Abstracts*)

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POLICIES, REQUIREMENTS, GUIDELINES IN POCT MANAGING

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Definition of POCT is important in defining field of application and conditions of use. Guidelines for POCT have been produced by a number of organization and POCT-related ISO Guidelines are briefly reported below.
ISO 22870 Point of Care Testing – requirements for quality and competence
ISO 17593 Clinical laboratory testing and in vitro medical devices - requirements for in vitro monitoring system for self-testing in oral anticoagulant therapy .
ISO 15197 –In vitro diagnostic test systems - requirements for blood glucose monitoring systems for self-testing in managing diabetes mellitus
Patient self-testing is excluded from ISO 22870, but is covered specifically in ISO 17593 and ISO 15197
International standard ISO 22870, *Point-of-care testing* (POCT) - Requirements for quality and competence, defines POCT as: "testing that is performed near or at the site of a patient with the result leading to possible change in the care of the patient".
In Europe, in Germany the institution of a POCT coordination group was demanded for supervising the quality assurance of all decentralized POCT devices under RiliBÄK (Richtlinien der Bundesärztekammer), that is German Guidelines for Quality rules, adopted also in Austria and Switzerland as a base for National requirements (QUALab in Switzerland) (1). In Norway National Government and the Norwegian Medical Association established an organization to take care of improving laboratory quality of POC instruments (NOKLUS – 2012) with a large number of participants (2).
Point-of-care testing management protocol using ISO 22870 guidelines in France will be mandatory by 2016 (3). So, in Europe most countries already legislated for POCT management
In Italy a national law was not enacted still now, and only fifty percent of Italian Regions established regional rules for POCT. Actually, despite technology improvements, the successful implementation of POCT is still dependent on the effective organization and management of staff; as a consequence policies, requirements and guidelines are tools strongly required for POCT governance.

1. Schweizerische Kommission für Qualitätssicherung im medizinischen Labor
   Commission suisse pour l'assurance de qualité dans le laboratoire médical Commissione svizzera per l'assicurazione di qualità nel laboratorio medico
   CONCEPT QUALAB Concept d'assurance qualité dans le laboratoire médical
   Directives d'application et partie intégrante de la convention de base pour l'assurance de qualité entre assureurs et prestataires dans le cadre de la liste fédérale des analyses
2. External quality assessment of-point-of-care methods, model for combined assessment of method bias by the use of native patient samples and noncommutable control materials – Clin Chem 2013 Feb 7;59(2): 363-71. Anne Stavelin, Per Hyltoft Petersen, Una Ø Sølvik, Sverre Sandberg
3. Pernet P, et al. Point-of-care testing management protocol using ISO 22870 guidelines and French requirements. Clin Biochem 2009; 42: 324.

PERFORMANCE ANALYSIS IN POINT-OF-CARE. HbA1c TESTING IN A CLINICAL SETTING

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Background and aims: HbA1c test reflects glycemic control over past three months, predicts diabetic complications and can be used for diabetes diagnosis and screening. Although POCT HbA1c assays may be NGSP-certified the ADA doesn't recommend them for diagnostic purposes. PTS Diagnostics (Indianapolis, USA) has recently introduced POCT HbA1c monitoring system (A1cNow), NGSP and IFCC-certified, CLIA-waived, that provides results in 5 minutes and requires a 5 µl blood sample. Decentralised testing may contribute to optimal patient care but quality assurance programs are required to ensure the quality of results over time. We investigated A1cNow performance in out clinic diabetic patients.
Materials and methods: HbA1c levels of 101 diabetic subjects were measured with A1cNow devices, using capillary blood samples, and laboratory Tosoh G8 HPLC Analyzer (CV% from external quality assessment of low and high HbA1c levels: 2.1 and 2.6%, respectively) using EDTA venous blood samples. A1cNow precision was evaluated by the CV of ten replicates in two consecutive days using low (5.4%) and high (10.0%) NOD HbA1c control solutions (Nova-One Diagnostics, Woodland Hills, USA).
Results: Diabetic patients Tosoh results were 7.6#1.2% (range 5.3-11.0%) vs A1cNow 7.4#1.3% (5.1-10.5%). The A1cNow results correlated with laboratory results ($r=0.95$, $p<0.001$), but mean difference between A1cNow results minus Tosoh results was -0.24#0.39 (from -1.6 to 1.1, $p<0.001$); the 95% confidence intervals (CIs) of mean difference were -0.16 and -0.32. The relative error (bias/reference x 100) was 3.1#5.1% and showed a non-normal distribution: skewness 0.57 and kurtosis 3.84 ($p<0.0001$). The within- and between-run CVs were well <4% for both levels of control solutions.

Discussion and conclusions: Thus, although the majority of A1cNow measurements were accurate in comparison with results ofthe reference method, a small percentage (5%) of mismatched results could lead to inappropriate medical decision. Research is in progress to determine system factors contributing to error. A high quality POCT couldhave a big impact on diabetes screening promotion as well as on diabetes care at home, in the hospital or office setting.

PRESEPSIN AND PROCALCITONIN FOR SEPSIS DIAGNOSIS AND APPROPRIATENESS OF ANTIBIOTIC THERAPY PREDICTION IN CRITICALLY ILL PATIENTS

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Aim: Evaluation of Presepsin (PSEP) and Procalcitonin (PCT) as early biomarkers of sepsis diagnosis and clinical severity and as early predictors of appropriateness of empiric antibiotic therapy.
Methods: Prospective, observational study on adult critically ill patients with suspected sepsis. Exclusion criteria were trauma and surgery within the first 72 hours. Age, Charlson Index, APACHE II score, sepsis severity, source of infection, PSEP (PathFas Presepsin, Mitsubishi) and PCT levels (Liaison BRAMS PCT II GEN, Diasorin) on days 1, 2, and 3 were recorded. Appropriateness of antibiotic therapy was based on microorganisms isolated in cultures. Data are median and IQ range, number and percentage. Statistical analysis was performed by the chi-square and the Mann-Withney U tests and by the ROC curve analysis.
Results: Twenty-five patients with sepsis (28%), severe sepsis (40%) and septic shock (32%) due to pneumonia (60%), intra-abdominal (16%), urinary tract (12%) and bloodstream infections (12%) were enrolled. Patients aged 59 (51-70) years, APACHE II score was 18 (14-24) and Charlson Index was 2 (1-3.5). On day 1, PSEP levels were 1402 (924-2277) pg/mL and PCT levels 1.6 (0.7-17) ng/mL. PSEP levels ≥600 and PCT levels ≥0.5 were observed in 100% and 75% patients, respectively ($p < .05$). The diagnostic accuracy for severe sepsis/shock was higher for PSEP (AUC 1, $p < .0001$, cutoff value >1400, sensitivity 100%, specificity 100%), as compared to PCT (AUC 0.84, $p < .0001$, cutoff value >0.4, sensitivity 92.3%, specificity 66.7%), with a significant difference between the two AUCs ($p < .001$). In patients who received first-line appropriated antibiotics (n=16), PSEP levels dropped from 1701 (1401-2419) pg/mL on day 1 to 1181 (653-1849) on day 2 and to 1009 (571-1511) on day 3 ($p < .05$); PCT levels were 1.3 (0.1-54) ng/mL on day 1, 3.3 (0.5-31.4) on day 2 and 2.35 (0.43-16.8) on day 3. Conclusions: In critically ill patients with sepsis due to deep-seated infections, PSEP seems to be more useful for early diagnosis, clinical severity definition and early prediction of antibiotic therapy appropriateness, as compared to PCT.
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CLINICAL BIOCHEMISTRY AND IMMUNOCHEMISTRY OF CAVITARY FLUIDS

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Other fluids, rather than blood and urine, can be used as substrate for the measurement of analytes useful as interpretative tools in order to establish presence or absence of disease and its severity and prognosis. Most fluids are ultra-filtrates of blood that have undergone processing by the relevant tissues while some are produced by active transport. These fluids may contain bio-markers that are not found in blood or are at different concentrations than in blood. Many types of fluid can be considered for biochemical investigation, some are naturally present in the healthy population and their content is modified under pathological conditions, others are generated as a consequence of a given disease (1).
Two different issues are encountered when measuring analytes in cavitary fluids with methods commonly employed for their measurement in blood or urine: the first based on the knowledge of the behavior of the analytical methods with different types of sample; the second for interpretative aspects of the concentrations measured in particular for fluids where reference ranges have not been established.
From the analytical point of view physical variables, such as viscosity and density, and method's performances, sensitivity and specificity, should be considered. The former can influence the accuracy of sample handling when automated platform are used, the latter must be adapted to the concentrations that are expected in cavitary fluids that could be really different in composition of type and amount for every kind of molecules. In addition we have to face with the variability in comparing results obtained from different instrument manufacturers (2).
For assays of biochemical parameters generally the use of colorimetric and enzymatic methods can be exploit for fluids, other than blood and urine, more easily since these reactions are mainly driven by analyte's concentration, much more attention must be paid when immunoassays are used for fluids other than those for which they are approved, the antigen-antibody binding is critically influenced from the reaction environment.

The issues related to immunoassay is relevant since some tumor marker assays have also been applied to measurements in cyst fluid or other fluid associated with a mass for diagnosis or malignancy. The use of tumor markers in fluids from pancreatic cysts is particularly important for diagnosis of malignant vs. benign lesions that are often discovered through imaging techniques that facilitate fine-needle aspiration of the fluid. The use of existing tumor markers in fluid from relatively inaccessible sites might prove to be extremely helpful for early diagnosis to avoid surgery in cases of benign lesions. This is based on the hypothesis that malignant tumors are more likely to synthesize large amounts of specific tumor markers than other disease processes, such as inflammation (e.g., chronic pancreatitis) (3-4).
Another example of the utility of analyzing a biological fluid is the determination of some steroid hormones (e.g. testosterone and cortisol) in the saliva: in fact their concentration in saliva appears closely related to the concentration of their free fraction in blood (5).
Notwithstanding the difficulties potentially arising from the lack of manufacturers' validation of biochemical analyses in fluids, the laboratory has to check performances of routine methods for the most commonly tests in cavitary fluids and using them appropriately they can provide useful information to clinicians (1-2).
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CELLULAR ANALYSIS OF BODY FLUIS

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The cellular analysis of body fluids (BFs) is important for the diagnosis and follow-up of a large number of pathological conditions (1-3).
In liquor (CSF) an increased number of leucocyte (WBC) (>5x10^6/L in adults, >7x10^6/L in children or >27x10^6/L in neonates) could be diagnostic for a lot of different neurological disorders (1, 2).
In ascitic fluids the presence of neutrophils (NE) higher than 250x10^{6}cells/L is diagnostic of spontaneous bacterial peritonitis (1, 2). In pleural fluids, a total cell (TC) counting higher than 1000x10^{6}cells/L characterizes a pleural exudative effusion and NE >50% is indicative of acute inflammation or infection, whereas with lymphocytes (LY) >50% is suggestive of tubercular infection, neoplastic disorder or chylous effusion (1, 2). In synovial fluid diagnostic criteria of infection differ between subjects with and without prosthetic implants (2).
The CLSI document H56-A (1) describes all the standard operating procedures for BFs: the sample must be collected in adequate tube (with anticoagulant or without only for CSF), transported to the laboratory at room temperature and processed within one hour of collection for CSF and two hours for other BFs.
The cell analysis may be made on manual method by optical microscopy (OM) (counting chamber and cells differentiation on cytocentrifuge preparation Romanowsky stained) or in automation, with appropriate analytical instrumentation.
Manual method by OM commonly used in laboratories showed a high risk of different types of mistakes: wrong samples mixing, choice and execution of the appropriate dilution of the sample, wrong type of counting chamber , wrong calculations of correct cell concentration (1).
Moreover, OM counting showed high inaccuracy as well as poor standardization and reproducibility, above all in absence of skilled and well-trained technical people. Despite of these limitations manual counting by OM is still the "Golden Standard".
The new generation of fully automated cell counters (hematological analyzers, flow cytofluorimeters or automated urinalysis) has also been developed for cell counting and differentiation in BFs (2, 3).
It is noteworthy that the automated analysis of BFs cellularity is mostly challenged by the presence of macrophages, mesothelial cells or other abnormal pathological cells (e.g. solid cancer cells) so that the accuracy and differentiation of cellular elements may be impaired. Some automated cell count analyzers are capable to provide TC count but not the cells differentiation, whereas specific information about the accuracy of cell recognition is unavailable for others (1-3).
CLSI document H56-A (1) and ICSH guidelines (4) requires that all laboratories apply the complex procedures for validating the automatic systems count adopted (low limits of blank, detection and quantification; carryover; imprecision; bias; linearity and range of clinical application). Finally validation rules of the results, obtained by automated counting, must be defined, also providing possible reflex tests (i.e. microscopic review) (1).
The standardization of cytometric analysis of BFs is still too poor, because EQA programs and analytical goals are now not available.
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CEREBRO SPINAL FLUID ANALYSIS AS A MODEL OF BODY FLUIDS ANALYSIS

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Cerebro Spinal Fluid (CSF) is one of the body fluids of CLSI C49-A document (1). This important document describes very well CSF physiology but for diagnostic procedures it suggests Fishman's 1992 work, dated and difficult to find. European Federation of Neurological Societies (EFNS) published two important guidelines, the first one on routine CSF analysis (2) and the second one on disease-specific CSF investigations (3). Italian Society of Clinical Biochemistry (SIBioC) Body Fluids Working Group published a recent comprehensive document on CCF analysis (4). Like other body fluids CSF can be considered as a plasma filtrate with specific molecules added, but in contrast with other fluids, in CSF proteins are highly diluted compared to plasma, having total protein concentration 200 times lower. In such solution, matrix effect is generally negligible and sensitivity of a test is usually the main problem with detection limits of an analyte far lower than those claimed for plasma use. CSF is secreted by Choroid plexus that add to plasma filtrate specific proteins and is enriched by interstitial fluid of the brain. A figurate global interface between plasma and CSF is the so called "Blood CSF barrier", a concept very useful in valuating CSF proteins. According to their origin, CSF proteins have been divided in three groups: plasma derived proteins, brain derived proteins, plasma and brain derived proteins (5). CSF Plasma derived proteins are present in CSF only by passive transfer, so CSF measurement doesn't give any information more than barrier function, Albumin, and mainly it's CSF/Serum quotient (QAlb) gives reference values for barrier function, as Albumin has only hepatic synthesis. Brain derived proteins have brain origin and directly get in CSF, their concentration has to be measured only in CSF and not in plasma: Alzheimer biomarkers are nowadays' well known analytes of this group. Plasma and brain derived proteins represent a real challenge for CSF analysis as only the brain derived fraction, the so called "intrathecal synthesis (IS)" fraction has clinical utility. Some IS proteins have an isoelectric point different from plasma proteins and can be identified by isoelectricfocusing as it happens for oligoclonal IgG and asialo transferrin. By quantitative point of view studies on CSF flow dynamics have identified the quantity of a specific protein that has to be expected according to barrier function, given by QAlb measurement. Each protein has it's CSF physiological concentration limit according to it's molecular size, permeability across barrier varies according to QAlb value complying with an hyperbolic function. Intrathecal synthesis concept has very high sensitivity and specificity and has been applied to a lot of analytes ranging from immunoglobulins to tumor markers, it is possible to make diagnosis also when pathological analytes concentration has a minimal difference from physiological ones, rather that using "the results from fluids in direct comparison with concurrent results in serum or plasma to establish whether the fluid has a very high concentration of the analyte or a very low one" as stated in C49-A document.

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PYROGLUTAMATE AMYLOIDβ_{42} OLIGOMERS IN CEREBROSPINAL FLUID: A NEW CANDIDATE MARKER FOR ALZHEIMER'S DISEASE

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The analysis of cerebrospinal fluid (CSF) is likely the best way we have to come closer to the brain modifications occurring in neurodegenerative diseases in vivo. In Alzheimer's Disease (AD), formation of oligomers composed by amyloid β_{42} ($A\beta_{42}$) provokes neuronal damage and alters synaptic plasticity; in CSF, reduction of $A\beta_{1\text{-}42}$ is the result of aggregates' sequestration in plaques, even preceding the onset of

clinical symptoms. Indeed, analysis of CSF $A\beta_{1-42}$ has been included in the recommendations for the diagnosis of AD and prodromal AD. The post-translational N-terminal truncation of $A\beta_{1-42}$ to $A\beta_{3-42}$ and the oxidation to pyro-glutamate (pE) $A\beta_{3-42}$ are some of the modifications that produce more toxic forms of $A\beta_{42}$ with higher aggregation propensity, that localize in amyloid plaques of AD brain. The detection in CSF of such modified $A\beta pE_{3-42}$ monomers or oligomers should be helpful for diagnostic and research purposes. We developed a flow cytometry and fluorescence resonance energy transfer (FRET)-based method to isolate Aβ aggregates from CSF (n=69) and to evaluate the presence of hetero oligomers composed by $A\beta_{1-42}$ and $A\beta pE_{3-42}$. Oligomers were identified by energy transfer between the two monoclonal specific antibodies against $A\beta_{1-42}$ (6E10 and 4G8) labeled with the fluorophores Alexa 594 and Alexa 488. Sorted oligomers were evaluated for the presence of $A\beta pE_{3-42}$ by their blotting with a monoclonal antibody specific for linear $A\beta pE_{3-42}$(2-48). We found that 12 /51 CSF with AD were positive for $A\beta pE_{3-42}$. Interestingly, all cognitively normal patients with other neurological diseases were negative for the presence of $A\beta pE_{3-42}$ (n=18). Our βdata suggest that $A\beta_{42}$ oligomers are heterogeneous and can include momomer $A\beta_{1-42}$ as well as modified form $A\beta pE_{3-42}$. In particular, the presence of hetero-oligomers containing $A\beta pE_{3-42}$ seems to be specific to AD, probably characterizing a subgroup of patient. Further larger and longitudinal studies should address the clinical relevance of $A\beta pE_{3-42}$ oligomers in CSF.

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SYNOVIAL TOTAL AND DIFFERENTIAL WHITE BLOOD CELLS COUNT FOR PERIPROSTHETIC JOINT INFECTION DIAGNOSIS: IS HARMONIZATION NEEDED?

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Background: Periprosthetic joint infection (PJI) is a relatively frequent complication after arthroplasty. Total and differential white cell count performed in synovial fluid (SF), is an important diagnostic tool (1). When laboratory test diagnostic accuracy is evaluated, information on preanalytical, analytical and postanalytical phases is relevant to assess risk of multiple biases. Pooling data originated from laboratory tests based on different methodology, may influence the magnitude and significance of results. The aim of the present study is to verify if in the present literature detailed information on SF assay, from sampling to white cells counting, are consistently provided.
Materials and methods: Medline was searched for articles published from May 1990 to May 2015, on PJI synovial total and differential white blood cells count. A total of 19 works were selected and reporting of synovial liquid analysis methodology was appraised.
Results: The majority of papers (89%) indicates whether the SF was drained before, during or after the surgical procedure; other preanalytical phase aspects, such as aspiration techniques and quantity of fluid, are reported just in 15% of the papers. Interestingly, only 21% of the studies mention the type of tube used and none describes the transport and sample conservation protocol. Similarly, the time between sampling and analysis is reported in a minority of works (21%).The analytical phase details are generally neglected in almost all studies. Cell count technology is systematically poorly described (26%), and a no information on quality of results, personals training, expertise and data reproducibility are given in any study (100%). Nevertheless, cut off values, as well as diagnostic accuracy parameters are defined in all studies. Given the lack of methodological details, the risk of bias can't be excluded and the cut off values suggested or recommended, as well as the diagnostic accuracy data, appear to be questionable. A multicenter study, in which a detailed protocol on laboratory testing is defined, will help to eliminate residual biases.

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APPROPRIATENESS OF TEST REQUEST

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Current pressures on appropriateness are principally due to two main reasons: the difficulties in sustainability of health systems, and the concern about the harm of unnecessary treatment. There is growing evidence that many people are over-diagnosed and over-treated for a wide range of conditions, and consequently, a concern is growing about escalating healthcare spending (1); moreover, at the same time there is an increasing effort to avoid harm when a patient is treated.

However, large uncertainties remain about where and how the lines between appropriate and inappropriate care should be drawn in any individual case. For laboratory diagnostic tests, distinguishing between appropriate and inappropriate action is even more difficult. In fact, the outcome deriving from whether a test is carried out or not depends on the appropriateness of the request, the appropriateness of pre-analytical, analytical and post-analytical phases, (right test, right patient, right time, right decision levels, etc.), but moreover, on the actions taken after the results, the therapy.
An unnecessary test could sometimes be harmful because of a false positive result, but it could be simply unhelpful or redundant. Redundancy is not always a waste. It is also a sort of internal control, like an audit or sometimes an excessive monitoring, but that should be contextualized in helping physicians to reduce their possible errors and to support them in their decisions, in an environment with increasingly defensive medicine.
On the contrary, an omitted test is always dangerous, causing a possible delay in diagnosis or a misdiagnosis. The omission of request for necessary tests is often underestimated. Several possible causes can be recognized: deficiency in training programs or information; lack of communication between the clinics and the laboratory; unavailability of tests (laboratory "spoke"; delay in technology availability, asynchrony between levels of care and diagnostic levels, critical issues in the transport of samples, time, etc.).
When one is called to evaluate the appropriateness of a test request, or a new proposed test, one can consider the pre-test probability of that particular disease (avoiding the test if it is low) or can apply Evidence Based Medicine (EBM) methodology to define the clinical question, searching for and evaluating the available evidences, applying and finally auditing the effectiveness of the test in consideration.
As demonstrated by the Bayesian theorem, the post-test probability of disease depends on not only the sensitivity and specificity of the test (that we can express as likelihood ratio) but, significantly, also on the pre-test probability. A pre-test probability of less than 0.20 suggests the test should not be carried out.
The second way, to apply an EBM methodology, can be made according to the method of the 5 "As": Asking the question that describes the problem, Acquiring the evidence that addresses the question, Appraising the evidence, for relevance and quality, Applying the knowledge that is gleaned from the evidence and finally assessing or auditing the application (2).
Finally, we have to consider that preliminary basic tests could also help physicians to formulate their clinical questions and represent a kind of "biochemical" patient examination.
Consensus between laboratories could be found in order to define harmonized recommendations and guidelines (3), to improve efficiency, avoiding rationing (4).

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SIBioC GUIDELINES FOR VENOUS BLOOD COLLECTION AND PHLEBOTOMY CHECKLIST

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The largest number of laboratory errors emerges from manually-intensive activities of the preanalytical phase, especially those related to venous blood collection. Inappropriate or mishandled activities performed during this crucial step of the total testing process pose a great risk on the quality of the specimens and thereby can seriously influence the reliability of test results. Since the 2006, the Italian Society of Clinical Biochemistry and Laboratory Medicine (SIBioC) has instituted a specific working group (WG) on extra-analytical quality of testing, whose leading aims are to identify the most vulnerable extra-analytical activities and propose guidelines and recommendations to standardize venous blood collection. After release of official recommendations for collection of venous blood in 2008 (1), the WG has then acknowledged that the quality of phlebotomy could have been further improved by development of a so-called "phlebotomy checklist", designed to help phlebotomist to optimize and standardize their practice during collection of venous blood samples (2). A multicenter study to verify the effectiveness of this tool was then planned, involving seven phlebotomy centers and four emergency departments. The investigation, which was separated in two periods of three months, was based on the registration of the leading preanalytical errors before and after implementation of the checklist. Interestingly, the overall rate of errors decreased significantly in phlebotomy centers but was unvaried in emergency departments. More specifically, a significant decrease was observed in the rate of identification errors, clotted and hemolyzed specimens, whereas the burden of incorrect tube filling and use of inappropriate containers was unchanged. Overall, the risk of identification errors was reduced up to 70%, whereas that of clotted up to 45%.

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HARMONIZATION OF THE PRE-ANALYTICAL PHASE INDICATORS

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The need to reduce the error rate, especially in the pre- and post-analytical phases, the problems in identifying adverse events and the need to comply with the International Standard for clinical laboratories accreditation, ISO 15189: 2012, stimulated laboratory professionals to develop and introduce into practice the Quality Indicators (QIs). However, evidences demonstrated the use of different criteria and procedures in the management of QIs. In this context, and on the basis of the growing awareness that the laboratory results have a significant impact on patient safety, it has become mandatory to focus also on the harmonization of the activities concerning the evaluation, monitoring and quality improvement.
The proposal of a model for the QIs management, already in use in the Department of Laboratory Medicine of University Hospital of Padova (MedLab) since 2009, includes the use of an internal assessment system and the participation in the interlaboratory comparison (EQAP) of the Working Group "Laboratory Errors and Patient Safety" of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC WG-LEPS).
The internal assessment system includes: a) a list of QIs identified both among those proposed by IFCC WG-LEPS and based on the need to monitor some specific activities; b) a form for each indicator, describing what have to be measured, how data have to collected, the acceptability limits of the results, the areas where QIs have to be used, the responsibilities; c) a working procedure.
Participation in the EQAP allows the comparison of each laboratory results with those of other laboratories involved, at national and international level.
The analysis of results, collected in 2009 and 2014 in the MedLab and expressed as yearly average, demonstrates the effectiveness of the system. It highlights, for example, a decrease of: errors concerning the requests with erroneous test entry for outpatients, from 2.2% to 0.7%, and for inpatients, from 0.64% to 0.10%; use of inappropriate containers, from 0.64% to 0.042%; hemolyzed samples, from 1.69% to 0.92%; samples with insufficient volume, from 0.09% to 0.013%. A general improvement for all QIs is observed thanks to continuous monitoring that allows to identify areas with action needs.
The use of a QIs system complying with effectiveness and harmonization requirements is a suitable tool to support the quality management choices and a fundamental element of the continuous quality improvement.
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DEVELOPMENT AND IMPLEMENTATION OF A "POP-UP" ALERT SYSTEM FOR IMPROVING APPROPRIATENESS OF LABORATORY TESTING

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Background: Appropriateness in laboratory diagnostics is a key driver for optimal use of laboratory resources and for improving patient's outcome. There is consolidated evidence that the burden of inappropriate laboratory test requests may be as high as 50%. We have hence developed a "pop-up" alert system specifically designed to limit the number of inappropriate or incongruent laboratory test requests.
Methods: A specific system of alert based on "pop-up" appearance has been developed and implemented in the computerized physician order entry of the University Hospital of Parma. A representative number of tests were identified and their electronic request was linked to the appearance of specific pop-up alerts when objective criteria of congruence and timing were violated, as follows: C reactive protein (CRP; repetition <24 hours), glycated hemoglobin (repetition <2 months), beta-human chorionic gonadotropin (beta-HCG; incompatible with age <9 and >60 years), prostatic specific antigen (PSA; incompatible with PSA reflex and female gender; repetition <3 months), thyroid-stimulating hormone (TSH; incompatible with TSH reflex; repetition <6 weeks), protein electrophoresis (repetition <7 days), cholesterol (repetition <2 months), brain natriuretic peptide (BNP; repetition <24 hours), procalcitonin (repetition <24 hours), ferritin (repetition <1 week), vitamin B and folate (repetition <1 year), immunoglobulins and albuminuria (repetition <3 months). Upon pop-up appearance, the alert could be

ignored and the test normally requested, or else the test request could be annulled. The system was implemented and monitored for 6 months in two clinical wards (Geriatric Unit and Section of Geriatrics).
Results: A total of 771 test requests generated a pop-up alert throughout the study period, leading to cancellation of 597 (77%) prescriptions. The percentage of test withdrawal was remarkably high for PCR, immunoglobulins, cholesterol, TSH, PSA, folate, vitamin B12 and ferritin (all >75%), and the lowest for BNP and PCT (~60%).
Conclusions: The introduction of a "pop-up" alert system was effective to consistently reduce the burden of inappropriate or incongruent laboratory test requests. Importantly, no complain was received by the personnel of the wards.

THE EFFECTS OF PRE-ANALYTICAL VARIABLES IN FAECAL IMMUNOCHEMICAL TESTS FOR HAEMOGLOBIN: AS "LOST WORLD" IN LABORATORY MEDICINE

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Introduction: Sampling of faecal material is one of the fields of laboratory medicine most affected by lack of harmonization, with differences in the mass of faeces collected for faecal immunochemical tests for haemoglobin (FIT-Hb), used in screening for colorectal cancer and other clinical purposes varying by up to twenty times. The aims of the study were to acquire information on faecal sampling and on the interaction between faeces and analytical methods to obtain a reference design for sample collection devices for FIT. Methods: Bias and imprecision of sample collection devices were measured using gravimetry. Dissolution times of faeces was monitored throughout the study. The effect of an increased amount of faeces on the haemoglobin (Hb) concentrations was investigated in NaCl and the buffers of different manufacturers using a single analytical methods (OC Sensor Diana, Eiken Chemical Co. Ltd, Tokyo, Japan). Results: Faecal mass recovered with different devices ranged from 56-121 % of theoretic (CV range: 9.7-31.1%). A dissolution time of up to 2h was observed when lumps (about 1 mg) of materials were collected. In NaCl, rapid decreasing of Hb concentrations was observed and the time of decrease was related to the overall mass of faeces. Increased Hb concentrations were observed adding faeces to manufacturers' buffers; recoveries of 103% and 125% of theoretic (dilutions) were obtained after the addiction of faeces. Different responses were obtained for Hb concentrations on adding an increasing amount of faeces to manufacturers' buffers. Conclusion: Solubilization time, bias and imprecision of sampling are related to device design. Analytical methods are designed to use a specific ratio between faeces and buffer. Introduction of a standardized specimen collection device design may be an useful step to harmonization of faecal tests. This, if supported by regulatory authorities, would reduce some sources of pre-analytical variability of faecal tests.
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INTERFERENCE BY ANTI-CANCER BIOLOGICAL DRUGS: A CASE OF ACCUMULATION OF BEVACIZUMAB

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Recently several works have been published about interference by anti-cancer biological-drugs (1). Few are the studies about their possible interference in patients treated with prolonged and repeated therapeutic cycles of this drugs. We reported a clinical case of a patient followed at the "National Cancer Institute Regina Elena" of Rome treated with bevacizumab that showed interference in electrophoretic (CZE) and immunofixation (IFE) techniques after repeated cycles of this anti-cancer biological drug. A 51 years old man, affected by multiple liver metastases caused by a primary tumor of the colon, was subjected at two cycles of chemotherapy protocol Folfiri (folinic-acid, fluorouracil, irinotecan) + bevacizumab 355 mg (02/10/2013 and 15/10/2013) than suspended because of several complication. The patient followed the chemotherapy treatment every 15 days with 14 cycles of protocol Folfox (folinic-acid, fluorouracil, oxaliplatin) + bevacizumab 390 mg (from 21/12/2013 to 11/07/2014) and than with 15 cycles of protocol capecitabine and bevacizumab 390 mg (from 25/07/2014 to 13/05/2015). Before the

first treatment with bevacizumab the CZE was normal while during treatment Folfox + bevacizumab, the CZE showed a marked alteration in the gamma globulin zone suggesting the presence of a suspected monoclonal component (MC). A third CZE was performed during treatment with capecitabine and bevacizumab showed a MC of 1.8 mg/L typed as IgG Kappa by IFE. The experiences acquired during our study on interference "in vitro" and "in vivo" by chemotherapy drugs and a consolidated cooperation with clinicians, allow us to evaluate this MC as an interference due to the accumulation of bevacizumab in the serum of the patient. This clinical case confirm the interference of a false-positive MC in electrophoretic and immunofixation techniques by anti-cancer biological drugs when these are used for repeated cycle treatment. The reporting of MC involves the patient in a further clinical and therapeutic procedure that are expensive and time loosing. This emphasized the importance of collaboration between clinical and medical laboratory in the management of patients undergoing repeated cycles of biological drugs.

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COMBINED VON WILLEBRAND FACTOR AND LUPUS ANTICOAGULANT ABNORMALITIES: A CASE REPORT

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We present a case concerning a 74 year old woman with negative bleeding history, idiopathic myelofibrosis, splenomegaly and bladder incontinence waiting for intervention of reconstruction of the pelvic floor. The patient had prolonged APTT (70 seconds, ratio=2.11), which resulted in further investigations because of an expected high risk of bleeding. Prolongation of APTT was found to be due to the presence of lupus anticoagulant (LA) based on the following observations: mixing tests with normal plasma did not alter APTT values neither when APTT was re-tested immediately (aPTT mix ratio=1.55) or after incubation of the sample at 37 °C (aPTT mix after 2h 37°C ratio=1.68). Moreover, LA testing with Silica Clotting Time (SCT, IL-Werfen) and diluted Russel's viper venom test (DRVVT, IL-Werfen) revealed a strong LA activity [SCT screen ratio=2.36, SCT mix ratio=1.61, SCT confirm Lupus ratio=1.65, DRVVT screen ratio=1.53, DRVVT mix ratio=1.25, DRVVT confirm Lupus ratio=1.45). Anticardiolipin and anti beta2Glycotprotein I IgG and IgM were found to be negative (EliA, Thermo Fisher). Further investigations showed normal levels of factor VIII (76.1%), XI (73.3%), IX (75.2%), XII (62%), von Willebrand factor antigen [immunoturibimetric HemosIL Werfen VWF: Ag=89.6% and Acustar IL-Werfen chemiluminescent (CliA) VWF: Ag=79.1%], strongly reduced immunoturbidimetric von Willebrand Ristocetin Cofactor (IL-Werfen VWF: RCo=11%) and slightly reduced CliA (CliA VWF: RCo=38%, Acustar IL-Werfen) and agglutination (VWF: RCO=41% Biodata Corporation, USA). In addition PFA-100 was found to be abnormal (closure time Collagen/ADP=170 s and closure time Collagen/Epinephrine=228 s). Interference of LA in vWF assays is described in literature. The abnormally strongly low immunoturbidimetric test based VWF: RCo measurements were disproportionate with CliA and agglutination VWF: RCo assays suggesting an interference of LA on immunoturbidimetric VWF: Ag RCo assay. No LA interference on immunoturbidimetric VWF: Ag was observed. The negative bleeding history, the myeloproliferative chronic disease with splenomegaly and the laboratory findings (abnormal PFA-100 and VWF: RCo) are consistent with the presence of acquired VWF Disease in a LA positive patient with high hemorragic and thrombophilic peri and post surgery risk.

SULPHAMETHOXAZOLE CRYSTALLURIA: HOW TO IDENTIFY IT

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In everyday practice, most urinary crystals can be identified by the combined knowledge of their morphology, birefringence features by polarized light and urinary pH. We describe a case of unusual crystalluria which could be identified only after Fourier transform infrared microscopy (FTIRM) was performed. On July 15th 2014, the urine sediment of a 50-year-old man, who three months before had been submitted to a kidney transplant from a codaveric donor, was examined. His serum creatinine was 1.7 mg/dL (0.5-1.20) (eGFR by MDRD equation: 46/ml/min/1.73 m^2), whilst urine by dipstick was normal (pH 5.0, density 1.030; glucose, haemoglobin, albumin, leukocyte esterase and nitrites all absent). Urine sediment, examined in blind conditions with phase contrast microscopy and polarized light, showed two types of crystals both with the shape of hexagons. One type was identical to cystine, consting in irregular plates of variable size heaped one upon another; however by polarized light they were strongly birefringent and polychromatic, as uric acid crystals usually are. The other type of hexagons consisted

in thin and irregular plates with a smooth surface, which under polarized light were either non birefringent or just whitish, as cystine crystals usually are. Due to the unusual birefringence features of crystals, and after cystinuria was ruled out on the basis of the clinical history of the patient, the residual urine was investigated by FTIRM. This demonstrated that the crystals were due to N-acetylsulfamethoxazole chlorhydrate (SMX), which is the main component of co-trimoxazole. The clinical notes confirmed that the patient was taking co-trimoxazole (trimethoprim 160 mg + SMX 800 mg) 1 tablet/day as prophylaxis of Pneumocystis carinii infection, which in the first months after kidney transplantation is frequent. Very few cases of SMX crystalluria have been reported so far, all of which, as our case, occurred in acidic urine. A correct identification of unusual urinary crystals such as those described here can be achieved only by a correct diagnostic approach. This consists in the use of phase contrast microscope coupled with polarized light and FTIRM.

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CASE REPORT: A TWIN PREGNANCY IN A MODY3 AFFECTED WOMAN

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Maturity Onset Diabetes of the Young (MODY), are rare familiar forms due to genetic mutations resulting in β-cells dysfunction. MODY 3 is due to mutations in the gene transcription factor HNF-1α, leading to progressive b-cells failure and increasing hyperglycaemia, with diabetes diagnosis in adolescence or early adult life. A few data are available about MODY 3 in pregnancy. F.C., a 36 years old Italian women, was referred to "Diabetes and Pregnancy" outpatient' s unit, for expert consultation, at the 5th week of pregnancy. She was affected from diabetes mellitus since the age of 18 years, with negative pancreatic islet autoantibodies. She had a strong familiarity for diabetes (her father and a 32 years brother, treated with OHA, a 38 years sister, treated with insulin). She was followed for a previous pregnancy in witch she had been treated with insulin, giving birth at 38 weeks of gestation to a 3.210 kg baby-girl, who showed neonatal hypoglycaemia. Her pre-gestational BMI was normal (24.5 kg/m^2) and her pre-gestational HbA1c was 60 mmol/mol (7.6%). At the first visit, she was already under a basal bolus insulin treatment. Because of her strong familiarity for diabetes mellitus, the early age of onset, negative autoimmunity and her normal BMI we asked for genetic testing that resulted in the c.812G>A mutation (p.Arg271Gln) in the exon 4 of HNF-1α gene. At 37 weeks of gestation, she gave birth to two normal weight baby-girls, one of which showed neonatal hypoglycaemia. The genetic test revealed that the father, the sister, the first daughter and only the newborn with neonatal hypoglycaemia resulted carrier of the same HNF-1α mutation. By contrast of MODY2 (GCK deficit), a fetus carrying a HNF-1α mutation has a normal birth weight, but seems to show an earlier age of diabetes onset, because of the exposure to intrauterine hyperglycaemia. Neonatal hypoglycaemia was generally observed in MODY1 infants, on the other hand MODY1 and MODY3 share similar clinical and metabolic features due to the relationship between HNF-4α and HNF-1α. It is possible to hypothesize that in our affected fetus, HNF-1α mutation leads to a functionally impaired protein that might cause dysregulated expression of HNF4α determining neonatal hypoglycaemia.

CEREBROSPINAL FLUID (CSF) ANALYSIS AND CSF FREE LIGHT CHAINS (FLC) INDICES IN A PATIENT WITH VARICELLA ZOOSTER ENCEPHALITIS

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A 3-year-old child was admitted to our Emergency Department with asthenia, hyporeactivity, drowsiness, slowed speech, severe headache, worsening of physical conditions. There was history of chickenpox 8 days before with fever for 48 hours and blisters on head, scalp and trunk. At the time of admission he showed crusty vesicles, ataxic gait and slowed speech. Neck stiffness was not detected. Cranial diffusion magnetic resonance imaging (MRI) showed pulvinar and lenticular nucleus involvement. After taking lumbar puncture the patient was hospitalized with a preliminary diagnosis of viral encephalitis and treated with intravenous acyclovir 30 mg/kg every 8 hours. His condition worsened with progressive decrease of motor control on the trunk, failure to maintain the sitting position, ambulate and feed, but on the 3rd day he started to show signs of clinical improvement. MRI performed one week later confirmed previous involvement in absence of new lesions. CSF was negative for albumin quotient, quantitative IgG synthesis, Isoelectricfocusing (IEF) and PCR for enteroviruses, HSV, EBV, VZV and CMV. Very high CSF intrathecal IgM fraction (48%) was detected and highly positive CSF VZV specific Antibody Indices IgM (8,74 n.v. <1,5) and IgG (2,41 n.v. <1,5). Thus VZV encephalitis was diagnosed. CSF FLC Kappa and Lambda Indices, recently proposed as CNS immune activation markers, were highly positive: respectively 34,12 n.v.<6,39 and 17,78 n.v.<5,51. Patient showed progressive improvement and was dismissed from hospital after 3 weeks. This is a case of VZV encephalitis, confirmed only by specific CSF serology and CSF FLC. CSF PCR is usually positive only in first days of VZV encephalitis, later on specific antibodies are synthesized. IgG IEF is considered best screening test to detect immune

activation in Central Nervous System, in our case it was negative because there was a mainly IgM response. In multiple sclerosis CSF FLC indices have shown the same sensitivity of IEF in detection CNS inflammation and authors noted this test is more likely as can be performed in emergency on automatic instruments. Our case proves that CSF FLC is the best CNS immune activation screening test, as it is positive also in case of specific or predominantly CNS IgM intrathecal synthesis.

POWERFUL OF INFRARED SPECTROSCOPY FOR STONE ANALYSIS: A CASE STUDY

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A 22-year-old male with recurrent bilateral stone disease and positive family history for stones (father) was referred in January 2015 to General Nephrology Clinic. This subject had been already treated with Endoscopic lithotripsy (right kidney) in 2010 and with Percutaneous Nephrostolithotomy (PCNL) (right kidney) in 2013 for staghorn stone. On physical exam, he has a seated blood pressure of 140/90 mmHg, with body mass index of 22.6 kg/m^2. Biochemical analysis showed that serum creatinine was 1.22 mg/dL, glomerular filtration rate (eGFR) 83 ml/min/1.73 m^2, uric acid 6.5 mg/dL, calcium 11.2 mg/dL, sodium 141 mEq/L, 25-OH-vitamin D 51.9 μg/mL, and intact parathyroid hormone (PTH) 4.5 pg/mL. The following parameters were measured in 24-hour urine collections: calcium 477 mg/24h, uric acid 1100 mg/24h, phosphate 1500 mg/24h, citrate 950 mg/24h, sodium 396 mEq/24h, pH (spot) 6.0, urine specific weight (spot) 1025, hemoglobin ++. In March 2015, the patient was referred to the Metabolic Stone Clinic. In this center metabolic and renal function exams were performed and mostly confirmed previous findings. In addition, the kidney stone sample was sent to the clinical laboratory of Policlinico Gemelli Hospital for the biochemical analysis by gold standard FT-IR method. Stone was grey, oval, rough, 11 mm in diameter and composed of 100% brushite and traces of carbonate apatite. FT-IR analysis identifying brushite and carbapatite, together with the finding of high urine pH, raised the suspect of distal renal tubular acidosis which was subsequently confirmed by urine acidification test with a 50 mg/kg load of NH 4Cl after which urine pH was 5.69 and blood pH 7.30.
Conclusion: The semiquantitative method of stone composition analysis would have yielded a mixture of calcium, ammonium, phosphate and magnesium, thus directing the diagnostic work-up, together with the abnormally high urine pH, towards infectious stones (e.g., by urease-producing bacteria). The information obtained by FT-IR analysis allowed the clinicians to correctly hypothesize a urinary acidification deficit, which was subsequently treated with thiazide and potassium citrate supplements.

TAILORED THERAPIES AND BIOMARKERS IN OVARIAN CANCER: STATE OF THE ART

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Epithelial ovarian cancer (EOC) is the most lethal gynecologic malignancy. During the last 15 years, there has been only marginal improvement in 5 year overall survival. Therapy rather depends on tumor stage, grade, than on histological type. Despite striking heterogeneity of all subtypes, their systemic management is today, still identical and consists of surgery and combination of taxane and platinum agents. Nevertheless there is growing evidence that, as epithelial OC is a heterogeneous disease, it needs a tailored approach based on the underlying molecular genetic changes. Several phase III studies investigating targeted therapies are underway. The most promising target agent in ovarian cancer treatment are antiangiogenics, poly (adenosine diphosphate [ADP]-ribose) inhibitors. Currently, the only targeted therapy agent approved for ovarian cancer is the VEGF (vascular endothelial growth factor) inhibitor bevacizumab. Response to bevacizumab is correlated with some biological predictive biomarkers like VEGF-A levels, the combined values of Ang1 and Tie2 and some clinical parameter like hypertension. Another hot topics in ovarian cancer treatment regards the deficit of homologous recombination mechanism, especially related to BRCA1 and BRCA2 gene mutations (10-20% of OC) and other genes (6% of OC), that occur up to 50% of high grade serous ovarian cancer (HGSOC). This mutations are associated to a major chemo sensibility to platinum based treatment, to a better prognosis compared to sporadic ovarian cancer and they are predictive of the efficacy of PARP inhibitors. HGSOC is an histopatological diagnosis but it may represent multiple disease at a molecular level. Recently it has been distincted in four different molecular subgroups that may need to be validated for influencing treatment choices. A more individual approach for treating OC will be selected in the future. In conclusion, a large-scale coordinated effort is needed for the robust validation of the numerous biomarker candidates available in EOC therapy. Furthermore, there is an urge to develop subtype-specific studies in an attempt to improve outcomes, which currently remain poor.

DIAGNOSTIC PERFORMANCE OF CA125, HE4 AND ROMA SCORE IN PATIENTS WITH PELVIC MASS

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Introduction: Ovarian cancer is a frequent malignancy in women, accounting for 239,000 new cases/year and as many as 152,000 related deaths worldwide. Timely diagnosis and treatment of this condition are necessary to reverse the otherwise unfavorable outcome of disease. For decades the diagnostic approach to patients with suspected ovarian cancer has been challenging, and the measurement of CA125 was found to be highly unreliable for initial screening of pelvic masses. The recent discovery of human epididymis protein 4 (HE4) and further development of the so-called ROMA score (incorporating both CA125 and HE4 values) was found to be a promising alternative, that we have further investigated in this case-control study.
Materials and Methods: The population consisted in 58 female patients with pelvic mass (age 18-80 years, 26 in postmenopausal state) admitted to the Obstetrical and Gynecological department of the University Hospital of Parma between March and December 2013. Blood samples were collected upon patient admission for biopsy into 6.0 mL primary blood tubes without additives (Becton Dickinson, Franklin Lakes, NJ, USA), and immediately centrifuged according to manufacturer's specifications. Serum was separated and tested for CA125 (reference range <35 U/mL) and HE4 (reference range <74 and <104 pmol/L according to menopausal state) on Roche Cobas ELECSYS (Roche Diagnostics, Mannheim, Germany). The ROMA score was calculated according to manufacturer's indications (reference range <11.4 and <29.9% according to menopausal state).
Results: The final diagnosis was: benign conditions (n=35; fibromas, endometriosis, endometriotic or ovary cysts), endometrial cancer (n=17) and ovarian cancer (n=6). The sensitivity, specificity, negative and positive predictive values for diagnosing ovarian cancer were: 0.50, 0.69, 0.92 and 0.16 for CA125; 0.83, 0.88, 0.98 and 0.45 for HE4; 0.67, 0.88, 0.96 and 0.40 for ROMA, respectively. In patients with ovarian cancer, ROMA better discriminated cancer severity than either CA125 or HE4 alone.
Conclusions: Compared to CA 125, HE4 and ROMA exhibit higher sensitivity and specificity for screening of ovarian cancer in women with pelvic mass. ROMA also displays higher accuracy for identifying advanced cancer stage.

ANALISYS OF BRCA1, BRCA2 AND PALB2 IN PATIENTS WITH HEREDITARY OVARIAN CANCER

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Ovarian Cancer (OC) represents 3,7% of all female cancers. More than 10% of OC are hereditary with a dominant autosomic genetic predisposition. Mutation carriers in BRCA1/2 genes have a risk of developing OC about 39-40% in BRCA1 and 11-18% in BRCA2. Moreover, these carriers have in their family cases of other malignancies, such as breast and colon cancer. Inherited mutations in PALB2 are known to be associated with increased risks of breast, pancreatic and likely, ovarian cancer. We recruited 40 hereditary OC patients (29-76 years), 26 affected with ovarian cancer, 1 with bilateral ovarian cancer, 10 with breast and ovarian cancer and 3 patients with ovarian and bilateral breast cancer. BRCA1, BRCA2 and PALB2 mutational analysis was conducted. We found 14/40 (35%) mutations carrier patients, moreover, we identified 7 unclassified variants in BRCA1 and BRCA2 genes. All patients were negative for PALB2 mutations. Particularly, we found 9 mutations in BRCA1 gene (172delC, 917delTT, IVS8+2t>a, 3470insT, 5083del19, 5382insC, E1172X, A1708E and Q1811X) and 4 in BRCA2 gene (1377insXG, 6696delTC, K944X and NS1742del). Out of 14 patients, 1 woman with bilateral breast cancer showed double heterozygosity in BRCA1 (IVS8+2t>a) and BRCA2 (K944X). The NS1742del is a novel mutation of BRCA2 was identified in a woman affected with hereditary OC. The 5382insC mutation of BRCA1 gene was recurrent and observed in two patients. Our results showed an highly frequency of mutations in BRCA1 gene, 10/14 (71,4%), in OC patients. It's important to make a genetic counseling and analysis in OC patients related to hereditary conditions to identify mutations in BRCA1, BRCA2 and PALB2 genes. Other genes, like TP53, ATM, CHEK2, RAD51, BRIP1 and mismatch repair (MMR) genes in Lynch syndrome, have been associated with hereditary OC. Therefore, in patients negative for BRCA1/2 and PALB2 mutations, these genes, less involved in hereditary OC, can be analyzed with NGS technologies. In conclusion, genetic testing in hereditary OC patients is an efficient way to identify mutation carriers and their families, it may result in more personalized cancer risk management and help to guide treatment decision-making.
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METROLOGICAL TRACEABILITY AS A TOOL FOR THE MEASUREMENT STANDARDIZATION

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There is now a global agreement that laboratory results should be traceable to higher-order references (1). This permits to improve their trueness and promote their equivalence. For establishing traceability some basic requirements should be fulfilled. First, it is essential to establish a calibration hierarchy through an unbroken metrological traceability chain starting from the unequivocal definition of the measurand as the quantity subject to measurement. The selectivity of analytical methods for the measurand as defined and the stability and commutability of reference materials employed at each level of the traceability chain are also crucial. The applied implementation strategy should reliably transfer the measurement trueness from the highest level of the chain to values of commercial calibrators, permitting to obtain unbiased results on clinical samples. Last but not least, an adequate estimation of measurement uncertainty should be performed. To fulfil this aim, IVD manufacturers should identify the higher-order material or method against which to trace their calibration. Moreover, they should estimate the combined uncertainty (including uncertainties of the previous steps of the metrological chain) associated with their calibrators when used in conjunction with other components of their analytical system (2). End-users (i.e. clinical laboratories) should verify that the alignment of analytical systems to higher-order references has been correctly implemented and survey their performance through appropriately structured quality control (QC) programs, which should be redesigned to meet metrological criteria (3). Particularly, the internal QC (IQC) has to be reorganised into two independent components: one devoted to checking the alignment of the analytical system and to verify the consistency of declared traceability during daily operations performed in accordance with the manufacturer's instructions (IQC component I) and the latter structured for estimating the measurement uncertainty due to measurement random effects (IQC component II) (4). Important barriers to the correct implementation of traceability concepts have been described: lack of full information about traceability and uncertainty of commercial calibrators, poorly defined analytical specifications and lack of appropriately identified reference intervals/decision limits (5).

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CALCULATION AND USE OF MEASUREMENT UNCERTAINTY IN THE IMPLEMENTATION OF INTERNAL QUALITY CONTROL

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Measurement uncertainty is a metrological concept to quantify the variability of individual results; its presence means that the measurement result is not a point (only one value), but an interval in which the measurement result is located with a certain probability. Because it is quite common to compare laboratory results with a reference interval, with a cut-off or with a result of the same test obtained in the same patient in a previous day, the uncertainty estimation is critical for their clinical interpretation.
For these reasons, the estimation of measurement uncertainty is now an accreditation requirement of ISO 15189, although its use by clinical laboratories is hampered by the fact that an agreed method for its calculation is still absent. Measurement uncertainty can be easily calculated from Internal Quality Control (IQC) data and a proposal for its estimation based on a top-down approach will be presented. The random component of the uncertainty derives from the intermediate imprecision of the IQC results and the systematic component from the calibrators' uncertainty.
Measurement uncertainty can be used to decide whether a measurement result indicates compliance or non-compliance with a quality specification. On the basis of this assumption, recently we proposed an IQC alarm system based on expanded uncertainty (U) estimation (1). Briefly,

this approach uses the uncertainty information to verify the conformity of each measured IQC point to a predefined quality goal: an alarm is generated if the complete IQC result (i.e. result ± U) is not entirely within the maximum permissible error (TEa).
We developed further this approach calculating a sort of power function curves based on the ratio between the quality specification and U and the number of IQC replicates; for this purpose, a Monte Carlo simulation was accomplished, calculating the percentage of non conformities in relation to different systematic shifts of a theoretical analytical procedure.
The power function curves analysis shows that the mean (95%CI) probability of error detection at the critical systematic error is 63.60% (63.54÷63.66) with N=1, 86.82% (86.77÷86.86) with N=2 and 98.26 (98.24÷98.28) with N=4.
We applied this system to real analytical situations. To estimate the alarm frequency, IQC results for glucose, creatinine and total cholesterol were collected from 6 different clinical laboratories and from 9 different analyzers: 3 Siemens Dimension Vista 1500, 2 Ortho Vitros 4600, 1 Siemens Advia 2400, 1 Beckman Coulter AU5800, 1 Abbott Architect c16000 and 1 Roche Cobas 6000. For each analyte U was estimated as described before. Two "alarm zones" were established from TEa reduced on either sides by U, and the percentage of IQC points falling within these areas during a 3 months evaluation period was calculated.
This real world application demonstrates a correlation between the proposed procedure alarms and the number of defects expected on the basis of Sigma value (r=0.88).
Work is in progress to compare the performance of the proposed procedure versus the traditional choice of Westgard rules based on s-metric QC selection tool.

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THE ROLE OF EQAS: EXPERIENCES AND THOUGHTS AFTER THE 2014 SIBioC PILOT EQAS FOR HbA1c

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Two fresh blood samples collected with EDTA were distributed by courier in December 2014 to 206 Italian laboratories asking for the determination of their HbA1c content. Target HbA1c values were assigned by the IFCC reference measurement procedure based on HPLC-capillary electrophoresis. The results, collected from 193 laboratories using kits from five different manufacturers (Bio-Rad Laboratories, A. Menarini Diagnostics, Roche Diagnostics, Sebia and Tosoh), showed a global variability of 5.3% (in terms of CV, %) and of 3.8% at an HbA1c value of 37.4 mmol/mol (sample 1) and 62.0 mmol/mol (sample 2), respectively. Inter-laboratory CVs, calculated per group of methods, were between 3.3 to 5.0% and between 2.2 and 3.7% for sample 1 and 2, respectively. Tosoh users registered the smaller inter-laboratory CV in sample 1, and Sebia's in sample 2. With regard to trueness, all methods had a mean bias of ≤ 2.0% respect to the target values, with the exception of Menarini (bias of +2.5 % in sample 2) and of Tosoh (bias of +6.1 and +5.8 %, for samples 1 and 2, respectively). Globally, 84% of the participants reported HbA1c results within the total allowable error of 8.6% (sample 1) and 93% for sample 2. These percentages decreased to 70 and 77% respectively, when using a goal for the allowable total error (TE) of 6.0% as criterion.
In conclusion, this pilot study has proven that it is possible to organize and execute an EQAS for HbA1c by using the native biological sample, thus avoiding any possible problem of matrix effect. Moreover, the comparison with the target values has clearly evidenced the bias prob-lems of some methods, thus stimulating the manufacturers to further corrective actions.
The performances of a significant number of participants have still to be improved, in order to reach the more stringent target of the total error equal to 6.0%. Finally, the possibility of repeating, on a regular base, this kind of VEQ is under investigation. In principle, such kind of VEQ could be extended also to other measurands.

VERIFICATION OF EXAMINATION PROCEDURES IN ROUTINE CLINICAL LABORATORY: A PROPOSAL FOR ISO15189:2012 ACCREDITATION

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Introduction: In order to comply with requirements of ISO 15189, clinical laboratories (CL) have to collect evidences to demonstrate that the analytical methods (AM) operate according to the performances claimed by manufacturers. As several AM are already in use, it is necessary to identify an operative flow to verify the performances considering also that the quality results can be monitored by Internal Quality Control (IQC) and External Quality Assessment (EQA) procedures. Aim of this study is to propose an operative flow to verify the AM performances for all the methods typologies used in a clinical laboratory.

Methods: After the evaluation of the available scientific documents, the following steps have been considered as major topics for the verification procedure: a) the typology of AM; b) performance characteristics to consider; c) the workflow; d) criterion for the results acceptability; e) the feasibility.
Results: Quantitative, semi-quantitative and qualitative AM were identified. For quantitative AM, imprecision and trueness are evaluated in terms of CV% and bias%, respectively. IQC and EQA may be used to verify the performance claims by the manufacturer. For qualitative AM, diagnostic accuracy is evaluated, in terms of sensibility and specificity. For semi-quantitative AM, imprecision and diagnostic accuracy are assessed.
Conclusions: A model for the verification of the examination procedures is proposed, considering all kind of methods commonly carried out in a routine clinical laboratory.
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SAMPLES COLLECTION FROM HEALTHY VOLUNTEERS FOR BIOLOGICAL VARIATION DATA: A NEW PROJECT OF THE EUROPEAN FEDERATION OF CLINICAL CHEMISTRY AND LABORATORY MEDICINE BIOLOGICAL VARIATION WORKING GROUP

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Background: Biological variation (BV) data are used to multiple purposes in laboratory medicine. BV data reliability and limitations were extensively discussed during the 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) and it was suggested that the BV data should be improved. The BV Working Group of EFLM is working to promote new studies on BV data.
Aim: To establish a biobank of samples obtained from healthy individuals to be used to establish data on BV. Design, Subjects and Methodology: The project consists in a multicenter study involving 6 European labs (Milan Italy; Bergen Norway; Madrid Spain; Padova Italy; Istanbul Turkey; Assen the Netherlands). The samples collection was made from 98 volunteers (44 men 18-60, 44 women 18-50, 10 women 55-69 years old) selected according inclusion/exclusion criteria to guarantee that they can be considered healthy individuals. The participants completed a first questionnaire in order to verify their health status. A shorter health questionnaire was completed and some biochemical and hematological tests were performed at each sampling. Once a week in 10 weeks, 1 venipuncture per subject was made to collect serum, plasma EDTA and plasma citrate samples. The serum indexes of lipemia, hemolysis and icterus were measured to guarantee the acceptability of the samples.
Results: A total of 19600 aliquots were collected: 120 aliquots of serum, 40 of plasma EDTA, and 40 of plasma citrate for each subject. The samples are stored at -80 °C until the delivery to the coordinating laboratory, where they will be stored at -80 °C in a dedicated freezer until analyses. A large number of tests, including enzymes, substrates, proteins, electrolytes, hormones, vitamins, tumor markers and coagulation tests will be performed in duplicate. The data will be treated according to recommended procedures for calculating BV including tests for homoscedasticity and a nested ANOVA.

Conclusions: The European project will provide new data on BV based on a recommended methodology to be included in a new database on the EFLM website.
Acknowledgements: Project realized thanks to the financial support of the Italian Society of Clinical Biochemistry and Clinical Molecular and of Becton Dickinson.

UPDATES ON NOA (NON-VITAMIN K ORAL ANTICOAGULANTS): WHAT ROLE TODAY FOR LABORATORY COAGULATION IN ROUTINE AND IN EMERGENCY?

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One ongoing assumption that has gained widespread consensus among several health-care professionals is that NOA are more practical than VKAs or LMWH because they do not require laboratory monitoring. Although NOA generally display a highly predictable pharmacokinetic profile, so that their anticoagulant effect is reasonably stable under controlled conditions, the metabolism of NOA is complex and multifaceted, entailing enteric adsorption and liver or renal clearance. Therefore, acute renal or liver impairment may also substantially impair the blood levels of all NOA and expose the patients to a significant risk of over- or under-coagulation; moreover, some drugs that compete with NOA for the permeability glycoprotein (P-gp) may variably increase or decrease the blood levels of the anticoagulant drugs. In total, despite generalized highly predictable pharmacokinetic profiles, a wide range of plasma concentrations may actually be evident in population studies, ranging from below 20 to as high as ≥400 ng/mL in different patients at different times post-dosage. Measurement of anticoagulant activity of NOA may be desirable in special clinical settings such as bleeding, the pre-operative state, breakthrough thrombosis, suspected overdose, noncompliance, drug interactions and in certain populations, including those with extremes in body weight and in the elderly and patients with renal insufficiency in whom there is a risk of drug accumulation. Assessment of anticoagulant effect may also be important in patients with AF presenting with acute ischemic stroke before administration of thrombolytic therapy. According to the most recent guidelines it can be stated that for dabigatran, the recommended screening assays are activated partial thromboplastin time (aPTT) and/or thrombin time (TT), and the quantitative assays (using a dabigatran standard) are dilute TT/direct thrombin inhibitor assay (Hemoclot Thrombin Inhibitor) or an ecarin-based assay such as the ecarin clot time (ECT); for rivaroxaban, the recommended screening assay is the prothrombin time (PT), but this was not endorsed by all guidelines, and the quantitative assay (using a specific rivaroxaban standard) is an anti-FXa assay; for apixaban, the general insensitivity of PT and APTT prevented most groups from providing recommendations and instead there was generalized support for direct quantitative assessment using anti-FXa assays and specific apixaban standard.
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HIGH-SENSITIVITY TROPONIN ASSAY: INSTRUCTIONS FOR USE IN ITALY

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In 2000, the First Global Myocardial Infarction Task Force recommended a new definition of myocardial infarction (MI), which implied that any necrosis (as small as it is) in the setting of myocardial ischemia should be labeled as MI (1). These principles were further refined by the Second Global MI Task Force, which emphasized the different conditions, which might lead to an MI, in three consecutive documents entitled "Universal Definition of Myocardial Infarction" (published from 2007 to 2012) (2). In the same time, the progressive implementation in clinical laboratory practice of highly sensitive immunoassays for cardiac troponin I (cTnI) and T (cTnT) allowed the detection of very small myocardial necrosis (3). The concomitant application of international guideline recommendations (1,2) and the routine use of highly sensitive cTnI and cTnT immunoassays in clinical practice produced in the last years an increase in diagnosis of MI of about 25-55% (3). Although increased cTnI or cTnT values always indicate myocardial tissue damage, ischemia may be not responsible of cardiac injury. The international guidelines recommend that the decision level, identified as the 99th percentile of the reference population, must be measured with an imprecision less than or equal to 10% (1, 2). Unfortunately, several commercially available immunoassay methods in Italy for cardiac troponins do not yet fit these recommendations (3, 4). Furthermore, several patients presenting to emergency department with thoracic pain, especially those with advanced age, heart failure, severe co-morbidities or assuming cardio-toxic drugs, actually have cTnI or cTnT increased circulating levels, but recent studies indicate that only about 30% of these patients has truly MI (3). The clinical interpretation of results of highly sensitive troponin assays may be difficult in the setting of acute coronary artery syndromes (3, 4). Recently, an expert consensus document was prepared with the purpose to develop recommendations on the use of the latest generation of cardiac troponins in emergency room setting for the diagnosis of MI in patients with suspected acute

coronary syndrome, taking into considerations the specificities of clinical health care system in Italy. In particular, the main points discussed in this document were the quality specifications of high sensitivity immunoassays for cTnI and cTnT measurement, the appropriateness of the request and the clinical criteria for the diagnosis of MI. The aim of the present relation is to discuss in details these points.

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INTERNAL QUALITY CONTROL: PRACTICAL GUIDANCE ON HOW TO HANDLE "OUT OF CONTROL" SITUATIONS

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The management of out of control situations is one of the key activities in the application of the Internal Quality Control (IQC) (1) procedure. Root cause analysis of an alarm is an opportunity for revisiting the processes under ICQ, to put in place immediate actions to fix the problem and to design and implement corrective actions of long-term improvement. The latter will lead to standardize the operations with the advantage of eliminating the variability introduced by the different operators and thus stabilizing or even improving the performance of the different analytical systems.
Proper and systematic approach to "out of control" situations is one of the specific expertise that Laboratory operators should acquire in order to ensure the necessary analytical quality of the results produced, but also an easier management of the daily workflow.
Usually the failures of ICQ belong to two categories:
False alarms due to:
– the probability of false rejections (pfr) of the implemented rules
– poor management of the control material (storage and/or use)
– improper management of control charts statistics (mean, standard deviation, target and analytical goals)

Real alarms caused by:
– instrument's malfunction
– "malfunctioning" of the reagent due to manufacturing defects or unsuitable conditions of transport and storage
– incorrect procedures
– insufficiently trained operators

To distinguish between the two types of alarm is a skill that is acquired through experience. The variables to be taken into account during the resolution of the "out of control" situations are many and sometimes difficult to find out. It is essential to approach the resolution of cases with method and order, to optimize the investment of resources , being able to deal only with real problems which have a negative impact on the quality of the clinical data provided to the clinician.
For this reason the investigation process needs two elements: the first is the "process map" describing all steps of the implementation of the ICQ procedure; this document describes how the operator should behave properly to perform the ICQ. The second element is a check lists (2) with standard questions which are able to guide the operator in a backward analysis through all the phases of the production process of the analytical data, in order to find the last change made before the out of control situation.

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VALIDATION CRITERIA FOR AUTOMATED HEMATOLOGY

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The automated complete blood count is at the origin of Diagnostic Hematology.

Latest generations of fully automated hematology analyzers (in use in modern labs) combine both counting accuracy and high analytical rate performances.
Moreover, they can provide (the user with) further qualitative informations like cell scattergams, morphological flags, and even additional sets of parameters strictly instrument-related.
In order to optimize resources while maintaining quality, check-in and validation rules for automation counting have been defined long ago, with particular attention to discrimination between normal and pathological samples needing further diagnostic investigations (e.g. microscopic examination; retic count, platelets or red blood cells count performed with alternative methods by instruments merging different technologies.
In 2005, the International Consensus Group for Hematology proposed a panel of 41 validation rules (REF), now outdated from various points of view, mainly as because of the analyzers then considered are now by far technologically improved. It is also well documented in the literature a great performance variability in such analyzers when a set of rules is applied depending on particular instrument characteristics (REF).
An Italian survey directed by the Diagnostic Hematology Study Group (GdSDE – Gruppo di Studio Diagnostica Ematologica), made it clear that there was an uneven application in defining, applying and verifying the validation criteria into automated complete blood count on italian territory.
In agreement with the 2013-2015 objectives proposed by SIBioC, the GdSDE has planned a multicentric study based upon the application of validation criteria in Automated Hematology, to pursue consolidation and standardization of the whole analytical phase throughout Italy.
With the aim of protecting the subjects that undergo analysis, it is fundamental to standardize and evaluating the global analytical process that takes place in Automated Hemocitometry. This means a deep knowledge of all the analytical performances of the instrument used, a good match between chek-in criteria and validation rules, especially when related to a specific case-mix, and finally the selection of outcome indicators for an adequate follow-up.
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LEUKOCYTE AND RETICULOCYTE EXTENDED COUNT

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The term extended differential count (EDC) means the counting of additional parameters inside of complete blood count (CBC) (1). Regarding leukocytes these additional parameters are represented by NRBC (nucleated red blood cells), IG (Immature Granulocytes), variants and/or atypical lymphocytes, blasts (2). Regarding reticulocytes EDC includes IRF (immature reticulocytes fraction) and the reticulocyte indexes.
NRBC
Erythroblasts are normally absent in the peripheral blood of normal adults; their presence is correlated to medullar stress conditions or to ineffective erythropoiesis. During fetal and perinatal period, erythroblasts are normally present at low concentrations in the peripheral blood but usually disappear within the first week of life. They can cause pseudoleukocytosis and pseudolymphocytosis therefore they must always be subtracted from the count of WBC (white blood cells). Recent studies have shown that NRBC have a negative predictive value, that are related to hypoxic and inflammatory stimuli, independent from reticulocytes, renal and hepatic lesions. NRBC are reported as a ratio of NRBC to WBC. The analytical performances of modern hematology analyzers show low imprecision, low inaccuracy, high sensitivity.
IG
The IG fraction includes promyelocytes, mielocytes and metamielocytes, while band cells and blasts are excluded (3). Blood levels of myelocytes and metamyelocytes were elevated in patients who died within the first week of ICU (intensive cure unit) stay, and their appearance in the peripheral blood may be associated with increased mortality, but IG is not recommended as screening test in infections due to its low diagnostic sensitivity.
IRF and reticulocytes index
The majority of modern automated hematology analyzers provides data concerning IRF (immature reticulocytes fraction), divided into 3 fractions based on the amount of RNA contained in these cells. It is a parameter that includes the youngest reticulocytes, adapting the Heilmeyer formula based on emitted fluorescence or on absorbance. Reticulocyte indexes include the determination of the volume, concentration and content average of reticulocytes hemoglin. Automated methods have undoubted advantages compared to manual methods, however, the

different sensitivity of the dyes and of the techniques of detection, the diversity in the choice of the thresholds of readings (gating), the unavailability of a standard and a reference calibrator, the lack of a single reference range have generated differences of results and these issues are not yet solved (4).

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MICROSCOPIC MORPHOLOGY OF CIRCULATING PERIPHERAL BLOOD CELLS

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The morphological identification of circulating peripherals blood cells as well as their quantitative and qualitative anomalies still represent a hinge of the hematological diagnosis. Other pillar are the flow cytometry as well as the cytogenetic and molecular analysis. The microscopic morphological aspects related to some reactive or neoplastic diseases can be observed even in asymptomatic subjects. This fact can improve the patient outcome because it allows us an earlier diagnosis.

However, for these purposes, the microscopic ability by itself is not enough if the professional is unable to communicate the clinical information with adequate and appropriate language. Right now, diagnostic laboratory hematology seems to suffer a kind of "Babel Tower Syndrome" caused by a lack of standardization and sharing regarding the nomenclature and description of morphological features of leukocytes, erythrocytes and platelets. The lymphocytes in infectious and in lymphoproliferative diseases were yesterday "atypical lymphocytes, suspect reactive or suspect neoplastic or uncertain nature" (1). The same lymphocytes are today "reactive lymphocytes" and "abnormal lymphocytes" respectively.

The same shape changes of an erythrocyte can be called "bite cell" in the G6PDH deficiency or keratocyte in presence of a microangiophatic hemolytic anemia (2). In this way the diagnostic process appears to be upside down: we must use the disease to diagnose the symptom. As a result, the diagnostic hematology is almost disappeared from clinical laboratories (3).

Today, only few laboratory perform the morphological examination of bone marrow whose expertise was transferred to Clinical Hematology. Meanwhile the clinical laboratory pursues the productivity. The shortening of turn around time and a low microscopic review ratio represents a good quality marker. Because this new "professional philosophy", the automated digital cell morphology systems not only as complementary tool but also as substitute of expertise are used.

It's not surprising in this scenario if someone can affirm that "traditional Romanowsky stain may be past the apogee of its ascent and beginning its trip into history along with the hemocytometer counting chamber and the Sahli pipet (4). It's not like that and the right way is in opposite side: only an aware back to the past can restore the professional dignity to Laboratory Medicine.

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DETECTION OF PERIPHERAL BLOOD EOSINOPHILS BY MEANS OF MULTIPARAMETER FLOW CYTOMETRY

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We report a simple method to identify peripheral blood eosinophils by means of multiparameter flow cytometry (MFC), using the association of CD157 plus FLAER. These markers are used to detect clones of paroxysmal nocturnal hemoglobinuria (PNH), which are

characterized by partial or total absence of glycosylphosphatidylinositol (GPI)-anchored proteins. FLAER is a fluorochrome-conjugated derivative of the bacterial toxin aerolysin, which specifically binds the GPI molecule and so is the best marker for recognizing PNH clones. According to recent studies which provided evidence about high sensitivity of MFC assays based on FLAER plus CD157 combination, we added this reagent in routine examination of patients with known or suspected PNH. Flow cytometric assays were carried out by a FacsCanto II cytometer. In the course of analysis of cytograms, we noticed the presence of variable percentages of granulocytes which were positive for FLAER, but lacked CD157; since this molecule is expressed on the surface of neutrophils, but not by eosinophils, we hypothesized that the simultaneous analysis of CD157 and FLAER might be able to distinguish eosinophils from neutrophils. Therefore, we studied a series of 31 subjects with either normal or high number of circulating eosinophils, with (n: 4) or without (n: 27) PNH. The whole granulocyte population was analyzed in a CD157/FLAER cytogram, and granulocytes positive for FLAER but negative for CD157 were expressed as percentage of CD45+ cells. This percentage was compared with the percentage of eosinophils enumerated by ADVIA 2120 (and confirmed by manual counting), and a very high correlation was found (r: 0.981, Spearman linear correlation test). After setting a specific gate to include CD157-/FLAER+ events, followed by CD45/SSC back-gating, this population was found to be characterized by higher CD45 expression and higher SSC properties , as expected in the case of eosinophils. To demonstrate that the CD157-/FLAER+ granulocytic population consisted of eosinophils, we performed sorting studies by means of a Bio-Rad S3 Cell Sorter: the sorted CD157-/FLAER+ population consisted of well-recognizable eosinophils, while the sorted CD157+/FLAER+ population was represented only by neutrophils.

MORPHOLOGICAL ALTERATIONS OF MEGAKARYOCTYTES IN MYELODYSPLASTIC SYNDROMES WITH CHROMOSOMAL ABNORMALITIES

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Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic stem cell disorders characterized by ineffective and inefficient hematopoiesis and a high risk of progression in acute myeloid leukemia (1). The MDS diagnosis is based on morphological evaluation of dysplasia. The choice of therapy depends on the prognostic stratification that is based on peripheral number of cytopenias, on the percentage of blasts and on karyotype according to the criteria IPSS (international prognostic scoring system). Some aspects of megakaryocytes dysplasia correlate with specific chromosomal abnormalities. In myelodysplastic syndromes with the del(5q), megakaryocytes have slightly smaller with an eccentric and not lobed nucleus. Monosomy 7 is associated with evidence to micromegakaryocytes and it has a negative prognostic significance. Isolated deletion of the 20q associated with dysmorphic megakaryocytes has favorable prognosis, especially when to diagnosis it is present as isolated alteration and it has been recently associated with refractory thrombocytopenia(4). We evaluated the morphological characteristics of the megakaryocytes in bone marrow of 8 patients with MDS presenting a complex karyotype in which at least one of the following anomalies were present: -7, del (7), del(5) and del(20)). Megacaryocytes dysplasia has been confirmed on at least thirty elements for each sample according to WHO2008 criteria. We have shown that each of this genetic alteration is associated with a specific megacaryocytic morphology and that the morfology can be often predictive of the cytogenetic and of the prognosis in MDS.

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MASS SPECTROMETRY: APPLICATIONS IN CLINICAL BIOCHEMISTRY

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It is incontrovertible as mass spectrometry is a compelling tool in many life sciences. Some research domains like the “omics” (e.g. proteomics, metabolomics) could not have made any progress without mass spectrometry. Even in the routine clinical investigations, mass spectrometry, mainly through LC-MSMS, becomes competitive in respect to the traditional immuno-assays in terms of accuracy and running costs (like for example in the steroid hormone, vitamin D metabolite, aminoacid, biomarker measurements).
Not only LC-MSMS is exploited for quantification of metabolites but it is nowadays highly appreciated in the therapeutic drug monitoring (TDM) context. Taking benefit of what developed for more than 25 years in applying mass spectrometry in pharmaco-kinetic and pharmaco-metabolism studies, LC-MSMS has started to be extensively used in the immunosuppressant measurements, and now extended to the monitoring of chemotherapeutic, antiretroviral, anticoagulant, psychotropic drugs.
Introduced some 25 years ago in the clinical domain with the extended neonatal screening application, mass spectrometry and more specifically LC-MSMS can be regarded as a big contributor not only in the routine clinical laboratory but also in the new concept of personalized medicine.

NEW TRENDS IN CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY APPLIED TO PHARMACOTOXICOLOGY

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Measuring concentrations of psychotropic drugs and doping agents in human conventional and non conventional matrices is important for both therapeutic drug monitoring and forensic toxicology. Similarly, also determination of unknown psychoactive principles in herbal preparations and non biological matrices is a crucial task in pharmacotoxicology.
We will provide a critical overview of the analytical methods for detection and quantification of psychotropic drugs and doping agents in the resolutions of pediatric and adult intoxications of unknown origin occurring at the Emergency rooms and to disclose unknown substances seized at illegal internet web sites and sexy or herbal shops.
Focus lies on advances in sample preparation, analytical techniques and alternative matrices. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is the most used technique for quantification of psychoactive drugs and doping agents and their metabolites. This sensitive technique makes it possible to determine low concentrations not only in serum, plasma or whole blood or urine, but also in alternative matrices like oral fluid, dried blood spots, hair, nails and non biological matrices. Ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS), recently introduced in our analytical laboratory makes it possible to quantify a high number of compounds within a shorter run time with a minimum use of solvents and simple matrices extraction. This technique is widely used for multi-analyte methods. Only recently, high-resolution mass spectrometry has gained importance when a combination of screening of (un)known metabolites, and quantification is required.
Another worthy application of UHPLC-MS/MS is the measurement of ethanol biomarkers such as ethylglucuronide or fatty acid ethyl esters or psychoactive drugs (eg. drugs of abuse, antipsychotics, antidepressants) in maternal (eg. hair) and neonatal biological matrices to disclose gestational consumption and consequent prenatal exposure to those xenobiotics.
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FULLY AUTOMATED MULTI-METHOD LC-MS: APPLICATION IN CLINICAL LABORATORY

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Clinical mass spectrometry laboratory could be ideally divided into 2 main groups: big laboratories who perform more than 20'000 test/year per method, and medium-little laboratories who perform less than 20'000 test/year per method. If the former group could use one LCMS per one method, the second need to apply different methods on the same LCMS in order to optimize the time machine and cost. In the last years several LCMS homemade methods or IVD kits became available into the market but, unfortunately, each one define their own configuration: Isocratic vs Gradient, single vs binary pumps, different connections, single column vs online SPE, etc. This is a big problem for medium-little laboratories in order to: identify the best instrument configuration when buy an LCMS, and instrument management during routine due to intra-day changes. So, it's easy to understand that the main request into medium-little clinical laboratories is the instrument flexibility. In the last 4 years we setup a flexible configuration able to do different methods on a single LCMS. This configuration, unavailable on the market up to now, is able to load simultaneously: up to 24 solvents into the primary pump, up to 4 solvents into the secondary pump and up to 15 columns, divided into 2 independents thermostats. Thanks to this configuration, this LCMS is able to work with different approaches without any hardware changes like: direct injection (e.g. new-born screening), single analytical column method (e.g. typical home-made assay), online SPE (e.g. typical IVD kits for Vitamin) and method development. This

system is able to manage all instrument changes (solvent selection, column selection, etc.) directly from the work-list. In addition, due to the little dead volume of the overall system, the method to method changes require only one warm-up (circa 5 minutes), instead up two hours for hardware changes plus 6/7 warm-up as usual. This is a real huge result that open the possibility to process urgent samples just adding them into the work-list also while the LCMS is processing other methods. In conclusion we build up an LC-MS configuration that make: 1) the work for technicians really easy; 2) the instrumentation more flexible, robust and stable; 3) the analysis more reliable and fast.

CSF MALDI PROFILING FOR THE IDENTIFICATION OF NOVEL BIOMARKERS IN DEMENTIA PATIENTS

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Aim: The increase of dementia incidence and the lack of effective therapies have promoted the search for early markers able to discriminate and prevent this pathology (1). In this context we adopted MALDI-MS to profile the CSF proteins in old patients with cognitive impairment affected by Alzheimer's disease (AD) and idiopathic Normal Pressure Hydrocephalus (iNPH) compared to controls.
Methods: Ten CSF from full-blown AD and iNPH patients diagnosed according to Aβ, tau and p-tau proteins values (ELISA evaluation, Innogenetics), respectively, and 12 age-matched cognitively healthy controls, were profiled by MALDI-MS (Ultraflex III mass spectrometer, Bruker Daltonics) for the detection of proteins discriminating the two forms of dementia. Statistics (Wilcoxon test $p < 0.01$, PCA and ROC AUC > 0.800) and classification models were performed by ClinProTools software (Bruker Daltonics). Putative biomarkers (peaks discriminating the different classes) were identified after SDS-PAGE coupled to MALDI-MS. Protein identification was validated by 2D-DIGE both in serum and in CSF of iNPH and AD patients. An independent samples cohort (n=50) was adopted for results validation.
Results: Similarity between iNPH and controls was assessed by MALDI profiling (absence of discriminating peaks). Furthermore, 21 differentially changed peaks in the acquisition range of 4-33 kDa, discriminated iNPH from AD patients. Among them, 6 were selected on the basis of p-values, ROCs and box-plots, and combined to build models to classify a blind set of CSFs. A differentially changed protein, under-expressed in AD vs iNPH patients, was identified by SDS-PAGE and MALDI-MS. The down-regulation of this specific protein was confirmed in CSF by 2D-DIGE. Two under-expressed isoforms were detected, in AD, whereas serum analysis revealed an opposite trend: 4 isoforms were present in sera and, among those, the most abundant was over-expressed in AD.
Conclusion: CSF MALDI profiling can be adopted to identify protein differences in various types of dementia to obtain a specific pattern able to support clinical and biochemical diagnosis. This approach can also provide new putative biomarkers to be coupled to molecules currently evaluated in clinical practice.
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EXPRESSION OF RESULTS: THE ISSUE OF MEASUREMENT UNITS

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The concept of measure is part of the heritage of primordial ideas common to all men since the dawn of civilization. The measures were at the beginning completely subjective: the first cubits, palms, feet, were those of the person that measured. Over the centuries the intensification of trade, the development and increasing use of measurement systems led to an effort to unify them. In the late eighteenth century in France a commission appointed to study for a fundamental unit of length choose the forty millionth of the terrestrial meridian, and put for rules for deriving other units from it and for the nomenclature: the foundations of the metric system. Since then the metric system spread to countries of Europe and South America. In order to align established references, that were dissimilar between countries,17 of them convened in 1875 at the International Convention of the Metro. The Bureau International des Poids et Mesures, established there, was entrusted with the task of preserving the primary prototypes of the meter and the kilogram, of making copies and comparing to them the national standards. The name International System of Units, SI, was given during the 1960 Conference, when the rules for prefixes, derived units and other aspects were also set out. In 1971 the SI was completed by adding the mole as unit for amount of substance.

The use of SI units to express the results of measurements in the clinical laboratory has long been a major concern of IUPAC and IFCC. They jointly approved in 1966 a Recommendation on the use of Quantities and Units in Clinical Chemistry:
- the use of the liter as the denominator in concentration units;
- the use of the mole for the amount of the substance;
- the use of multiples of the unit as powers of 10.

The mole is appropriate on the scientific basis: most of the analytical methods are based on measurement of the amount of molecules and not on their mass; the concentration of a calibration standard is not influenced by the chemical state of the material used; the physiological relationships between substances commonly occur on a molar basis. Therefore the use of the mole is preferred whenever it is possible; when the molecular weight or the homogeneity of the measured analyte are not sufficiently known, the gravimetric units are still used and acceptable within the SI.
Despite the recommendations, in many countries the SI units have not yet been fully adopted: a recent survey on the use of SI units in Europe revealed that, next to countries with full adoption, there are countries with SI units in less than 10% of their laboratories, including Italy. Many different units to express the concentration of the same analyte are in use: eg. the survey has recorded up to 6 different units for some hormonal analytes. This may lead to misunderstandings and erroneous interpretations.
The strongest argument against the use of the mole is the fear of the confusion caused by the change, but this is an argument against the change in itself. The strongest argument for the change is the move towards uniformity: if we agree on the need for uniformity, the mole is the unit more scientifically founded.
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HARMONIZATION OF REFERENCE INTERVALS

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Reference intervals (RI) are probably the most commonly used medical decision tool. Unfortunately their utility is hampered by several problems, the most important being the low individuality index typical for a large number of components. A second relevant aspect is the high heterogeneity of the RI adopted by the clinical laboratories. This heterogeneity derives essentially by the common statement: “each laboratory has to define its own RIs”. This statement, probably valid 30 years ago, when the method-dependency of the results was the rule, has now two negative consequences: when a laboratory really performs a RI study probably it does not follow the complex and demanding protocol, or, due to those difficulties, it does not perform any activity, but just adopts manufacturers’ or literature data usually without any verification. The final result is: methods precision and comparability improved, but RIs quality did not, so decreasing, or at least not improving accordingly, the quality of the information provided by the laboratory report. Harmonization of the RIs have to parallel the harmonization of the analytical methods to assure a real amelioration of the clinical information provided.
There are two possible ways to improve the situation: a pragmatic consensus approach, based on existing literature data, mediating the most frequently used values according to some experts’ opinion to define a common set of RIs to be adopted by every laboratory, and a more scientific approach setting the analytical quality pre-requisite for a sufficient standardization level, followed by the definition of common RIs based on a multicenter study to be applied by all the laboratories able to fulfill the analytical quality requirements (provided that the population served has similar characteristics: typically pediatric population requires different RIs for most of the analytes).
The first approach is faster to reach and easier to implement, but lacks of a real scientific basis and suffers for the risk of frequent amendments as a different consensus develops.
The second approach is more complex and requires longer times, but has the ambition of being somewhat “definitive” and represents the effective fourth pillar of the standardization process.
Examples of application of this second approach are momentarily limited to few analytes: creatinine, AST, ALT, GGT, but work is in progress and large multicenter studies are close to the end.
However, even if we are still not ready to propose sound common RIs to be applied by all the laboratories, at least for the most common clinical chemistry and hematology analytes, there are few simple things that can contribute to improve harmonization: a) provide sex partitioned RIs when needed (Albumin, ALT, ALP, apolipoproteins, CK, creatinine, ferritin, iron, transferrin, uric acid just as examples); b) clearly distinguish RIs from decision limits and do not use both, but only decision limits, when the message would be confounding (e.g. lipids); c) provide pediatric RIs.
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HARMONIZED REPORTING OF CLINICAL LABORATORY RESULTS

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Harmonization of the post analytical phase is a powerful weapon to decrease incorrect interpretations of the Laboratory tests performed both in ambulatory setting and emergency department, thus increasing Patient's safety.
The following items must be taken into consideration and every Laboratory should ensure in its test reporting:

- A clear, unambiguous identification of the test required and at least the method employed
- Which tests were performed in the Laboratory that deliveries the final report and which ones in a referral Laboratory (if there is any)
- The name and the surname of the Patient should be reported on every page of the report
- The name of the Doctor who ordered the test and his qualification
- The date and the time of the samples collection. If the samples were collected in different time, each of them should be indicated
- The type of material on which the test is performed, so correctly identifying the measurand
- The measurement procedure when the method is thought to be source of ambiguity
- The examination results should be reported in SI units, at least when the results are identical to those of the conventional units (e.g. in the case of Na and K)
- Biological reference values, appropriate for age and sex
- Reference changes values might be reported for those tests used in the follow-up of a Patient
- Clinical decision limits, when existing, should be preferred to biological reference values. They may be of great value for General Practitioner, in order to induce him/her to refer the Patient to a Specialist. Do not report both decision limits and reference intervals
- Diagrams might be useful when many mnemonic rules must be taken in mind for the correct interpretation of a test
- Interpretative comments are a unique opportunity to add value to the laboratory reports. Comments should be clear both for the clinician and the Patient and so they are a very sensitive area
- It might be useful to indicate the name of the Specialists involved in the analytical process and not only of the one responsible of the final report

References
ISO 15189:2012 Requirements for quality and competence.

A LABORATORY PUZZLE: WHAT IS LAC POSITIVITY?

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Aim: While there is agreement on overall diagnostic laboratory criteria for lupus anticoagulant (LAC), there is still discrepancy between specific recommendations regarding pre-analytical, analytical and post-analytical issues. The Clinical and Laboratory Standards Institute (CLSI) published a new lupus anticoagulant detection guideline document on April 2014. The aim of the present work is thus to assess the impact of the new guidelines on LAC identification and reporting in our laboratory.
Methods: Plasmas from forty-three healthy donors were analysed to obtain reference intervals (RI) as the 97.5 th percentile, according to CLSI. The analysis required for LA testing were performed with ACL TOP® 700 (Instrumentation Laboratory®). Parameters measured were: dRVVT Screen, dRVVT Confirm, dRVVT Screen on 1:1 mix with normal plasma pool (NPP). Normalized ratios were calculated against RI mean of each clotting time, according to CLSI. Twenty-three patients were randomly selected among those with LAC testing requests from our laboratory and results were evaluated according to old and new criteria.
Results: The RI obtained from dRVVT Screen ratio (dRVVTs), dRVVT Confirm ratio (dRVVTc), dRVVTs/ dRVVTc ratio and dRVVTs mix ratio were: 0,83-1,28; 0,91-1,14; 0,87-1,18 and 0,87-1,10, respectively. Our locally derived cut-off for percentage of correction ratio was: <17%. Following the ISTH 2009 guidelines currently in use in our laboratory, 7 out of 23 would be reported as positive for LAC, while according to CLSI 2014, 13 out of 23 would be reported as positive for LAC.
Conclusions: Even though conducted on a small number of samples and on a single LAC test (dRVVT), our study suggests that LAC testing following the new CLSI guidelines could determine a relative increase of positive results, the impact thereof should be critically verified with clinical data. This is, in our opinion, a relevant issue in laboratory armonization, since it may lead to incogruous results between laboratories following one or the other published guidelines and this may be especially critical in weak/borderline cases.
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DEFINITION OF RELIABLE ELF SCORE CUT-OFFS FOR NON-INVASIVE PREDICTION OF LIVER FIBROSIS STAGE IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

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Background: Fibrosis identification and staging in patients with chronic liver diseases is crucial for prognosis definition, risk stratification and therapeutic management. Although invasive, painful and hazardous, liver biopsy remains the reference standard for liver fibrosis detection. Recently, several non-invasive diagnostic tests have been developed as surrogates of biopsy, including the Enhanced Liver Fibrosis (ELF) Test. Aim of the study was to evaluate the ELF Test performance in non-alcoholic fatty liver disease (NAFLD) adult patients and to define reliable ELF score cut-offs for non-invasive prediction of liver fibrosis stage. Methods: After an informed consent, 82 patients (62/20 men/women) with suspected NAFLD underwent percutaneous liver biopsy and serum sampling. The modified Brunt classification was used to score fibrosis (F0=no fibrosis; F1=perisinusoidal/periportal; F2=perisinusoidal and portal/periportal; F3=bridging fibrosis; F4=cirrhosis). The ELF score was determined combining hyaluronic acid, amino-terminal propeptide of type III collagen and tissue inhibitor of metalloproteinase 1 levels. In order to distinguish among fibrosis stages, reliable cut-offs were determined as discriminant scores applying canonical discriminant functions to binary outcomes (F0 vs F1-4, F0-1 vs F2-4, F0-2 vs F3-4, F0-3 vs F4).
Results: ELF scores were distributed among fibrosis stages as follows: F0 median 8.38 (interquartile range - IQR- 7.69-9.48), F1 median 8.51 (IQR 8.21-9.02), F2 median 8.53 (IQR 8.24-9.05), F3 median 9.93 (IQR 8.86-10.96), F4 median 11.51 (IQR 10.84-13.45). Discriminant cut-offs were obtained: 8.56 for the detection of any fibrosis (F0 vs F1-4) with 57% of patients correctly classified; 9.09 for significant fibrosis (F0-1 vs F2-4, 61% of patients correctly classified), 9.95 for severe fibrosis (F0-2 vs F3-4, 91% of patients correctly classified), 10.33 for detection of cirrhosis (F0-3 vs F4, 96% of patients correctly classified). Conclusion: In our cohort of NAFLD patients, ELF Test represents a useful alternative to biopsy for the diagnosis and staging of liver fibrosis, especially in patients with more advanced disease.