SIBioC 2008

40° Congresso Nazionale
Società Italiana di Biochimica Clinica e
Biologia Molecolare Clinica

Riassunti Sessioni Scientifiche

Codice Abstract	Titolo Sessioni Scientifiche
• **LM1**	L'errore in medicina
• **SP1-SP3**	Update sull'evidence-based laboratory medicine
• **SS1-SS2** **CO1-CO2**	Il laboratorio nell'urgenza del pronto soccorso
• **SS3-SS5** **CO3-CO4**	Le emoglobinopatie, diagnosi clinica e di laboratorio
• **SP4-SP7**	Marcatori cardiovascolari
• **SS6-SS8** **CO5**	Progressi in citofluorimetria
• **SS9-SS11** **CO6-CO7**	Vecchi esami, nuovi scenari
• **SP8-SP11**	Standardizzazione
• **SS12-SS14** **CO8-CO9**	La diagnostica molecolare del prossimo decennio
• **SS15-SS17** **CO10-CO11**	La spettrometria di massa nel laboratorio clinico
• **SS18-SS19** **CO12**	Il tecnico di laboratorio: un ruolo da protagonista nel governo del cambiamento

Legenda:

LM Lettura Magistrale
SP Sessione Plenaria
SS Sessione Scientifica
CO Comunicazione Orale

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

LM1
ERROR IN MEDICINE

L.L. Leape

Harvard School of Public Health, Boston, Massachusetts, USA

Studies from the early 1990's suggested that 3-4% of patients hospitalized in America suffered an injury caused by their treatment, and that more than half of these injuries were caused by errors or systems failures. It was estimated that as many as 98,000 people die annually in U.S. hospitals because of medical errors. If the same rates apply in Italy, 19,000 people die needlessly in Italian hospitals every year. The American studies have since been repeated in 6 other countries, where the average rate of injury has been found to be close to 10%, suggesting the problem is even worse than imagined.
Studies from cognitive psychology and human factors engineering, as well as everyday life experience, show that errors are normal part of human existence. But studies also show that most errors result from faulty systems, not from carlessness or incompetence. That is, the design of the tasks and processes that we work in leads us to make mistakes. For example, two medication containers that have similar appearing labels are an invitation to a mix-up. Requiring a nurse or a doctor to work a full shift after they have just been awake working all night increases the chance they will make a mistake. Excessive work loads make errors more likely. To prevent errors, the systems need to be redesigned. This is what commercial aviation has done with such great success. This is what health care must do.
A major effort is underway around the world to redesign health care systems to improve patient safety. A number of new safe practices have been developed that will significantly reduce medical errors. They now need to be implemented widely in hospitals. This is part of the mission of the World Alliance for Patient Safety. Other examples of recent successes will be described. The time has come for a world-wide effort to reduce medical mistakes.

SP1
TEACHING EVIDENCE BASED LABORATORY MEDICINE: HOW TO SPREAD THE WORD?

R.H. Christenson

Rapid Response Laboratories, University of Maryland School of Medicine, Baltimore, USA

There is a changing paradigm in clinical and laboratory medicine that has been driven by the easy availability and rapid access to information. This paradigm is coined evidence-based laboratory medicine (EBLM); stated simply, EBLM involves the process for use of best quality information on which patient decisions are made. The EBLM process involves the A5 cycle: Ask a well-formulated question; Acquire the evidence to address the question; Assess the evidence critically; Apply the evidence in practice; Assess (or Audit) the impact of change. The purpose of this lecture will be to address how the specific skill set needed for EBLM should be presented to effectively spread the word on this new paradigm.
Be prepared to discuss the context of EBLM. When EBLM is first introduced to experienced individuals, they may find it difficult to accept that former methods of practice are no longer sufficient for keeping up with the best the information available for care of patients. Teachers must emphasize that EBLM does not substitute or replace skills acquired through practice; rather, EBLM complements and supplements previous knowledge and skill with valid information. Positive reinforcement must focus on the confidence gained from being able to answer one's own clinical and technical questions with best available evidence through systematic use of the EBLM process.
Address any anxiety about acquiring the skills needed to use EBLM. Experts and teachers in evidence-based practice often have advanced training in research methodology or statistics. Learners often find these areas esoteric, and can be difficult to see how these specialized skills can be relevant to routine practice. Teachers must demonstrate that EBLM skills can be acquired within a reasonably short time frame and used in everyday practice. Teachers must try to build on the knowledge and experience that learners already have in these areas by using examples.
Learning needs to be connected to how EBLM will be used in practice. Teaching is most effective when it is clearly connected with how it will be used in a practical sense; teaching must be oriented toward real situations that will be familiar to the learners. Teaching must focus on how to use the EBLM steps to improve practice. Again, practical examples of how EBLM can be used in practice are a powerful way to connect with learners. Answering questions from clinicians and laboratorians about the interpretation of diagnostic and monitoring tests or how to decide which tests and assay formats are appropriate for local practice helps learners realize the value of EBLM. Effective teaching connects new knowledge with old, and facilitates how learners can build on their current expertise.
Learning needs to balance passive versus active methods. Passive methods of learning (such as lectures) should be balanced with active methods (such as small group work and practical exercises). Active learning through exercises and discussion build confidence and are necessary for developing practice skills. Small group work is an excellent way for providing peer and facilitator feedback for formulating clinical questions, developing searching strategies, and critical appraisal of the evidence for use in decisions and in applying the evidence in practice.
Make EBLM education fun. Humor facilitates learning by reducing tension, holding the learners' attention, and increasing enthusiasm for the topic. Humor can promote a positive image of EBLM, however teachers must remember that "humor is serious business" and they must be cautious to avoid being viewed as comedians. Carefully selected cartoons and pictures can rapidly get a message across and invigorate the learning environment.
Teaching must be focused on learners' needs. The teaching materials presented and the technique used must match the learners' stage of training and learning needs. Teaching post-graduate practitioners versus individuals early in training can be complicated because of differences in learners' aptitudes, previously acquired skills and enthusiasm for the subject. Small group facilitation skills are necessary to manage the different skill levels. Identification of more confident and experienced members of a group can provide assistance in group learning. There must be balance, however, and teachers must be careful to avoid having any member of a group dominate the opinions and participation of other members.
Seek feedback and evaluation of your performance as a teacher. Clearly individuals have different styles and natural aptitudes for lecturing and small group interaction. Individual skill can benefit from evaluation and feedback on what worked well and what needs improvement during a session. Teachers should always seek written feedback on their educational activities.
Use EBLM skill is becoming essential for routine practice at all levels. This is because laboratory professionals must provide reliable and valid information on the application and interpretation of diagnostic, monitoring and prognostic

laboratory procedures and communicate this information effectively. Objective evidence is critical for optimizing medical decisions and patient outcomes at all stages. For laboratory medicine, this includes the entire pre-analytical, analytical and post-analytical testing phases. Effective teaching of EBLM can help clinicians choose the right test, for the right patient at the right time, and to interpret the results in meaningful way that benefits patients.

SP2
CLINICAL GOVERNANCE AND EVIDENCE BASED LABORATORY MEDICINE

T. Trenti

Patologia Clinica-Tossicologia e Diagnostica Avanzata, Ospedale Nuovo Sant'Agostino Estense, Dipartimento di Patologia Clinica, AUSL di Modena, Italy

Clinical Governance, "a framework through which the NHS organizations are accountable to continue to improve the quality of the service and safeguarding high standards of care by creating an environment in which excellence in clinical care would flourish", is aimed to improve the overall standard of clinical care, ensuring that clinical decisions are based on the most up to date evidence in terms of effectiveness. The Clinical Governance principles seem to be of value in healthcare system worldwide in terms of quality, effectiveness and accountability. In Italy, these principles are at the base of the health care reform that has the goal to promote the effectiveness and appropriateness of the use of health services.
Clinical Effectiveness, a vital part of Clinical Governance, is a term that refers to measuring and monitoring the quality of care and also comprises various activities that include: Evidence Based Practice, Research & Development, Clinical Audit, Clinical Guidelines, Integrated Care Pathways, Total Quality Management. Clinical effectiveness are based on the best available research evidence, as assessed by meta-analyses and systematic reviews and made more understandable and operational, in the case of diagnostic process in Laboratory Medicine, by the use of expressions as likelihood ratios, area under the ROC curve, sensitivity and specificity. The best available research evidence are usually derived from clinically relevant research, often from the basic sciences of medicine, but especially from patient centred clinical research into the accuracy and precision of diagnostic tests, the power of prognostic markers, and the efficacy and safety of the diagnostic intervention or consequent treatment. New evidences from clinical research both invalidate previously accepted diagnostic tests and procedures and replace them with new ones that are judged more powerful, more accurate, more efficacious, and safer.
The application of EBM in Laboratory Medicine or Evidence Based Laboratory Medicine (EBLM) aims to advance clinical diagnosis by researching and disseminating new knowledge, combining methods from clinical epidemiology, statistics and social science with the traditional pathophysiologic molecular approach. EBLM, by evaluating the role of diagnostic investigations in clinical decision-making process, can help in translating the results of good quality research into every day practice. Clinical Guidelines EBLM oriented are pivotal in training and maintaining performance in a Clinical Governance policy because they center the attention on the efficacy to support the decision-making process required in good clinical practice, in this view both the introduction of a new diagnostic procedure or the currently traditional routine activities should be subject to a regular audit practice to evaluate the quality and appropriateness of care in Laboratory Medicine.

SP3
EVIDENCE-BASED LABORATORY REPORTING TO CLINICIANS

D. Giavarina

Clinical Pathology Department, San Bortolo Hospital, Vicenza, Italy

The difference between an 'Information' and a 'notice' lies in the former's ability to remove or significantly reduce someone's uncertainty about something. In Laboratory Medicine, you have to know exactly how to ask a clinical question, i.e. what are you looking for, in order to get a plausible answer. A test result, the same test result, is going to get completely different information depending on the clinical context where you decided to order it, namely for screening or diagnosis rather than for monitoring or follow up. The first step in Evidence Based Laboratory Medicine is asking an answerable clinical question, e.g. using a popular tool as the Fagan's nomogram you have to get some clinical (pre-test) information in order to evaluate the post-test probability. As a laboratory physician you must understand the clinical question; the other way round, clinicians must be aware of the more relevant pre-analytical, analytical and post-analytical issues. How the laboratory reports its results is paramount for clinicians to understand their real meaning.
A typical Laboratory report is made of a list of figures sided by reference intervals, set to dimension the biological signal. Common practice and professional standards such as ISO 15189 and CPA UK both suggest the application of appropriate comments, accuracy indicators, quality specifications, decision levels based upon biological variation or expert advice. Further improvements of Lab reports are expected adding information on pre-analytical issues, such as serum indices of hemolysis, lipemia and jaundice, managing analytical information about tests and their performance, add on testing (reflex), autoverification (faster reporting of results under defined conditions) delta checks or range checks (helping in detection of possible erroneous results), abnormality flags (high, low, critical results), correlation to other relevant results (both electronic and manual), interpretative comments, computerized decision supports (artificial intelligence). All suitable tools to generate added valuable clinical information in order to modify behavioral habits and favor a more efficient and appropriate use of lab test results. Unfortunately evidence supporting the clinical advantage of these tools are still fair. Most often added information derives from previous experience and is consensus-based.
Sometimes local or national regulations and reccomandations from professional bodies help defining a more evidence based approach but up to now a clear benefit to the patient from an interpretative comment or by data on test accuracy has never been proved, even though the single physician likes to receive advice. In a survey about the order of multiple coagulation tests whose results were reported also by narrative interpretation, Laposata et al could demonstrate time savings and/or improved diagnostic workups in 80% of cases. While documenting clinician's needs is of importance, we still lack evidence that answering these needs will get improved clinical outcome fot the patient. In summary, Laboratory physicians are well aware that translational knowledge goes far beyond the analytical quality and a sound laboratory report will play a major role in the patient-centered healthcare of the near future.

SS1

MARKERS OF SEVERE BACTERIAL INFECTION IN FEBRILE CHILDREN

L. Da Dalt, B. Andreola, S. Bressan

Department of Pediatrics, University of Padova, Italy

Management of fever in infants is a common dilemma for clinicians. Actually, fever is one of the most common reasons of visits in the Emergency Department (ED) of children younger than three years of age, accounting for approximately 10% to 35% of admissions. Severe bacterial infections (SBI) represent 10% to 25% of febrile illnesses in this age group and the diagnosis is often not straightforward, especially if a localizing finding is absent. Because clinical parameters (body temperature, clinical score) often provide inadequate information, there is a need for sensitive (enough to detect the presence of infection) and specific (enough to discriminate SBI from other infections) laboratory markers of infection.

One of the first indicators of SBI examined by researchers, the White Blood Cell count (WBC) is ubiquitous with infant fever work-ups. It is universally available and historically useful as an indicator of serious infections; however, studies in the past have indicated that WBC is not a reliable indicator of SBI in febrile infants.

Much progress has been made in recent years in finding more accurate indicators of SBI than WBC. Several studies have suggested that additional markers such as C-reactive protein (CRP) and, more recently, procalcitonin (PCT) may be useful. CRP is an acute-phase reactant synthesized by the liver in response to the elevated levels of the cytokines; it is produced within 4–6 hours after onset of tissue injury or inflammation, it doubles every 8 hours before peaking around 36 hours and it has long been studied as a sensitive marker of bacterial infection. Although the range of proposed cutoff values for CRP is rather large, it appears to have potential as a reliable indicator of bacterial infection in pediatric populations.

Since the early 1990s, there has been mounting interest in the question of whether PCT, a 14-kDa protein prohormone of calcitonin, is an earlier and more specific marker; PCT levels rise more rapidly than do CRP levels, given the triggers, and it is known to rise slightly in viral infections, but it can increase nearly a 1000-fold in the very invasive bacterial infections. Overall, PCT appears to be a superior single screening test for severe bacterial infections, with generally better sensitivity and specificity than WBC or CRP. However, findings from different studies range widely and, actually, definitive data are lacking to validate CRP and PCT as screening tools in the ED.

In our experience, in order to assess the value of PCT and CRP, compared to WBC count, in predicting the presence of SBI in children with fever a prospective was conducted study including 408 children aged 7 days to 36 months admitted for fever and no localizing sign of infection to our ED. We found that CRP and PCT are both valuable markers in the prediction of SBI in patients with fever without a source and both parameters offer a better sensitivity and specificity than WBC. However, whilst PCT seems to be a more accurate predictor at the beginning of an infection, CRP, if correctly employed by taking into account the time needed for its rise in the bloodstream, seems to be a better screening test in emergency settings similar to ours, because of its overall better sensitivity and feasibility (i.e. lower cost, better availability and better historical practice).

Reference

1. Hsiao AL, Baker MD. Fever in the new millennium: a review of recent studies of markers of serious bacterial infection in febrile children. Curr Opin Pediatr. 2005;17:56-61.
2. Maheshwari N. How useful is C-reactive protein in detecting occult bacterial infection in young children with fever without apparent focus? Arch Dis Child 2006; 91:533-5.
3. Hsiao AL, Chen L, Baker MD. Incidence and predictors of serious bacterial infections among 57- to 180-day-old infants. Pediatrics 2006 May;117:1695-701.
4. Andreola B, Bressan S, Callegaro S, Liverani A, Plebani M, Da Dalt L. Procalcitonin and C-Reactive Protein as diagnostic markers of severe bacterial infection in febrile infants and children in the Emergency Department. Pediatr Infect Dis J 2007;26:672-7.

SS2

THE ROLE OF PHARMACOTOXICOLOGICAL LABORATORY IN EMERGENCY MEDICINE

S. Pichini

Department of Therapeutic Research and Medicines Evaluation, Italian National Institute of Health, Rome, Italy

Exposure to drugs and toxins is a major cause for patients' visits to the Emergency Department (ED). For drugs of abuse intoxication, analytical determination made within the ED requires the involvement of the pharmaco-toxicological laboratory. In the emergency setting, drug screening should be performed in less than one hour and the accuracy of analytical result is striking to be life-saving. In the current practice of pharmaco-toxicological laboratory, analysts have at their disposal immunoassays (including on-site testing) or chromatographic methods (HPLC or GC with a variety of detectors). Comparing turnaround time for drugs analyses performed with several methods, one-site testing are the fastest: they require 10-15 minutes, while classical immunoassays lasts approximately 1 and half hour; GC/MS or LC/MS assays require 3 hours turnaround and the intervention of skilled staff. Despite their easiness and speed, on-site testing and immunoassays have to take into account several drawbacks. First of all, false positive results due to substances similar to the one investigated by the kit and the selection of appropriate cut-off concentrations. Indeed, if certain tests are included in a general drug-screening panel, the possibility exists that immunoassays exhibit a significant cross-reactivity toward other drugs, as observed for phencyclidine (PCP) and its cross-reactivity with some anti-inflammatories. Laboratory should clearly explain to the ED staff the extent of available toxicology services (such as the menu of analysed substances or cross-reactivity data) and indicate in a final report (e.g. in the "notes" section) that a negative result does not indicate absence of substances not specifically included in the assay. When reporting results of an immunoassay screening, a note has to be included that assay used is a screening test and that positive results are to be considered as "presumptive". GC/MS or LC/MS are the only definitive methods for identification of specific drugs of abuse, pharmaceuticals or toxins. Moreover, when patient is unconscious, he cannot report substance responsible of intoxications and hence in the majority of pediatric intoxications, extensive systematic toxicological analysis (STA) by GC/MS or LC/MS is necessary. While immunoassay screens may be helpful in detecting sedative-hypnotics and opioid analgesics, most paediatric ingestions do not involve a drug of abuse. The number of drugs that can be detected by immunoassay is typically much less than those obtained by a properly configured GC/MS or LC/MS. Different 10-drug immunoassay are capable to detect a total of approximately 99 drugs and metabolites, while GC/MS searches in a library of over 10.000. A technique, which relies among immunoassays and

chromatographic methods, is the Remedy system. Remedy (Rapid Emergency Drug Identification) is an automated drug profiling system that employs high-performance liquid chromatography with a multicolumn design. Reliable results are given in about 20 minutes with automated analysis. Remedy can be useful complementary technique in the clinical toxicology laboratory.

CO1
TROPONIN TURN AROUND TIME (TAT) IN EMERGENCY: A ONE YEAR EXPERIENCE

D. Cosseddu, S. Musolino, L. Gallo, R. Ronchi, L. Andrini

Laboratorio Analisi, A.O. Ospedale Mauriziano, Torino, Italy

International guidelines suggest that troponin (T) measurement in the presence of ischaemic cardiac event should be available for the clinician within one hour from the patient arrival in the emergency unit.
Aim of the present study has been the evaluation of our laboratory adherence to the guidelines and the identification of improvement strategies of our behavior, if necessary. During 2007, 12439 T measurements have been performed in our laboratory, of which 5701 coming from the emergency unit; three time intervals (expressed in minutes) have been evaluated for the latter ones:
Total TAT: time interval between the clinician request and report.
Delivery TAT.: time interval between the clinician request and the laboratory check-in Laboratory TAT time interval between check-in and report. For each parameter the 10th, 25th etc percentiles have been calculated. Serum T was measured with AIA-360 (Tosoh) after sample centrifugation for 10 minutes at 4000 g. The assay running time is 18 min, so that the minimum laboratory time for the whole procedure is 28 min.
Conclusion. On the ground of our data guidelines requirements have been satisfied only in 25% of cases (gold standard 60 minutes). This discrepancy can be ascribed to several factors: excessive delivery time (49 min at 90th percentile) prolonged laboratory time not related to the procedure but to staff inadequacy (the minimum technical time of 28 min has been achieved in less than 10% of cases). In our opinion possibiles solutions are: a) taking into account the use of POCT, supervision, b) a better training of the laboratory staff involved in this topic.

CO2
RECOMMENDATIONS FOR APPROPRIATE LABORATORY TESTS FOR PATIENTS WITH FEVER IN AN EMERGENCY DEPARTMENT

S. Valenti[1], P. Carraro[2]

[1]*Lab. Analisi, Osp. S.M. delle Croci, Ravenna, Italy*
[2]*Lab. di Patologia Clinica, Ospedale S. Antonio, Azienda, ULSS 16, Padova, Italy*

Fever, a common symptom of patients at presentation in emergency rooms, is most frequently due to a minor disease. In some cases, however, it can hide a serious condition,
in particular, complications from sepsis, in adults, and meningitis, in children. Fifty percent of children encountered in the emergency room have so-called fever, which is less common in adult patients; in all cases, laboratory tests are required to rule out serious underlying diseases. Therefore, a review was made of evidence-based scientific sources, the guidelines of scientific societies and current scientific literature in order to differentiate the diagnostic work-up for infants, children and adults presenting at emergency units.
Results. It was found that recommendations for the identification of four degrees of acute inflammation in adults (Severe Inflammatory Response Syndrome (SIRS), Sepsis, Severe Sepsis and Septic Shock) for which laboratory tests play a decisive role, are based on limited scientific evidence. In several papers, the importance of laboratory tests is acknowledged for total Leukocytes, Neutrophil Granulocytes, C-reactive protein, and Pro-Calcitonin, in identifying localized and systemic infections. Quick and rapid microbiological tests are also of crucial importance. For children and infants, strictly "evidence-based" guidelines are provided. Finally, diagnostic tests for the patient with fever are also reported. Moreover, conditions such as fever associated with respiratory symptoms, bleeding (petechiae), dehydration, neurological symptoms and shock are dealt with exhaustively in the literature.
Conclusions. In scientific literature, adequate definitions are given of appropriate laboratory tests in patients with sepsis; we propose a tentative of the most appropriate approach for these cases.

Reference

1. Dellinger RP, et al. Surviving Sepsis Campaign guidelines for the management of severe sepsis and septic shock. Crit Med Care 2004;32:858-73.

SS3
HEMOGLOBINOPATHIES: EPIDEMIOLOGY AND CLINICAL MANIFESTATIONS

L. De Franceschi

Dipartimeto di Medicina Clinica e Sperimentale, Sezione di Medicina Interna, Università di Verona, Policlinico G.B. Rossi, Italy

Hereditary hemolytic anemias due to mutation of hemoglobin chains represent a novel emergency in world public health related to immigration of people from endemic areas to Europe and North America as well as to the amelioration of life conditions in the low developed countries (Modell B et al Scand J Clin Lab Invest 67: 39, 2007; Model B et al Bulletin of the WHO 86: 480, 2008; Roberts I et al 92: 865, 2007). Hemoglobinopathies are present in almost 71% of the 229 countries all over the world and affect 89% of the newborns in total population with similar incidence when considered it in ethnic minorities (Modell B et al Scand J Clin Lab Invest 67: 39, 2007; Model B et al Bulletin of the WHO 86: 480, 2008; Roberts I et al 92: 865, 2007). Studies of population have shown that 13% of the newborn carrying hemoglobinopathies is present in the Western countries such Europe and Italy, where immigrants from the endemic areas are moving to. OMS has identified the following endpoint for the present millennium: to develop educational programs for medical doctors and nurses to recognize and successfully treat these hereditary red cell disorders. These endpoints will be persecuted by multidisciplinary tasks acting at different complexity levels: (i) to create educational programs for family medical doctors; (ii) to promote educational programs and continuative educational strategies for medical doctors and nurses in the Emergency departments; (iii) to create multidisciplinary groups as reference group for treatment of acute and chronic events; (iv) to create national programs of screening and population genotyping. The final goal is the generation of an effective network that will be able to offer an holistic treatment of the patients with hemoglobinopathies to improve their quality of life.

SS4
DIAGNOSTIC APPROACH TO THALASSEMIA SYNDROMES

F. Manzato

A.O. Istituti Ospitalieri Carlo Poma, Lab. Analisi Chimico Cliniche e Microbiologiche, Mantova, Italy

The thalassemias constitute a heterogeneous group of naturally occurring, inherited mutations characterized by abnormal globin gene expression resulting in absence or reduction of α- or β-globin chain synthesis. This will result in an overall deficit of haemoglobin in red cells and cause a hypochromic microcytic anemia with a low mean corpuscular hemoglobin concentration (MCH). Microcytic anemia is the most frequent type of anemia encountered by physicians in general hospitals and outpatient clinics. The two most common causes are iron deficiency and thalassemia minor. The differentiation between these two diagnosis is of great consequence. An important way to improve the referral of at-risk pregnancies is to ensure adequate screening procedures in peripheral laboratories so that carriers are detected and referred to the central facility for DNA analysis. Several discrimination indices have been proposed to distinguish between iron deficit anemia and thalassemic trait,. These indices are derived from several simple red blood cell indices, like red blood cell count (RBC), mean cell volume (MCV) and RBC distribution width (RDW), as these are provided by electronic cell counters. However, none of these indices can provide a safe diagnostic tool for the discrimination between these two conditions and total body iron status and hemoglobin A2 level should be obtained for accurate differential diagnosis. The hemoglobin chromatography by HPLC can quantitate HbA2 and HbF and can detect the most common, clinical relevant, hemoglobin variants that can interact with β-thalassemia to produce a wide range of disorders of varying degrees of severity. A low MCV (< 78 fl) and MCH (< 27 pg) and elevated HbA2 levels (> 3.5%), are consistent with a presumptive diagnosis of heterozygous β-thalassemia. However, several environmental and genetic factors may modify this hematologic phenotype and confound carrier identification. The co-inheritance of α-thalassemia trait and β-thalassemia mutation may result in the patient having normal red cell indices but, fortunately, high HbA2 levels. A hypochromic microcytic anemia with normal-borderline HbA2, and normal HbF, may result from α-thalassemia trait, mild β-thalassemia mutation, compound heterozygous inheritance of both δ- and β-thalassemia, and the unusual γδβ-thalassemia. The level of HbA2 may be reduced in association with coexistent iron deficiency, but it remains elevated in the β-thalassemia trait range. There are conditions characterized by normal MCV and MCH, and normal HbA2 and HbF levels, that can be identified only by gene analysis: silent β-thalassemia mutation, α-globin gene triplication and the rare presence of α-, δ-, β-thalassemia together. Other rare cases show normal or low MCV and MCH, normal or reduced HbA2 levels and high HbF; the differential diagnosis is between δβ-thalassemia trait and HPFH. A Kleihauer test should be performed since in the former case HbF is usually heterocellular, in the second is pancellular. DNA analysis must be utilized for a definitive diagnosis.

SS5
LABORATORY INVESTIGATION OF HEMOGLOBINOPATHIES

M.R. Carta

Laboratorio di Chimica Clinica ed Ematologia, Presidio Ospedaliero San Bortolo, Vicenza, Italy

The hemoglobinopathies, or structural Hb variants, are attribuible to aminoacid substition in either α or non- α chain. More than 800 hemoglobinopathies have been described to date, the majority of which are benign and fortuitously discovered. Mutations resulting in abnormal hemoglobin structure may affect the function of the hemoglobin molecule; if this occurs, there may be significant clinical implication for an individual who has such a hemoglobin. A first level's laboratory ought to do a screening programme for the detection of haemoglobin variants. Majority of Italian laboratories uses High Performance Liquid Chromatography (HPLC) for the screening, but this methods will detects but not identifies the haemoglobin variants (1). It is possible to stop here the analytical procedure and to send the patient in a referral laboratory. Nevertheless in these laters years the italian population is changed and there are many persons coming from Africa or from others countries. In these cases is very important to try to give a presumptive diagnosis, at least. The nature of any variant hemoglobin detected by HPLC should be confirmed by an alternative tecnique like the hemoglobin electrophoresis at alkaline and acid pH, but also others parameters have to estemeed, like full blood count and blood film, sickling test and iron metabolism (2). Is very important also the study of the parents if this is possible and the patient's origin.
In many case it's possible, also for a first level's laboratory, to obtain a reliable diagnosis, which is essentially a presumptive diagnosis. If an unequivocal, definitive diagnosis is required, characterisation methods based on DNA analysis must be utilized (3).

Reference

1. Wild BJ, Bain BJ. Detection and quantitation of normal and variant haemoglobins: an analytical review. Ann Clin Biochem 2004;41:355-69.
2. Guideline. The laboratory diagnosis of haemo-globinopathies. Br J Haematol 1998;101:783-92.
3. Traeger-Synodinos J, Old JM, Petrou M, Galanello R. Best Practise guidelines for carrier identification and prenatal diagnosis of haemoglobinopaties. CMGS and EMQN 2002.

CO3
CHANGES IN BETATHALASSAEMIA DIAGNOSTICS AS A CONSEQUENCE OF IMMIGRATION

A. Amato, M.P. Cappabianca, P. Grisanti, M. Perri, I. Zaghis, D. Canzoniere

ANMI Onlus, Centro Studi Microcitemie, Roma, Italy

Purpose. In Italy, in the past years, the internal migration gave rise to the increase of the presence of thalassaemia (thal) where it was negligible, changing a regional phenomenon
in a national instance. However during last years, the percentage of immigrated populations, examined in our Molecular Biology Laboratory at Centro Studi Microcitemie of Rome (CSMR), has been progressively increasing. Since 1994 up to today, among 5230 individuals analyzed, we have detected 224 foreign subjects with a wide heterogeneity of mutations: 29 point mutations, 13 abnormal haemoglobins (Hb) and 4 deletional defects shared between 96 healthy ß-thal carriers, 74 carriers of Hb variant and 54 homozygous or compound heterozygous patients.
Method. The wide spectrum of origin among immigrated populations forces us to diversify the molecular analysis for detecting the unknown genotypes, although the primary survey consists in using ARMS-PCR technique. At present we need to select the suitable ARMS primers to screen the mutations that are most common in original country of the patients. The mutations that remain uncharacterized may

be detected by direct sequencing analysis of DNA.
Result. Owing to the great ethnic heterogeneity of patients, it is fundamental to discover which mutations are present in each ethnic group, designing specific primers that recognize the most common defects of original countries of all patients: cd 82-83 (-G) in Albanians, cd 10 (C#A)/cd 16 (-C) in Afghans, cd 36-37 (-T) in Dominicans, cd41-42 (-TCTT) in Sri Lankans, cd 51 (-C) in Romanians.In addition, it is always important to perform family studies both to identify heterozygous mutations and to understand the segregation of different globin genes (a, d and g) that may modify the phenotype.
Conclusion. At present, the main objective of the CSMR is to inform the immigrant people regarding the prevention, to provide means of haematological and molecular diagnosis and genetic counselling and to offer the opportunity of performing prenatal diagnosis with the eventual support of other medical structures or organizations.

Reference

1. Amato A et al. Need of implementing thalassaemia prevention. Haematologica 2007;93(3):15.

CO4

DETERMINATION OF BETA-GLOBIN GENE CLUSTER HAPLOTYPES IN SICKLE CELL AND β-THALASSEMIC PATIENTS

M. Montagnana[1], E. Danese[1], L. De Franceschi[2], P. Marradi[3], F. Bellorio[1], G.L. Salvagno[1], G. De Matteis[1], P. Solero[1], G.C. Guidi[1], G. Lippi[1]

[1]Sez. di Chimica Clinica, Dip. di Scienze Morfologico-Biomediche, Università degli Studi di Verona, Italy
[2]Sez. di Medicina Interna B, Dip. di Medicina Clinica e Sperimentale, Università degli Studi di Verona, Italy
[3]Oncoematologia Pediatrica, Policlinico GB Rossi, Verona, Italy

Background. The human ß-globin gene cluster contains over 20 fragment length polymorphisms. Five haplotypes have been associated with the mutation of Sickle Cell Disease (SCD): Benin, Senegal, Bantu, Cameroon, and Arab-Indian, according to the geographical area in which they are most commonly encountered. Moreover, nine different haplotypes (I-IX of Orkin) are observed in ß-thalassemic patients, related to specific mutations. The analysis of ß-haplotypes seems useful for genetic and epidemiological studies and to appreciate the heterogeneous spectrum of clinical manifestations between SCD patients. The aim of our research was to develop a reliable and clinically useful method to determine the haplotype pattern.
Methods. 47 African SCD patients resident in Veneto region were studied: 15 homozygous (SS), 23 heterozygous (AS), 4 HbSC, 5 HbSß-thalassemia, along with 15 patients with heterozygous ß-thalassemia. Seven regions in the ß cluster were amplified by polymerase chain reaction (PCR), using specific primers and treated with the appropriate enzymes. The following restriction sites were studied: HincII, HindIII, HincII, AvaII and HinfI.
Results. Among the 47 SCD patients, 25 (53%) were Benin-Benin, 6 (13%) Benin-Bantu, 1 (2%) Benin/type I, 3 (6%) Benin/type II, 3 (6%) Benin/type IX and 2 (4%) Benin/type 1. Seven SCD patients (15%) showed an heterozygous haplotype characterized by one common (Benin or Bantu) and a rare haplotype. Among ß-thalassemic patients, the haplotype more frequently detected was the type I (30%), followed by type V (23%) and II (20%). Mutation ß0 39 showed a strong association with type II.
Conclusions. Our data, the first obtained on SCD Africans patients living in Italy, confirm the haplotype frequencies observed in other European Countries. We have also observed that the coupling of haplotypes and specific mutations in ß-thalassemia reflects the origin of specific mutations in particular chromosomal background. Although further studies characterizing the association between clinical manifestation and haplotype pattern are undergoing in our institution, these preliminary results confirm that this method is reliable, inexpensive and rapid enough to genotype SCD patients.

SP4

"UNIVERSAL DEFINITION OF MYOCARDIAL INFARCTION": LE NUOVE LINEE-GUIDA 2007

M. Galvani

UO Cardiologia, Ospedale Morgagni, Forlì

Un nuovo documento di consenso [1], elaborato congiuntamente dalle quattro principali Società Cardiologiche mondiali (American Heart Association, American College of Cardiology, European Society of Cardiology, World Heart Federation) ha recentemente rifinito ed espanso la definizione di "infarto miocardico" aggiornata l'ultima volta sette anni fa, individuando cinque categorie distinte di infarto miocardico basate su differenze fisiopatologiche e sul fatto che l'evento sia collegato a procedure di rivascolarizzazione coronarica percutanea o chirurgica. La nuova definizione di infarto, che per le caratteristiche di condivisione a livello mondiale è chiamata "Universale", afferma definitivamente l'importanza dell'individuazione del danno cellulare per la corretta diagnosi della malattia. Ciò può essere ottenuto misurando nel sangue un marcatore specifico di morte della cellula miocardica, la troponina cardiaca. Tuttavia è il contesto clinico e l'informazione che viene dall'elettrocardiogramma, talora completati da tecniche di "imaging" del cuore, che permettono di accertare se la morte delle cellule miocardiche è dovuta ad un evento coronarico acuto. E' stato dimostrato negli anni precedenti la prima stesura del documento, ovvero il 2000, che in Italia (come nel resto del mondo) venivano impiegati 7 differenti criteri biochimici per stabilire se il paziente con malattia coronarica acuta aveva avuto un infarto [2] cosicché il raggiungimento di un consenso globale rappresenta il presupposto per una classificazione omogenea della malattia coronarica su scala mondiale. Il documento sottolinea comunque che, laddove la misurazione della troponina non è disponibile per motivi di costo, per porre la diagnosi possono essere sufficienti i marcatori tradizionali come la creatin-chinasi. Altri aspetti innovativi del documento comprendono la elaborazione di criteri per porre la diagnosi allorché l'evento coronarico è fatale prima che i dati biochimici siano disponibili, ed il peso che viene riconosciuto per la prima volta a tecniche di "imaging" cardiaco come l'ecocardiografia e la scintigrafia.
L'elemento chiave della diagnosi, con alcune importanti eccezioni, è l'elevazione nel sangue di un marcatore di danno miocardico provvisto di elevata sensibilità e specificità, la troponina, una proteina dell'apparato contrattile della cellula cardiaca. E' stato infatti dimostrato, in epoca precedente al ricorso sistematico alla rivascolarizzazione e all'uso di terapie anti-trombotiche aggressive, che i pazienti con ischemia miocardica acuta di gravità tale da portare ad un incremento anche piccolo della troponina hanno una prognosi simile a quella dei pazienti in cui la diagnosi di infarto veniva posta secondo i criteri tradizionali [3]. Questo concetto, ora unanimemente condiviso, ha ispirato nuove modalità di trattamento di questi pazienti. Oggi infatti l'approccio terapeutico è basato sull'uso di farmaci anti-trombotici potenti e sulla stabilizzazione meccanica in tempi rapidi, preferibilmente

mediante angioplastica, della lesione coronarica responsabile dell'evento. La sensibilità della troponina nell'individuazione del danno miocardico (è possibile rilevare perdite di solo 1-2 grammi di muscolo cardiaco) è tale da consentire la diagnosi anche quando lo strumento tradizionalmente utilizzato per questo scopo, l'elettrocardiogramma, non è alterato. Questa sensibilità si traduce nel fatto che il numero di infarti individuati mediante la misurazione del marcatore cardiaco aumenta di almeno il 25%. Ciò consente di focalizzare l'attenzione sui pazienti più ad alto rischio, risparmiando potenzialmente risorse su quei soggetti che, pur affetti da malattia coronarica acuta, hanno un rischio di eventi sostanzialmente inferiore. Al contrario, l'uso "routinario" della troponina consente l'individuazione di un danno miocardico in malattie diverse dalla sindrome coronarica acuta; in queste situazioni l'informazione che ne deriva è comunque utile nella gestione del paziente, dal momento che riflette la gravità della malattia che ha portato danno al muscolo cardiaco o permette di svelare la presenza di una importante malattia coronarica fino a quel momento misconosciuta.
La nuova definizione di infarto fornisce anche criteri quantitativi per individuare l'infarto che si può verificare in contesti iatrogeni in cui in danno miocellulare si verifica inevitabilmente, come in corso di procedure di rivascolarizzazione coronarica sia percutanea che chirurgica (angioplastica e by-pass).
La tabella illustra la nuova classificazione dell'infarto miocardico proposta.

Nuova classificazione dell'Infarto Miocardico (IM)

Tipo	Descrizione
1	IM spontaneo da ischemia miocardica acuta causato da un evento coronarico primario, come l'erosione e/o rottura, fissurazione o dissezione di una lesione aterosclerotica coronarica *
2	IM secondario a ischemia per sbilanciamento tra apporto e richiesta di O2, come risultato di spasmo o embolismo coronarico, anemia, aritmie, ipertensione o ipotensione *
3	Morte cardiaca improvvisa e inattesa, compreso l'arresto cardiaco, spesso accompagnato da sintomi suggestivi di ischemia e con rilievo elettrocardiografico di nuovo sopraslivellamento del tratto ST, nuovo blocco di branca sinistro, evidenza angiografica o anatomo-patologica di trombo coronarico fresco – in assenza di dati biochimici attendibili
4a	IM associato con intervento di rivascolarizzazione coronarica percutanea **
4b	IM associato con trombosi documentata (all'angiografia o all'anatomia patologica) di stent coronarico
5	IM associato a chirurgia cardiaca di by-pass aorto-coronarico ***

* criterio biochimico: elevazione del marcatore cardiaco (preferibilmente la troponina) superiore al 99° percentile della popolazione di riferimento
** criterio biochimico: elevazione del marcatore cardiaco (preferibilmente la troponina) superiore di 3 volte il 99° percentile della popolazione di riferimento
*** criterio biochimico: elevazione del marcatore cardiaco (preferibilmente la troponina) superiore di 5 volte il 99° percentile della popolazione di riferimento

La classificazione proposta supera la vecchia distinzione tra infarto transmurale verso non-transmurale (altrimenti detto infarto ad onde Q verso infarto non Q), e la più moderna separazione tra infarto associato a sopraslivellamento del tratto ST verso infarto senza sopraslivellamento del tratto ST. Ciò in quanto la prognosi a lungo termine di queste diverse forme di infarto risulta ora sovrapponibile.

Bibliografia

1. Thygesen K, Alpert JS, White HD; Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction, Jaffe AS, Apple FS, Galvani M, Katus HA, Newby LK, Ravkilde J, Chaitman B, Clemmensen PM, Dellborg M, Hod H, Porela P, Underwood R, Bax JJ, Beller GA, Bonow R, Van der Wall EE, Bassand JP, Wijns W, Ferguson TB, Steg PG, Uretsky BF, Williams DO, Armstrong PW, Antman EM, Fox KA, Hamm CW, Ohman EM, Simoons ML, Poole-Wilson PA, Gurfinkel EP, Lopez-Sendon JL, Pais P, Mendis S, Zhu JR, Wallentin LC, Fernández-Avilés F, Fox KM, Parkhomenko AN, Priori SG, Tendera M, Voipio-Pulkki LM, Vahanian A, Camm AJ, De Caterina R, Dean V, Dickstein K, Filippatos G, Funck-Brentano C, Hellemans I, Kristensen SD, McGregor K, Sechtem U, Silber S, Tendera M, Widimsky P, Zamorano JL, Morais J, Brener S, Harrington R, Morrow D, Lim M, Martinez-Rios MA, Steinhubl S, Levine GN, Gibler WB, Goff D, Tubaro M, Dudek D, Al-Attar N. Universal definition of myocardial infarction. Circulation. 2007 Nov 27;116(22):2634-53.
2. Ottani F, Galvani M, Dolci A, Plebani M, Tubaro M, Zaninotto M, Panteghini M; Gruppo di Studio Interdisciplinare Intersocietario ANMCO-SIBioC-SIMeL "Marcatori di Lesione Miocardica". Markers of myocardial damage in the diagnosis of acute myocardial infarction: the Italian reality in the year 2000. Ital Heart J Suppl. 2002 Sep;3(9):933-42.
3. Galvani M, Ottani F, Ferrini D, Ladenson JH, Destro A, Baccos D, Rusticali F, Jaffe AS. Prognostic influence of elevated values of cardiac troponin I in patients with unstable angina. Circulation. 1997 Apr 15;95(8):2053-9

SP5

HIGH SENSITIVE METHODS FOR CARDIAC TROPONIN ASSAY: ANALYTICAL CHARACTERISTICS AND PATHOPHYSIOLOGICAL ASPECTS

A. Clerico, C. Prontera

Fondazione Toscana G. Monasterio and Scuola Superiore Sant'Anna, Pisa, Italy

In 2000, the European Society of Cardiology/American College of Cardiology (ESC/ACC) consensus conference along with the American Heart Association (AHA)/ACC guidelines for differentiating acute myocardial infarction (AMI) and unstable angina codified the role of cardiac troponin monitoring by advocating that (a) diagnosis of AMI and (b) risk stratification are based on the increase in cardiac troponin I (cTnI) or T (cTnT), in an appropriate clinical setting; in 2007, these recommendations were confirmed by the so-called "Universal Definition of Myocardial Infarction". Moreover, in 2001 the IFCC C-SMCD established recommended quality specifications for cardiac troponin assay. In particular, these guidelines recommend that an increased concentration of cardiac troponin should be defined as a measurement exceeding the 99th percentile of the distribution of cardiac troponin concentrations in the reference group,. A total imprecision (CV) at this decision limit of 10% is recommended. Unfortunately, at present time, analytical imprecision is not uniform among different commercial immunoassays for cardiac troponins, mainly within the low concentration range, and therefore some troponin assays do not fit the goals recommended for functional sensitivity. As a result, a new generation of more sensitive and standardized cTnI immunoassays should show a ratio of 10% CV concentration to 99th percentile limit equal or even less than 1. In our laboratory we evaluated the analytical and clinical performance of the TnI-Ultra immunoassay for cardiac Troponin I (cTnI) measurement, carried out on the fully automated ADVIA Centaur CP® platforms (Siemens Medical Solutions Diagnostics SrL). The distribution of cTnI values was calculated in a population including 692 healthy

subjects (311 males and 381 females; age range from 11 to 89 years); log-transformed values of original cTnI concentration approximated to a symmetrical distribution with a calculated 99th percentile of 0.072 µg/L. As a result, the ratio between 10% CV concentration and 99th percentile values was less than 1 (i.e., 0.057/0.072= 0.79). A significant difference was found between the cTnI values in men and women (men: median 0.012 µg/L, range from undetectable values to 0.196 µg/L; women: median 0.008 µg/L, range from undetectable values to 0.130 µg/L; $p < 0.0001$ by Mann-Whitney U test). When a multiple regression analysis was performed, NT-proBNP, gender and age significantly contributed to the regression with cTnI ($R= 0.444$, $p < 0.0001$). Furthermore, a close linear regression was found between the cTnI values obtained with the TnI-Ultra method and the Access AccuTnITM, carried out on the UniCell® DxI 800 platform (Beckman Coulter, Inc., Fullerton) in 230 patients with cardiovascular diseases, including 70 samples with a cTnI value higher than the decision level for acute myocardial infarction (TnI-Ultra= -0.192 + 1..434 AccuTnIT; R= 0.976; n= 230).
Our data indicate that cut-off values, based on 99th percentile of cTnI distribution in apparently healthy subjects, can significantly vary according to age and gender of the reference population. From a physiological point of view, cTnI circulating levels are independently related to gender, age, and NT-proBNP values. As a work hypothesis, we can assume that, if heart dysfunction is an inevitable, ultimate fate, the measurement of cardiac troponins and natriuretic peptides should be used to detect people, who are at risk of a more rapid progression toward symptomatic cardiac failure, thus needing a specific clinical care.

SP6

QUALITY SPECIFICATIONS IN CARDIAC BIOMARKERS ASSAYS: WHICH UPDATINGS?

M. Zaninotto

Department of Laboratory Medicine, University-Hospital, Padova, Italy

The recommendations recently issued by clinical and biochemical Scientific Societies, about the measuring of biochemical markers of myocardial damage, underline again the relevance that such measurings hold in strategical clinical spheres for the outcome of the patients, particularly in diagnosis of acute coronary syndrome, in the evaluation of the efficacy of the therapy, in risk stratification and in prognosis with relevant consequences both on living quality and time of survival. Therefore, it comes out that the quality of the results is always more critical both in reproducibility, accuracy, time to report results and evidence-based decisional limits. The evolution of biochemical knowledges should be added to all these considerations: in fact, nowadays, it is underlining unexpected preanalytical problems (development of autoantibodies against troponin I, glycosilation of circulating fragments of natriuretic peptides) which have relevant consequences on the accuracy of the results obtained with different methods commercially available. Therefore, it is to regard that, in spite of all the analytical problems which should be more considered and discussed (standardization of the methods to measure troponins and to assay the more recently studied natriuretic peptides as well as the new biochemical markers), the interest should be directed towards all the phases that concur to the production of a relevant biochemical information. The possibility, for example, of using in routine diagnosis of acute coronary syndrome some high-sensitive methods for troponins measurement, open an interesting debate on the presence of measurable troponins concentrations also in healthy subjects, that seems to be sex and age-dependent. Consequently, the criteria to select the healthy population in order to calculate the reference range as well as to evaluate the significant troponins change in different cardiac diseases should be updated. The definition of quality specifications, according to the recent biochemical and physiopathological knowledges, should be both checked, following the actual standards, as well as improved considering the analytical performance, the specificity of new proposed methods, the informations which should be present in a laboratory report as well as the criteria to valute the results and the opportunity to provide a multimaker strategy using diagnostic algorithms. These are the most important factors which concur to an efficient and useful biochemical information provided by laboratory medicine in several clinical fields, such as that of acute coronary syndrome, were the biochemical markers represent a validated and essential diagnostic support.

SP7

CARDIOVASCULAR MARKERS: WHAT DOES IT CHANGE IN THE ROUTINE DAILY CARE OF PATIENTS WITH NEW "GOLD STANDARD"?

M. Cassin, D. Rubin

Cardiology Unit and Clinical Phatology Unit, AO S. Maria degli Angeli, Pordenone, Italy

In the past years WHO defined myocardial infarction from symptoms, ECG abnormalities, and enzymes. However, the development of more sensitive and specific biomarkers allows detection of ever smaller amounts of myocardial necrosis. Accordingly it requires a more precise definition of myocardial infarction. In response to this issue ESC and ACC in 2000 re-examined the definition of myocardial infarction, with recent update (2007) by ESC, AHA, WHF in order to refine the ESC/ACC criteria for the diagnosis of myocardial infarction from various perspectives. The term of myocardial infarction should now be used when there is evidence of myocardial necrosis in a clinical setting consistent with myocardial ischemia. Under these conditions, from biochemical point of view, there must be detection of rise and fall of biomarkers, preferably troponin, with at least one value above the 99th percentile of the upper reference limit, together with evidence of myocardial ischemia. The preferred biomarker for myocardial necrosis is cardiac troponin which has nearly absolute myocardial tissue specificity as well as high clinical sensitivity, thereby reflecting even microscopic zones of myocardial necrosis. An increased value for cardiac troponin is defined as a measurement exceeding the 99th percentile of the normal reference population and detection of a rise and/or fall of the measurements is essential to the diagnosis of acute myocardial infarction, and it needed to distinguish background elevated troponin levels, e.g. patients with renal failure. Optimal precision at the 99th percentile for each assay should be defined as $\leq$10%. To establish the diagnosis of myocardial infarction, one elevated value above the decision level is required.
Evolution of the definition of the diagnosis of myocardial infarction has a number of implication for society and for individual patient, specially in his routine daily care. Shift in criteria results in:

- the new defintion will impact epidemiological data
- consequences for the patients and their families with respect to psycological status, life insurance, professional career, driving licences
- a substantial increase in case identification that will have significant health resource and cost implications (increased number of myocardial infarction DRG, hospital

reimbursement, disability attestation)
- a substantial impact on treatment (a number of risk score include troponin neasurement for treatment algorithms)
- awareness of the presence of myriad conditions, other than myocardial infarction, that can lead to myocardial necrosis with consequent elevation of biomarkers.Clinical, therapeutic and prognostic implication of these pathological conditions are not always definite

SS6

THE IMPACT OF THE NEW INTERNATIONAL CONSENSUS GUIDELINES ON CLINICAL FLOW CYTOMETRY

M. Stetler-Stevenson

Chief, Flow Cytometry Laboratory, Laboratory of Pathology, National Cancer Institute, National Institute of Health, Bethesda, USA

Introduction

Flow cytometric techniques are widely utilized in diagnosis, determination of prognosis and monitoring of patients with leukemia and lymphoma and related disorders. An international consensus conference was assembled to address issues relevant to the diagnostic applications of flow cytometry of leukemia and lymphoma. This conference extended work previously presented in two consensus guidelines, updating recommendations in light of new data, and clarified issues left unresolved in earlier documents.

Medical indications

The new consensus guidelines approach medical indications from the perspective of a patient's clinical signs, symptoms and laboratory abnormalities as this approach more closely resembles actual clinical practice. A specimen is generally sent to the laboratory not with a diagnosis, but because of a clinical finding such as pancytopenia or lymphocytosis, with the goal of establishing a diagnosis of a particular disease.

1. Flow Cytometric immunophenotyping is indicated in anemia, leukopenia, monocytopenia, thrombocytopenia, and pancytopenia. Isolated cytopenias such as anemia should be evaluated for benign causes first. All lineages should be evaluated.
2. Flow cytometric immunophenotyping is indicated in the diagnostic evaluation of persistent lymphocytosis, monocytosis, and eosinophilia. Flow cytometry evaluation of unexpected neutrophilia, basophilia, or polycythemia is not recommended. Flow cytometric immunophenotyping is only useful in the evaluation of thrombocytosis when abnormal platelets forms are present.
3. Identification of blasts in a specimen is an indisputable indication for flow cytometry in essentially all cases.
4. Flow cytometric immunophenotyping is useful in the analysis of atypical cells in marrow, blood and body fluids such as CSF, serous effusions and ocular fluid.
5. Flow cytometric immunophenotyping is recommended in the evaluation of monoclonal immunoglobulinemia and diagnostic evaluation of unexplained marrow plasmacytosis. Flow cytometry is not recommended in the evaluation of polyclonal immunoglobulinemia as an isolated finding in the absence any other indications of hematolymphoid neoplasia.
6. Flow cytometric immunophenotyping is indicated in the diagnostic evaluation of primary lymphadenopathy, organomegaly and tissue masses by biopsy, fine needle aspiration or needle core biopsies. Evaluation of peripheral blood for hematolymphoid neoplasia may be sufficient for diagnosis and remove the need for more invasive procedures.
7. Flow cytometric immunophenotyping is indicated in the diagnostic evaluation of appropriate skin rashes (erythroderma) or cutaneous masses suspected to represent cutaneous hematolymphoid disease.
8. Flow cytometric immunophenotyping should always be performed on lymph node biopsies in patients with a history of leukemia or lymphoma or in the presence of clinical or laboratory changes suggestive of hematolymphoid neoplasia (B symptoms, multiple adenopathy and/or splenomegaly, or laboratory changes such as lymphocytosis, abnormal lymphocytes in the peripheral blood or cytopenias).
9. Flow cytometric immunophenotyping is indicated in the evaluation of CSF or other fluids from patients with a history of hematolymphoid neoplasia.
10. Flow cytometric immunophenotyping is indicated in bone marrow staging of lymphoma. There is no evidence that flow cytometric evaluation of bi-lateral bone marrow aspirates is better than a pooled specimen from both sides.
11. Flow cytometric immunophenotyping is useful for detecting potential therapeutic targets (e.g. CD20, CD52).
12. Flow cytometric immunophenotyping is recommended for assessment of response to treatment (including minimal residual disease testing). Examples of diseases in which FCI has been shown to be useful in assessing response to therapy include ALL, multiple myeloma and CLL.
13. Flow cytometric immunophenotyping is useful for documentation of progression or relapse.
14. Flow cytometric immunophenotyping is useful for evaluation of disease acceleration or transformation. Examples include detecting CML blast crisis or diffuse large B cell lymphoma in low grade lymphoma or CLL.
15. Flow cytometric immunophenotyping is recommended for prognostication in CLL (detection of ZAP-70 and CD38), plasma cell processes (detecting circulating plasma cells, proliferation rate) and acute leukemia.
16. Diagnosis of additional intercurrent hematolymphoid neoplasm, either treatment-related (such as MDS/AML or PTLD) or coincidental.

Optimal reagents for the flow cytometric diagnosis of hematopoietic neoplasia

Panel Design: Limited Specimen Specific Panels verses Larger Standard Panels. Specimen specific panels may be designed based upon morphology, sample type, medical indication, prior testing, and clinical history. However it may not be possible to use morphology and clinical information may be inaccurate or incomplete. Larger panels allow more comprehensive evaluation and provide improved efficiency but are more expensive. Minimizing initial work on all specimens and reserving more extensive evaluation for specimens that need it will decrease costs. Therefore the use of a primary panel to gather information that directs a secondary panel can reduce costs.

Basic Principles of Panel Design:

1. The panel should allow the analysis to account for all major cell populations present in the specimen, but does not need to identify all major hematopoietic cell types.
2. The flow cytometric testing performed should be comprehensive enough to identify all major categories of hematopoietic neoplasia. The initial panel does not need to contain all reagents necessary to completely characterize all diagnostic possibilities.

Panel Selection- Should be based primarily on specimen type with consideration of information provided from morphology, medical indication and/or clinical history. Proposed Reagents- Based upon Survey of Participants

Table 1
Cell lineages to be evaluated for each medical indication.

Medical Indication	Lineage to be Evaluated
Anemia	B, T, M, P
Leukopenia	B, T, M, P
Thrombocytopenia	B, T, M, P
Pancytopenia	B, T, M, P
Neutrophilia	M (limited)
Monocytosis	M
Lymphocytosis	B, T
Eosinophilia	T, M
Erythrocytosis	M (limited)
Thrombocytosis	M (limited)
Blasts in blood or marrow	B, T, M
Lymphadenopathy	B, T
Extranodal masses	B, T
Splenomegaly	B, T, M (limited)
Transformation of chronic leukemia - B cell	B
Transformation of chronic leukemia - T or NK cell	T
Staging for non-Hodgkin lymphoma - B cell	B
Staging for non-Hodgkin lymphoma - T/NK cell	T
Skin rash	B, T
Atypical cells in body fluids (CSF, serous, ocular, etc.)	B, T, M (limited)
Monoclonal gammopathy	B, P
Unexplained Plasmacytosis of bone marrow	B, P
Monitoring of Rx response (unknown diagnostic immunophenotype)	
Mature B cell neoplasm	B
Mature T or NK cell neoplasm	T
Acute lymphoid leukemia - B cell	B
Acute lymphoid leukemia - T cell	T
Acute myeloid leukemia	M
MDS / MPD / Overlap Syndrome	M
Plasma cell neoplasm	P

B = B cell, T = T cell, M = Myeloid, P = Plasma cell

Table 2
Consensus reagents for initial evaluation for hematopoietic neoplasia.

Lineage	Primary Reagents
B cells	CD5, CD10, CD19, CD20, CD45, Kappa, Lambda
T cells and NK cells:	CD2, CD3, CD4, CD5, CD7, CD8, CD45, CD56
Myelomonocytic cells:	CD7, CD11b, CD13, CD14, CD15, CD16, CD33, CD34, CD45, CD56, CD117, HLA-DR
Myelomonocytic cells (limited):	CD13, CD33, CD34, CD45
Plasma cells	CD19, CD38, CD45, CD56

Table 3
Reagents for secondary evaluation of specific hematopoetic cell lineages.

Lineage	Secondary Reagents
B cells	CD9, CD11c, CD15, CD22, cCD22, CD23, CD25, CD13, CD33, CD34, CD38, CD43, CD58, cCD79a, CD79b, CD103, FMC7, Bcl-2, cKappa, cLambda, cMPO, TdT, Zap-70, cIgM
T cells and NK cells	CD1a, cCD3, CD10, CD16, CD25, CD26, CD30, CD34, CD45RA, CD45RO, CD57, $\alpha\beta$-TCR, $\gamma\delta$-TCR, cMPO, cTIA-1, T-beta chain isoforms, TdT
Myelomonocytic cells	CD2, cCD3, CD4, cCD22, CD25, CD36, CD38, CD41, CD61, cCD61, CD64, CD71, cCD79a, cMPO, CD123, CD163, CD235a
Plasma cells	CD10, CD117, CD138, cKappa, cLambda

The next phase in the consensus process will be to design and validate specific panels utilizing the suggested reagents in a multi-site trial

In Conclusion, the medical indications for flow cytometric testing for hematolymphoid malignancy are based upon signs and symptoms in the patient. Guidelines have been developed for each medical indication as to the hematolymphoid lineages (e.g. B cell, T cell, NK cell, Myelomonocytic, and plasma cell) flow cytometric testing should evaluate and appropriate primary as well as secondary panels.

SS7

THE STANDARDIZATION IN FLOW CYTOMETRY; PROS AND CONS OF A LIGHT APPROACH

C. Ortolani[1], M.C. Sanzari[2]

[1]Presidio Ospedaliero Umberto I, Lab. Analisi Chimico-Cliniche, Mestre (VE), Italy
[2]Azienda Ospedaliera di Padova, Lab. Analisi Chimico Cliniche e Microbiologiche, Padova, Italy

In the diagnosis and follow up of hematopoietic disorders flow cytometry is a technique of paramount importance. In spite of its diffusion, its standardization is very difficult, because of the plenty of pre-analytical, analytical and post-analytical variables, such the existence of deeply different instrumentations, the possibility of diverse antibody combination, and the uncertainty of final data interpretation. This situation beget the creation of exhaustive guidelines; nevertheless, too complex guidelines risk to get poor compliance.
Considering our past experiences, we think that it could be useful to identify the most relevant variables, reducing the final number of instructions to be shared between different laboratories. These semplified guidelines should be graduated according the type of instrumentation available and the type of mainly observed diseases, and should not disregard some pivotal topics like the appropriateness of the request, the choice of the most suitable antibody combination, and the best way of reporting diagnostic results.
In other word, this “light” approach highlights the role of the operator, stressing its responsibility in choosing between interchangeable solutions on the base of his/her needs, but relies on a broadly diffused “good cytometry practice”, and consequently requires a high level of continuous professional training.

SS8

OPTIMISATION AND MEASUREMENT OF FLOW CYTOMETER PERFORMANCE

B. Brando, A. Gatti

Hematology Laboratory and Transfusion Center, Legnano Hospital (MI), Italy

Flow cytometry (FCM) workstations implemented in the accredited clinical laboratory must undergo the same maintenance and long-term checkings as any other analytical instrumentation. FCM analyses still imply a certain degree of operator-dependance, that is partly due to the great complexity of phenotypic and functional multicolor procedures and partly to the limited automation currently available to the clinical FCM platforms.
Optical-fluidical alignment stands as the basis of a well functioning FCM. This delicate procedure is periodically performed by field engineers, and its aim is to ensure the highest degree of optical signal collection with the minimum coefficient of variation (CV) for any available light scatter and fluorescence channel. Optical alignment and efficiency of light signal collection are therefore critical variables that should be kept recorded into a strict monitoring protocol. This constitutes the internal quality assessment (IQA) that must precede any clinical analysis on a daily basis. The operators must record any change occurring with time to such performance indicators, since many untoward factors may significantly interfere with it even in 2-3 month time lags. Optical alignment and efficiency of light collection are easily checked by the use of very small (1-2 micron), intensely hard-dyed latex microbeads (i.e. type 1-A beads) endowed with very low intrinsic CV (i.e. 1-2%). Operators must verify daily that the same magnitude of scatter and fluorescence signals (as high as possible with a predefined instrument setup) and of the CVs of each channel (as low as possible) are kept similar to those obtained at the time of field servicing. Daily checks are to be recorded in Levey-Jennings plots, with indicated predefined tolerance ranges (typically +/- 50 linear channels on a 1024 channel scale). As for any instrumental IQA, any significant drift from the expected values or any important setup variation to compensate such changes must prompt a new field servicing to check what is going on.
A good optical alignment is not however the only critical factor to ensure an optimal FCM performance. The optimisation of the electronic instrument setup is the second major influencing factor. Light scatter detectors are relatively neutral to the amplification and photomultiplier (PMT) settings, whereas fluorescence detectors are very sensitive to that.
PMTs can be adjusted over a wide range of high voltage values (typically 300-900 V), but each fluorescence detector displays a restricted range of voltage in which the sensitivity to low-intensity signals and dim event resolution are maximal. Each operator must ensure that the FCM is set to the minimal achievable fluorescence threshold, which also correlates with the maximum resolution index between background signal and dimly stained events. This can be easily obtained using fluorescent latex particle mixtures (type 3-A or 3-B beads) endowed with known and scaled fluorescence intensities (typically 4 or 5 different peaks plus a blank). Many different calibrated intensity multicolor bead mixtures are commercially available, that can be used to check simultaneously all the FCM fluorescence channels. The optimisation of electronic setting can be checked routinely every six months or so, but must be repeated every time a major change occurs to the FCM hardware (i.e. a new alignment procedure, replacement of laser, PMT, flow chamber, electronic amplifiers etc.).
Great care should be given to the optimisation of FCM performace, since it has marked effects on the detection of cells displaying low-intensity markers, such as leukemic cells, with critical diagnostic consequences.

CO5

FLOW-CYTOMETRY FOR THE EVALUATION OF PLATELET ACTIVATION PROFILE AND CLOPIDOGREL RESISTANCE IN PATIENTS WITH STABLE CORONARY ISCHEMIC DISEASE UNDERWENT ELECTIVE PERCUTANEOUS CORONARY INTERVENTION (PCI)

E. Volpi[1], J. Gianetti[3], S. Sbrana[2], S. Berti[3], A. Clerico[1]

[1]*Scuola Superiore Sant'Anna, Pisa, Italy*
[2]*Lab. Ematologia e Coagulazione, Ospedale del Cuore, Fondazione Toscana G. Monasterio-CNR, Massa, Italy*
[3]*U.O. Cardiologia, Ospedale del Cuore, Fondazione Toscana G. Monasterio-CNR, Massa, Italy*

Platelet hyperreactivity, despite a standard anti-thrombotic therapy, is emerging as a risk factor for recurrent myocardial ischemia after PCI. High (600 mg) loading dose of clopidogrel before elective PCI is now recognized as effective for the prevention of periprocedural ischaemic complications, without significant increase in the risk of bleeding complications. However, most patients after PCI still remain at substantial risk of thrombotic events due to insuf#cient inhibition of platelets by loading dose of clopidogrel. The aim of this study is to evaluate the platelet activation state and the clopidogrel efficacy before PCI, using flow cytometry. Twenty patients with stable angina taking aspirin (100 mg/die) for at last a month were enrolled in the study. Blood samples were collected at baseline before coronary angiography (T0), 12 h after 600 mg of clopidogrel (T1) and 24 h after PCI (T2). Platelet reactivity was assessed by flow cytometric measurements of P-selectin (CD62P) and activated GpIIb/IIIa complex (PAC-1). Clopidogrel response was assessed both with flow cytometric measurements of vasodilator-stimulated phosphoprotein (VASP) phosphorylation (platelet reactivity index, PRI) and with light transmittance aggregometry (LTA) after stimulation with adenosine diphosphate (ADP 5 mmol/l). Clopidogrel resistance was defined as a decrease <10% of the aggregation peack in response to 5 mmol/ l ADP, using LTA. In resistant patients (n=2), ADP-induced aggregation by LTA, PRI measured with VASP-P and the expression levels of PAC-1 and CD62P were significantly higher between the three collection times, whereas in the sensitive group (n=18) we found a significant reduction of platelet reactivity between T0, T1 ($p<0.0001$) and T2 ($p<0.0001$) reflecting the efficacy of clopidogrel loading dose.
In conclusion, our results suggest that by comparison of different tests we are capable to evaluate if the platelet activation state is adequately inhibited by clopidogrel loading dose and so we can recognize the “low-responder” patients even before the procedure. In fact all tests performed have shown higher value of platelet reactivity in resistant patients compared with the patients who demonstrate a sufficient level of inhibition.

Reference

1. Angiolillo D et al. J Am Coll Cardiol 2007;49:1505-16.

SS9

GAMMA-GLUTAMYLTRANSFERASE

M. Franzini[1], A. Pompella[2], A. Paolicchi[2], M. Emdin[3]

[1]*Scuola Superiore Sant’Anna, Pisa, Italy*
[2]*Dip. Patologia Sperimentale B.M.I.E., Università di Pisa, Italy*
[3]*Fondazione G. Monasterio, CNR Pisa, Regione Toscana, Italy*

Serum gamma-glutamyltransferase (GGT) activity is a sensitive test of liver function, its increase being associated

with conditions such as liver steatosis, cholestasis, viral hepatitis, and hepatocellular carcinoma. Serum GGT determination by automated analyzers, is a quick, reproducible and low cost test, but its clinical use is limited by its low specificity. Epidemiological studies of the last fifteen years have shown that GGT values within the reference range are positively associated with the risk of onset and evolution of atherosclerotic process and related diseases such as hypertension, type II diabetes and metabolic syndrome. Evidence of the association between serum GGT activity and coronary artery disease related events, infarction and stroke was achieved either in unselected populations, or in patients with ascertained cardiovascular disease. The relevance of the predictive value of serum GGT has been recently increased by studies showing the association of GGT levels with the risk and prognosis of cancer, even unrelated with hepatic involvement, and renal disease. GGT has been proposed also as marker of oxidative stress and of exposure to environmental xenobiotics which may directly induce oxidative stress. All these new epidemiological informations are not supported yet by a detailed understanding of the biogenesis and functions of serum GGT and its different fractions, and of the pathogenetic mechanism(s) of their increase. Recently, we have devised a procedure that allows the sensitive and reproducible determination of four gamma-glutamyltransferase fractions in human serum and plasma (1), and we have provided the reference values for them. The study of biological characteristics of serum GGT and of its heterogeneity is of great interest for a better diagnostic/prognostic use of GGT and for improving the comprehension of the pathogenesis of the disease associated with GGT (hepatic, cardiovascular and metabolic).

Reference

1. Franzini M, Bramanti E, Ottaviano V, Ghiri E, Scatena F, Barsacchi R, Pompella A, Donato L, Emdin M, Paolicchi A. A High Performance Gel Filtration Chromatography Method for γ-Glutamyltransferase Fraction Analysis. Anal Biochem 2008;374:1-6.

SS10
URIC ACID

G. Lippi

Sezione di Chimica Clinica, Università degli Studi di Verona, Verona, Italy

Uric acid (urate) is an organic compound consisting of carbon, nitrogen, oxygen and hydrogen with the scientific formula $C_5H_4N_4O_3$. In human, primates and in a particular species of dog (Dalmatians), uric acid is the end product of oxidation of purine catabolism. In most other mammals, the enzyme uricase further oxidizes uric acid to allantoin. The variability of uric acid in plasma is multifactorial, influenced by environmental variables and genetic factors. The biosynthesis is catalyzed by the enzyme xanthine oxidase; nearly two-thirds of its catabolism is sustained by urinary excretion, while the remaining is eliminated by the intestine through the faeces.
The uric acid has traditionally attracted the interest of clinical and laboratorists for his deterministic role in gout, a dysmetabolic disease caused by increased production of uric acid, or a decrease in its excretion. The following hyperuricemia cause the deposition of crystals in the periarticular tissues, painful inflammation in joints and can develop into chronic forms of arthritis deformans. Deposits of uric acid can also grow in the kidneys and cause renal calculi, or in the subcutaneous tissue with generation of nodules (tofi).
For decades it has been assumed that the antioxidant properties of uric acid could assign an essential role to this molecule in protecting against aging and oxidative stress. However, recent clinical and epidemiological findings suggest that hyperuricaemia might also represent a risk factor or a marker of cardiovascular disease, in which the pathophysiology of oxidative stress plays a pivotal role. It was also suggested that the hyperuricaemia could be involved in heart failure and metabolic syndrome. This apparent paradox between protective and toxic effects is supported by clinical and experimental trials, showing that some antioxidant compounds can actually act as pro-oxidant in certain situations, particularly when they are present in excess in plasma. Due to the complex interaction with other cardiovascular risk factors such as obesity, metabolic syndrome, diabetes and chronic kidney disease, it remains questionable however whether hyperuricemia might be an independent risk factor or rather a marker of cardiovascular disease. Nevertheless, considering the clear association between hyperuricemia and increased risk of mortality for cardiovascular disease and heart failure, it might be advisable to adopt population screening programs to better define the risk on the basis of plasma concentration of uric acid. If the harmful role of hyperuricaemia will be confirmed, and because uric acid measurement in plasma or serum is simple, cheap and affordable to all clinical laboratories, the screening might be ultimately sustained with a favorable cost-benefit ratio.

SS11
THE ERYTHROCYTE SEDIMENTATION RATE

M. Plebani, E. Piva

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

The erythrocyte sedimentation rate (ESR) is the laboratory test that, assessing the acute phase response to inflammation, provides to clinicians a useful “sickness index” (1). Since the first descriptions by Fåhraeus and Westergren in the 19th century, the erythrocyte sedimentation rate becomes one of the most popular laboratory tests (2,3). Over the years, with a few modifications relatively to the original description, the manual technique introduced by Westergren was recommended as the method of reference by the International Council for Standardization in Haematology (ICSH) and by the Clinical and Laboratory Standard Institute (CLSI) (4,5). It still remains the benchmark against which all new instruments and methodologies should be tested for validation.
Until now, the erythrocyte sedimentation is just a partly understood phenomenon. It is transient and confined to fresh blood. ESR is the measurement of the suspension stability of red blood cells in plasma under specified test conditions. If the descent of the plasma-red cell interface is plotted against time, it forms a typical sigmoid curve with three distinct phases. The initial portion of the curve, the lag phase, reflects red cells rouleaux formation. In this phase there is little sedimentation and the size of the aggregates is dependent both on plasma (extrinsic) factors, mainly acute phase proteins and on cellular (intrinsic) factors.
Erythrocyte aggregation is critical for the outcome of sedimentation. During the second, decantation phase, the plasma-red cell interface falls more rapidly. During the final phase, the cell aggregates pile up on the bottom of the tube. The two main determinants of ESR are the degree of red cell aggregation and the haematocrit (packed cell volume). The Westergren method evaluates the length at which erythrocytes settle, as measured by the distance that red blood cells fall in a tube within a

specific amount of time. The sedimentation time for each phase may vary from patient to patient and the ESR test result will depend on varying contributions from the three phases. This method measures the final contributions from the three phases, i.e. the fall of erythrocytes at 60 minutes (5).

ICSH declared that the term “erythrocyte sedimentation rate” is retained because of traditional usage, although a single measurement of the amount of fall of the red cells after 60 minutes is not truly a rate. Given that this method estimates the amount of sedimentation at a fixed time-interval, IFCC-IUPAC deemed improper the term “sedimentation rate” and decided to re-define ESR as “length of sedimentation reaction in blood” (LSRB) (6). This definition recognizes that the Westergren method and its modified versions measure neither the kinetics nor the rate of erythrocyte sedimentation, but only the final phenomenon described by Fåhraeus.

The new millennium has consolidated the ESR automation, with an improvement of working analytical techniques, laboratory workflow, biohazard and standardization. Methodology for ESR has improved thanks to technical advances that allowed marketed automated instruments with good correlation to Westergren reference method as well as advantages like safety for operators, reduced turn-around time and limited analytic imprecision. In clinical laboratories the Westergren method is not and it could not be used as “practical” routine technique even if it is the reference against which all currently marketed systems should be compared. While the automated and semi-automated instruments now available have shorted the testing time, this variety of methods have increased variation in the reported results making more difficulty to compare procedures. Thus, there is an obvious need to evaluate all the proposed working methods against the reference in order to document their reliability, accuracy and robustness.

Some automated techniques address other important issues; in particular, these attempt to measure the rate of red cells sedimentation by selecting an appropriate time interval, or by employing multiple readings in a selected phase of the reaction (7,8,9,10). Some instruments require anticoagulated blood with sodium citrate, whereas the most recent ones measure ESR from EDTA undiluted samples (reference specimen) (11). EDTA anticoagulated sample has several advantages: 1) it preserves the red blood cell morphology, 2) does not interfere with mechanisms that lead to erythrocyte sedimentation, 3) increases specimen stability, and 4) does not incur problems related to sample dilution with sodium citrate, in view of the fact that the ratio between blood and anticoagulant is of great importance and failure to respect the standard may explain specimen rejection in routine practice. Finally, EDTA fifth advantage is that it allows the laboratory to measure the ESR after 24 hours without any significant change in comparison to the “fresh” sample, using the same tube for the blood cell counts (CBC), decreasing the number of tubes and blood collected from patients (12). Since the ESR was made using manual and semi-automated methods, the procedure has been judged so simple that it could be performed without quality control (13). According to this view, the ESR was considered a semi-quantitative test instead as a haematological analysis with a great clinical importance in different diseases. Accreditation and regulatory requirements have prompted clinical laboratories to adopt internal and external quality control procedures that cannot be ignored. The poor suitability of stabilized materials for ESR measurement is well recognized (14). In 1993 the official documents of ICSH and NCCLS have described how patient specimens can be used for quality control; the specimen should be collected in EDTA, have a packed cell volume of 0.35 or less, and an elevated ESR in the range of 15 to 105 mm/h. Before testing, these must be inverted, at least, 16 times (4,5). In 2002 we described a procedure based on the use of fresh human whole blood for the daily quality control of ESR and we demonstrated how this procedure is reliable and inexpensive. Therefore, we concluded that fresh samples do not represent a useful warning for the drift control or Levey-Jennings plot and “efforts should now be made to assure that adequate control materials and procedures are provided” (14). Commercially quality control materials are now available, but usually these ESR-check are stabilized whole blood specimens, are not suitable for use in all analyzers and should not be considered “commutable” for simulating the human whole blood. In conclusion, we underline that accurate ESR testing is based on the choice of a reliable working method. Moreover, quality control represents a fundamental procedure to obtain reliable and precise laboratory results; also for the ESR, in the clinical laboratory, a quality control plan must ensure reliable results for patients care. With the commercial development of control materials, it should be feasible to include the ESR test in inter-laboratory proficiency testing programs.

While increasing automated techniques improved analytical precision and accuracy, the open question is whether these technologies have been developed merely to maintain and promote the use of an obsolete and useless test.

ESR has little value as a screening test for hidden diseases. The routine use of ESR without non-evidence-based medical procedure was considered an indicator of the presence of unconscious defensive medicine among hospital internists (15). Indiscriminate requests for ESR in healthy subjects and in other clinical situations are inappropriate. Other laboratory tests show greater clinical efficiency (e.g., C-reactive protein in the first 24 hours from tissue damage), and also because the phenomenon of erythrocyte sedimentation is known to be affected by physiological and pathological factors including anaemia or abnormally shaped red cells, independent of acute phase reactants. While some laboratory scientists have described ESR as an “obsolete test”, much evidence has been collected to demonstrate its usefulness in clinical practice (16). Since 1990 the ESR is indicated in the diagnosis and therapeutic monitoring of temporal arteritis and polymyalgia rheumatica (17,18,19). It may be helpful in resolving conflicting clinical evidence in patients with rheumatoid arthritis (20) and with the evaluation and management of patients with specific autoimmune, inflammatory or infectious disorders (e.g. pelvic inflammatory disease, bacterial endocarditis, septic arthritis and osteomyelitis). Recently, the relation between erythrocyte sedimentation rate (ESR) and risk of developing coronary heart disease or fatal cerebrovascular accident was assessed in the Reykjavik Study (21), while another study has evaluated ESR as a screening tool for the presence of low-grade inflammation in people with atherothrombotic risk factors (22).

Continuing improvements of technologies allow the in depth investigation of the kinetics of red cell aggregation and biological variables affecting the erythrocyte sedimentation; with clinical appropriateness, the ESR become far obsolete in either the near or distant future.

References

1. Koepke JA. Welcome Innovation in Erythrocyte Sedimentation Testing. Am J Clin Pathol 2002;118:11-2.
2. Fåhraeus R. The suspension-stability of the blood. Acta Med Scand 1921;55:1-228.
3. Westergren A. Studies of the suspension stability of the blood in pulmonary tuberculosis. Acta Med Scand 1921;54:247-82.
4. International Committee for Standards in Haematology. Recommendations for measurement of erythrocyte

sedimentation rate. J Clin Pathol 1993;46:198-03.
5. NCCLS. Reference and selected procedures for the erythrocyte sedimentation rate (ESR) test; approved standard, 4th ed. H2-A4. Wayne, PA: NCCLS, 2000.
6. Pure and Applied Chemistry; Properties and units in general chemistry. IUPAC, 2000;72:945.
7. Plebani M, De Toni S, Sanzari MC, Bernardi D, Stokreiter E. The TEST 1 automated system: a new method for measuring the erythrocyte sedimentation rate. Am J Clin Pathol 1998;110:334-40.
8. Happe MR, Battafarano DF, Dooley DP, Rennie TA, Murphy FT, Casey TJ, *et al.* Validation of the Diesse Mini-Ves erythrocyte sedimentation rate (ESR) analyzer using the Westergren ESR method in patients with systemic inflammatory conditions. Am J Clin Pathol 2002;118:14-7.
9. Romero A, Munoz M, Ramirez G. Length of sedimentation reaction in blood: a comparison of the test1 ESR system with the ICSH reference method and the sedisystem 15. Clin Chem Lab Med. 2003;41:232-7.
10. Ajubi NE, Bakker AJ, van den Berg GA. Determination of the length of sedimentation reaction in blood using the TEST 1 system: comparison with the Sedimatic 100 method, turbidimetric fibrinogen levels, and the influence of M-proteins. Clin Chem Lab Med. 2006;44:904-6.
11. Piva E, Sanzari MC, Servidio G, Plebani M. Length of sedimentation reaction in undiluted blood (erythrocyte sedimentation rate): variation with sex and age and reference limits. Clin Chem Lab Med 2001;39:451-4.
12. Piva E, Fassina P, Plebani M. Determination of the length of sedimentation reaction (erythrocyte sedimentation rate) in non-anticoagulated blood with the Microtest 1. Clin Chem Lab Med 2002;40:713-7.
13. Bull BS. Quality assurance strategies. In: Koepke JA. Ed. Practical Laboratory Hematology. New York, NY: Churchill Livingstone; 1991.
14. Plebani M, Piva E. Erythrocyte sedimentation rate: use of fresh blood for quality control. Am J Clin Pathol. 2002 Apr; 117:621-6.
15. Asher E, Parag Y, Zeller L, Yerushalmi R, Reuven H. Unconscious defensive medicine: the case of erythrocyte sedimentation rate. E J Int Med 2007; 18:35-8.
16. Plebani M. Erythrocyte sedimentation rate: innovative techniques for an obsolete test? Clin Chem Lab Med. 2003; 41:115-6.
17. Hunder GG, Bloch DA, Michel BA. The American College of Rheumatology 1990 criteria for the classification of giant cell arteritis. Arthritis Rheum 1990; 33:1122-8.
18. Salvarani C, Cantini F, Boiardi L, Hunder GG. Polymyalgia reumatica and giant-cell arteritis. N Engl J Med 2002;347:261–71.
19. Salvarani C, Hunder GG. Giant cell arteritis with low erythrocyte sedimentation rate. Arthritis Care Res 2001; 45:140-5.
20. Combe B, Dougados M, Goupille P, Cantagrel A, Eliaou JF, Sibilia J. Prognostic factors for radiographic damage in early rheumatoid arthritis: a multiparameter prospective study. Arthritis Rheum 2001;44:1736-43.
21. Andresdottir MB, Sigfusson N, Sigvaldason H, Gudnason V. Erythrocyte sedimentation rate, an independent predictor of coronary heart disease in men and women: the Reykjavik Study. Am J Epidemiol 2003; 158:844-51.
22. Steinvil A, Shapira I, Arbel Y, Justo D, Berliner S, Rogowski O. Determinants of the erythrocyte sedimentation rate in the era of microinflammation excluding subjects with elevated C-reactive protein levels. Am J Clin Pathol 2008;129:486-91.

CO6

SERUM CA125 CONCENTRATION CORRELATES WITH THE SEVERITY OF CARDIAC DYSFUNCTION AND THE PRESENCE OF SEROSAL EFFUSION IN AL AMYLOIDOSIS

P. Russo[1], G. Palladini[1], L. Montanari[2], F. Lavatelli[1], L. Obici[1], G. Sarais[1], R. Albertini[3], G.V. Melzi D'Eril[4], R. Moratti[3], G. Merlini[1]

[1]Center for Amyloidosis, Biotechnology Labs, "Fondazione I.R.C.C.S. San Matteo" and University of Pavia, Italy
[2]Dept. of Obstetrics and Gynecology, "Fondazione I.R.C.C.S. San Matteo" and University of Pavia, Italy
[3]Clinical Chemistry Laboratory, "Fondazione I.R.C.C.S. San Matteo" and University of Pavia, Italy
[4]Dept. of Medicine and Surgery, University of Milan, Italy

Introduction. In AL amyloidosis the deposition of fibrils formed by monoclonal immunoglobulin light-chains causes organ dysfunction. Cardiac involvement is common; N-terminal natriuretic peptide type B (NT-proBNP) and cardiac troponins (cTn) are the most powerful prognostic determinants. Fluid retention, caused by heart failure (HF), nephrotic syndrome (NS) or both is frequent. Severe refractory pleural effusion has been reported in patients with amyloid deposition in serosal membranes. CA125, produced by serosal epithelium, is used as marker of ovarian cancer, but its concentration is known to increase also in HF. However, so far this marker has not been investigated in AL amyloidosis. We investigated the correlation between serum CA125 concentration, cardiac dysfunction and fluid retention in 21 consecutive patients with AL amyloidosis.
Methods. The serum concentration of CA125 was measured with a direct chemiluminescence sandwich immunoassay (CA 125II TM ADVIA Centaur, Bayer). The upper reference limit is 35 U/mL. The patients were classified as having either peripheral edema or severe fluid retention with serosal effusion at physical examination and by X-ray and ultrasonography. The differences between subgroups were tested by the Mann-Whitney U-test. Ovarian cancer was excluded in female patients (10/21).
Results. The heart was involved in 20 patients (95%) with HF in 16 cases (76%), the kidney in 19 (90%), with NS in 13 cases (62%). The median (range) CA125 concentration was 96 U/mL (10-1169 U/mL), NT-proBNP 8186 ng/L (225-58091 ng/L), cTnI 0.07 ng/mL (0.02-0.35 ng/mL), proteinuria 4 g/24h (0.1-18 g/24h). Peripheral edema was present in 10 patients (48%) and pleural effusion in 11 cases (52%), 1 of whom also had ascites. CA125 was >35 U/mL in 15 patients (71%). The concentration of CA125 correlated with that of NT-proBNP (p=0.02) and of cTnI (p=0.01), did not correlate with proteinuria, and was higher in patients with serosal effusion than in other patients (median 145.9 vs. 39.3 U/mL, p=0.02). There was no correlation between serosal effusion and NT-proBNP or cTnI.
Conclusions. In AL amyloidosis the concentration of CA125 independently reflects both the severity of cardiac dysfunction assessed by biomarkers and the presence of serosal effusion.

CO7

THE ERYTHROCYTE SEDIMENTATION RATE (ESR): AN OLD TEST WITH NEW CONTENTS

R. Pajola, E. Piva, B. Robecchi, F. Tosato, M. Plebani

Dep.of Lab. Medicine, Padua University School of Medicine, Padua, Italy

Introduction. ESR evaluates the suspension stability of red blood cells in plasma under specified test conditions. Consequently, it should not be considered a measure of

speed but rather as an evaluation of the length of sedimentation of red blood cells. The term “sedimentation rate” has been deemed improper by the IFCC-IUPAC and has been redefined as “length of sedimentation reaction in blood” (LSRB) (1).
Materials and methods. TEST1 is a closed automated analyzer that determines the erythrocyte sedimentation in EDTA vacuum tube using a sensing area with a standard temperature of 37°C. The method studies the aggregation capacity of red blood cells by telemetry. A mathematical algorithm converts the raw data obtained from evaluation of optical density signals into ESR results, with a satisfactory statistical comparison to Westergren values. The telemetry applied to TEST1 estimates mainly the aggregation of erythrocytes. The new TEST1 TH provides three derived factors, RBC Aggregation Factor (Ag.F), Viscosity factor (Vs.F) and anemia factor (An.F). In order to evaluate these new ESR correlated indices they were studied in a group of reference healthy subjects and in a patient group suffering from anemia. At later stage, the ESR indices and RBC aggregation using the manual Mirenne RBC aggregometer were evaluated in two patient groups suffering from monoclonal and lymphoproliferative diseases vs a reference group. The correlation of RBC Ag.F with Mirenne RBC aggregometer measurements was also verified using reference healthy subjects.
Results. The results of the preliminary study demonstrated statistical differences between the reference group and the group affected by monoclonal disease both as far as RBC aggregation and Vs.F is concerned ($p<0.0001$). The An. F. and Vs.F. also showed significant differences in value between reference and anemic groups ($p=0.05$). The Ag.F with Mirenne RBC aggregometer measurements also show a good correlation ($r=0.81$).
Conclusion. Continuing improvements in technologies allow the investigation of the kinetics of red cell aggregation and biological variables affecting erythrocyte sedimentation.

Reference

1. Pure and Applied Chemistry; Properties and units in general chemistry. IUPAC, 2000; 72:945.

SP8

STANDARDIZATION IN LABORATORY MEDICINE: ACTIVITIES, GOALS, AND FURTHER ISSUES

M. Panteghini

Centre for Metrological Traceability in Laboratory Medicine (CIRME), University of Milan, Milano, Italy

The standardization of measurements is of high priority in Laboratory Medicine, its purpose being to achieve closer comparability of results obtained using routine analytical systems. In order to achieve standardization, an approach is required that provides reliable transfer of the measurement values from the highest hierarchical level to methods which are routinely used in the clinical laboratories. Such a structure is presented by the reference measurement system, based on the concepts of metrological traceability and a hierarchy of analytical measurement procedures. Since the development of metrologically sound reference systems is a complicated and expensive process, it is clear that the objective of improving standardization in Laboratory Medicine will only be achieved if the problems are dealt with not on a national level but through international cooperation. This was the reason for the creation of the Joint Committee on Traceability in Laboratory Medicine (JCTLM), which has made publicly available a list of higher order reference materials and reference methods for analytes measured in Laboratory Medicine, identified by a thorough review process for conformity with appropriate ISO standards (http://www.bipm.org/en/committees/jc/jctlm/jctlm-db).
JCTLM has also published the list of reference laboratories that fulfil established selection criteria and are able to deliver a reference measurement service.
As soon as a new reference measurement system is implemented, clinical validation of the correctly calibrated routine methods should take place. In specific cases, in order to maintain the value of clinical experience, correlation of measurement results obtained with the new traceable calibration to results of measurements obtained with the previous not standardized calibration should be established. Other important issues concerning the implementation of a metrologically-correct approach for result standardization should also be defined. Firstly, a clear definition of the clinically allowable error of measurements is required. Secondly, a post-market surveillance of the performance of diagnostic products should be established through the organization of appropriate External Quality Assessment Schemes (EQAS). The applicability of the true value concept in EQAS requires, however, the availability of control materials with target values assigned by reference methods and that these materials behave exactly as human patient specimens. True value assignment to commutable EQAS materials will allow an objective evaluation of the performance of commercial systems, together with an accuracy-based (instead of inferior consensus group-based) grading of the competency of participating clinical laboratories.

SP9

STANDARDISATION OF HbA_{1c} MEASUREMENTS: PRACTICAL CONSEQUENCES AND COURSE OF ACTIVATION

A. Mosca

CIRME, Dip. di Scienze e Tecnologie Biomediche, Università degli Studi di Milano, Segrate, Milano, Italy

The measurement of hemoglobin A_{1c} has been the gold-standard measurement of chronic glycemia for over two decades. Since elevated HbA_{1c} values increase the likelihood of the microvascular and perhaps macrovascular complications of diabetes, clinicians have used HbA_{1c} test results to guide treatment decisions, and the assay has become the cornerstone for the assessment of diabetes care. To achieve a more uniform standardization of HbA_{1c} measurements, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) developed a new reference method that specifically measures the concentration of only one molecular species of HbA_{1c}. Results by the new reference method have also been compared with the results obtained by current methodologies, and the relation between the assays can be expressed by simple regression equations (“master equations”). A network of IFCC Reference Laboratories is now in place, and it has been proved that the Reference system is robust and stable over time (1).
All manufacturers should implement worldwide the traceability to the IFCC reference system for HbA_{1c}. In the European Union (EU) the implementation of calibration traceability in laboratory medicine to higher-order standards is already mandatory. The EU directive 98/79/EC on in vitro diagnostic (IVD) medical devices explicitly requires manufacturers to ensure and document metrological traceability of their products. The IFCC WG-HbA_{1c} is willing to review the proposed and will be able, to provide an expert scientific opinion about the suitability of a manufacturer’s proposed HbA_{1c} traceability chain and to

offer some metrological advice and guidance if appropriate.
A consensus statement was published last year (2), which resulted in three main recommendations: a) the IFCC systems represents the only reference to implement the standardisation of HbA_{1c}; b) the HbA_{1c} result should be reported in both SI units (mmol/mol) and NGSP derived unit (%) using the IFCC-NGSP master equation; c) the calculation of an HbA1c derived average glucose (eAG) is suggested as an aid for the interpretation of HbA1c result, based on the results of a recently published international study (3).
In the presentation an overview of the above mentioned issues will be performed, focussing on the different responsibilities (IFCC, Scientific Societies, Manufacturers, Laboratory professionals, other stakeholders) along the way of the implementation of the IFCC reference system.

Reference

1. Weykamp C, John GW, Mosca A et al. The IFCC Reference Measurement System for HbA1c: A 6-Year Progress Report. Clin Chem 2008;54;240-8.
2. Consensus Statement. Consensus Statement on the Worldwide Standardization of the Hemoglobin A1c Measurement. Diabetes Care 2007;30:2399–400.
3. Nathan DM, Kuenen J, Borg R et al. Translating the A1c assay into estimated average glucose values. Diabetes Care 2008;31:doi:10.2337/dc-0545.

SP10
WILL STANDARDIZATION ASSIST BEST PRACTICE IN THYROID FUNCTION TESTING?

G.H. Beastall

Department of Clinical Biochemistry, Royal Infirmary, Glasgow, UK

Assays for thyroid function tests (TFT) have been used in the clinical chemistry laboratory for thirty years but external quality assurance schemes reveal that there is still considerable variability in results from different methods. This variability creates difficulty in the transferability of patient data and has caused patient interest groups to question the value and effectiveness of TFT.
Recent international evidence-based guidelines have been prepared to promote best practice and harmonization of the use of TFT. However, the application of such guidelines for TFT is challenged by method dependent differences in results.

The IFCC Working Group on the standardization of thyroid hormones (WG-STFT) has been established to develop new reference materials and reference measurement systems for thyroid hormones and to investigate the use of synthetic or recombinant materials for mass calibration of thyroid stimulating hormone (TSH). With expert direction from Professor Linda Thienpont (University of Gent, Belgium) WG-STFT has overseen the following achievements:

- International clinical thyroid association members have made the clinical case for greater harmonization of TFT results.
- Reference materials and reference measurement procedures have been published to assist the standardization of total thyroxine (TT4) and total tri-iodothyronine (TT3).
- A reference measurement system for free thyroxine (FT4) has been adopted based on equilibrium dialysis and isotope dilution liquid chromatography/tandem mass spectrometry. Forty donor were selected with FT4 results across the normal range and these have had FT4 measured using the reference measurement procedure. Nine IVD manufacturers and two mass spectrometry laboratories also measured FT4 in these specimens. The results confirmed the anticipated between method differences in results but for the first time these could be compared with the results from the reference measurement procedure. Discussion with IVD manufacturers has commenced and it is hoped that this will lead to greater harmonization of FT4 results in the future.
- TSH is a complex, heterogeneous glycoprotein and standardization presents challenges. Measurement of TSH in the forty specimens used for the FT4 project has confirmed the method dependent differences. WG-STFT is currently discussing whether to undertake a primary standardization project and/or development of a reference method procedure.

SP11
LA STANDARDIZZAZIONE IN EMATOLOGIA

M. Buttarello

Dipartimento Interaziendale di Medicina di Laboratorio, Azienda Ospedaliera-Azienda USL16, Laboratorio Ospedale S. Antonio, Padova, Italy

Introduzione e concetti generali
Martin Rowan, all'epoca segretario esecutivo dell'International Council for Standardization in Haematology (ICSH), scrisse nel 1998, che il compito principale del laboratorio (clinico) è la produzione di test che supportino il Clinico nel prendere decisioni relativamente alla diagnosi, alla scelta della terapia e al suo controllo, e alla formulazione di una prognosi. Il risultato di un test deve pertanto fornire informazioni utilizzabili dal punto di vista clinico (1).
Tutti i test di laboratorio sono peraltro soggetti ad incertezze ed errori che ne diminuiscono l' utilità. La possibilità di errore inizia già nel momento in cui l'esame viene richiesto, continua durante le fasi preanalitica, analitica propriamente detta, e postanalitica, per finire quando il Clinico assume una decisione in seguito all'informazione ricevuta sottoforma di referto (2,3). Tutte la azioni messe in atto per prevenire e identificare gli errori rientrano negli obiettivi della sicurezza di qualità. All'interno del più ampio concetto di sicurezza di qualità rientra il processo di standardizzazione, definito, dal punto di vista del laboratorio, come " la descrizione di sistemi di misura uniformi e riproducibili al fine di assicurare precisione, accuratezza, specificità, e armonizzazione dei risultati" (4). L'ICSH elaborò nel 1991 una propria definizione di programma di sicurezza di qualità inteso "come l'insieme delle azioni intraprese dal direttore di un laboratorio al fine di assicurare l'attendibilità dei risultati, e di aumentare l'accuratezza, la riproducibilità e la comparabilità fra laboratori. Questo include la proficiency surveillance, l'uso costante di controlli interni, e la partecipazione a uno schema di valutazione esterna di qualità. Deve anche essere prevista la partecipazione a corsi di formazione e aggiornamento, a studi collaborativi su strumenti e metodi di laboratorio, nonché altre attività collaborative intese a migliorare le prestazioni di laboratorio. Un programma di sicurezza di qualità in ematologia deve anche riguardare gli aspetti clinici dell'ematologia" (5).
Come si può notare è particolarmente enfatizzato il controllo della parte analitica ed interpretativa, tuttavia, la consapevolezza che il maggior numero di errori avviene durante le fasi extra analitiche ha portato a meglio definire la proficiency surveillance come "la supervisione e l'azione tali da assicurare una buona pratica di laboratorio. Importanti aspetti sono il controllo di qualità interno e la valutazione esterna di qualità, ma devono anche prevedersi attenzione e controllo sulla raccolta ed etichettatura dei campioni, sulla consegna degli stessi al laboratorio, sugli effetti dovuti all'ambiente e alla conservazione, sull'interpretazione dei

risultati e sulla rilevanza dei vari test rispetto alle informazioni cliniche richieste. Devono essere compresi anche il mantenimento e il controllo degli equipaggiamenti e degli apparecchi e la formazione, protezione e sicurezza del personale" (5).
Queste definizioni sono alla fine riconducibili al "Sistema Qualità" dell'ISO definito come: " le strutture organizzative, le responsabilità, le procedure, i processi e le risorse per attuare la gestione della qualità" (6).

Storia e attualità

L'esigenza di procedure per la standardizzazione in ematologia iniziò con l'avvento dell'automazione dei conteggi cellulari. Inizialmente i conteggi erano eseguiti con metodi manuali (camere di conta previa diluizione/lisi del campione di sangue), ma, a partire dai primi tentativi ad opera di Moldavan del 1934, i metodi automatizzati sono diventati insostituibili. Due differenti metodologie si svilupparono: il metodo ad impedenza (a partire dal brevetto di Wallace Coulter presentato nel 1949), e il metodo a dispersione di luce che vide le prime applicazioni commerciali negli anni' 60 principalmente ad opera della Technicon Corporation.
L'automazione oltre ad aumentare la velocità nell'esecuzione dei test comportava anche sensibili miglioramenti nell'accuratezza e nella precisione superando gli errori dovuti alla preparazione del campione. Con la diffusione dei metodi automatizzati cominciarono tuttavia ad emergere significative differenze di conteggio a seconda dello strumento e del principio su cui era basato.
Si vide inoltre che nel caso di differenze nei risultati era difficile se non impossibile stabilire quali analizzatori risultassero in errore e quali no.
Queste problematiche e la necessità di procedere alla standardizzazione in emocitometria furono discusse per la prima volta al Congresso della Società Europea di Ematologia tenutosi a Lisbona nel 1963 in un simposio dal titolo " Erythrocytometric methods and their standardization".
Iniziarono quindi i primi trial per verificare le differenze esistenti fra i vari laboratori. Una prima sorpresa negativa si ebbe quando uno stesso campione distribuito a 50 laboratori Europei di riferimento riportò una concentrazione di emoglobina compresa fra 11 e 18 g/dL. Le cose miglioravano sensibilmente nei controlli successivi dopo che i laboratori vennero dotati di linee-guida e di una preparazione di calibrazione. Analoghi problemi si manifestarono in un trial sul conteggio degli eritrociti che vedeva coinvolti 40 laboratori: i risultati erano compresi fra 4.1 e 5.4x1012 /L.
La dimostrazione della grande variabilità dei risultati e l'esigenza di trovare una soluzione furono lo stimolo per la costituzione dell'International Committee (oggi Council) for Standardization in Haematology (ICSH) che attraverso differenti gruppi di studio (Expert Panel on…), produsse, a partire dal 1965, numerosi documenti anche per conto dell'OMS, con lo scopo di identificare le cause della variabilità delle misure, di elaborare raccomandazioni (fino a veri e propri metodi di riferimento) e, ove possibile, produrre materiali di riferimento.
Qualche anno più tardi (1968), negli Stati Uniti, in risposta alle istanze presentate dal College of American Pathologists (CAP) si costituì il National Committee for Clinical Laboratory Standard (NCCLS, oggi CLSI) come federazione fra tutte le parti interessate nel campo del laboratorio clinico (organizzazioni professionali, scientifiche, industriali e governative). L'NCCLS iniziò a produrre degli standard (intesi come documenti normativi) relativi anche alle determinazioni ematologiche utilizzando gruppi di lavoro e Sottocomitati di Esperti.
Alcuni documenti sono stati accettati trasversalmente dalle 2 organizzazioni, mentre altri sono stati prodotti congiuntamente.
I documenti più rilevanti per l'ematologia automatizzata tutt'ora considerati di attualità sono:
-The assignement of values to fresh blood used for calibrating automated blood cell counters (ICSH, 1988)
-In Vitro Medical Devices. Measurement of quantities of Biological Origin – Metrological Traceability of values assigned to Calibrators and Control Materials.
(EN ISO 17511, 2001)
-Recommendation for reference method for haemoglobinometry in human blood and specification for international haemiglobincyanide standard (ICSH, 1995)
-Guidelines for the evaluation of blood cell analysers including those used for differential leucocyte and reticulocyte counting and cell marker applications (ICSH, 1994)
-Reference method for enumeration of erythrocytes and leucocytes (ICSH, 1994)
- Proposed reference method for reticulocyte counting based on determination of the reticulocyte to red cell ratio (ICSH, 1998).
- latelet counting by the RBC/Platelet ratio method: a reference method (ICSH/ISLH, 2001).
- Reccomendations for "surrogate reference" method for packed cell volume (ICSH, 2003)
- Methods for reticulocyte counting (flow cytometry and supravital dyes); approved guideline (NCCLS/ICSH H44-A2, 2004)
- Reference leukocyte (WBC) differential count (proportional) and evaluation of instrumental methods (NCCLS H20-A2, 2007)
- Calibration and Quality Control of automated hematology analyzers; proposed standard (NCCLS/ICSH, H38-P, 1999)
- Guidelines for organization and management of external quality assessment (EQA) using Proficiency testing (ICSH 1998)

Problemi non risolti e ipotesi per il futuro

Alcuni dei documenti citati probabilmente necessitano di una revisione e altri aspetti dell'ematologia di laboratorio dovrebbero essere affrontati. Si va dalla necessità di elaborare criteri uniformi e maggiormente condivisi per l'organizzazione e l'interpretazione dei risultati dell'EQA, alla proposta di programmi relativi ad indagini morfologiche su striscio di sangue periferico e su aspirato midollare.
Opportuna sarà la revisione del protocollo per la valutazione degli analizzatori ematologici sia dal punto di vista statistico (modalità di comparazione differenti per parametri dotati di metodo di riferimento rispetto agli altri), che per l'esigenza di considerare l'extended differential count.
Sono necessarie raccomandazioni per l'analisi e la refertazione dell'aspirato midollare.
E'auspicabile l'individuazione (con relativa validazione) di anticoagulanti alternativi al K2EDTA tali da aumentare la stabilità del campione nel tempo senza compromettere volume, forma e funzionalità cellulare.
Non da ultimo dovranno essere definiti criteri per fissare gli obiettivi di precisione e accuratezza (o di errore totale) per i parametri ematologic i(7).
Infine, saranno necessarie raccomandazioni per la standardizzazione del referto ematologico sul sangue periferico. In questo contesto va ribadito che la trasmissione dei soli dati numerici non opportunamente commentati (quando necessario) potrebbe mascherare informazioni utili. Al riguardo si può concordare con una affermazione di Maxwell Wintrobe scritta nel 1981:"there is danger as well as value in numbers. The physician must not permit himself to be mesmerized by the magic of numbers" (8).

Bibliografia

1. Rowan RM. The significance and issues of Quality Assurance Sysmex. J Int 1998;8:63-6.
2. Lundberg GD. How clinicians should use the diagnostic laboratory in a changing medical World. Clin Chim Acta 1999;280:3-11.
3. Plebani M. Errors in clinical laboratories or errors in laboratory medicine? Clin Chem Lab Med 2006;44:750-9.
4. Rowan RM. The standardization of calibration and control

of instruments and kits. In: Lewis SM, Verwilghen RL, eds. Quality assurance in haematology. London: Bailliere Tindall, 1988.
5. International Council for Standardization in Haematology (ICSH): Rules and operating Procedures. ICSH Secretariat, 1991.
6. International Organization for Standardization (ISO): Vocabulary. ISO 8402. Geneva 1986.
7. Buttarello M. Quality specification in haematology: the automated blood cell count. Clin Chim Acta 2004;346:45-54.
8. Wintrobe MM. To measure or not to measure? That too is the question. In: Ross DW, Brecher G, Bessis M, eds. Automation in hematology: what to measure and why? Berlin: Springer-Verlag, 1981.

SS12

CURRENT STATUS AND POTENTIAL OF PHARMACOGENOMICS IN CLINICAL PATHOLOGY AND MEDICAL PRACTIC

K.T.J. Yeo[1], H.K. Lee[2§,] L.D. Lewis[2*]

[1]Department of Pathology, Pritzker School of Medicine, The University of Chicago, Chicago, IL, USA
Departments of Pathology§ and Medicine, [2]Dartmouth-Hitchcock Medical Center & Dartmouth Medical School, Lebanon, NH, USA*

Approximately two million hospitalized patients/year are estimated to have severe adverse drug reactions (ADRs) in the United States. ADRs were ranked between the fourth and sixth leading cause of death in the United States (JAMA 1998;279:1200). Additionally, drug therapy is only effective in 40-75% of patients. Some ADRs and therapeutic failures are caused by genetic variation in the drug metabolism, distribution and protein target. These adverse therapeutic outcomes may now be preventable due to recent advances in the understanding and application of pharmacogenomics (PGx) for personalized drug treatment. In the United States, current PGx biomarkers include: TPMT (for 6-mercaptopurine), UGT1A1 (for irinotecan), CYP2D6 (for codeine and tricyclic antidepressants), CYP2C9/VKORC1 (for warfarin), and more recently, HLA-B1502 (for carbamazepine), and HLA-B5701 (for abacavir). For example, homozygous variants for TMPT is at 100% risk for hematopoietic toxicity and thus require only 5-10% of the standard 6-mercaptopurine dose. A newborn infant that was breastfed by a mother taking codeine died of opioid toxicity due to the mother being a fast metabolizer of codeine (3 copies of CYP 2D6). Genotyped-guided (2C9 and VKORC1) dosing of warfarin that includes other clinical factors has been shown to account for 50-60% dosing variability and algorithms exist for improving warfarin dose prediction (e.g. www.warfarindosing.org) rather than the current empiric dosing of "trial-and-error" adjustments based on INR. Asians with the HLA-B1502 allele who are taking carbamazepine are at an extremely high risk (odds ratio of 1357-2504) for the potentially fatal Stevens-Johnson syndrome. Despite good scientific evidence and the US-FDA drug re-labeling/alerts regarding specific PGx tests available in conjunction with the use of these drugs, adoption and utilization of these biomarkers in clinical practice is currently low. There are numerous barriers preventing wider adoption of clinical pharmacogenetic testing in routine clinical pathology and medical practice: lack of education and understanding by prescribing physicians regarding PGx, lack of consensus guidelines for use of PGx markers, lack of prospective studies regarding the cost-effectiveness of performing pharmacogenotyping, reimbursement issues, and tension between patient knowledge via web-based information/advertisement surrounding genetic testing versus physicians readiness to adopt patient-driven testing. Potential solutions need to include major educational efforts for all health-care professionals, more evidence-based studies and guidelines, institutional expertise and leadership and stronger FDA drug labeling before the promise of PGx can be fully realized.

SS13

PHARMACOGENOMICS FOR PERSONALIZED MEDICINE OF ADDICTION, AND FOR FORENSIC TOXICOLOGY AND FOR THE EMERGING PERSONALIZED JUSTICE

S.M.Y. Wong[1,2] and the Forensic Pathology/Toxicology Methadone Pharmacogenomics Study Group (FPTMPGxSG), USA

[1]Dept. Pathology, Medical College of Wisconsin, and [2]Milwaukee County Medical Examiner's Office, Milwaukee, USA

Personalized Medicine using adjunct "omics" biomarkers such as pharmacogenomics, proteomics and others., and molecular imaging is enabling Personalized Justice. Optimized therapy, in the context of personalized medicine, would apply the above biomarkers to identify the right patient by using the right diagnostic tests, matching with the right drug or treatment with the right dose at the right time. For approved drugs such as warfarin, preliminary outcome studies demonstrated increased efficacy by genotyping CYP2C9, CYP 4F2, and VKOCR1. These approaches are also being used for drug discovery/development, such as 6 SNPs identified with increased efficacy for the newly developed iloperidone for the treatment of schizophrenia. Depending on the patients' genotype, altered drug metabolism may result in side effects and behaviour such as driving performance encountered in driving under the influence of drugs (DUID) cases. This will give impetus to the future use of adjunct molecular " omics " biomarkers in case deliberation, ushering in the practice of the emerging Personalized Justice. In assessing the use of PGx for molecular autopsy, 9 coroners/medical examiner offices formed the FPTMPGxSG: British Columbia, Cuyahoga (Cleveland, OH), Milwaukee (Milwaukee, WI), New Hampshire, North Carolina, Suffolk (NY), Washington, Washington, D.C., and Wayne (Detroit, MI), with Universities of Utah and Louisville providing additional consults. Genotyping of polymorphic CYP genes (CYP 2D6, 2C9, 2C19, and 3A4/5) mediating methadone metabolism would be accompanied by using Microsoft Access for data base entry of pertinent case/clients history. Using PyrosequencingTM, 652 to 927 samples of 1100 samples collected during 2002-3 were genotyped. The study demonstrated the feasibility of coordinated planning of the nine sites., sample collection without incident., and the feasibility of routine multiplex genotyping reliably performed on previously collected postmortem whole blood samples. Another study correlated methadone enantiomers concentrations with CYP genotypes. Investigations of DUID cases involve observations of the arresting officer, standardized evaluations, and toxicology analyses to determine blood-drug concentrations, with litigation for such cases often relying on expert testimony to define and to prove impairment of the individual on a case-by-case assessment. As adjunct "omics " biomarkers, PGx testing can be utilized in DUID cases to explain the effect of genetic variations (gene-dose) on observed blood-drug concentrations and may distinguish therapeutic misadventure from overdose or abuse. Thus, the use of PGx as an adjunct to DUID investigation might be regarded as the emerging practice of Personalized Justice – a logical extension of personalized medicine.

Reference

1. Caldwell MD, Awad T, Johnson JA, Gage BF, Falkowski M, et al. CYP4F2 genetic variant alters required warfarin dose. Blood. Blood; 2008;111:4106-4112.

2. Cutler AJ, Kalai AH, Schooler NR, Hamilton J, Polymeropouos MH, Wolfgang CD. Iloperidone: Placebo- and ziprasidone-controlled study in patients with schizophrenia. (Abstract) 46th Annual meeting of the American College of Neurolopsychopharmacology; Dec. 2007, Boca Raton, FL.
3. Wong SHY, Gock SB, Shi RZ, Jin M, Wagner MA, et al.(2006) Pharmacogenomics as an aspect of Molecular Autopsy for Forensic Pathology/Toxicology. In Wong SHY, Linder M, Valdes R Jr. Pharmacogenomics and Proteomics: Enabling the Practice of Personalized Medicine. AACC Press. Washington, D.C. pp. 311-320.
4. Wong SH, Forensic Pathology/Toxicology Pharmacogenomics Methadone Study Group, Jentzen JM, Schur C, Gock S, et al. A multi-center study of applying pharmacogenomics as molecular autopsy for certifying methadone fatality in nine counties/states. Clin Chem 2008;54:A19.(Supplement - Abstracts).

SS14

AUTOMATION AND NANOTECHNOLOGY: THE FUTURE OF MOLECULAR DIAGNOSTICS

M. Ferrari

Università Vita-Salute San Raffaele, Genomic Unit for the Diagnosis of Human Pathologies, San Raffaele Scientific Institute, Milan, Italy and Diagnostica e Ricerca S. Raffaele SpA, Milan, Italy

The completion of human genome project and the development of new technologies for DNA testing started the revolution of the diagnostic laboratory. For the diagnosis of genetic diseases, DNA-based diagnostics provides a sensitive alternative to protein-based diagnostics. Every day, researchers discover the functions of new genes and increase the knowledge that can be translated into clinical practice. This growth in knowledge fuels, in turn, the expansion of DNA testing both for diagnosis and prediction of disease susceptibility. Moreover in the post genomic era, the screening of many different genetic polymorphisms in large populations represents a major goal that will facilitate the understanding of individual genetic variability in the development of multi factorial diseases and drug response and toxicities. For the future of genomics is demanding the rapid evolution ofautomation, miniaturization and high-throughput genotyping technologies toward increased speed and reduced cost.

Through miniaturization of the test platform, microchip-based nucleic acid technologies allow rapid analysis of genetic information in large sample populations thus reducing time and manual work.

Nanotechnology is the development of engineered devices at the atomic, molecular and macromolecular level in nanometer range.

Nanotechnology-the creation and utilization of materials, devices, and systems through the control of matter on the nanometer-has been applied to molecular diagnostics.

Nanotechnologies enable the diagnosis at single cell and molecule level and some of these can be incorporated in the current molecular diagnostics such as biochips. Nanoparticles, such as gold nanoparticles and quantum dots, are the most widely used but various other nanotechnologies for manipulation at nanoscale as well as nanobiosensors are reviewed. These technologies will extend the limits of current molecular diagnostics and enable point-of-care diagnosis as well as the development of personalized medicine. Although the potential diagnostic applications are unlimited, most important current applications are foreseen in the areas of biomarker research, cancer diagnosis and detection of infectious microorganisms.

CO8

HIGH-THROUGHPUT MUTATIONAL ANALYSIS, COMPLEX ALLELES AND GENOTYPE – PHENOTYPE RELATIONSHIP IN CYSTIC FIBROSIS

M. Lucarelli[1], G. Ferraguti[1], L. Narzi[2], S.M. Bruno[1], S. Pierandrei[1], A. Stamato[2], F. Ceci[1], D. Frattolillo[1], S. Quattrucci[2], R. Strom[1]

[1]Sez. Biochimica Clinica, Dip. Biotecnologie Cellulari ed Ematologia, Sapienza Università di Roma, Italy
[2]Centro di Riferimento Regione Lazio per la Fibrosi Cistica, Dip. Pediatria, Sapienza Università di Roma, Italy

Cystic Fibrosis (CF) is an autosomal recessive disease, caused by mutations in the CFTR gene. The relationship between genotype and phenotype in CF is poorly understood. The occurrence of complex alleles, with more than one mutation in cis on the same allele, can produce a combined effect on CFTR functionality, thus introducing further genetic variability. Due to the large number of CFTR mutations and polymorphisms (over 1500) and of their combinations, both in trans or in cis, extensive CFTR mutational analysis appears as a crucial topic.

We are presenting here an original automated approach including: 1) a 2-days multiplexed minisequencing test (CF-SNAP+20, based on primer-extension principle), for the search of a regional-specific panel of CFTR mutations, and 2) a 5-days extensive CFTR re-sequencing test (based on the cycle sequencing principle).

This methodology was applied to a cohort of 335 CF patients. By coupling the automated CF-SNAP+20 minisequencing to the commercial CF-OLA test (Abbott) for the 32 worldwide most frequent mutations, the detection rate rose from 78% to 88%, at a supplementary cost of 15 euros / subject. By adding the CFTR extensive re-sequencing step, the detection rate increased to 96%, at a cost that never exceeded 250 euros / subject. This approach was also used to tackle the problem of cases with apparently identical mutated genotypes but highly discrepant biochemical and clinical presentation. Four different complex alleles were so far detected by this detailed analysis. By examining, e.g., 11 subjects with the controversial L997F mutation on one allele and a classic mutation on the other, 3 out of the 4 subjects that exhibited the highest sweat test values and most severe clinical presentation were found to have, on the allele with the L997F substitution, an additional R117L mutation.

Our automated methodology, characterized by rapidity of execution and a relatively low cost, allows not only a high mutational detection rate but also a more complete genetic characterization of affected subjects. The occurrence of complex alleles, revealed by this approach, may lead to a better comprehension of genotype – phenotype relationship in CF.

P.S. and S.A. were partially supported by a grant from “Associazione Laziale Fibrosi Cistica”.

CO9

DETERMINATION OF HER2 STATUS IN BREAST CANCER PATIENTS BY TWO METHODS: REAL-TIME QUANTITATIVE PCR (QPCR) AND IMMUNOENZIMATIC ASSAY (EIA)

M. Savino[1], P. Parrella[2], M. Copetti[3], R. Barbano[2], R. Murgo[4], V.M. Fazio[2], V.M. Valori[5], M. Carella[6], M. Garrubba[1], S.A. Santini[1]

[1]Clinical Analysis Laboratory, CSS Hospital, IRCCS, San Giovanni Rotondo (FG), Italy
[2]Oncology Laboratory, CSS Hospital, IRCCS, San Giovanni Rotondo (FG), Italy

[3]*DSEMS, University of Foggia), Italy*
[4]*Department of Surgery, CSS Hospital, IRCCS, San Giovanni Rotondo (FG), Italy*
[5]*Department of Onco-ematology, CSS Hospital, IRCCS, San Giovanni Rotondo (FG), Italy*
[6]*Medical Genetics Service, CSS Hospital, IRCCS, San Giovanni Rotondo (FG), Italy*

Background. Over expression and/or amplification of the HER2 oncogene, occur in up to 20-30% of breast cancer patients and genomic alterations are associated with poor prognosis and more aggressive tumor phenotype. Actually, HER2 determination on tumour tissues is reliably performed by immunochemistry assay. However the development of non invasive procedure to determine HER2 status may represent a powerful method for monitoring disease progression and response to the treatment in breast cancer patients.
Methods. Serum samples and RNA from peripheral blood were evaluated in 85 breast cancer patients and 22 healthy controls. Of those patients 49 were HER2 positive and 36 were negative. HER2 mRNA levels were measured by real time quantitative PCR (QPCR) and serum HER2 protein by immunoenzimatic assay (EIA). ROC curve analyses were used to determine the optimal cut off values.
Results. A statistically significant difference was detected for both QPCR and EIA in HER2 positive patients as compared with both healthy controls and HER2 negative tumours. QPCR showed a 91% (CI95%: 84%-98%) specificity and a 78% (CI95%: 68%-88%) sensitivity for an optimal cut off value of 4.74. The optimal cut off value for EIA was 22 ng/ml yielding a 95% (CI95%: 90%-100%) specificity and a 59% (CI95%: 48%-70%) sensitivity. The QPCR assay was slightly less specific than EIA in discriminating HER2 positive breast cancers from HER2 negative tumours, but it was more sensitive.
Conclusions. Our results indicate that QPCR is suitable alternative method for the determination of HER2 status in peripheral blood of breast cancer patients. Thus, QPCR could be used as diagnostic tool when primary tumour samples are unavailable or to monitor the outcome of the disease and the response to therapy during follow up of breast cancer patients.

Reference

1. Savino M, Garrubba M, et al. Development Of Real-Time Quantitative Reverse Transcription-PCR for Her2 Detection in Peripheral Blood from Patients with Breast Cancer. Clin Chim Acta 2007 Sep;384(1-2):52-6.

SS15
TANDEM MASS SPECTROMETRY: THE NEW FRONTIER OF NEWBORN SCREENING

C. Corbetta, M. Grossi, L. Guidi, E. Pratiffi, M. Tavazzani, V. Ravazzani, L. Alberti

Lab. di Riferimento Regionale per lo Screening Neonatale, Osp. dei Bambini "V. Buzzi", A.O. Istituti Clinici di Perfezionamento, Milano, Italy

Today the Newborn Screening is a complex and highly specialized branch of public health, consisting primarily of a sequence of actions: screening or selection; diagnostic follow-up; clinical follow-up and therapeutic treatment of the patient in a specialized clinical centre; professional training and people informing.
In the late'60s, the World Health Organization (WHO), thinking mainly about the adult chronic diseases, defined principles and criteria for identify the diseases to select as target of the newborn screening programs.
This model has to date a highly critical aspect, because it do not consider the family weight in newborn screening, in particular the value of the genetic information and the opportunity for prenatal diagnosis, the helpful feedback that a early postnatal communication of a disease, even if incurable, could have in the family management of a rare congenital disease, avoiding the problem of so-called "diagnostic odyssey". Recent advances in technology have enabled clinical laboratories, providing newborn screening services, to improve and to expand testing to include additional (treatable) and rare disorders.
Starting from the end of the 80's, the introduction in clinical laboratories of the so-called "Tandem Mass Spectrometry" (MS/MS) technology, has resulted in the "dramatic" increase in the number of disorders technically detectable in accordance with the "new" rules established for the neonatal screening. In fact, this technology, in contrast with conventional methods applied previously, has the ability to measure, in very short analyses, more than one compound simultaneously, defining a biochemical profile (acylcarnitines and amino acids) that allows - in a single analytical session and in a single biological sample (Guthrie card) - the identification of many different errors of metabolism (amino acid metabolism disorders, fatty acids oxidation disorders, organic acidoses); the other diseases (hemoglobinopathies, congenital hypothyroidism, biotinidase deficiency, congenital adrenal hypoplasia, galactosemia, classical cystic fibrosis) are individually identifiable with the others, traditional, analytical approachesnologies.
In recent years, in the U.S.A., the strategies for newborn screening programs have radically changed: in 2006, only 5 states are running neonatal screening for less than 10 diseases, and altogether 63% of U.S.A. babies is screened for more than 30 diseases, included inborn errors of metabolism.
In Europe, recent data reveal that the spread of the strategies of "expanded newborn screening", based on tandem mass technology, now involves approximately 33% of births.

SS16
PROTEOMIC: A NEW TOOL FOR CLINICAL STUDIES

G. Federici

Department of Internal Medicine University of Rome Tor Vergata and Department of Laboratory Medicine, University Hospital Tor Vergata Rome, Italy

Our Proteomics Group is focused in the investigation of differential protein expression profiles and metabolites pattern in neurodegenerative diseases. We have actively established a set of novel mass spectrometry investigations in order develop potential assays for molecular biomarkers identification and characterisation. 2D Electrophoresis approach has been complemented by MALDI-TOF mass spectrometry protein profiles either in the screening of complete protein extracts and in direct analysis of tissue samples. Novel bioinformatics tools have been developed to specifically analysed MALDI-TOF-MS molecular profiles and possibly outline spectra differences (Mantini et al., BMC Bioinformatics, 2007).
We have investigated the cerebral spinal fluid (CSF) of Alzheimer Disease affected patients versus subjects without cognitive impairment (Biroccio et al., Proteomics, 2006). Our results indicated a differential distribution of TTR-cysteine and TTR-cysteinglycine adducts. A sensitivity > 90% and a specificity >70% was outlined in ROC curve analysis when the overall cohort is discriminated by the TTR-Cysteine signals. Transthyretin posses binding capacity of the beta-amyloid peptide and a number of amyloidosis diseases have been associated with its allele variants. Transthyretin has a free reactive sulphidryl moiety located on the Cys10 residue which might undergo to differential oxidation patterns.
Moreover we are pursuing comparative proteomics

analysis to disclose the key molecular mechanism of acquired chemo-resistance of Neuroblastoma. The SH-SY5Y (ATCC CRL-2266) is a thrice cloned subline of the neuroblastoma cell line SK-N-SH established from a metastatic bone tumour have been selected for resistance to etoposide (Urbani et al., Proteomics, 2005) and Cis-platin.In this investigation we applied both a classical proteomic approach based on 2DE gels differential analysis coupled to mass spectrometry protein identification and a more innovative label-free LC-MSE quantification method to identify proteins associated with cisplatin resistance by comparing proteome from sensitive and resistant human neuroblastoma cell lines. A number of potential protein biomarker candidate have been identified, these gene product have a common motif in the Nrf2 activation pathway as shown by meta-analysis of the gene ontology by Ingenuity Pathway Analysis.

SS17

MASS SPECTROMETRY: FROM CLINICAL TO FORENSIC TOXICOLOGY

R. Pacifici

Dipartimento del Farmaco, Istituto Superiore di Sanità, Rome, Italy

Mass spectrometric techniques, particularly gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS), are indispensable tools in clinical and forensic toxicology and in doping control owing to their high sensitivity and specificity.. Infact in clinical and forensic toxicology and in doping control, high specificity is demanded besides sensitivity, because in many cases the analytes are not known in advance and many other xenobiotics or endogenous biomolecules may interfere with their detection. The particular task in these fields is the analysis in complex biological matrices such as ante- or postmortem blood (plasma, serum), urine, gastric content and tissues, or alternative matrices such as hair, sweat and oral fluid, meconium or nails. Therefore GC/MS and LC/MS are indispensable tools in clinical and forensic toxicology and in doping control. They are used for screening, library-assisted identification and quantification of drugs, poisons and their metabolites, prerequisites for competent expertise in these fields. In addition, they allow the study of metabolism of new drugs or poisons as a basis for developing screening procedures in biological matrices, most notably in urine, or toxicological risk assessment. Today, GC/MS is still the most frequently used technique in analytical toxicology, but single-stage or tandem LC/MS with electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) have left the development stage and are becoming increasingly important in routine toxicological analysis, especially for quantification of the identified analytes. LC/MS with atmospheric pressure photoionization (APPI) facilitates the measurement of nonpolar compounds that are inefficiently ionized by ESI or APCI, but is only rarely used in analytical toxicology..Therefore GC/MS in the EI modewill play amajor role particularly in comprehensive screening procedures, because very huge collections of reference spectra are available and the apparatus costs are moderate. LC/MS with the different mass analyzer types will become more and more a standard technique for automated target screening procedures and particularly for high-throughput quantification. Modern hybrid mass analyzers, such as QTRAPs with much higher sensitivity, will allow determination drugs in trace volumes of biosamples or modern very-low-dosed drugs. QTOF mass analyzers will find their place in forensic toxicology for detection of very rare compounds via their empirical formula and in drug metabolism confirming the structural ch'ange during metabolism. However, before the final breakthrough of the different LC/MS techniques in analytical toxicology, the costs of the apparatus must markedly be reduced and the discussed disadvantages must be overcome

CO10

PLASMA 24S-HYDROXYCHOLESTEROL AND CAUDATE MRI IN PREMANIFEST AND EARLY HUNTINGTON'S DISEASE

V. Leoni[1], C. Mariotti[1], S. Gentrini[1], S. Tabrizi[2], M. Valenza[3], M.L. Mandelli[4], M. Grisoli[4], E. Cattaneo[3], S. Di Donato[1]

[1]Unit Biochemistry and Genetics, Fondazione-IRCCS Istituto Neurologico Carlo Besta, Milano, Italy
[2]Dept of Neurodegenerative Disease and Dementia Research Centre, Institute of Neurology, University College London, United Kingdom
[3]Dept of Pharmacological Sciences and Centre for Stem Cell Research, University of Milano, Italy
[4]Unit of Neuroradiology, Fondazione-IRCCS Istituto Neurologico Carlo Besta, Milan, Italy

Aim. 24S-hydroxycholesterol (24OHC) is a neuron-specific cholesterol catabolite which crosses the blood-brainbrarrier.
Its plasma levels are considered to reflect the number of metabolically active neurons in the brain.
Methods. To pursue the identification of putative biomarkers of neurodegeneration, we thus investigated plasma 24OHC and cholesterol by isotope dilution mass spectrometry, in 62 controls, 96 HD symptomatic patients at different disease stages (stage 1 to 3), and 33 HD premanifest subjects (pre-HD) have been investigated.
Results. Cholesterol and 24OHC plasma levels were not influenced by fasting or post-meal status. Cholesterol levels were similar in controls, pre-HD and HD patients.
Te plasma levels of 24OHC were significantly lower in HD patients at all disease stages compared with controls ($P<0.001$), in case of preHD patients levels were similar to those of controls, and thus significantly higher than those of HD patients at any disease stage ($P<0.001$). The pre-HD cohort was heterogeneous, and subjects closer to motor onset had 24OHC levels lower than those far from motor onset, and similar to those of HD stage 1 patients.
Caudate MRI morphometric evaluation showed that the caudate volumes in HD stage 1 patients were significantly lower ($P<0.001$) than those in preHD subjects. Also, caudate volumes of the pre-HD subjects were significantly lower than those of controls ($P=0.03$). Notably, 24OHC levels resulted significantly lower in HD stage 1 patients compared to preHD and controls subjects ($P<0.001$). A significant positive correlation was found between 24OHC levels and caudate atrophy measured by morphometric MRI ($r=0.42$, $P=0.002$).
Conclusion. Our data indicated that: a) the brain-specific 24OHC was reduced in HD patients at any disease stage compared to controls and pre-HD subjects; b) 24OHC plasma levels paralleled caudate atrophy in pre-HD and HD1 patients. We conclude that 24OHC might be a usefull biochemical marker to follow the evolution from pre-HD to HD with open motor disease, and should be added to MRI morphometry to follow disease progression in early Huntington disease.

Reference

1. Björkhem I. Crossing the barrier: oxysterols as cholesterol transporters and metabolic modulators in the brain. J Intern Med 2006;260:493-508.

CO11

PANCREATIC CANCER BIOMARKERS DISCOVERY BY SELDI-TOF-MS

A. Padoan[1], F. Navaglia[1], D. Basso[1], P. Fogar[2], E. Greco[1], L. Tonidandel[3], E. Fadi[1], C. Zambon[2], D. Bozzato[1], R. Seraglia[3], S. Pedrazzoli[2], M. Plebani[1]

[1]Dip. di Medicina di Laboratorio, Az. Osp., Padova, Italy
[2]Dip. di Scienze Mediche e Chir., Univ. Padova, Italy
[3]CNR-ISTM, Padova, Italy

Introduction. SELDI-TOF-MS is one of the currently used techniques to identify biomarkers for cancers. Our aim was to explore the application of serum SELDI proteomic patterns to distinguish pancreatic cancer (PC) from chronic pancreatitis (CP), type II diabetes mellitus (DM) or healthy controls (HC).
Methods. Sera from 12 HC, 24 DM, 126 PC (84 diabetics) and 61 CP (32 diabetics) were analyzed by SELDI-TOFMS. Spectra, generated on IMAC-30, were clustered and classified using Chipergen Biomarker Wizard® and Biomarker Pattern software®.
Results. Peaks present in at least 5% of all spectra were selected. Two decision tree classification algorithms, including or not CA19-9 as predictor, were constructed. In the absence of CA19-9 the splitting protein peaks were at 1526, 1211 and 3519 m/z. When CA 19-9 entered the analysis, the former two peaks were maintained as splitters while the 3519 was replaced by CA19-9. The two classification trees performed equally in classifying HC (Se=100%) and DM (Se=100%); CA19-9 tree classified better both CP (Se=89% vs 79%) and PC (Se=63% vs 57%). The specificity of this classification tree was 93%, better of CA 19-9 alone (Se=86%, Sp=65%). We then constructed a classification tree considering only diabetic patients. The optimal tree resulted from the following main splitters: 1211, CA19-9, 7903, 3359, 1802. 100% DM, 97% CP and 77% PC were correctly classified.
Conclusion. SELDI-TOF-MS allowed the identification of new peptides which, in addition to CA 19-9, allowed to correctly classify the vast majority of PC patients and to distinguish them from CP or DM.

Reference

1. Koopmann J, Zhang Z, White N, et al. Serum diagnosis of pancreatic adenocarcinoma using surface-enhanced laser desorption and ionisation mass spectrometry. Clinical Cancer Research 2004;10:860-8.

SS18

A NEW LABORATORY IN A NEW HOSPITAL: CHRONICLE OF AN EXPERIENCE

I. Bertoli

Lab. Analisi, Ospedale S. Angelo, Mestre, Italy

The moving of a complex service such as an analysis laboratory while maintaining services to users, requires a very ambitious and well organized project planning studied in minimum details.
The laboratory of Mestre supplies services for: emergency analyses as well as routine services for the Operational Units of the same hospital; six different district points of service and for several nursing homes. Moreover, Mestre collaborates with the Civil Hospital in Venice for immunochemistry and sierological investigations in the distribution of the diagnostic specialties among the two laboratories.
The transfer of the laboratory services with respect to the different wards present in the new hospital only from May 26th on, did result in a complete modification of the services in that the emergency services were maintained functional on a 24 hour basis in the old hospital in Mestre (VOM), while the routine analyses were completely transferred to the new hospital in Mestre (NOM).
The remarkable phases of the transfer have been documented and recorded according to the criterions of the UNI EN ISO 9002 certification and are the following:

i. individualization of the organizational model
ii. choice of the instrumentation
iii. organization of the spaces
iv. equipment installation
v. training of technical personnel
vi. comparison between old and new instrumentation
vii. transfer of laboratory reagents and material
viii. computer training of the personnel of the Operational wards in the hospital, day hospital and districts.

The routine activity was suspended for one day only on April 19th.
The most critical phases actually took place in the computer management system due to the complexity of the alignment of the hospital network as well as the necessity to manage databases and operating systems. These phases had to be integrated in input and output for accepting and response referrals among the collaborating units which consisted of the Laboratory in Venice, Old laboratory in Mestre (VOM), districts, wards and the new laboratory in Mestre (NOM).
Other critical points that were evidenced were the adaptments of personnel to a completely different work organization, the reorganization of the work load between the two different laboratories of Venice and Mestre and the organization of transport and interaction among the different emergency operational units.

SS19

THE REORGANIZATION OF MEDICAL LABORATORY. THE PLAN FROM THE REGION SARDINIA

N. Camusso

Service Laboratory Medicine P.O. "San Martino" A.S.L. N. 5 Oristano, Italy

The law December 27,2006 (Finance act 2007) Article 1, paragraph 796, letter "O" says that every districts must prepare a plan to reorganize the network of structures public and private accredited that provide specialist laboratory diagnosis in order to adjustment of levels of organization and personnel, conform to increase efficiency in consideration of automated methods. In relation to this request, region of Sardinia is divided into eight provinces with a low population square kilometres and a very inefficient road system. The medicine laboratory services are locally distributed inside every administrative division, inside each administrative division it can be demonstrate more than one service, often of little dimensions, most of the time that collect both clinical chemistry and microbiology samples. Very rarely, in the structures of great dimensions we assist to a differentiation between the several specialties. The plan previews the reorganisation with the transformation in Hub of the central laboratories and in spoke of those smaller and peripheral. It is scheduled the institution of regional reference laboratories ad high specialization. Hub laboratories should be only located at each administrative division where would be transferred also the examinations to high specialization, now little executed in the peripheral laboratories of the single ones administrative divisions. It will be developed or strengthened the net of the points of withdrawal of the blood and of delivery of the reports in the territory.Leaving the laboratories of the hospitals more little ones pertaining to the same one administrative division the execution of the

urgent detection of the patients in hospital and the management of the Point Of Care Testing. Are identified regional reference laboratories to perform analysis of high specialisation diagnosis: of rare diseases, execution methods RIA, centres of reference for determinations of toxicology and molecular biology, cytofluorometry examinations, immunogenetic of transplants. The criteria are to be determined, but have already been identified and are undergoing reorganization the centres for rare diseases and immunogenetic of transplants. The project involves improving the link-up services to regional telecom network and a more efficient and effective corporate computer network.Strengthening the network of sampling points of the territory will lead to the reorganization of the transport system of biological samples.This reorganisation should consider the road system which constitutes the main obstacle. In the vision of a rationalisation of resources, the plan previews the only management of the contests of contract and a regional system of management of the warehouses. This project involves the reorganization of facilities, transports, networks, but especially promote the participation of professionals who work within departments of medicine laboratory also through a rational training project. They will have to be characterized the formative contents, instruments and training methods facilitating dialogue between operators of different skill levels and territory.This objective can not be left solely to individual companies, but must be obtained through a collaboration with Scientific Societies

CO12

EMERGENCY LABORATORY AND TECHNICAL GOVERNANCE: AS A WELL ORGANISATION CAN CONTROL THE SAMPLES ACCESS

M. Ampollini, R. Perini, D. Bonfanti, R. Fontana, M. Bardi, G. Sinfonia, R. Capuano, M. Ferrari, C. Gnocchi, L. Ippolito, C. Monica

Lab. Diagnostica Ematochimica, Dip. Patol. e Medicina Lab., Azienda Ospedaliero-Universitaria, Parma, Italy

Aim. During last years the technical staff of the Clinical Pathology Laboratory of Parma Hospital, has observed a progressive but inappropriate use of emergency sector by clinical departments. The aim of our work was to gain a right use of this sector of laboratory and, as a consequence, a significant cut of emergency accesses with an improvement of appropriate requestes.
Methodology. The way used to carry out the plan was complex because required data collection, long analysis, same revision of the trial, clinical audit. This trial was managed by a multidisciplinary team including laboratory technicians, one biologist, one physician, some hospital nurses, the head of Laboratory.
The steps of the work were:
1. Analysis of the internal procedures
2. Statistics about results gained in a short period
3. Statistics about results consolidated in time
4. To arrange questions list
5. To organize audit
6. To collect data and answers valuation
7. To draw up a new organization involving laboratory, clinical departments, internal transports and Hospital Direction.
8. Final valuation of results.
Results. The new organization started on 8/10/07 but already from the end of November we pointed out a substantial change in numbers and kind of accesses. At the moment the emergency samples are received and are analysed at routine laboratory, not more at emergency section, with a remarkable improvement of human and technological resources. The TAT is better than before and it contributes to obtain a more appropriate request. We pointed out a request reduction of 40%.
Conclusions. Most Departments had shared and appreciated the new organization. Some of those using at the beginning emergency section in exclusive way, today are using routine laboratory without problems. We can affirm that a good, shared organization and a correct information are able to control requests number and their propriety.