 25 mmol/mol in linea con quello della capillare. La presenza della variante viene confermata anche con metodo specifico per la valutazione degli assetti emoglobinici (Capillarys Hemoglobine) e risulta migrare in Z12 in percentuale analoga a quella precedentemente riscontrata. Si rende pertanto indispensabile, ai fini della valutazione dell'appropriatezza dell'utilizzo di HbA1c per questo paziente, la caratterizzazione molecolare della variante presso un centro di riferimento. Il sequenziamento dei geni beta rivela la presenza in eterozigosi della variante Hb J-Calabria (beta 64(E8) Gly>Asp), la cui sostituzione amminoacidica cade al di fuori del sito di riconoscimento dell'anticorpo utilizzato con il metodo immunologico. Grazie all'elettroforesi capillare è stato possibile quantificare A1c non rendendosi necessario l'utilizzo della fruttosamina come marker alternativo da utilizzare per i futuri controlli.

PO142

Cellular proteolysis systems in Parkinson's disease: activity of proteasome and Acylaminoacyl-Peptide Hydrolase

C. Fusco[1,3], E. Cocca [1], R. Camerlingo[2], G. Palmieri [1], A. Di Costanzo[3], A. Angiolillo[3]

[1]Institute of Biosciences and BioResources, CNR, Napoli, Italy
[2]SC Cell Biology and Biotherapy, Istituto Nazionale Tumori-IRCCS-Fondazione G. Pascale, Naples, Italy
[3]Department of Medicine and Health Sciences, University of Molise, Italy

Over the last years, there has been a growing interest in identifying and characterising the factors involved in the etiopathogenesis of Parkinson's disease (PD). The proteasome complex, a major regulator of intracellular protein homeostasis, seems to be involved in many neurodegenerative disorders characterised by the formation of aberrant proteins in neuronal cell bodies. Besides the proteasome-system, the Acylaminoacyl-Peptide Hydrolase (APEH) was also reported to play a key role in the protein degradation machinery and antioxidant processes. APEH is a ubiquitous bifunctional enzyme, exhibiting exopeptidase activity on N-acyl-peptides and endoprotease activity on oxidised proteins. We have previously proposed the hypothesis of a combined action between proteasome and APEH activity in maintaining cellular homeostasis within the context of neurodegenerative disease, particularly Alzheimer's disease. The present study aimed to investigate the activity and the blood levels of proteasome and APEH in PD patients compared to healthy controls (HC). Forty-six participants were recruited and divided into two groups based on their clinical profiles: 23 PD and 23 HC. Venous blood samples were used to obtain erythrocyte hemolysates and measure the chymotrypsin-like proteasome and exopeptidase APEH activities by spectrofluorimetric analyses. A statistically significant increase in proteasome and APEH activities and gene expression was found in PD samples compared to HC. Therefore, this work suggests that dysfunctions within the cellular proteolysis systems could play a pivotal role in the onset of the disease. Although the sample is not very large, the correlation studies performed did not reveal significant relationships between the two catabolic processes, suggesting that they act independently. In light of what is reported both in the literature and in our findings, it is possible to hypothesise that the accumulation of α-synuclein, characteristic of the PD, could be linked to impairment of the proteasome and APEH systems. If these results are confirmed in larger prospective studies, the measurement of the activity of such systems, using a simple blood sample, could simplify the diagnostic approaches allowing the early diagnosis of PD.

PO143

MicroRNA detection in tears from dry eye patients atraumatically collected by aspiration

C. Ciavarella, P. Versura

Laboratory for Ocular Surface Analysis and Translational Research - DIMES – Alma Mater Studiorum University of Bologna

Aim: MicroRNA (miRs) play a posttranslational role regulating protein production, with an expanding interest in the pathogenesis of ocular surface diseases, but the techniques of collection for their retrieval and characterisation is still to be optimised for application to the clinical setting. We provide a protocol for ocular surface miR extraction using cells collected with tears.

Methods: A minimum of 10 µL tears was sampled by aspiration from the lower fornix of fifteen patients suffering from dry eye disease, centrifuged at 13.200 rpm for 15'. The cells in the pellet were quantified, and then processed for RNA extraction by lysis with 200µL Trizol. RNA quality and concentration of the final flow through collected was determined on the Nanodrop ND-1000 Spectrophotometer. A volume of 1.5 µL was used for cDNA reverse-transcription, by using the TaqMan microRNA Reverse Transcription kit. Then, cDNA was subjected to preamplification through TaqMan Preamplification Master Mix. miR expression was performed through Real Time PCR, using TaqMan MicroRNA Assay for miR-30a-5p and miR-30d and TaqMan Universal Master Mix II No AmpErase UNG, and normalized to non-coding small nuclear RNA (snRNA) U6. Real Time PCR was carried out in CFX Connect Real-Time PCR. The intensity of subjective discomfort at sampling was quantified using the 100-mm horizontal line length VAS score.

Results: Estimation of the pellet source was of 500±100 cells/µL. From each pellet we obtained an amount of total RNA of 69.3 ± 48.15 ng/µL, as an average on a final volume of 10 µl. The VAS score was 1.12±0.22.

Conclusions: Our data show this method is well tolerated by patients, with minimum discomfort. The amount of RNA retrieved is comparable to that obtained with more expensive and painful impression cytology collection methods, and represents an enhancement on RNA concentration, well above the minimum requirements. Supernatant of centrifuged tears can be further utilized for biochemical analysis.

PO145

The Lean methodology applied to risk assessment in the "Blood Gas Analysis" process

E. Tripodo[1], M. Fantacci[2], P. Sanchini[1], S. Di Mario[1], C. Donnini[1], A. Tarquini[2], C. Artini[1], S. Fabbroni[1], P. Magliocca [1], S. Gervino[1], M. Lorubbio[1], F. Cinci[1], A. Sereni[1], A. Ognibene[1]

[1]Clinical Chemical Analysis Laboratory, San Donato Arezzo Hospital

[2]Clinical Chemical Analysis Laboratory, Montepulciano Valdichiana Reunited Hospital

INTRODUCTION The BGA (Blood Gas Analysis) is a bedside testing that ensure results in a short time, usually performed in Emergency-Urgency for time-dependent clinical condition. Lean Thinking for "KAIZEN" or rather, continuous improvement in services provision increasing the value perceived by the user reducing of waste. The aim of the present analysis has been to assess, applying Lean methods, the likelihood of risk in the BGA testing, related to the increase in their use during the COVID19 pandemic.

MATERIALS AND METHODS To carry out the risk assessment, the context was studied through the use of distribution maps of the BGA analyzers in the Covid19 and non-Covid19 wards of the San Donato Hospital in Arezzo. Data was collected on the entire BGA process: activities, hours of greatest use, instrumental stops and maintenance, in the year 2020. The spaghetti chart, Lean mapping method, was used to calculate the distances between the instruments (used and backup) and patient beds. In addition, Lean methods were used: the Hishikawa Diagram with the cause-effect matrix, the HFMECA, the Swot, the monitoring indicators of the entire process and the A3 report.

RESULTS The analysis carried out using the Lean methodology has shown that the entire ABG process is well governed within the wards. However, there remain the risks associated with the new staff employed and their training on the which much of the implementation plan was focused. The risk is mainly linked to the high turnover, to the reorganization of human resources that had to be used to deal the pandemic.

CONCLUSIONS For optimal governance of BGA analyzers and reduce the risk in entire process, it is essential to maintain adequate and continuous training of personnel.

PO146
Evaluation of the Elecsys SARS-CoV-2 Antigen test

A. MARINO, A. BETTINARDI, G. BORRELLI, G. FIORDALISI, G. SAVERIAMPILLAI, P. CAMOSSI, G. FALOCCHI, F. GIMITELLI, E. PELAMATTI, V. MARETTI, L. MORANDINI, G. BONETTI

Laboratory of Clinical Pathology, ASST-Valcamonica, Esine, Brescia, Italy.

Background: In the context of the SARS-CoV-2 pandemic, the development and validation of rapid diagnostic methods are of high priority. This study was performed to evaluate the Elecsys SARS-CoV-2 Antigen test (Roche) on a Cobas e 602 system [antigen-detecting rapid diagnostic test (Ag-RDT)].Materials and Methods: The Ag-RDT was performed using nasopharyngeal (NP) swabs in UTM (Copan) according to manufacturer. Diagnostic accuracy was determined in NP samples in comparison rRT-PCR performed on a CFX96™ System (Bio-Rad) using ELITe MGB amplification Kit (ELITech) detecting the ORF8 and RdRP genetic loci of SARSCoV-2 after RNA extraction. Whitin-laboratory imprecision was evaluated according to CLSI EP-15 A3 protocol using pooled UTM of NP samples previously treated with Extraction Solution C stored at -20°C. A recovery study was performed by duplicate analysis of serial dilutions ranging from 1:10 to 1:5120 of a high viral load sample (ORF8 Ct 17.3,RdRP Ct 18.2).Results: A total of 114 NP samples were included; 99 (86.8%) were rRT-PCR-positive. The median patient age was 49 years, 56 were male. The area under the ROC curve (AUC) for rRT-PCR positivity was 0.952 (95% CI, 0.895-0.983; p<0.0001), with an optimal cut-off of 0.531 COI, associated with 81.8% sensitivity and 100.0% specificity. The suggested cut-off of 1.0 was associated with 62.6% sensitivity and 100.0% specificity.In positive rRT-PCR samples a strong correlation was found between Ct of target genes and Ag-RDTI: log(COI Ag-RDT)= 21.576 + -13.809 log (Ct ORF8), r=0.94, P<0.001 and log (COI Ag-RDT)= 25.243 + -16.257 log (Ct RdRP), r=0.95, P<0.001.An agreement of 100%, 100%, 69.6%, 7.7% and 0.0% was found between Ag-RDT and rRT-PCR in subgroup of samples with Ct<=25 (n=22), 25-30 (n=23), 30-35 (n=23), 35-40 (n=26) and >40 (n=5), respectively.At a 4.84 COI a SD of 0.237 and a within-laboratory CV of 4.9% was found slightly higher than that indicated from the manufacturer (CV 3.5%).A linear response was found from 8.0 to 3996 COI with a recovery within ±10%.Conclusions: Elecsys SARS-CoV-2 Antigen showed a good performance in particular in samples with high viral loads. This test has the potential to become an important tool for a rapid diagnosis in situations with limited access to rRT-PCR.

PO147
EVALUATION OF IL-6 LEVELS IN COVID-19 PATIENTS WHIT SMELL DISORDERS.

R. De Bellis, M. Antonucci, V. Boccassini, D. Musaico, R. Lovero, L. Varraso, R. Contino, L. Loiodice, A. Schirinzi, F. Di Serio

1 AOU Policlinico Consorziale di Bari - Ospedale Giovanni XXIII, Clinical Pathology Unit, Bari 70124, Italy.

Smell dysfunction is one of the most frequent symptoms in COVID-19 patients. In the early stages of the disease it allows to identify positive subjects. The odorous substances recognize two different systems in the olfactory epithelium: the “olfactory” and the “trigeminal” systems that coexist and interact in the processing of sensory information. In COVID-19 patients there is an inflammatory reaction of the nasal mucosa. Infected supporting cells of the nasal mucosa release molecules that activate the local antiviral innate immune response. In fact, macrophages spread inflammatory mediators, in particular TNF-¤, IL-6 and IL-1. In this study we compared IL-6 levels with the degree of olfactory disorders and with the type of unperceived odour.Materials and methodsFrom 15 March to 30 November 2020 have been selected 82 patients (45 men age 62.3 ±14.2 and 37 women age 57.1± 12.8) with only smell dysfunctions were divided into mild and moderate patients. The evaluation of the smell disorder was carried out with a 14 questionnaire relating to the perception of domestic odorous: 6 questions for olfactory sensitivity (own perfume usually sprayed, oregano, olive oil, nutella, coffee aroma, orange juice) and 8 for olfactory-trigeminal sensitivity (alcohol, fish odor, vinegar, mint (gum), toothpaste, shampoo, cheese, ammonia).The IL-6 (v.n. 0 – 7 pg/ml) was measured with chemiluminescence assay using Cobas e801 (Roche Instrumentation). Statistical analyses were performed with Wilcoxon Rank test, and Mann-Whitney test (p <0.05). ResultsThe trigeminal and olfactory sensitivity are more compromised in moderate than mild patients (p <0.05). The statistically significant differences there were in IL6 levels in moderate versus mild patients when there was an impairment of trigeminal sensitivity (p <0.05). Conclusion In this study suggested that the smell disorders in Covid-19 patients couldn’t be a deficit of the olfactory central nervous pathways but could be rather than mainly associated with the inflammatory process of the nasal mucosa and that deficit of the type of domestic unperceived odour ("olfactory" or "trigeminal" sensitivity) could indicate the degree of severity of the disease.

PO148

HCV (genotype) distribution in the area of Arezzo: epidemiological data in the eradication strategy

A. Sereni, M. Mafucci, P. Magliocca , S. Gervino, G. Viti, P. Sanchini, S. Fabbroni, M. Lorubbio, E. Tripodo, A. Moncada, A. Ognibene

Clinical Chemical Analysis Laboratory, San Donato Arezzo Hospital

INTRODUCTION Infection with hepatitis C virus (HCV) is a major cause of chronic liver disease worldwide. HCV is enveloped, small circular, positive-sense and single stranded ribonucleic acid (RNA) virus from genus Hepacivirus, family Flaviviridae. The ability of the HCV to mutate has resulted in the existence seven main genotypes (1–7) with multiple subtypes. Genotyping is most significant for planning of HCV treatment and helps to cure HCV infection. The Tuscany region has declared its intention to proceed with the eradication of the Hepatitis C virus in the population. The aim of this retrospective study is to investigate the incidence of HCV in a population screened in the area of Arezzo from 2016-2020. Furthermore, genotype distribution in those subjects positive for screening was studied. MATERIALS AND METHODS The subjects included in the study 5012(31.3%) were positive for HCV-RNA quantification, so they were enrolled for viral genotyping. Blood sample collected in BD Vacutainer® PPT™ Plasma Preparation Tube were used to perform the viral load quantification of the HCV and for genotyping analyses. RESULTS In the patients examined from 2016 to 2020 years we have been note a reduction in positive cases of HCV: 1250(35.4%) in 2016 respect 521(25.4%) in 2020. Among the patients examined from 2016 to 2020 years have been seen a major incidence for genotype 1(55.8%), comparable fraction for type 2(19.2%) and 3(19.1%) and lower incidence for genotype 4(0.05%). The distribution of the HCV genotypes in the years showed that genotype 1a reach the peak of incidence in the 2018 year, while the trend of 1b genotype was almost constant in the period 2016-2020. The genotype 2a/2c reach a peak in the years 2018 and 2019, the genotype 3a were observed an increase in the 2017, then a reduction. The genotype 4 was constant in the years. Conclusion The data from the present study seem to suggest that there has been a slight change in the epidemiology of HCV genotypes in the Arezzo, although the genotype 1b remain the most prevalent in Italy, the genotype 3a in surprisingly increased in the area of Arezzo. This study show a reduction of positive cases during the years, as a result of the Tuscany's new eradication strategy, despite the reduced requests during covid emergency.

PO149

Evaluating anti-aging protein α-Klotho in serum and CSF of Parkinson's disease patients

E. Giannella[1], M. Alwardat[2], P. Grillo[2], M. Pieri[3], S. Bernardini[3], N.B. Mercuri[2,6], T. Schirinzi[2], M.G. Sancesario[1]

[1]Biobank Unit, IRCCS Fondazione Santa Lucia, Rome – Italy

[2] Department of Systems Medicine, University of Roma Tor Vergata, Rome, Italy

[3]Department of Experimental Medicine, University of Roma Tor Vergata, Rome, Italy

[4]Department of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy

[5]IRCCS Mondino Foundation, Pavia, Italy

[6] IRCCS Fondazione Santa Lucia, European Centre for Brain Research, Rome, Italy.

ABSTRACT One of the challenges of laboratory medicine is the identification of reliable biomarkers to obtain an early and non-invasive diagnosis. In neurological diseases, the cerebrospinal fluid (CSF) represents the main source for biomarkers, but the invasiveness of collection represents an obstacle to the analysis. α-Klotho (KL) is a recently discovered biomarker involved in several age-related and neurodegenerative disorders, but its role in Parkinson's disease (PD) is still poorly explored.

In our study, we assess the level of α-Klotho in CSF and serum of 36 PD patients and 9 controls; moreover, we evaluated the level of CSF α-synuclein and other biomarkers of neurodegeneration.

We found KL was increased in CSF in PD compared to controls, independently from age and sex, and inversely associated with CSF α-synuclein. Oppositely, serum KL was reduced, in the absence of correlations with respective CSF values or blood-brain-barrier integrity index.

In PD, CSF and serum KL represent two distinct pools, the first activated probably to counteract accumulating synucleinopathy, the second instead down-regulated.In conclusion, KL emerges as a biomarker or candidate target for neuroprotective interventions in PD. Future studies need to confirm the accuracy of KL and other serum biomarkers, to investigate PD pathology and reach an early and non-invasive diagnosis.

PO150
Importanza delle frazioni reticolocitarie nella gestione dell'anemia del neonato

M.P. Monaco, L. Frascogna, V. Federigo, A. Lombardo, R. Riccio, I. Piccirillo

Lab.di Patologia Clinica, Osp. San Giuliano, Giugliano

Introduzione
Nel neonato nei primi giorni di vita si può avere una anemia normocromica-normocitica, determinata dall'aumento dell'ossigenazione, condizione fisiologica, che non richiede particolari trattamenti.
Tuttavia, il perdurare di una anemia, talora severa, deve indirizzare il clinico verso il sospetto di una condizione patologica degna di essere approfondita, soprattutto nel neonato pretermine.
L'anemia si manifesta più frequentemente in bambini di <32 settimane di gestazione, a causa di una emorragia prenatale(emorragie materno-fetali, malformazioni cordonali, anomalie placentari, procedure diagnostiche), perinatali (parto precipitoso,errori ostetrici, coagulopatie) o emorragie post-partum, e, si presenta con reticolocitosi, ipocromia, macrocitosi.
Il laboratorio può fornire il proprio contributo grazie ad alcuni indici ematologici, spesso trascurati, i reticolociti e le frazioni reticolocitarie che permettono un migliore inquadramento diagnostico terapeutico del paziente.
Scopo del lavoro
Gli indici reticolocitari sono stati usati con ottimi risultati nel monitoraggio delle terapie con eritropoietina, ferro o trasfusioni nei pazienti adulti, non ci sono in letteratura valori di riferimento in campo neonatale, motivo per cui abbiamo creato intervalli di riferimento dei diversi stadi di maturazione reticolocitaria nel neonato nei primi 30 giorni di vita ed abbiamo utilizzato tali valori nel monitoraggio della risposta terapeutica in un caso clinico di neonato pretermine (33 settimane), giunto alla nostra osservazione con diagnosi di anemia.
Materiali e metodi
Abbiamo esaminato con esame emocromocitometrico e conta reticolocitaria eseguiti su ADVIA 2120 Siemens circa 120 campioni di neonati a termine.
La nostra attenzione si è rivolta al piccolo nato pretermine in cui si è sviluppata una severa anemia da emorragia perinatale in cui abbiamo valutato la risposta alla terapia con ferro dalla seconda settimana di vita e la ripresa degli indici reticolocitari.
Conclusioni
Il conteggio del numero totale espresso in percentuale dei reticolociti non fornisce informazioni utili e dirimenti circa la terapia cui il paziente è stato sottoposto, la valutazione delle variazioni delle frazioni reticolocitarie permette, invece, al clinico un migliore orientamento diagnostico terapeutico.
Sarà, però, visto il piccolo numero di pazienti osservati, utile e necessario approfondire con ulteriori studi le frazioni reticolocitarie nei piccoli pazienti anemici.

Bibliografia
1. Weatherall DJ.et al.; 2011 System biology and red cell, The New England J.Med.364;376-377
2. Chung NG. et al. Current insights into inherted bone marrow failure syndrome. Korean J. Pediatr. 2014;57:337-44
3. Colombatti R. et al. Anemia and trasfusion in the neonate. Seminars in Fetal and Neonatal Medicine 2016;21:2-9
4. Gallagher P. G. The neonatal erytrocyte and its disorders. In: Nathan and Oski's Hematology and Oncology of Infancy and Childhood, 8th ed, Orkin S H, et al. Philadelphia. WB Saunders 2015, p.52

PO151

INTERPRETATION OF HIGH CT VALUES OF N GENE OF SARS-COV-2 BY REAL-TIME PCR

V. FEDERIGO, A. SORRENTINO, L.A. PICCOLO, E. MAURIELLO, C. ROMANO, R. RICCIO, I. PICCIRILLO

P.O. San Giuliano ASL Napoli 2 Nord Lab. di Patologia Clinica, Giugliano, Italy

We examined the impact of the molecular diagnosis of CoViD-19 based on only one target gene detected at high Ct. About 1600 nasopharyngeal swabs, collected from both male and female patients, were analyzed everyday for the detection of N and RdRp genes of SARS-CoV-2 by Real-Time PCR with NeoPlex CoViD -19 (GeneMatrix, Eurospital). About 11% of samples were found positive to N gene at high Ct (Ct > 37) and negative to RdRp gene.However, patients were retested after 24 hours, 48 hours or 1 week and about 2% of samples were found negative for both N and RdRp genes.These data suggest that probably samples were initially found positive to only N gene because of very low viral RNA loads.In coronaviridae, including SARS-CoV-2, subgenomic RNAs (sgRNA) are replicative intermediates, whose presence could correlate with viral replication activity and with the type or stage of the infection.Moreover, lower levels of RNA were detected in asymptomatic patients with post-CoViD-19 conditions.Therefore, to get an accurate diagnosis, samples resulted positive for only N gene at high Ct has to be carefully interpreted and clinical correlation is highly recommended to define the most right and effective therapy.

PO152

Implementation of a Next-Generation Sequencing (NGS) analysis for the diagnosis of metabolic and rare diseases and application in a paediatric population: results after one year of activity

F. Barretta[1,2], B. Mirra[1,2], F. Uomo[1,2], S. Fecarotta[3], G. Parenti[3], C. Mazzaccara[1,2], G. Frisso[1,2]

[1]Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università Federico II, Napoli (Italy)
[2]CEINGE Biotecnologie Avanzate, s.c.a r.l., Napoli (Italy)
[3]Dipartimento di Medicina Translazionale-Sezione di Pediatria, Università Federico II, Napoli (Italy)

In recent years, advances in genomics and high-throughput sequencing technologies, such as Next Generation Sequencing (NGS), have revolutionized the molecular diagnostics of the Inherited Metabolic (IMD) and Rare (RD) Diseases. This approach allows for the simultaneous analysis of different samples for several genes, potentially involved in these disorders, with low costs and optimizing analysis times. The aim of this study was to demonstrate how the implementation of the NGS methodology has improved the molecular diagnosis of IMD and RD. Between June 2020 and May 2021, we performed, in 64 patients, the NGS molecular analysis for searching of genetic mutations in genes associated with over 200 IMD/RD, including the IMD of the Newborn Screening (NBS).Molecular testing was carried out by using NextSeq (Illumina), technology and data analysis was performed by BaseSpace (Illumina) software. The detected variants were confirmed by Sanger sequencing and classified according to American College of Medical Genetics guidelines.Thirty-six/64 patients were new-borns, with a positive test for NBS and 28 were patients with biochemical alterations and/or a clinical phenotype suggestive of IMD/RD. Genetic analysis showed pathogenic or likely pathogenic variants in 47 (73%) of the analysed patients. Mutations in genes considered not frequently related to clinical phenotype, were identified in 10% of cases. These uncommon genes would not have been identified by using Sanger sequencing approach. Genetic test represents a fundamental tool to confirm or exclude the patients' clinical suspicion which is based on the NBS results and/or the presence of laboratory alterations. Furthermore, the early diagnosis of IMD/RD is fundamental in preventing serious and permanent complications on the new-born health. Finally, the NGS approach has consistently increased the sensitivity of the genetic test.

PO154

Next-Generation Sequencing Gene Panels in inheritable cardiomyopathies and channelopathies: Prevalence of Pathogenic Variants and Variants of Unknown Significance in uncommon genes

B. Mirra[1,3], V. D'Argenio [1,2], F. Barretta[1,3], F. Uomo[1,3], M. Caiazza[5,6], M. Losi[4], G. Limongelli[5,6], R. Lombardi[4,7], G. Frisso[1,3], C. Mazzaccara[1,3]

[1]CEINGE Advanced Biotechnologies, Via Gaetano Salvatore 486, 80145 Napoli, Italy

[2]Department of Human Sciences and Quality of Life Promotion, San Raffaele Open University, Via di val Cannuta 247, 00166 Roma, Italy

[3]Department of Molecular Medicine and Medical Biotechnologies, University of Naples Federico II, Via Sergio Pansini 5, 80131 Napoli, Italy

[4]Department of Advanced Biomedical Sciences, University of Naples Federico II, Via Sergio Pansini 5, 80131 Napoli, Italy

[5]Monaldi Hospital, AO Colli, Italy

[6]Department of Translational Medical Sciences, University of Campania 'Luigi Vanvitelli', Italy

[7]Division of Cardiology, University of Colorado Anschutz Medical Campus, 12700 E 19th Ave # F442, Aurora, CO 80045, USA

The knowledge of the molecular bases of cardiomyopathies and channelopathies has increased due to the diffusion of Next Generation Sequencing (NGS)-based approaches, which have allowed identifying causative, pathogenic mutations in more than 200 different genes. Consequently, since also genes considered “uncommon” for a clinical phenotype are now included in molecular testing, the detection rate of disease-causing variants is increased. We report the prevalence of genetic variants, detected by using a NGS custom panel, in a cohort of 134 patients with an inherited cardiomyopathy or channelopathy and 11 healthy subjects with a positive family history for Sudden Cardiac Death, enrolled during an observation time of 3 years. Interestingly, 59 out of the 96 detected variants (61%) were identified in genes considered without a strong or definitive evidence of disease association, according to the NIH-funded Clinical Genome Resource (ClinGen), a standardized evidence-based framework to systematically assess gene-disease relationships, and here named “minor genes”. In particular 6/59 (10%) variants were reported as pathogenic/likely pathogenic (P/LP) and 49/59 (83%) uncertain significance (VUS) and 4/59 (7%) as benign or likely benign (B/LB) variants, according to ACMG classification. Thirty three /145 (23%) patients carried at least one L/LP variant; six out of thirty-three (18%) showed P or LP variants in “minor genes”. Furthermore VUS variants were identified in 50/145 patients (34%), 41/50 patients (82%) showed VUS in “minor” genes. These data reinforce the need for the screening of uncommon genes, in order to identify the possible genetic cause of inherited cardiomyopathies/channelopathies and to increase the diagnostic sensitivity of molecular testing. The extended molecular screening allows bettering managing the borderline cases, to identify the at-risk family members and start early their management. However, the use of NGS-based approach in diagnostics is increasing the yield of VUS, whose clinical interpretation is still challenging, because the functional and structural impact of many variants is difficult to establish.

PO155

Territorial Diagnosis: Project by the Lombard coordination of the professional registers of Medical Laboratory Technicians

N.A. Saletta[1], A. Di Lorenzo[2], A.M. Stefanone[3], A. Carobbio[4], A. Alberti[5], R. Dalzini[6], S. Lambiase[7], D. Antognoli[8], G. Morstabilini[9]

[1]*SMeL, Osp. Bassini, ASST Nord Milano, Cinisello B.*
[2]*SIMT, ASST Valle Olona, Busto Arsizo*
[3]*SIMT, Osp. Voghera, ASST Pavia*
[4]*Lab. Microbiologia, ASST Papa Giovanni XXIII, Bergamo*
[5]*CdS Tecniche di Laboratorio Biomedico, ASST Spedali Civili, Brescia*
[6]*SMeL, ASST Mantova*
[7]*SMeL, ASST Cremona*
[8]*S.C. Anatomia e Istologia Patologica, ASST Sette Laghi, Varese*
[9]*DAPS, ASST Cremona*

The Regional Law 23/2015 redesigned the Healthcare System of Lombardy, re-evaluating territorial and proximity medicine whose basic principle is to organize and manage the processes of taking care of people in a coordinated and integrated way, through the creation of centrally defined clinical protocols, making a sum of interorganizational and interprofessional connected actions.
Hospital and territorial integration provides a perspective of development and enhancement even for those professions that had not, so far, actively participated in territorial diagnostic therapeutic paths such as the Medical Laboratory Technician (MLT), who can become part of care and prevention system in the territorial context, through diagnostic tests performed at patient's home (POCT) or at decentralized structures from central laboratories (community hospitals, clinics, family doctor, pharmacy), ensuring immediate and safe use of the analytical data avoiding late diagnosis. Type of MLT intervention models on the territory could be the following:
- Management: functional and organizational management with identification of intervention areas for chronic diseases;
- Coordination of activities: surveillance responsibility and management with optimization of resources;
- Performing of sampling and analysis: analytical phases management with control of the analytical data conformity;
- Training for patients and Caregivers: training to guarantee the good use of tools and analysis quality.
The POCT methodology, with the contribution of MLT, promotes the improvement in the clinical management of patients, thanks to rapid replies of controlled and verified analytical data to the clinicians and, from a logistical point of view, to a decrease in overcrowding in the healthcare area, increasing the diagnostic activity effectiveness.
The introduction of MLT skills in the Care Networks will bring the added value of all the operators involvement in the Health System, with a continuous exchange of knowledges and information to better guide the patient in the different diagnostic care paths, optimizing performances and correct use of the available services, in order to guarantee the continuity of the integrated care between Hospital and Territory.

PO156

The effect of direct oral anticoagulants on the prolongation of ACT induced by unfractionated heparin. An in vitro study.

M. Casini[2], F. Negro[3], P. Caravelli[3], R. Morganti[3], L. Ruocco[2], R. De Caterina[3]

[1]*Lab. Analisi Chimico Cliniche Azienda Ospedaliero Universitaria Pisana*
[2]*Med. Cardiovascolare 1 Azienda Ospedaliero Universitaria Pisana*

Background and aims - Unfractionated heparin (UFH) is the first choice and the most often used parenteral anticoagulant during percutaneous invasive procedures such as catheter ablation for the therapy of atrial fibrillation (AF); its farmacological effect is monitored by Activated Clotting Time (ACT). Catheter ablation in patients with AF may be associated with the risk of thromboembolic and hemorragic complications. Many studies show how non-interruption of oral anticoagulant therapy is safe and effective in reducing the risk of embolic events. The effects of direct oral anticoagulants (DOAC) on the ACT and its prolongation induced by unfractionated heparin are not clear yet. We evaluated the longthening of the ACT caused by different concentrations of UFH added in samples of patients receiving DOAC and in a group of non-anticoagulated control patients.
Methods and Results - We measured the ACT in patients on dabigatran (BID, n=10), rivaroxaban (SID, n=10), apixaban (BID, n=10) and edoxaban (SID, n=10) at peak plasma drug concentration, before and after the in vitro addition of three increasing doses of UFH, corresponding to administered in vivo concentrations of 2000 IU, 5000 IU and 10.000 IU. Seven patients not taking DOAC served as a control group. Patients in the five groups did not differ significantly for age, body weight and estimated glomerular filtration rate, baseline means ± standard deviations ACT (seconds) were 192±31, 124±14, 133±13, 161±30 and 134±7 in dabigatran, rivaroxaban, apixaban, edoxaban and control patients, respectively; ACT on dabigatran was significantly higher ($p<0.05$) than for the other DOAC. We found a linear prolongation of the ACT with the in vitro addition of UFH ($p<0.001$), but prolongation was similar between the DOAC and the control group.
Conclusions – Our study shows that, except dabigatran, DOAC do not modify the basaline ACT. None of the four drugs affect the prolongation of the ACT due to the scalar additions of heparin and there are no differences when this effect is compared to the control group. ACT can therefore be used to mesure the anticoagulant activity of heparin even in patients taking DOAC.

PO157

Rapid molecular diagnostics for COVID-19 in Emergency Room

G. BONETTI[1], A. BETTINARDI[1], G. FIORDALISI[1], G. BORRELLI[1], G. SAVERIAMPILLAI[1], M. BARDELLI[1], L. BONFATTI[1], L. MORANDINI[1], S. BONETTI[2], F. MANELLI[2]

[1]*Laboratory of Clinical Pathology, ASST-Valcamonica, Esine, Brescia, Italy*
[2]*Emergency Department, ASST-Valcamonica, Esine, Brescia, Italy*

Background: In this SARS CoV-2 pandemic, there is a need of rapid and reliable diagnostic tools for highly urgent cases. Antigen tests lack of sensitivity at low viral load so there is the need of molecular tools allowing a rapid diagnosis. In this study we evaluated VitaPCR™ SARS-CoV-2 Assay (Credo), a rapid (20 min) POC nucleic acid amplification test used for patients admitted to Emergency Department.Materials and Methods: The VitaPCR™ detects: the human ¤-globin gene, a specific sequence on the nucleocapside N-encoding gene and a conserved sequence (common to SARS-CoV2, SARS-CoV, and SARS-like bat coronavirus) located on the N encoding gene. Diagnostic accuracy was determined in 73 nasopharyngeal specimens (NP) samples in comparison to the routine and STAT rRT-PCR methods in use. In particular, 24 NP samples, 11 positive for ORF8 (Ct 17.5-35.3) and RdRP (Ct 18.9-33.6) genes were tested on EliTech diagnostic line and 21 NP samples, 13 positive for E (Ct 16.7-35.3) and RdRP (Ct 18.9 -36.4) genes were tested on Roche diagnostic line, requiring a previous RNA extraction, with total assay time of 5 hours. 28 NP samples, 18 positive for E (Ct 11.7-34.6) and N2 (Ct 14.3-37.0) genes were tested on STAT GeneXpert System (Cepheid) diagnostic line, with total assay time of 1 hour. To evaluate if VitaPCR™ assay influenced the management times of patients in the emergency room (ER) a comparison of a 45 days period (from 1 June to 14 July) with (year 2021) and without (year 2020) the use VitaPCR™ was performed.Results: An agreement of 100% in SARS CoV-2 RNA detection for ELITe MGB amplification Kit (ELITech) on a CFX96™ System (Bio-Rad) and of 95.2% with weighted k of Cohen of 0.901 (95% CI 0.714 – 1.000) for LightCycler z-480 (Roche) using LightMix ® Modular Wuhan CoV RdRP-gene and LightMix ® SarbecoV E-gene plus EAV control kits (Roche) was found. An agreement of 96.4% with weighted k of Cohen of 0.924 (95% CI 0.778 – 1.000) was found between VitaPCR™ and Xpert Xpress SARS-CoV-2. For systems targeting N gene (VitaPCR™and Xpert Xpress) P/B regression was: Ct VitaPCR™ = 1.012 (0.918-1.196) Ct Xpert Xpress + 0.000 (95% CI: -1.230 – 0.000). A reduction in ER stay of 35min (-16%), 13min (-7%), 9min (-8%) and 6min (-8%) was recorded for Red (n=151), Yellow (n=703), Green (n=2621) and White (n=466) codes patients, respectively in 2021 vs 2020.Conclusion: The VitaPCR™ SARS-CoV-2 represents an accurate molecular POCT suitable for a rapid diagnosis of COVID-19 also allowing a reduction in management times of critical patients in the ER.

PO158

Monocyte distribution width (MDW) in COVID-19: A pilot study

L. PIGHI[1], S. DENITTO[1], D. BRAGANTINI[3], A. COMEL[5], W. MOSANER[6], P. BAZZONI[4], F. FACCINCANI[6], G. FIORIO[3], G. GIANFILIPPI[2], G. LIPPI[1], G.L. SALVAGNO[1,7]

[1]Section of Clinical Biochemistry, University Hospital of Verona, Piazzale L.A. Scuro, 10, Verona

[2]Medical Direction, Pederzoli Hospital, Peschiera del Garda, Italy

[3]Infectious Diseases Unit, Pederzoli Hospital, 37019 Peschiera del Garda, Italy

[4]Internal Medicine Unit, Pederzoli Hospital, 37019 Peschiera del Garda, Italy

[5]Pneumology Unit, Pederzoli Hospital, 37019 Peschiera del Garda, Italy

[6]Emergency Department, Pederzoli Hospital, 37019 Peschiera del Garda, Italy.

[7]Service of Laboratory Medicine, Pederzoli Hospital, 37019 Peschiera del Garda, Italy.

Introduction: The monocyte distribution width (MDW) reflect volume variation of circulating inflammatory monocytes, cells with high plasticity capable to react against viral infections. Since a few studies have been published on the correlation between coronavirus disease 2019 (COVID-19) and MDW, we further explored this association during the second and third wave of COVID-19 infection in Italy.Materials and Methods: A retrospective study was conducted in two groups of COVID-19 patients, 126 testing positive from November to December 2020 (mean age, 71±15; 41 women, 32.53%) and 59 from March to April 2021 (mean age, 67±17; 25 women, 42.37%). A third group was also included, composed of 123 non-COVID-19 hospitalized patients (mean age, 62±22, ; 62 women, 50.41%). All these patients were hospitalized at the Pederzoli Hospital (Peschiera del Garda, Verona, Italy). UniCel DxH800 Hematology Analyzer (Beckman Coulter Inc., CA, USA) was used to analyze whole blood venous samples previously collected in K2-EDTA, according to routine methods for routine blood cell count (thus including MDW). Results were expressed as median and interquartile range (IQR).Results: MDW values were significantly higher in the entire cohort of COVID-19 patients (median and IQR, 21.8 and 4.4 fL; $p<0.001$), as well as in the first-wave COVID-19 positive group (median and IQR, 22.0 and 4.1 fL; $p<0.001$) and in the second-wave COVID-19 positive group (median and IQR 21.5 and 4.1 fL; $p<0.001$) compared to the control cohort (median and IQR, 17.8 and 2.7 fL). No significant differences in MDW were observed between the two COVID-19 positive groups ($p=0.251$).Conclusion: Monocytes are cells of innate immunity strongly involved in the pathogenesis of COVID-19 and their size variation, as result of infection and/or activation, is reflected by MDW changes. Overall, our data show that MDW is higher in COVID-19, thus mirroring the hyper-inflammatory condition triggered by SARS-CoV-2 infection. Routine assessment of MDW could hence be useful for diagnosing and monitoring patients with SARS-CoV-2 infection.

PO159

Neutrophil/Lymphocyte ratio (NLR): A probable new marker for SARS-CoV-2 infection

S. DENITTO[1], L. PIGHI[1], W. MOSANER[2], F. FACCINCANI[2], D. BRAGANTINI[7], A. COMEL[6], P. BAZZONI[5], G. FIORIO[7], G. GIANFILIPPI[3], G. LIPPI[1], G.L. SALVAGNO[1,4]

[1]Section of Clinical Biochemistry, University Hospital of Verona, Piazzale L.A. Scuro, 10, Verona 37134, Italy;

[2]Emergency Department, Pederzoli Hospital, Peschiera del Garda, Italy

[3]Medical Direction, Pederzoli Hospital, Peschiera del Garda, Italy

[4]Service of Laboratory Medicine, Pederzoli Hospital, Peschiera del Garda, Italy.

[5]Internal Medicine Unit, Pederzoli Hospital, Peschiera del Garda, Italy.

[6]Pneumology Unit, Pederzoli Hospital, 37019 Peschiera del Garda, Italy

[7]Infectious Diseases Unit, Pederzoli Hospital, 37019 Peschiera del Garda, Italy

Introduction: The neutrophil-to-lymphocyte (NLR) is a readily available biomarker reflecting systemic inflammation, derived from a ratio of absolute blood neutrophil and lymphocyte counts. Since coronavirus disease 2019 (COVID-19) requires the identification of early and efficient diagnostic and prognostic biomarkers, we compared the NLR index between subjects with or without positive COVID-19 molecular swab.Materials and Methods: A retrospective study was conducted in two groups (CoV1 and CoV2) of COVID-19 patients, 126 testing positive from November to December 2020 (CoV1; mean age, 71±16; 32.5% women) and 59 testing positive from March to April 2021 (CoV2; mean age, 68±18; 42.4% women). A third group (CoN, controls) included 123 non-COVID-19 ostensibly healthy subjects (mean age, 63±22; 50.4% women). All patients were hospitalized at Pederzoli Hospital (Peschiera d/G, VR, Italy). Whole blood venous samples previously collected in K2-EDTA were analyzed with UniCel DxH800 Hematology Analyzer (Beckman Coulter Inc., CA, USA). Results were expressed as median and interquartile range (IQR).Results: Subjects in group CoV1 had significantly higher NLR values (median and IQR, 6.33 and 9.38; $p=0.029$) compared to subjects testing negative for COVID-19 (median and IQR, 4.10 and 6.44), whilst no significant difference was found between NLR values of group CoV2 (median and IQR, 4.90 and 5.54; $p=0.414$) and the control cohort. Accordingly, NLR values in group CoV1 were significantly higher than in CoV2 ($p=0.031$).Conclusion: In this study, NLR was found significantly higher in COVID-19 patients during the November-December 2020 outbreak, but not during the following March to April 2021 “wave”. We hypothesize that this may be due to the more pronounced inflammatory state in the former period, which was also associated with a much higher hospitalization and death rate in our region.

PO160

Aneuploidy and Translation Initiation Factors in Colorectal Cancer

C. Scuderi[1], A.P. Privitera[1], V. Di Bella[1], D.F. Condorelli[1], V. Barresi[1]

[1]*Università di Catania, Dip. di Scienze Biomediche e Biotecnologiche, Sez. di Biochimica medica*
[2]

The aneuploidy pattern recurrence in cancer is not fully understood and many cancer types show distinctive models of aneuploidy [1,2]. Colorectal cancer (CRC) is often characterized by chromosomal instability defined by an increased percentage of Broad Genomic Aberrations, including many Broad Copy-Number Abnormalities (BCNAs), distinguished in gain- and loss-type. Previous data on correlations between aneuploidies and transcriptomic profiles in CRC have identified genes that are upregulated in cancer and further overexpressed in BCNA-bearing tumors (Over-UpT genes) [3]. Over-UpT genes are enriched for "Fitness Genes" (Fitness-OverT genes) which are indispensable for cell growth and viability in colon cancer cell lines. Fitness-OverT genes are enriched in the Eukaryotic Initiation Translation Factor (EIF) signaling pathway [4]. In this study the anticancer effects of single and combined downregulation of EIF3H (chr8q) and EIF2S2 (chr20q) Fitness-OverT genes, belonging to the EIF pathway, were investigated by multiple silencing. Chr20-gain and Chr8-gain are among the most common chromosomal aberrations in CRC. The RNA interference experiments have been conducted on three colorectal cancer cell lines: HT-29 and CACO-2, characterized by chromosomal instability, and HCT-116, typically with microsatellites instability and near-diploid karyotype. The EIFs gene silencing produced a decrease of their expression levels at 24h and 48h from transfection. siRNA transfection resulted in a significant decrease of survival rate in transfected cells versus control group. In addition, the combined silencing of EIF3H and EIF2S2 produced synergistic effects at 24h and 48h, suggesting a functional cooperation of the two subunits. This study provides results in agreement with the role of eIF3H and eIF2S2 in supporting the growth of cancer cell lines bearing chromosome 8 and 20 gains. A specific eiF3H role in the translation of a subset of oncogenic mRNAs through a mRNA looping mechanism has been suggested [5]. In conclusion, the results obtained support the role of Fitness-OverT genes in the promotion of the malignant phenotype in human CRC and the hypothesis that they might represent candidate targets for transcriptional therapy in tumours bearing chr8 and chr20 gains.

References:

[1] Davoli, Teresa, Andrew Wei Xu, Kristen E. Mengwasser, Laura M. Sack, John C. Yoon, Peter J. Park, e Stephen J. Elledge. 2013. «Cumulative Haploinsufficiency and Triplosensitivity Drive Aneuploidy Patterns and Shape the Cancer Genome». Cell 155 (4): 948–62. https://doi.org/10.1016/j.cell.2013.10.011.

[2] Taylor, Alison M., Juliann Shih, Gavin Ha, Galen F. Gao, Xiaoyang Zhang, Ashton C. Berger, Steven E. Schumacher, et al. 2018. «Genomic and Functional Approaches to Understanding Cancer Aneuploidy». Cancer Cell 33 (4): 676-689.e3. https://doi.org/10.1016/j.ccell.2018.03.007

[3] Condorelli, Daniele F., Giorgia Spampinato, Giovanna Valenti, Nicolò Musso, Sergio Castorina, e Vincenza Barresi. 2018. «Positive Caricature Transcriptomic Effects Associated with Broad Genomic Aberrations in Colorectal Cancer». Scientific Reports 8 (1): 14826. https://doi.org/10.1038/s41598-018-32884-3.

[4] Condorelli, Daniele Filippo, Anna Provvidenza Privitera, e Vincenza Barresi. 2019. «Chromosomal Density of Cancer Up-Regulated Genes, Aberrant Enhancer Activity and Cancer Fitness Genes Are Associated with Transcriptional Cis-Effects of Broad Copy Number Gains in Colorectal Cancer». International Journal of Molecular Sciences 20 (18). https://doi.org/10.3390/ijms20184652.

[5] Choe, Junho, Shuibin Lin, Wencai Zhang, Qi Liu, Longfei Wang, Julia Ramirez-Moya, Peng Du, et al. 2018. «MRNA Circularization by METTL3-EIF3h Enhances Translation and Promotes Oncogenesis». Nature 561 (7724): 556–60. https://doi.org/10.1038/s41586-018-0538-8.

PO162

RISCONTRO OCCASIONALE DI UNA CONDIZIONE EMOLITICA MEDIANTE LA MISURA DI HbA1c IN ELETTROFORESI CAPILLARE

J. Turkman, S. Altinier, C. Artusi, M. Marinova , M. Plebani

U.O.C. Medicina di Laboratorio, Azienda Ospedale - Università di Padova

Una donna di 38 anni con pregressa storia di dermatite ocra e xerosi degli arti inferiori ad eziologia sconosciuta si rivolge presso l'U.O.C. Medicina di Laboratorio per esami di routine che includono emocromo, emoglobina glicata (HbA1c) e indici biochimici. La presenza di anemia ipocromica microcitica e gli indici biochimici alterati (ferritina, recettore solubile della transferrina, transferrina, bilirubina, LDH) risultano compatibili con uno stato di emolisi cronica. L'analisi dell'HbA1c ottenuta tramite elettroforesi capillare (CE) (Capillarys HbA1c kit - Sebia) evidenzia un "profilo atipico" caratterizzato dalla presenza di una frazione anomala "veloce" dell'emoglobina (Hb). Il successivo assetto emoglobinico eseguito in CE (Capillarys Hemoglobine kit - Sebia) conferma la presenza di una frazione di Hb con un picco pari al 27,3% in "Z15", evidenziata anche con metodica alternativa HPLC (Menarini ADAMS A1c HA-8160), sebbene con percentuali discordanti. Gli elettroferogrammi ottenuti depongono per la presenza di emoglobinosi H (Hb H), ipotesi supportata dal quadro di emolisi cronica osservato nella paziente. Il campione viene inviato presso un Centro di Riferimento dove viene eseguita l'analisi molecolare tramite tecnica MLPA che conferma l'ipotesi diagnostica con il riscontro di tre geni α-globinici mutati, non funzionanti.La determinazione di HbA1c richiesta dal medico curante per sospetta diagnosi di diabete mellito (glicemia a digiuno pari a 6.5 mmol/L) eseguita tramite tecnica separativa ha consentito di osservare una frazione di Hb anomala associata alla malattia da Hb H responsabile dell'emolisi cronica e alla quale è possibile ricondurre le manifestazioni cutanee degli arti inferiori.

In conclusione, l'utilizzo di tecniche separative per la determinazione di HbA1c è risultato fondamentale per identificare la presenza di un difetto talassemico che può interferire nella corretta valutazione dell'HbA1c. La presenza di una frazione di Hb anomala deve essere opportunamente segnalata sul referto poiché, in alcuni casi come quello descritto, l'HbA1c non può essere utilizzata per la diagnosi di diabete e per il monitoraggio glicemico a lungo termine.

PO163

Un aPTT insolitamente allungato rivela un LAC in corso di terapia con rivaroxaban. Studio mediante l'utilizzo di DOAC-stop.

M. Casini[2,3], T. Pavia[2], B. Fedi[2], M. Martelloni[4], S. Gonnelli[3], L. Macchia[4], G. Pellegrini[2,3]

[1]Lab. Analisi chimico Cliniche Azienda Ospedaliero Universitaria Pisana

[2]Centro Antitrombosi FCSA n.281 Azienda Ospedaliero Universitaria Pisana

[3]SD Patologia Clinica Azienda Ospedaliero Universitaria Pisana

Donna di 73 anni con tempo di protrombina (PT) e tempo di tromboplastina parziale attivato (aPTT), prolungati in modo inusuale rispetto alla terapia in atto, rivaroxaban 20 mg sid, prescritta per una fibrillazione atriale.Le analisi previste dal nostro protocollo di follow-up hanno dimostrato emocromo, creatinina e transaminasi all'interno dei valori di riferimento; la concentrazione del rivaroxaban a valle (a 24 h dall'ultima assunzione) è risultata 84 ng/mL, il PTr 1,29 e l'aPTT 1,98. Al momento del picco, a circa due ore dall'ultima assunzione di rivaroxaban, la concentrazione ematica del farmaco è risultata 428 ng/mL, al limite alto dei valori attesi, il PTr era 2,37 e l'aPTTr 2,87. Abbiamo deciso di ridurre la dose del rivaroxaban da 20 a 15 mg/die. Al controllo successivo, con l'assunzione del dosaggio più basso, abbiamo misurato una concentrazione di rivaroxaban a valle di 113 ng/mL, con PTr 1,25 ed aPTT 1,85; il rivaroxaban al picco era 261 ng/mL. Le misure a valle e a picco, sia dopo assunzione della dose di 20 che quella di 15 mg/die, mostrano, contrariamente a quanto si osserva usualmente, un prolungamento dell'aPTT decisamente più marcato rispetto a quello del PT, test normalmente più sensibile alla presenza di rivaroxaban. Il dato ci ha fatto sospettare la presenza di un'alterazione della coagulazione di base indipendente dall'assunzione del farmaco anticoagulante.Abbiamo deciso approfondire, incubando il plasma della paziente con DOAC-stop, sperimentando questo presidio per la prima volta. Il trattamento si è dimostrato efficace nel rimuovere il rivaroxaban nel plasma abbiamo assistito ad una completa normalizzazione del PTr (1,08), l'aPTTr invece continuava ad essere allungato (1,59). Il test di miscela ha dato esito incerto, accorciamento non soddisfacente dell'aPTT. Il dosaggio dei fattori XII, XI, VIII e IX e di quelli della fase di contatto, mediante aPTT a 10' sono risultati nella norma, ai limiti bassi il FXII (60%). Il test del LAC ha dato esito positivo; gli anticorpi antifosfolipidi sono risultati negativi. Nell'arco di due settimane abbiamo assistito ad un rialzo delle due transaminasi intorno a 90 U/L. Studieremo ulteriormente la paziente.

PO164

Studio diagnostico delle emoglobinopatie presso il Laboratorio di Patologia Clinica del P.O. Pellegrini - A.S.L. Napoli 1 negli ultimi 5 anni

M. Caldora[1], A. Di Domenico[1], M.A. Fernando Kurukulasuriya[1], M. Acunzo[1], F. Balzamo[1], G. Cardiero[2], G. Cangiano[1]

[1]U.O.C. Patologia clinica, P.O. Pellegrini, A.S.L. Napoli 1, Napoli
[2]Institute of Genetics and Biophysics "Adriano Buzzati Traverso" (I.G.B.- A.B.T., C.N.R.)

Presso il laboratorio di Patologia Clinica del P.O. Pellegrini – A.S.L. Napoli 1, negli ultimi 5 anni sono stati analizzati più di 2000 campioni di sangue per lo studio delle emoglobinopatie provenienti da reparti e da ambulatori dei vari P.O. dell'azienda.
Sono stati identificate e classificate forme di talassemia di tipo alfa, beta, delta, delta - beta e varianti comuni (Hb S, Hb Lepore, Hb C, Hb E) e meno comuni (Hb Policoro, Hb Neapolis, Hb NYU, Hb A2', …).
Considerato l'incremento del numero e della tipologia ed anche il riscontro di due o più difetti emoglobinici nello stesso individuo, gli autori suggeriscono un algoritmo diagnostico - operativo conforme alla maggiore frequenza e complessità dei casi esistente oggi nel nostro Paese a causa dei recenti flussi migratori da territori ove queste alterazioni ematologiche sono endemiche.

PO165

MDW (Monocyte Distribution Width): between sepsis and Covid-19

M. Lorubbio, V. Bartolini, S. Bassi, E. Benigni, A. Beucci, S. Carniani, C. Donnini, S. Fabbroni, P. Giustini, A. Nicolopoulos, C. Palazzi, M. Pettinari, D. Verdelli, A. Ognibene

Clinical Chemical Analysis Laboratory, San Donato Arezzo Hospital

Introduction
As part of the " Lotta alla sepsi - Call to Action" project of the ARS (Regional Health Agency) of Tuscany, a study was undertaken on sepsis which had the aim of evaluating the biomarker MDW (Monocyte distribution width) of all enrolled patients of the PS with suspected sepsis. MDW is a hematological parameter that measures the dispersion around the mean of the monocyte volume population and has been proposed as an early indicator of sepsis. Given the timeliness and ease of measurement with complete blood count (CBC), MDW can quickly help the clinician in risk stratification. Patients who tested positive for Covid-19 were also enrolled during the study. This contribution reports on how the MDW biomarker behaves in patients with similar presentation who may progress to sepsis or Covid-19.
Materials and methods
For the study, 397 patients (178 F + 219 M) aged between 18 and 98 years (mean 62.6 SD ± 21.7) were enrolled by the Emergency Department of the San Donato Hospital in Arezzo between 5 July 2019 and 30 April 2020 with suspected sepsis. Of these, 133 tested positive and 264 negative for the nasopharyngeal molecular swab for SARS-CoV-2. Diagnostic stratification for sepsis was performed according to the definition of Sepsis-3. Complete blood count (CBC), which includes MDW, was performed on the UniCel DxH 900 analyzer (Beckman Coulter) using whole blood collected in tubes with K2 EDTA anticoagulant. Data analysis was performed by IBM SPSS statistics version 20.0 (SPSS, Chicago, IL).
Results
For Covid-19 patients, MDW AUC is 0.85 (95% CI 0.80-0.90) and PCT (procalcitonin) AUC is 0.81 (95% CI 0.75-0.87). The PCT at the cutoff of 0.5 ng / mL had a sensitivity of 13% and specificity of 92%, while the MDW at the cutoff of 22 had a sensitivity of 86% and specificity of 74%. In patients with sepsis MDW AUC is 0.83 (95% CI 0.76-0.90) and PCT AUC is 0.84 (95% CI 0.78-0.91). The PCT at the cutoff of 0.5 ng / mL showed a sensitivity of 66% and specificity of 82%, while the MDW at the cutoff of 22 had a sensitivity and specificity of 90% and 55% respectively.
Conclusion
In both sepsis and Covid-19 infection, the two parameters convey different information and have complete effectiveness in terms of sensitivity and specificity.

PO166

Misura della Bilirubina Neonatale: due metodi a confronto

F. Veroni[1], M.F. Messina[1], F. Cetica[1], S. Rapi [2], E. Stenner[1]

[1]Dipartimento delle Diagnostiche, Azienda USLNordOvest, Ambito Livornese

[2]Dipartimento dei Servizi, Azienda Ospedaliero-Universitaria Careggi, Firenze

INTRODUZIONE

L'iperbilirubinemia neonatale può essere associata a neurotossicità con conseguenze anche gravi (encefalopatia acuta). L'immediato intervento terapeutico, che dipende dai livelli di bilirubina, è alla base di un outcome favorevole. L'EGA può essere un utile ausilio in quanto in un unico step analitico, su minime quantità di sangue, permette il dosaggio della bilirubina. Scopo di questo studio è di valutare la congruità dei dati di Bilirubina sierica totale (TBili) rilevati mediante EGA collocato presso il reparto di Pediatria di un'ospedale periferico isolato, con quelli dello strumento presente nel Laboratorio Centrale dello stesso presidio.

MATERIALI E METODI

Studio osservazionale retrospettivo monocentrico: dati di TBili forniti dall'EGA (GEM 5000, Werfen) su 66 campioni di prelievo capillare di 35 neonati (età<7gg), sono stati confrontati con quelli dello strumento COBAS 6000 (Roche) su sangue periferico dello stesso soggetto. La correlazione tra i due metodi è stata valutata mediante regressione lineare ed analisi di Passing Bablock e Bland Altman (MedCalc versione 12.7.7.0).

RISULTATI

PB: y=-1,49(95°IC-3,04 to -0,21)+1,29(95°IC 1,17 to 1,44)x; BA (ng/mL): media= 1.73 (95%CI: -2,13 a -1,34); limite inferiore=-4,62 (95%CI: -5,29 a -3,95); limite superiore= 1,15 (95%CI: 0.48 a 1,82). CC 0,91 (95°IC 0,8384 a 0,9413).

CONCLUSIONI

I dati evidenziano un errore sistematico e proporzionale con un bias medio assoluto che non permette di definire i due metodi interscambiabili. Occorre quindi cautela nella interpretazione dei risultati con EGA, soprattutto a cutoff. La sovrastima del dosaggio con EGA presuppone la possibilità di utilizzare un cutoff superiore rispetto allo strumento Roche, limitando così i falsi positivi, anche se la priorità è ridurre al minimo i falsi negativi. Necessari ulteriori dati per individuare un cutoff adatto all'EGA. Tale discrepanza può essere, in parte, spiegata dalle differenti matrici utilizzate, dalle differenti metodologie impiegate dalle due strumentazioni ed anche dal fatto che la misurazione non è avvenuta sullo stesso campione. Le linee guida internazionali di Pediatria considerano il prelievo capillare accettabile per la gestione del paziente e non raccomandano la conferma mediante campione venoso, non tenendo conto dei problemi legati alla mancanza di standardizzazione tra metodi, che possono portare ad interventi e trattamenti, che potrebbero non rivelarsi necessari utilizzando metodiche più accurate.

PO167

Dosaggio dell'attività anticoagulante in pazienti trattati con DOAC: studio multicentrico sul confronto di metodi

C. Bulato[1], B. Morelli[2], M. Vidali[3], M. Albertini[4], P. Calzoni[5], B. Casetta[4], M. Casini[6], L. Cerutti[7], S. Marzatico[4], B. Montaruli[8], C.A.E. Novelli [9], A. Papa[10], P. Pradella[11], F.G. Viola[12], P. Simioni[1]

[1]Medicina Generale, MTE, AOUPD, Padova

[2]Laboratorio Synlab - Castenedolo (BS)

[3]Lab. Analisi, Fondaz. IRCCS Osp. Maggiore Policlinico, Milano

[4]R&D Laboratory, B.S.N., Castelleone (Cremona)

[5]Lab. Patologia Clinica, AOUS, Siena

[6]Lab. Analisi Chimico-Cliniche e Microbiologia, AOUP, Pisa

[7]Lab. Analisi Chimico-Cliniche e Microbiologia, ASST Papa Giovanni XXII, Bergamo

[8]Lab. Analisi Chimico-Cliniche e Microbiologia, Osp. Mauriziano, Torino

[9]Centro Immuno-Trasfusionale, ASST Ovest Milano, Legnano (MI)

[10]UOC Medicina di Laboratorio, Fondaz. Toscana G. Monasterio, Pisa

[11]SC Medicina Trasfusionale, ASUGI, Trieste

[12]UOC Biochimica Clinica, Fondaz. Policlinico Tor Vergata, Roma

Introduzione e scopo. Gli anticoagulanti orali diretti (DOAC) generalmente non richiedono un aggiustamento della dose in base ai risultati di laboratorio, ma il loro monitoraggio è necessario in alcune condizioni cliniche. I metodi includono la spettrometria di massa (LC-MS/MS) (gold standard), i test funzionali (tempo di trombina diluito o dTT per il dabigatran e il dosaggio cromogenico dell'attività anti-Xa per gli xabani), o più recentemente il test di generazione della trombina (TGT). In questo lavoro sono stati confrontati i valori ottenuti con i test funzionali, LC-MS/MS e TGT per dabigatran (DABI) e apixaban (API).Metodi. I campioni di pz in trattamento con DABI (n=105) e API (n=120) sono stati raccolti in 7 ospedali e analizzati con i relativi test funzionali. Le rimanenti aliquote sono state conservate a -80°C e successivamente trasportate in altri 2 centri per le analisi con LC-MS/MS e TGT.Risultati. I metodi in LC-MS/MS e funzionali per DABI presentavano una moderata correlazione (rho=0,699; p<0,001); l'analisi di Passing-Bablok (PB) ha evidenziato un errore sistematico proporzionale significativo (slope: 2,15; 95%IC 1,63-2,73). Le correlazioni (n=81) tra i vari parametri TGT e i valori misurati con il metodo funzionale o LC-MS/MS erano rispettivamente 0,769 (p<0,001) e 0,721 (p<0,001) per la lag phase, 0,690 (p<0,001) e 0,667 (p<0,001) per il time to peak, -0,708 (p<0,001) e -0,611 (p<0,001) per il peak height e -0,662 (p<0,001) e -0,527 (p<0,001) per ETP. Tra i due metodi per API era evidente un'elevata correlazione (rho=0,871; p<0,001) e un errore sistematico proporzionale significativo (slope: 0,74; 95%IC 0,69-0,80). Le correlazioni (n=77) tra i vari parametri TGT e i valori misurati con il metodo funzionale o LC-MS/MS erano minori rispetto a quanto

osservato per DABI e pari rispettivamente a 0,236 (p=0,041) e 0,336 (p=0,003) per la lag phase, 0,463 (p<0,001) e 0,493 (p<0,001) per il time to peak, -0,530 (p<0,001) e -0,499 (p<0,001) per il peak height. Nessuna correlazione significativa era presente tra metodo funzionale (-0,073; p=0,534), o LC-MS/MS, ed ETP (0,050; p=0,665).Conclusioni. Sono state rilevate importanti differenze tra i metodi funzionali e LC-MS/MS. Il dabigatran correla maggiormente rispetto all'apixaban con i parametri del TGT.

PO168

DEFINIZIONE DI SPECIFICHE DI PERFORMANCE ANALITICA BASATE SULLO STATO DELL'ARTE DERIVATE DA UN PROGRAMMA DI VALUTAZIONE ESTERNA DELLA QUALITÀ (VEQ) PER SARS-COV-2: RISULTATI PRELIMINARI DI UNO STUDIO COLLABORATIVO TRA UN'ISTITUZIONE E UNA SOCIETÀ SCI

S.M. Mattioli[1,2], D. Brugnoni[1,3], F. Pasotti[4], E. Rizzetto[4], G. Liga[4], M. Quercioli[5], C. Ottomano[6], S. Buoro[4]

[1]Gruppo di studio SIBioC "Qualità analitica"

[2]Laboratorio di Patologia Clinica ASST Valcamonica, Esine (BS)

[3]Laboratorio Centrale di Analisi Chimico cliniche ASST Spedali Civili, Brescia

[4]Centro di Riferimento Regionale per la Qualità dei Servizi di Medicina di Laboratorio, ASST Grande Ospedale Metropolitano Niguarda, Milano

[5]Synlab Italia

[6]I.R.C.C.S. SDN, Napoli

INTRODUZIONE: La necessità di contrastare la pandemia Covid-19 ha accelerato l'iter di acquisizione delle autorizzazioni necessarie per l'immissione in commercio dei test molecolari per la ricerca di SARS-CoV-2, accentuando il bisogno, da parte dei laboratori clinici, di verificare e monitorare l'accuratezza dei risultati prodotti. Poiché tali test forniscono risultati di tipo qualitativo che derivano da dati quantitativi (Threshold Cycle, Ct), la qualità analitica di questi test può essere tenuta sotto controllo verificando che la variabilità dei Ct sia contenuta all'interno del range "valore atteso ± massimo errore accettabile (TEa)". Pertanto, non esistendo, allo stato attuale, specifiche di qualità (APS) basate sui modelli proposti nella 1a Conferenza strategica EFLM tenutasi nel 2014 a Milano, il GdS "Qualità analitica" SIBioC, in collaborazione con il Centro di Riferimento Regionale per la Qualità dei SMeL della Regione Lombardia, ha ritenuto utile proporre APS basate sul modello dello stato dell'arte, calcolando traguardi di TEa per i Ct dai dati inviati dagli 86 Laboratori lombardi e toscani partecipanti al programma VEQ "RNA SARS CoV-2 (diagnostica molecolare)" gestito dal Centro.
METODI: 2608 risultati prodotti in 8 esercizi positivi per 6 geni di SARS-Cov-2 sono stati elaborati come segue: suddivisione per gene, per esercizio e per gruppi di pari (definiti dalla coppia estrattore/amplificatore; almeno 7 utilizzatori della stessa coppia); calcolo della deviazione % di ciascun risultato di Ct dal valore atteso (media robusta del gruppo di pari, dopo la rimozione degli outlier secondo l'approccio di Huber); calcolo del 90° percentile di tutti gli scostamenti.
RISULTATI: Assumendo come TEa il 90° percentile di tutti gli scostamenti dal valore atteso di Ct, i limiti definiti sono i seguenti: gene E: ±11% (numerosità del campione (n) pari a 349 risultati), gene N: ±8,3% (n=300), gene Orf1ab: ±11% (n=90), gene RdRp: ±9% (n=181), gene RdRp/S: ±13,2% (n=45) e gene S: ±8,3% (n=80)).
CONCLUSIONI: I traguardi di prestazione analitica dei Ct desunti dal programma VEQ gestito dall'istituzione lombarda possono essere utilizzati come limiti di

accettabilità da applicare alla procedura per il Controllo di Qualità Interno dei test molecolari per SARS-CoV-2.

PO169

Il laboratorio delle emoglobinopatie alla luce dei recenti flussi migratori.

M. Caldora [1], A. Di Domenico[1], M.A. Fernando Kurukulasuriya[1], M. Acunzo[1], F. Balzamo[1], G. Cardiero[2], G. Cangiano[1]

[1]U.O.C. Patologia clinica, P.O. Pellegrini, A.S.L. Napoli 1, Napoli

[2]Institute of Genetics and Biophysics "Adriano Buzzati Traverso" (I.G.B.- A.B.T., C.N.R.)

Presso il laboratorio di Patologia Clinica del P.O. Pellegrini – A.S.L. Napoli 1, negli ultimi 5 anni sono stati analizzati più di 2000 campioni di sangue per lo studio delle emoglobinopatie provenienti da reparti e da ambulatori dei vari P.O. dell'azienda.

Sono stati identificate e classificate forme di talassemia di tipo alfa, beta, delta, delta beta e varianti comuni (Hb S, Hb Lepore, Hb C, Hb E) e meno comuni (Hb Policoro, Hb Neapolis, Hb NYU, Hb A2', …).

Considerato l'incremento del numero e della tipologia ed anche il riscontro di due o più difetti emoglobinici nello stesso individuo, gli autori suggeriscono un algoritmo diagnostico - operativo conforme alla maggiore frequenza e complessità esistente oggi nel nostro Paese a causa dei recenti flussi migratori da territori ove queste alterazioni ematologiche sono endemiche.

PO170

Medicina di prossimità e medicina di laboratorio: l'esperienza dell'Azienda Provinciale per i Servizi Sanitari di Trento

M. Fontanari[1], G. Ventimiglia[1], P. Calvi[1], C. Di Natale[1], S. Molon[1], E. Rampoldi [2], A. Anesi[1]

[1]Laboratorio di Patologia Clinica, Ospedale S. Chiara di Trento, APSS di Trento
[2]Coordinatrice Gruppo di Studio POCT per SIBioC

La Medicina di Laboratorio ha registrato nell' Azienda Provinciale per i Servizi Sanitari di Trento una improvvisa evoluzione organizzativa dall'inizio della pandemia SARS-Cov-2. Uno degli ambiti più interessati è legato all'emogasanalisi (EGA) per la valutazione della funzionalità respiratoria. Un incremento del 20% nel numero degli emogasanalizzatori e del 25% delle emogasanalisi in POCT ha comportato di conseguenza anche un aumento del lavoro di supporto tecnico fornito ai reparti e la necessità di offrire attività formativa al personale medico e infermieristico attraverso l' organizzazione di corsi in situ e Webinar per gli aspetti clinico-diagnostici della emogasanalisi. Per le attività di laboratorio analisi della rete ospedaliera territoriale si è verificata una veloce accelerazione verso il completamento del modello hub&spoke con l'implementazione nei PS degli ospedali di valle (Arco, Borgo, Cavalese, Cles, Tione) di strumentazione per POCT. Nella fascia oraria giornaliera tra le 20 e le 8, i tests in urgenza vengono eseguiti direttamente dal personale dei PS (2 infermieri e 1 OSS). Esistono grandi differenza stagionali legate al turismo, ma, in media, si registrano 10 accessi per notte con richieste di 60 tests di laboratorio. Il 97,5% delle richieste viene coperta dalla dotazione strumentale implementata, che consiste in uno strumento per troponina T convenzionale, betaHCG, PCT e D-dimero (AQT90 Flex); uno strumento per 12 parametri di chimica clinica (Piccolo Xpress); uno strumento per PT e PTT (Hemochron), uno strumento per ematologia (Icon5). Per ogni PS, il costo complessivo dei canoni strumentali, reagenti e consumabili è intorno ai 32.000 Euro per quadrimestre. Le principali criticità sono derivate dalla necessità di dover creare un percorso diagnostico per il follow-up dei pazienti con dolore toracico che comprenda sia l'utilizzo della troponina T convenzionale in POCT che di quella ultrasensibile eseguita in laboratorio e l'interferenza dei farmaci DOAC con il metodo di misura del PT. Per la mancanza di back-up degli analizzatori e la non completa copertura dei tests richiedibili in urgenza con gli strumenti in POCT, viene mantenuta la reperibilità di un tecnico di laboratorio. La notevole resistenza del personale all'introduzione del POCT in PS è diminuita progressivamente dalla prima installazione, non solo per la costante disponibilità dei tecnici di laboratorio nel garantire assistenza e riferimento per ogni criticità/ problematica e per il miglioramento delle capacità di comunicazione e didattiche dei POCT manager che si sono affinate nel tempo, ma anche per il passaparola tra PS sui vantaggi portati dalla riduzione del TAT analitico nella gestione dei pazienti. In conclusione, il bilancio delle esperienze ad oggi maturate sostiene l'idea di un POCT integrativo e non sostitutivo del laboratorio analisi.

PO171

MESSA A PUNTO DI UNA PROCEDURA PER LA VERIFICA DELLA COMPARABILITÀ DEI RISULTATI OTTENUTI CON STRUMENTI POINT-OF-CARE (POCT) E STRUMENTI AUTOMATIZZATI DEL LABORATORIO UTILIZZANDO I DATI DEI PAZIENTI

S.G. Signorini, A. Franzoni, A. Lonati, A. Melcore, E. Bianchini, A. Camossi, A. Rossi, S. Bellintani, E. Orlandi, D. Brugnoni

Laboratorio Centrale di Analisi chimico cliniche ASST Spedali Civili di Brescia

INTRODUZIONE. Tenere sotto controllo la comparabilità dei risultati ottenuti, per lo stesso misurando, con strumenti e metodi diversi, risulta particolarmente complesso quando si prendono in considerazione i POCT e gli strumenti automatizzati del laboratorio a cui gli stessi fanno riferimento. Per questo motivo, abbiamo messo a punto una procedura mutuata dal modello 4 proposto nel documento SIBioC di riferimento (Biochimica clinica, 2019;43:228-43) per comparare i risultati ottenuti con lo strumento per emogasanalisi Siemens Rapidpoint 500 con quelli prodotti dagli strumenti automatizzati presenti nel Laboratorio Centrale dell'ASST Spedali Civili di Brescia (Roche Cobas 8000 e Sysmex XN-9000).
METODI. La procedura prevede: 1) Determinazione dei parametri selezionati sugli strumenti Rapidpoint 500 del Laboratorio ed esecuzione su Cobas 8000 e Sysmex XN-9000 di analoghe determinazioni sullo stesso campione. 2) Estrazione dal database del Laboratorio dei risultati ottenuti su campioni di Pazienti analizzati sugli emogasanalizzatori dei reparti ospedalieri e dei risultati ottenuti su campioni differenti prelevati nella stessa occasione ma inviati in Laboratorio per essere eseguiti sulla strumentazione automatizzata. La preparazione dei dati per l'effettuazione dei confronti è stata realizzata mediante un software in Access, mentre la loro analisi è stata effettuata con il software Analyse-it, utilizzando i test di regressione di Passing Bablok e il test di Bland-Altman.
RISULTATI. L'analisi di comparazione ha messo in evidenza che potassio, glucosio, emoglobina ed ematocrito misurati con gli emogasanalizzatori RapidPoint 500 vengono sottostimati o sovrastimati (presenza di bias costante) rispetto ad un'analoga misurazione effettuata sulla strumentazione del Laboratorio.Usando i criteri, per altro molto stringenti, della variabilità biologica, tale differenza risulta per la maggior parte dei casi clinicamente significativa, impedendo una trasferibilità dei risultati fra il sistema analitico POCT e quello della strumentazione di Laboratorio.
CONCLUSIONI. Utilizzando strumenti informatici di facile realizzazione è possibile utilizzare i dati dei Pazienti ricoverati per verificare l'allineamento fra gli strumenti POCT e quelli presenti nel Laboratorio di riferimento.

PO172

VALIDATION OF AN AUTOMATED IMMUNOASSAY FOR THERAPEUTIC DRUG MONITORING OF LEVETIRACETAM

M. Stornaiuolo, R. Spataro , C. Artusi , M. Marinova , M. Zaninotto , M. Plebani

1Department of Laboratory Medicine University-Hospital, Padua, Italy

Introduction: TDM of Levetiracetam drug levels is a relevant tool to support appropriate treatment choice in patients suffering from epilepsy. Laboratory workload has increased over 5000 samples in the last one year and a rapid response system is an essential key to ensure safe patient treatment. Aim of the study was to validate the ARK Diagnostics Levetiracetam immunoassay on Ilab Taurus automatic platform. All data obtained were compared to UPLC-MS/MS laboratory routine method accredited in agreement with ISO 15189. Methods: The study was conducted according to FDA and EMA guidelines. Linearity was evaluated by performing in duplicate serial dilutions of the highest calibrator stock solution and a patient sample with concentration above the upper limit of quantification (ULLOQ). The lower limit of quantification (LLOQ) was obtained by serial diluting EQA samples. Three different control materials and blood specimens from patients treated with Levetiracetam were employed to evaluate the between-day precision (SWL) and assessed once for 20 days. Trueness were obtained using three different EQA samples tested in triplicate for three days. Finally, 130 serum samples were compared for both methods. Passing–Bablok regression, Pearson correlation coefficient and Bland–Altman statistical analysis were performed to define the agreement between the two methods. Results: The calibration curves was found to ensure linearity in the range between of 0.5 and 100 mg/L. LLOQ was 0.5 mg/L. SWL values obtained were <10%. Trueness showed a bias% of +6.4% (at 4.0 mg/L), -2.2% (at 23.7 mg/L) and +4.8% (at 78.5 mg/L). Passing–Bablok regression equation was [ARK immunoassay]=0.98×[UPLC-MS/MS]+0.20 [mg/L], with a Pearson's correlation coefficient of r=0.995, the Bland–Altman plot showing no significant %bias (-0.3). Discussion: ARK Levetiracetam Assay applied on ILab Taurus automatic platform exhibits good efficiency and was chosen to replace the prevously method adopted in our laboratory. Key words: TDM, Immunoassay

PO173

Confronto tra metodi: troponina T COBAS e601 vs COBAS h232 (POCT)

M. Lorubbio[1], F. Baldelli[1], G.P. Caldarelli[3], M. Fantacci[2], L. Gasbarri[1], A. Maddalena[3], M. Mazzi[3], L. Ramazzotti[3], A. Saracini[1], A. Tarquini[4], E. Tripodo[1], A. Ognibene[1]

[1]Clinical Chemical Analysis Laboratory, San Donato Arezzo Hospital

[2]Clinical Chemical Analysis Laboratory, Montepulciano Valdichiana Reunited Hospital

[3]Clinical Chemical Analysis Laboratory, Misericordia Grosseto Hospital

[4]Clinical Chemical Analysis Laboratory, Alta Valdelsa Hospital

Introduzione

Secondo le linee guida, la troponina è considerata il marcatore di elezione per la diagnosi di infarto acuto del miocardio (IMA) utilizzando un valore soglia calcolato al 99° percentile della popolazione di riferimento con CV<10% nelle metodiche "high-sensitivity". I metodi "Point Of Care Testing" (POCT) che non sono raccomandati per la diagnosi di IMA, possono essere però utilizzati per un primo screening al letto del paziente, in ambulanza o nei Pronto Soccorso periferici per la stratificazione del rischio di Sindrome Coronarica Acuta (SCA) indirizzando rapidamente il paziente verso il reparto clinico specializzato. In questo contributo è stato effettuato il confronto tra metodi per la determinazione della troponina T eseguita su strumentazione Roche Cobas e601 e COBAS h232 (POCT).

Materiali e Metodi

E' stata effettuata la misurazione della troponina T in 43 campioni di plasma, provenienti dal Pronto Soccorso, raccolti in provette con anticoagulante eparinato ed analizzati in parallelo sulla strumentazione Roche Cobas e601 ad "high-sensitivity" e sul "Point Of Care Testing" Roche COBAS h232 nei laboratori periferici di Bibbiena, San Sepolcro, Cortona e Massa Marittima. Per l'analisi statistica sono stati utilizzati i test Bland-Altman, Passing-Bablok e K di Cohen.

Risultati

I risultati dell'analisi Passing-Bablok hanno mostrato una pendenza di 0.9303 (IC 95%= 0.7826, 1.1856) ed intercetta = 2,7787 (IC 95%= -25.0479, 18.4783). L'analisi Bland-Altman mostra Bias=10.714 (IC 95%= -2.290, 23.717). Per la concordanza dei valori di troponina T <40 ng/L la K di Cohen=0.80

Conclusioni

I risultati mostrano una buona correlazione tra i 2 metodi ed una buona concordanza anche per bassi valori di troponina T.

PO174

Valutazione dell'accuratezza diagnostica del test diagnostico rapido "CareStart Rapid kit" per la carenza di glucosio-6-fosfato deidrogenasi G6PD nell'era del COVID-19: uno studio preliminare

M.E. Onori[1], C. Ricciardi Tenore[1], S. Baroni[1], A. Urbani[1,3], A. Minucci [1]

[1]UOS di Diagnostica Molecolare e Genomica, IRCCS Fondazione Policlinico Universitario A. Gemelli di Roma
[2]Università Cattolica del Sacro Cuore, Roma, Italia

Introduzione: A seguito della pandemia da Coronavirus Sars-CoV-2, la carenza della Glucosio-6-fosfato-deidrogenasi (G6PDD) ha assunto un ruolo importante soprattutto in merito ai trattamenti a base di Clorochina e idrossiclorochina in pazienti G6PD carenti affetti da Covid-19. Lo scopo di questo studio è stato quello di valutare le performance del kit G6PD CaresStart Rapid Test nel laboratorio analisi dell'ospedale IRCSS Fondazione Policlinico Universitario A. Gemelli di Roma, centro di riferimento per diagnosi biochimica e molecolare di favismo.
Materiale e metodi: Un totale di 20 campioni di sangue periferico con attività della G6PD carente, intermedia e normale sono stati inclusi in questo studio. Tutti i campioni sono stati valutati anche a livello molecolare, confermando la carenza enzimatica con l'identificazione dell'alterazione molecolare nel gene della G6PD. Il dosaggio enzimatico della G6PD è stato eseguito mediante lo strumento Sentinel Diagnostics Advia XP della Siemens, mentre il dosaggio qualitativo con il kit G6PD CareStart Rapid test. La procedura del kit G6PD CareStart Rapid Test prevede l'uso del pungidito, il prelievo del sangue con la mini-pasteur e il trasferimento sulla card della goccia di sangue (circa 2 µl) nella finestra "S" e di 2 gocce di "Assay Buffer" (equivalente a 100 µl) nella finestra "A". Dopo 10 minuti dalla deposizione del campione, si procede alla lettura: colore bianco per i campioni carenti e rosa per quelli normali.
Risultati: Su 20 test, solo 16 hanno riportato perfetta concordanza tra il dosaggio enzimatico e il risultato prodotto dalle card del kit (procedura con l'utilizzo di pipette). Le 4 restanti non sono risultate leggibili a causa di un'eccessiva saturazione (senza l'utilizzo delle pipette).
Discussione: Il kit è rapido e facile da utilizzare. Nonostante la concordanza tra dosaggio enzimatico ed il risultato qualitativo prodotto dalle card, è necessario tuttavia sottolineare delle piccole criticità: per produrre risultati ideali è stato necessario l'utilizzo della pipetta sia per il campione di sangue che per l'Assay Buffer. Senza l'uso della pipetta la card risulta essere troppo saturata e di difficile interpretazione. I campioni con dosaggio normale o campioni fortemente carenti producono un risultato concordante ed evidente ad occhio nudo da parte dell'operatore. Per i campioni borderline, la valutazione dell'esito della card risulta difficoltosa. Tale evidenza suggerisce la necessità di un lettore di card (attualmente non disponibile dalla ditta) per produrre un risultato maggiormente confidente.Nonostante le criticità sopra riportate, riteniamo che il kit G6PD CareStart Rapid Test possa essere considerato un ottimo test di screening utile ad identificare rapidamente un paziente G6PD carente, sia in pronto soccorso che in sede di laboratorio, soprattutto in questo periodo in cui la correlazione tra la carenza di G6PD e Covid-19 è di vitale importanza.

PO176

Is the Human Neutrophil Lipocalin (HNL) a specific and rapiddiagnostic biomarker todistinguish between bacterial and viral infections? A scoping review

V. Pecoraro, T. Trenti

Dipartimento di Medicina di Laboratorio, AUSL Modena

Background: Acute infections, either caused by virus or bacteria, affect almost anyone at least once a year, with unspecific signs and symptoms.This distinction is very important in deciding whether to prescribe antibiotics or not. Sensitivity and specificity of clinical criteria alone are about 55%–60%. The accuracy could increase in association with serum biomarkers. Human neutrophil lipocalin (HNL) is released from secondary granules of neutrophil granulocytes after an activation of innate immune system. We investigated, through a systematic review, the accuracy of HNL to discriminate between viral and bacterial infections and its determination as point of care testing.

Methods: We systematically searched Pubmed and CENTRAL as well as a list of reference literature. We included observational studies investigating the diagnosticperformance of HNL and determining its concentration in serum. We collected data about the number of bacterial or viral infection, sensitivity, specificity and accuracy.

Results :In our preliminary analysis, we considered data on more than 1000 infected patients, evaluated in 7 studies.To distinguish between bacterial and viral infection, the sensibility of HNL ranged from 71% to 97%, and the specificity from 72% to 94%, the AUC from 0.91 to 0.97. The mean concentration was 348 ¤g/l for bacterial, and 116 ¤g/l for viral infection ($p<0.001$). Serum concentrations of HNL allowed a distinction between bacterial or viral infections with negative and positive predictive values of 90%. The clinical performance of HNL in fMLP-activated whole blood (procedure for point of care testing), was similar to HNL in serum, with a response times of 15-20 min.

Conclusions: The assay of HNL in serum seems to be accurate to distinguish between bacterial and viral infections, but the evidence are still scarce. Further studies areneeded to confirm its usefulness in the real critical settings. A possible point of care application would make it attractive in emergency and primary care settings ruling out a possible bacterial infection, reducing the antibiotic resistance with and a great impact on health care system.

PO177

Detection of Risk Factors associated to baseline Hypovitaminosis D in Breast Cancer patients from different Italian regions

A. Minopoli[1], C. Montagnese[2], G. Carioli[3], A. Fassio[4], G. Porciello[2], S. Vitale[2], E. Palumbo[2], B. Grilli[1], P.C. Fiorillo[1], M.A. Isgrò[1], S. Arpino[1], L. Falzone[2], A. Crispo[2], M. Grimaldi[2], R. Pica[2], M. Prete[2], A. Luongo[2], I. Calabrese[5], M. Pinto[6], M. De Laurentiis[7], D. Cianniello[7], C. Pacilio[7], M. Rinaldo[7], G. Thomas[8], D. Serraino[9], C. Evangelista[10], A. Steffan[10], F. Ferraù[12], F. Catalano[11], M. Libra[11], M. D'Aiuto[13], E. Celentano[2], L.S. Augustin[2], D. Gatti[4], E. Cavalcanti[1]

[1]Laboratory Medicine Unit, Istituto Nazionale Tumori - IRCCS "Fondazione G. Pascale", Napoli, Italy

[2]Epidemiology and Biostatistics Unit, Istituto Nazionale Tumori - IRCCS "Fondazione G. Pascale", Napoli, Italy

[3]Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Milan, Italy

[4]Rheumatology Unit, University of Verona, Verona, Italy

[5]Department of Clinical Medicine and Surgery, Federico II University, Napoli, Italy

[6]Rehabilitation Medicine Unit, Istituto Nazionale Tumori - IRCCS "Fondazione G. Pascale", Napoli, Italy

[7]Division of Breast Oncology, Istituto Nazionale Tumori - IRCCS "Fondazione G. Pascale", Napoli, Italy

[8]Clinica Mediterranea, Napoli, Italy

[9]Cancer Epidemiology Unit, National Cancer Institute Centro di Riferimento Oncologico, IRCCS, Aviano, Italy

[10]Immunopathology and Cancer Biomarkers Unit, National Cancer Institute Centro di Riferimento Oncologico, IRCCS, Aviano, Italy

[11]Department of Biomedical and Biotechnological Sciences Oncologic, Clinical and General Pathology Section, University of Catania, Catania, Italy

[12]Ospedale San Vincenzo, Taormina, Italy

[13]Clinica Villa Fiorita, Aversa,

Introduction: Vitamin D (Vit D) has been reported to have an impact on Breast Cancer (BC) onset, prognosis and risk stratification. The aim of the study was to detect risk factors associated to baseline Hypovitaminosis D (hypoVit D) in BC patients from different Italian regions.Materials and methods: baseline data regarding region origin, age, BMI, lifestyle, cancer therapy, lipid assessment were collected from 394 women with primary BC without metastasis at diagnosis. Patients were enrolled in a multicenter randomized clinical trial of lifestyle modifications and Vit D supplementation (DEDiCa) conducted in Friuli Venezia Giulia (FVG), Campania and Sicilia Italian regions. Their serum Vitamin D (25-hydroxyvitamin D) levels were analyzed by a chemiluminescent method on Liaison XL (DiaSorin). Variables were compared between two baseline Vit D levels' groups (<20 and >= 20 ng/ml) using Chi-squared or Fisher's test in the overall cohort and separately in previously Vit D supplemented and unsupplemented subgroups. Using a multiple logistic regression model, Odds Ratios (ORs) for Vit D deficiency (<20 ng/ml) and their corresponding 95% confidence intervals (CIs) were

estimated for considered variables.Results: hypoVit D resulted associated directly with higher BMI both in the unsupplemented subgroup and in the whole population. Moreover it was related with hypertriglyceridemia (OR 2.46; 95% CI 1.16-5.22), chemotherapy (OR 1.86; 95% CI 1.03-3.38) and inversely with cancer hormone therapy (OR 0.43; 95% CI 0.24-0.75) only in the whole cohort. Lower levels of Vit D were more prevalent in Sicilia (68.7%), in respect to Campania (32.2%) and FVG (36.7%). Only patients coming from Sicilia were found to be at higher risk of hypoVit D when compared to those living in Campania, either in the unsupplemented subgroup (OR: 2.40; 95% CI 1.09-5.29) or in the whole study population (OR: 2.50; 95%CI 1.22-5.13). No significant associations were found with other variables. Conclusion: our results showed that hypoVit D is still widely common in BC patients, showing chemotherapy and hypertriglyceridemia as risk factors. Above all, in our cohort, higher BMI and southernmost Italian region provenance showed a higher risk of hypoVit D independently from Vit D supplementation.

PO178

The assessment of high sensitivity cardiac troponin in patients with COVID-19: A multicenter study

M.A. Perrone[1,2], F. Spolaore[3], M. Ammirabile[4], P. Caciagli[3], F. Ceriotti[4], S. Bernardini[2]

[1]Department of Cardiology, University of Rome Tor Vergata, Rome, Italy

[2]Department of Clinical Biochemistry and Clinical Molecular Biology, University of Rome Tor Vergata, Rome, Italy

[3]Department of Laboratory Medicine, Santa Chiara Hospital, Trento, Italy

[4]Deparment of Clinical Chemistry, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

Background: Recent studies have shown that patients diagnosed with coronarivus disease 2019 (COVID-19) and also with previous cardiovascular diseases have a higher mortality due to worsening heart disease. At the same time, patients without previous cardiovascular disease may also have cardiac complications. The aim of this multicenter study was to assess high sensitivity cardiac troponin T (hs-cTnT) in patients with COVID-19 and to evaluate the incidence of myocardial injury. Methods: In this multicenter study we enrolled 543 patients, 57.8% males, median age 63 years (range 18-99) from three selected hospitals: University Hospital Tor Vergata in Rome, Fondazione IRCCS Ca 'Granda Ospedale Maggiore Policlinico, in Milan, S Chiara Hospital in Trento. We measured hs-cTnT in all patients to assess myocardial injury and correlations with patient's age, symptoms and disease course. Results: The data showed that, among the 543 patients studied, 257 patients (47.3%) had hs-cTnT values higher than the upper reference limit (URL) of 14 ng/L. Patients with high hs-cTnT had more frequently fever ($p < 0.01$) and respiratory symptoms ($p < 0.01$), compared to the group with hs-cTnT values below URL. The results showed also that patients with hs-cTnT above URL had a higher frequency of previous cardiovascular disease ($p < 0.01$) as well as of hypertension ($p < 0.01$). Instead, among 231 patients with no previous cardiovascular disease, 81 (31.5%) had hs-cTnT values above the URL. Finally. the majority of the patients with high hs-cTnT were admitted to the intensive care unit ($p < 0.01$). Conclusion: Our data suggest the assessment of high sensitivity cardiac troponin in patients with COVID-19 for early diagnosis of cardiac involvement.

53° Congresso Nazionale della Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica (SIBioC - Medicina di Laboratorio)

Virtual Edition, 11-13 ottobre 2021

Indice degli Autori

Snibe Diagnostic

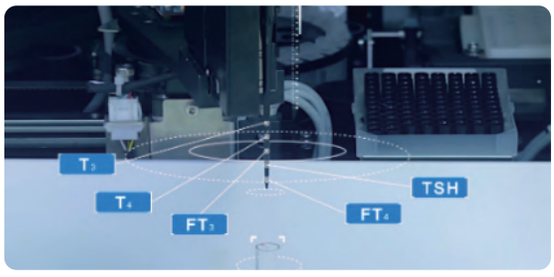
Modulo Singolo con la più Alta Produttività al Mondo

MAGLUMI® X8

X8 Vantaggi Tecnici

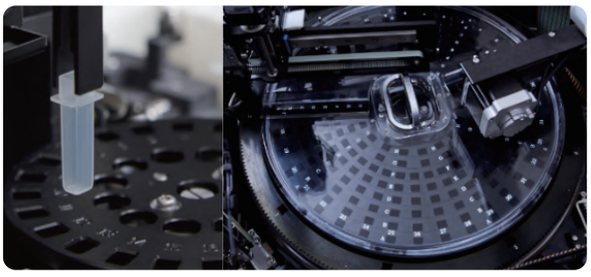
Campionamento

- Puntali monouso
- Un pipettamento per test multipli



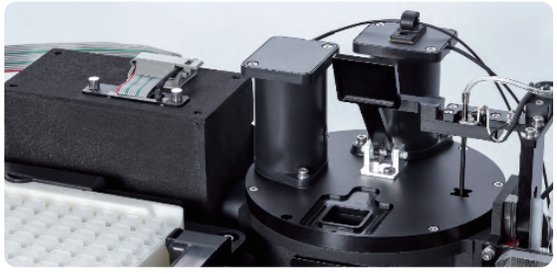
Incubazione

- Design di cuvetta singola
- Incubazione costante a 360° a 37±0,3°C



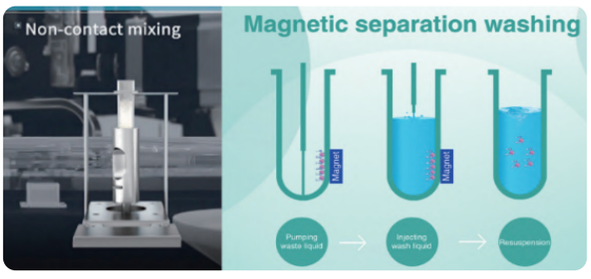
Misurazione

- Controllo bidirezionale della temperatura
- Misurazioni stabili ed accurate



Miscelazione e lavaggio

- Unità di miscelazione a vortice senza contatto
- Lavaggio ad alta efficienza in 4 fasi



*Per maggiori informazioni prego contattare Medical Systems S.p.A

MEDICAL SYSTEMS S.p.A.

www.medicalsystems.it

Follow Us on

www.snibe.com sales@snibe.com

Sistemi da sangue intero capillare per il Point of Care Testing
Soluzioni per la medicina territoriale in connettività

EMS Stat Data Link
GLU KET LAC Hb/Hct

Il Futuro dell'esame Pre-Ospedaliero

Allegro®

Un analizzatore multiparametrico con prelievo capillare

- I test includono: HbA1c, CREAT, PT/INR, Glu, UACR, CRP, Lipidi

NovaNet™

Sistema completo di connettività e gestione per Point of Care

Stat Strip®
GLUCOSIO/CHETONI

Glucosio/Chetoni POC Testing
- Nessuna interferenza clinica accertata
- L'unico glucometro certificato dalla FDA per il paziente critico

Stat Strip®
LATTATO
EMOGLOBINA/EMATOCRITO

Lattato, Hb/Hct POC Testing
- Screening e monitoraggio rapido di anemia e sepsi
- Misura sia emoglobina che ematocrito

Stat Sensor®
CREATININA

Creatinina, eGFR POC Testing
- Rapida identificazione di AKI

---

nova biomedical®

Contattaci per maggiori informazioni:
novabiomedical.com/it
Tel: +39 02 87071482
Email: it-info@novabio.com

BioMedia

La condivisione del sapere

“Da oltre vent'anni al servizio della comunità scientifica”

Provider ECM residenziale e FAD

Servizi di Segreteria Società Scientifiche

Casa Editrice

Servizi web e strategie di comunicazione

Family Health
Fascicolo Sanitario Digitale Personale

Biomedia progetta, organizza e coordina eventi formativi ECM medico-scientifici residenziali e a distanza. È partner di numerose Società Scientifiche, editore in ambito sanitario, ed è attiva nell'ambito della comunicazione digitale, realizzando servizi sviluppati e gestiti *in house*.

Per maggiori informazioni ■ www.biomedia.net ■ bm@biomedia.net ■ tel.: 02 45498282