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Abstracts*)

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GLYCATED HEMOGLOBIN IN 2012: ITS OPTIMAL USE FOR DIAGNOSIS, REPORTING, STANDARDIZATION

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The main part of this presentation will focus on the standardization of HbA_{1c}, a major prerequisite for its utilization in the clinical setting. The actions to be undertaken in order to obtain a standardized result have been indicated several times (1, 2). Essentially the first step concerns the choice of an IFCC aligned method with acceptable imprecision (overall CV ≤2.8%) and negligible bias (≤ 1mmol/mol) (3), followed by the calibration with IFCC units, then by a periodical evaluation of the imprecision by means of an adequate internal control quality program, and finally by a regular participation to external quality assessment schemes performed with commutable materials having the HbA_{1c} title assigned by the IFCC reference method. It has also recently suggested that, in order to give a precise indication that the measurement of HbA_{1c} was performed as indicated above, a short statement be included in the report ("glycated hemoglobin- HbA_{1c} IFCC-GLAD standardized") (4). In the past two years an active information campaign was undertaken within the two major societies of laboratory medicine (SIBioC and SIMeL), and our documents have been officially endorsed by the two major societies of diabetology (SID, AMD). At present an effort of information towards the national federation of physicians (FNOMCEO) and to other scientific societies interested to the care of diabetic patients (SIGO, SIMG, SIP) is under way. In conclusion, we recommend to the laboratory professionals, if they have not done it so far, to implement the recommendations as soon as possible. The date of October 1st 2012 was previously indicated as the date to report IFCC units only. The use of HbA_{1c} for the diagnosis (5) will certainly improve if the assay will be standardized as suggested.

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FASTING GLUCOSE AND ITS PRE-ANALYTICAL STANDARDIZATION

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Pre-analytical phase is an important component of total laboratory quality. This term involves specimen collection, handling and processing variables, physiological variables such as the effect of lifestyle, age, gender, pregnancy and endogenous variables such as drugs and circulating antibodies. The fasting plasma glucose (FPG) test measures blood sugar levels in basal condition and is used to diagnose diabetes. Even though simple and inexpensive, it could have important problems with the pre-analytical phase. Variables not taken into control can make it difficult to correctly apply the known levels of normality, prediabetes and diabetes. For instance, blood for fasting plasma glucose analysis should be drawn after the individual has fasted overnight (at least 8 h). Extended fasting may lead to significant decrease in glycaemia. Instructions to the patient, being prepared for specimen collection including fasting overnight, refraining from exercise and stressful activity the night before and just prior to blood collection, should be provided. Elevated stress can cause a temporary rise in blood glucose. Drugs, including corticosteroids, tricyclic antidepressants, diuretics, can increase glucose levels, while drugs such as acetaminophen and anabolic steroids can decrease the levels. Proper sample processing after blood collection is crucial. A great pre-analytical variability could be due to kind of sample (serum/plasma), temperature of sample storage, time between blood draw and centrifugation/separation and presence or not of a glycolysis inhibitor in the tube (1). These factors could cause the scarce reproducibility of IGT and IFG classifications (2). A study on the reproducibility of OGTT is now being carried out by our Study Group. The most practical way to control glycolysis is to measure glucose immediately in whole blood or to separate serum or plasma from cells within 30 min of collection, even if the specimen is collected in a tube that contains sodium fluoride. It has been well documented, but not widely recognized, that the rates of decrease of glucose in the 1st hour after sample collection in tubes with and without fluoride are virtually identical (9). Acidification should replace NaF alone as the recommended method in order to obtain an accurate glucose concentration (3). In conclusion, pre-analytical phase is an important component of total laboratory quality. Efforts towards the standardization of pre-analytical phase are important to improve a critical component such as FPG.

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FORT ASSAY: A RAPID METHOD TO DETERMINE OXIDATIVE STRESS DURING PROLONGED EXERCISE IN PATIENTS WITH TYPE 1 DIABETES

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BACKGROUND. Oxidative stress is a widely accepted component in the development and progression of type 2 diabetes and its complications. However, inconsistent results have been reported in

patients with type 1 diabetes (T1DM) for all the commonly measured markers of oxidative stress. Physical activity is widely encouraged to the T1DM patients; however, the impact on oxidative stress in these patients is largely unknown. We aimed at investigating the impact of prolonged moderate exercise oxidative stress during in a group of T1DM patients and a group of well-matched healthy controls.
METHODS. Nine patients (47±10 years, 73±15 kg, 170±10 cm; Hba1c 7.1±1.1%) and 15 healthy controls (46±10 years, 75±16 kg, 174±10 cm) performed a 3-hrs constant intensity walk at 30% of the heart rate reserve.
Patients were administered appropriate amounts of carbohydrates to avoid an excessive fall of glycemia [1, 2]. Venous blood samples were obtained before and at the very end of the trials for determination of glucose by means of a hexokinase based methodology (Olympus Diagnostic Systems AU2700) and insulin levels, which included the exogenous administered insulin by Immunoassay system (Beckman Coulter, Fullerton, CA). Capillary blood samples (n=240) were taken in duplicate at the start and the very end of the walks and as single measurements every 30 min throughout the exercise to perform the Free Oxygen Radicals Test (FORT, CR-2000 Callegari1930, Italy).
RESULTS. Glucose and insulin levels were higher in patients than in controls. Type 1 DM patients showed higher oxidative stress values as compared to healthy controls (380.1±14.7 vs 293.1±9.6 arbitrary units; $p<0.05$). Nevertheless, oxidative stress remained constant in both groups of volunteers throughout the whole exercise (p=NS).
CONCLUSIONS. The FORT assay is actually an easy method to determine the oxidative stress also during exercise. Our study showed higher oxidative stress values in type 1 diabetic patients show as compared to healthy people.
Nevertheless, prolonged moderate exercise does not exacerbate this potentially harmful condition.

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DIAGNOSIS OF DIABETES: WHICH WAY FORWARD?

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Background: Glycated haemoglobin (HbA1c) is considered the 'gold standard' for monitoring metabolic control in diabetes. New diagnostic criteria have been proposed by the American Diabetes Association (ADA) focusing on A1C for diagnosis of diabetes as well as for identification of the subjects at increased risk, being values >6,5% (48 mmol/mol) diagnostic for diabetes and values between 5,7–6,4%, (39- 47 mmol/mol) suggestive of a pre-diabete condition. Measuring HbA1c has several advantages over glucose measurements, but its particular adoption should be considered only if the test is carried out under standardized conditions taking into account its limitations, the impact of measurement on the epidemiology of diabetes and other categories of glucose intolerance is widely discussed.
Research design and Methods: The study, started on April 2010, includes all subjects presenting to outpatients department with request of OGTT in which the diagnostic performance of new proposed criteria and standard 75-g oral glucose tolerance test (OGTT) has been compared. Until now 455 subjects (males n=181, females n= 274, mean age (54,24±14.96)years) have been enrolled. Plasma glucose, lipid profile and creatinine have been measured using Cobas C 720, (Roche Diagnostics); Hb A1C with HPLC procedure (Adams HA-8180 Arkray, Kyoto, Japan) and serum insulin with Immulite 2000 (Simens). Results: OGTTs has identified pre-diabetic condition in 23.51% of subjects (n=107) while new diagnostic criteria in 39.56% (n=180), being serum insulin, median, 3.51 U/L and 3.67 U/L and HOMA index, median 0.95 and 0.98 (p=0.6493) respectively. The diagnosis of diabetes occurred in 16.26% of subjects (n=74, median serum insulin 4.31 U/L), vs 6.15% (n=28, median serum insulin 4.39 U/L) according to OGTT and new proposed criteria respectively.
The HOMA index were respectively 1.33 and 1.58 (p=0.7685). Discordant classification has been observed in 0.65% of cases (3 out of 455) showing HbA1C >6,5% (mean HbA1C 6,6%) and normal OGTTs.
Conclusion: The data obtained in our study evidence that the proposed new diagnostic criteria are questionable because 10.11% of diabetes's cases are misclassified.

ANAPHYLACTIC DEATH FROM RECRUITMENT OF MASTOCYTES AND BASOPHILS DUE TO PROLONGED INFUSION OF THYMOGLOBULIN CARBOHYDRATE EXCIPIENT DURING ORTHOTOPIC LIVER TRANSPLANTATION

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Background: Anaphylactic shock is a life-threatening allergic response characterized by severe hypotension, inducing tissue hypoperfusion with possible multi-organ failure and death. We describe the first case of fatal intra-operative anaphylactic shock due to prolonged infusion of Thymoglobulin(ATG) during Orthotopic Liver Transplantation (OLT), resulting from recruitment of both mastocytes and basophils,actived and degranulated.
Method: An adult patient (62 year old) without hystory of allergic reaction, affected by well-differentiated hepatocellular carcinoma,wich arose on HBV-related cirrhosis (MELD score 7), was submitted to OLT after the retrieval of a compatible donor cadaver. Immediately after ATG was administred, hemodynamic instability was observed with severe episodes of systolic hypotension which required continuos intraoperative infusion of noradrenalin. The patient was transferred to the Intensive Care Unit in a comatose state but he died few hours later.
Result: Post-mortem serological analysis on a preserved, pre-OLT sample of the patient'blood revealed S-IgE vs grass (2,66 kUA/L), latex (0,75 kUA/L), peanuts (2,16 kUA/L)(Thermo Fisher Scientific), whereas no significant IgE values were detected for the different classes of penicillin and cephalosporin antibiotics.S-IgE vs Carbohydrate Cross-reactive Determinants (CCD), suche as MUXF3 (1,78 KUa/L) and nAna c2 (1,94 KUa/L), proving that anaphylactic reaction was triggered by Thymoglobulin carbohydrate excipient (sugar alcohol mannitol) rather than anti-thymocyte globulin itself. Tryptase levels were investigated on pre and post-OLT sera of the patient: before transplantation their level was 5,72 µg/L, while 9 hours after shock beginning the level was 41.78 µg/L.
Conclusion: Glyco-epitopes can share significant structural homologies beyond the limits of protein families, they are prone to cause extensive antibody cross-reactivity. ATG with mannitol as excipient administered as immunosuppressant during OLT, were probably the trigger for anaphylaxis from IgE antibodies vs carbohydrate epitopes.

This case highliglts the need to pay particular attention in future not only to active substances but also to drug excipients, above all during intra-operative drug delivery.

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STUDY OF THE PREVALENCE OF CROSSREACTIVITY IN VITRO AND THEIR RESPONSIVENESS THROUGH COMPONENTRESOLVED DIAGNOSTICS (CRD).

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Background. CRD allows you to determine if the patient suffers from food allergies, present sensitization to foods independent or dependent of sensitization to aero-allergens.
Methods. 218 patients (2–80 years) with symptoms food and/or respiratory disease studied through Immucap1000 and ImmunoCAP microarray-ISAC after subjects tested on the allergen extract (Thermo Fisher Scientific).
We studied the following molecules: rPhl p1, rPhl p5, p7 rPhl, rPhl p12, Bet v1, Bet v2, v4 Bet, nder p1, p2, p10 rder, LTP, PR-10, Vicilin-like proteins, Legumin-like proteins, 2S Albumins, Profiline, nBos d4, nBos d5, nBos d8, Gal d1, d2, d3.
Results. Grass: positive pts to Phl p 1, 5 (56%) have a good responsivity to ITS; positive pts to rPhl p 1, p rPhl 5 and 7, 12 (37%) have medium responsivity to ITS; pts only positive Phl p Phl p 7 and 12 (7%) have a low responsivity to ITS; Betullacee: 65% pts has S-IgE vs Bet v 1, 48% vs Bet v 2 and 9.3% vs Bet v 4. Pts only sensitized vs Bet v 1 (44%), show good responsivity to ITS, patients (22%) with Bet v1,Bet v 2 /and Bet v 4 must be considered to be less responsive. Positive patients for Bet v 2 / Bet v 4 (34%), are not suitable for ITS. Mites: Pts with S-IgE vs Der p1, p2 = 52% they have a good responsivity to ITS; Der p1, p2, p10 (15%)medium responsivity to ITS; pts only positive vs. Der p10 (11%) have a low responsivity to ITS. For the following pan-allergens: 154 pts results symptomatic and 64 asymptomatic as follows: LTP = 16% positive pts(17% symptomatic, 7.8% asymptomatic), PR-10 = 24% positive pts (25% symptomatic, 23% asymptomatic); Vicilin-like proteins = 15% positive patients (14% symptomatic, 16% asymptomatic); Legumin-like proteins = 2% positive patients (1.9% symptomatic, 1.6% asymptomatic), 2S Albumins = 11% positive patients (12% symptomatic, asymptomatic 8%); Profiline = 24% positive patients (20% symptomatic, 34% asymptomatic) Milk = 52 positive patients (71% symptomatic, 29% asymptomatic) Egg = 51 positive patients (80% symptomatic, 20% asymptomatic).
Conclusion. Our work shows how essential is the use of CRD to differentiate: a) patients (35%) with an allergen sensitization genuine to food also; b) patients (65%) with crossreactivity to ubiquitous proteins present in pollen and common to food.

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ROLE AND CLINICAL SIGNIFICANCE OF MICROBIAL INFECTIONS IN CYSTIC FIBROSIS PATIENTS

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Cystic fibrosis (CF) is the most common life-limiting, autosomal recessively inherited disease in Caucasian populations. Although this is a multisystem disease, the primary cause of death in CF is respiratory failure resulting from chronic pulmonary infection.

The microorganisms that commonly infect the lower respiratory tracts of individuals with cystic fibrosis (CF) has evolved over time due to advances in clinical microbiology and therapeutic strategies and perhaps also due to changes in infection patterns. CF was recognized as a distinct disease entity in 1938, it was linked primarily to *Staphylococcus aureus* pulmonary infections. Following the availability of penicillin, children with CF and staphylococcal infections were for the first time given effective antimicrobial agents. Thus, it was recognized that antibiotic therapy could significantly modify the progression of CF lung disease.
Prevalence of several common respiratory pathogens in CF as a function of age, *S. aureus* is the most commonly isolated pathogen in infants and young children with CF, although *Haemophilus influenzae* and *Pseudomonas aeruginosa* are also prevalent. The frequency of positive cultures for *P. aeruginosa* increases with age, and growth of this bacterium from respiratory specimens is observed in approximately 80% of patients (adapted from the 2008 Annual Data Report of the Cystic Fibrosis Foundation (CFF) Patient Registry, Bethesda). *Burkholderia cepacia* complex (BCC) organisms are less common early in life, the CFF Patient Registry indicated that in 2008, respiratory cultures from 2.8% of individuals with CF grew BCC. The *Aspergillus fumigatus* is frequently isolated from CF patients, particularly older patients and those taking chronic inhaled antibiotics. Other organisms, such as *Achromobacter xylosoxidans*, *Stenotrophomonas maltophilia*, and nontuberculous mycobacteria (NTM), are being reported with increasing frequency and are now more commonly isolated from CF patients than *B. cepacia* complex bacteria. Thus, a growing and somewhat esoteric group of microbes appears to be well adapted to survival within the CF airways. The CFF Patient Registry data also indicate that the majority of infants with CF have positive cultures for respiratory pathogens in the first year of life.
Cystic fibrosis is an inherited condition where the airways frequently become blocked with mucus, often associated with respiratory infections. These infections may lead to progressive respiratory failure and death from breathing failure.

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THE INFLUENCE OF CFTR GENE MUTATION PATTERNS IN DIFFERENT FORMS OF CYSTIC FIBROSIS ON THE GENETIC TEST AND GENOTYPEPHENOTYPE RELATIONSHIP

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The Cystic Fibrosis (CF) is a monogenic autosomal recessive disease caused by over 1800 mutations of the Cystic Fibrosis Transmembrane

conductance Regulator (CFTR) gene. CF clinical manifestations are highly variable with an unclear relationship between genotype and phenotype. The mutational search is often unable to reveal all mutations.

We studied 610 patients clinically classified in 4 populations:

1) CF with pancreatic insufficiency (CF-PI, n=354);
2) CF with pancreatic sufficiency (CF-PS, n=138);
3) CFTR related disease (CFTR-RD, atypical forms of CF, n=71);
4) congenital bilateral absence of vas deferens (CBAVD, n=47). The mutational search was conducted by a multistep approach for the analysis of:
   a) the 32 worldwide most common CFTR mutations (CF-OLA, Abbott); b) the 14 CFTR mutations most frequent in our geographical area (by primer extension assay); c) the (TG)mTn variant tract (by sequencing); d) all exons and adjacent intronic zones of the CFTR gene (by sequencing); e) the 7 worldwide most frequent CFTR deletions (FC del assay, Nuclear Laser Medicine).

We evidenced 130 different CFTR mutated alleles leading to 228 different CFTR mutated genotypes. Within the mutated alleles, 11 were new and 10 were complex ones (with 2 or more mutations in cis on the same allele, detected although the protocol was not specifically pointed to their selection). The mutational patterns among the 4 populations were quite different. This heterogeneity influences the detection rate (DR, the proportion of mutated alleles the genetic test is able to select) of each step of mutational search. The search by mutational panels (steps a + b) results in a high DR value for CF-PI (0.890), in an intermediate value for CF-PS (0.725), but in a rather low value for CFTR-RD (0.535) and CBVAD (0.255). The remaining steps (c + d + e) enhance the overall DR in CFPI (0.993), CF-PS (0.967) and CFTR-RD (0.965); CBAVD showed the lowest overall value (0.585). In addition, 19 different mutated genotypes was found in at least 2 different populations, stressing the complexity of the genotype / phenotype relationship.

These findings have crucial outcomes for the organization and interpretation of the CF genetic tests, as well as for the comprehension of the genotype-phenotype relationship.

ANALYSIS OF METHYLATION LEVELS OF THE CFTR GENE PROMOTER

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The synthesis and the regulation of CFTR protein are poorly understood. The CFTR, disease gene of cystic fibrosis (CF), is regulated by epigenetic mechanisms, but the role of promoter methylation is not known. DNA methylation leads to gene silencing and is catalyzed by the DNA methyltransferases (DNMT). During development, the epigenome undergoes waves of demethylation and methylation changes, so there are cell type/tissue-specific DNA methylation patterns. A dense CpG island spans the main transcriptional start site (TSS). Low methylated state of CpG island in cell lines may be associated to both trancriptional activity or repression. However, high methylated state of CpG island is associated to repression, at least in cell lines. The TSS spanning CpG island was demethylated in different tissues independentely from the CFTR expression. Interestingly, in low expressing primary cells pHTE, specific CpG sites located upstream from CpG island are highly methylated. However few information are available about the CFTR methylation in primary cells from CF patients. We investigated the methylation status and the expression of the CFTR promoter in different primary cells, following 2 strategies: i) we analyzed the CFTR promoter by bisulphite sequencing to evaluate the methylated CpG islands; ii) to determine the role of DNA methylation in the regulation of CFTR mRNA expression, we investigated the CFTR reactivation after 5-Aza-2#-deoxycytidine treatment, which results in DNA demethylation. Expression levels of CFTR from treated cells were compared to untreated cells. The study demonstrated that in IMR-90 cells 68% of the CpG islands are methylated. Furthermore, we set-up a method for each different cell line: in IMR-90 cells CFTR mRNA levels increased with increasing 5-Aza-dC concentration, while in RPTEC cells we needed a higher concentration of DNAdemethylating to have an increase, suggesting that the DNA methylation could downregulate the CFTR expression. Our data suggest that CFTR methylation may have a relevant role in the regulation of gene expression at tissue level, contributing to explain the strongly discordant genotype-phenotype correlation observed in CF patients.

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LABORATORY MONITORING OF THE NEW ORAL ANTICOAGULANTS

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The new oral anticoagulants (NOAs) dabigatran (Pradaxa), rivaroxaban (Xarelto), and apixaban (Eliquis) have proved effective and safe when used in several clinical trials. Rivaroxaban and Apixaban are direct Factor Xa inhibitors; dabigatran etexilate, a pro-drug, is a direct Factor IIa inhibitor which has the same clinical indications as the direct FXa inhibitors. One of the main advantages of the NOACs is that their use does not require laboratory monitoring and dose adjustment. This demonstrated efficacy does not necessarily mean that the laboratory, considered the mainstay for the management of the old anticoagulants, will no longer play a role in treatment with OAs. Laboratories are involved in the management of anticoagulants in two ways. The first, by monitoring, implies laboratory testing to assess the drug's effect and to adjust the dosage to maintain anticoagulation within the therapeutic interval. This consideration applies to the old drugs (unfractionated heparins and the vitamin K antagonists (VKAs)). The second way, by measurement, applied to NOAs, implies laboratory evaluations of drug effect to determine whether patients are under- or over-anticoagulated, information that can be useful for decision-making in special circumstances. Measurements of the effect of NOAs are indicated in several situations: (1) patients with adverse events (i.e., thrombotic/hemorrhagic), particularly those who present with overdosage owing to excessive drug intake or decreased clearance; (2) patients undergoing surgical procedures for ensuring that no residual drug remains in the circulation; (3) patients requiring anticoagulation reversal because of life-threatening hemorrhage; (4) patients with renal insufficiency, who are likely to accumulate the drug in the circulation; (5) patients with liver failure, because NOAs are metabolized by the liver; (6) patients taking other drugs that might increase/decrease the effects of NOAs via drug-drug interactions; (7) elderly patients; (8) patients with a low body weight who can be hyper-responsive to the drug. The choice of tests is based on such characteristics as availability, linearity of the dose- response curve, standardization, and responsiveness to increasing the drug dosage. The test performed in patients on dabigatran treatment are PT, aPTT, TT and ECT (Echarin Clotting Time). The test proved to be the most suitable for dabigatran were ECT and TT (mainly for their high

responsiveness and good linearity). The test performed in patients treated with rivaroxaban were PT, aPTT, anti-Xa, Hep test, dRVVT, thrombin generation test and thromboelastography. Many studies have highlighted that is mainly PT the best test for monitoring dabigatran effects but the results are strongly dependent on the thromboplastin used. To minimize variability between the different thromboplastins it has been proposed the use of an INR rivaroxaban–related (Tripodi, JTH 2012). Practitioners also need to be aware that NOAs can interfere with the measurement of common hemostasis parameters as antithrombin, fibrinogen and APC resistance which must be interpreted with caution when measured during treatment with NOAs.

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APTT REFLEX ALGORITHM: EVALUATION WITH A SECOND APTT REAGENT MARKEDLY DECREASE INTRINSIC FACTORS INVESTIGATIONS

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Introduction
Prolongation of an APTT can be attributed to five common causes: medication (heparin and others), intrinsic factor deficiencies associated with hemorrage, intrinsic or contact factor deficiencies of little or no clinical significance, LA inhibitor, specific coagulation factor inhibitors.
Algorithm for investigation of a prolonged APTT is helpful in sorting the five potential clinical outcomes. A recent study suggests that investigation of a prolonged APTT with specific clotting factor assays is unnecessary if Actin FS APTT is normal. Aim of our study was to investigate whether, even in our laboratory, further investigations of a prolonged APTT with our routine reagent (STA APTT, Stago) should be undertaken only if a second APTT (Actin FS, Siemens) reagent is also prolonged, unless there is a history of hemorrage, in which case assays are indicated irrespective of the APTT.
Methods
71 (50 females and 21 males) consecutive patients referred to our Laboratory over a 3 month period (may-july 2012) with request for intrinsic coagulation factors and LA detection were investigated with Actin FS APTT.
Results
Only 22 out of 71 samples (31%) yielded an abnormal result with both, Actin FS and Stago, APTT and revealed a coagulation abnormality (4 LA, 2 VKA therapy, 3 von Willebrand type I, 4 factor XII, 3 factor XI, 3 factor VIII 2 factor IX and 1 factor X deficiencies,). 20 out of 71 (28%) showed abnormal Stago and normal Actin FS APTT. All of them were found to be positive to LA and gave intrinsic factors within the reference range. 27 out of 71 (38%) were found to be normal with both APTT (3 vWD typeI, 2 mild factor XII deficient and 1 LA). 2/71 (3%) yielded normal Stago and abnormal Actin FS APTT (1 factor X and 1 factor XII deficient).
Discussion
62% of all intrinsic factor requested in a 4 month period gave results within the reference range. Our study confirm that no significant coagulation factor deficiency would left undiagnosed if the protocol is followed and that von Willebrand investigation has to be done irrespectively of the APTT result. In conclusion the use of a second APTT reagent strongly reduce the cost and time spent performing these assays.

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EPIDEMIOLOGY AND CLINICS OF VIRAL HEPATITIS

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Hepatitis B (HBV) and hepatitis C (HCV) viruses are the two major causes of chronic liver inflammation world-wide. Despite distinct virologic features, both viruses are preferentially hepatotropic, not directly cytopathic and elicit liver diseases that share several aspects of their natural history. These viruses, however, are not the prevalent causes of acute viral hepatitis in Italy that is associated in more than half of the cases with hepatitis A virus infection that is generally self-limited.
As far as HCV is concerned, available estimates indicate that more than 9 million chronic carriers of HCV exist in Europe, with 27,000 to 29,000 newly diagnosed cases of infection and 86,000 deaths per year. The prevalence of infection in Italy is around 3% with approximately 1.5 million anti-HCV positive subjects. HCV infection accounts in the developed countries for approximately 20% of acute hepatitis cases, 70% of chronic hepatitis, 40% of end-stage cirrhosis, 60% of hepatocellular carcinoma and 30% of liver transplants. Since HCV infection is mostly asymptomatic (~90%), exposure to HCV is believed to be more frequent than hitherto recognized. Chronic persistence of the virus with progressive evolution of the associated liver disease is the most frequent outcome of HCV infection. Progression is however slow and overt liver disease occurs over decades with a higher rate of progression at older age (>40 years). Co-factors (HBV/HIV infection, alcohol, steatosis, haemocromatosis, etc.) accelerate significantly the progression of chronic hepatitis C. No preventive vaccines are available and therapy is based at present on the combined use of pegylated IFN-a (PEG-IFN) and ribavirin that can cure less than 50% of genotype 1 infected individuals, who represent the most difficult to treat patient population. The sustained virological response rate can be raised to ~70% in HCV-1 infection by the novel serine protease inhibitors that are however associated with severe side effects because they must be combined with pegylated IFN-a and ribavirin. Identification of novel predictor of response to therapy are therefore needed to avoid heavy and expensive treatments in those patients who have low chances to benefit from them.
As far as HBV is concerned, more than 300 million people worldwide are persistently infected by HBV. Most of them, especially in the endemic areas where HBV infection is more prevalent, have acquired infection at birth through vertical transmission from mother to neonate that results in chronic virus persistence in more than 90% of cases. The rate of chronic evolution of infection declines progressively as a function of the age of infection with a rate of chronic viral persistence lower than 5–10% when infection is acquired in the adulthood. Italy represents now a low endemicity area with HBsAg prevalence lower than 2%. Sexual contact, drug addiction and surgery/invasive procedures are the most frequent routes of HBV transmission. HBsAg prevalence (12%) among chronic hepatitis cases has

greatly declined in the last decades and HBeAg negative cases have become the most prevalent forms of chronic active hepatitis in Italy (~90%). A very effective prophylactic vaccine is available for HBV infection, while therapy is based on two different types of strategies. Short-term therapy with PEG-IFN that is effective in only 20–30% of patients and long-term therapy with 3rd generation nucleos(t)ide analogues (NUC) that can suppress HBV replication in most patients, avoiding progression to cirrhosis and decompensation. NUC must be however administered for indefinite time, with subsequent problems of compliance, cost and safety.

DIAGNOSTIC AND INTERPRETATIONAL ALGORITHMS IN SEROLOGY OF HEPATITIS

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The development and evolution of diagnostic algorithms is closely related to the concepts of appropriateness of the request, reflex testings, flow-charts and decision-making, diagnostic/therapeutic clinical pathways. These concepts are the paradigm of a new role for Laboratory Medicine, which is still under development, but that certainly is increasing within the Evidence based Medicine. In this context the perception of Laboratories as "test factories", providing nothing more than numerical results, without any commentary or interpretation, running uncritically any request, even if improper or redundant, without constantly interacting with hospital physicians and general practioners, contrasts strongly with the concept of algorithm.

The management of test appropriateness and the use of diagnostic algorithms are thus a resource for the new role of the professionals working within the laboratories, which must now take on the role of lead actors, knowing and sharing the efficacy characteristics of used tests (sensibility, specificity, likelihood ratio), using informatics approaches to achieve algorithms and finally proposing monitoring systems of interventions for improvement of appropriateness through clinical audits. Given the current epidemiological setting in Italy and the characteristics of the test in use, screening for B and C hepatitis markers, carried out in non-risk subjects, enhances the probability of false positives (very low pre-test probability) and therefore causes additional costs for confirmatory tests, psychological trauma for the patient etc., as with the screening for antibodies to viral agents HBV, HCV, HIV made on admission or before surgery.

With regard to subjects with risk behaviour In Italy[1] the 2005 Consensus Conference and in USA[2] the AASLD guidelines of 2009 recommend to screen all of them for antibodies to HCV. In august 2012 CDC[3] defined as additional target population for testing the persons born during 1945–1965, which in USA have an high prevalence of HCV infection and related disease. Test results, given as signal/cutoff (S/CO)[4], allow us to create confirmation algorithms based on clinical history and age. HBV algorithms also may be used in healthcare workers follow-up: for example in individuals born after 1991 quantitation of HBsAb is performed as the only marker of successful vaccination; HBsAg and HBcAb are evaluated to rule out chronic carriers when HBsAb resulted negative.

The use of multiple and simultaneously testing for A, B and C hepatitis must be avoided in case-finding for symptomatic subjects, since the risk factors differ for each hepatitis. Even in this scenario the use of algorithms allows cost-effective diagnosis. For example, in case of hypertransaminasemia without indication of risk factors, it is appropriate to require only IgM anti HAV, (ev. IgM anti HEV), HBsAg and HBcAb.

Follow-up of chronic hepatitis by serology and molecular assays can also be better managed and diversified, in relation to the patient typologies and disease stages, with the aim to standardize and optimize the effectiveness, with a proactive and perspective of "care" shared between GPs and hospitals, in which the Laboratory plays a central role.

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PHARMACOGENETICS OF HEPATITIS C

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Primary therapeutic failure (i.e. lack of Sustained Viral Response-SVR) in chronic Hepatits C virus genotype 1 (HCV1) infection, following current therapy with pegylated interferon α and ribavirin (INF-RIBV), can be predicted by several criteria comprising older age, high viral load, high serum GGT, low serum cholesterol: moreover a low platelet count is considered another criterion, being considered a surrogate marker of advanced liver fibrosis (1). However less than 50% of patients obtain SVR as reported in most studies. Recently genome-wide association studies evidenced that some single-nucleotide polymorphisms (SNPs) were significantly linked to the treatment outcome in HCV1 infection (2). A SNP on chromosome 19, 3 kb upstream of the IL28B gene encoding IFN-λ3 (C→T; rs12979860), appears strongly associated with SVR. Both INF-α and INF-λ activate the JAK-STAT signalling cascade thus participating in viral suppression, via a mechanism also able to inhibit HCV replication through a distinct pathway. The most favourable genotype (C/C) accounts for about 66% SVR rates, whereas the variant C/T and T/T genotypes show less than 30% SVR rates (3). Based on several clinical studies so far concluded, INF-RIBV response rates in HCV1 infected patients can successfully be predicted to be up to 80% or as low as 20% on the basis of a single patient genetic factor (4). More recently the addition of new direct acting antiviral agents (DAAs) to INF-RIBV (triple therapy) has shown further benefits in infected C/T and C/C patients. In summary data coming from IL28B SNPs allow to: a) personalize treatments in order to manage each patient in a specific manner; b) optimize clinical trials to better assess new DAAs, taking into account SNPs distribution in populations and comparison of treatments; c) possibly re-evaluate previous trials using stored samples; d) consider T allele as a possible risk factor for HCV-related carcinogenesis and post transplant fibrosis progression besides antiviral therapy failure (5).

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HHV-7 ASSOCIATED MENINGOENCEPHALITIS: AN "UNFORGETTABLE" CLINICAL CASE

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Introduction. Pathogenicity and treatment of Human Herpes Virus 7 (HHV-7) infection are still under discussion.
Aim of this study was to underline the importance of Real Time PCR (RT-PCR) in meningoencephalitis diagnosis and to appreciate how multidisciplinary integration can lead important clinical relapses for the patient.
Methods. A 25 years old woman, hospitalized with fever, otalgia, cephalea, photophobia and nuchal rigidity was subjected to rachicentesis, haematological and biochemical parameters evaluation, haemoculture and urine culture.
On cerebrospinal fluid (CSF) physical-chemical analysis and Real Time PCR (RT-PCR) for the detection of bacterial (Eurospital) and viral pathogens (Biomerieux) were performed. For RT-PCR, analytical sensitivity was >99% and specificity was 100%.
Results. Rachicentesis showed a clear CSF with high protein content, mixed pleocytosis (60% neutrophils, 40% lymphocytes), slight hypo-glycorrhachia; CSF Cryptococcus antigen results were negative, as well as RT-PCR detection of Herpes Simplex Virus 1 and 2, Epstein-Barr Virus, Cytomegalovirus, Varicella Zoster Virus, Enterovirus, Adenovirus, Neisseria meningitidis, Streptococcus pneumoniae, Haemophilus influenzae b-c, Streptococcus agalactiae, Escherichia coli, Lysteria monocytogenes and Klebsiella pneumoniae. Haemoculture, urine culture and Mycobacterium tuberculosis culture were negative. Six days after rachicentesis, with persistent high fever and cephalea, HHV-7 positivity was detected, with HHV-6 and HHV-8 negative results, both in CSF and in blood. After 21 days of treatment with valganciclovir 900 mg 2/die the patient was discharged without symptoms. HHV-7 was negative in blood 3 days after beginning antiviral therapy.
Conclusions. 1) Potential neurological pathogenicity of HHV-7 in immunocompetent patients was confirmed; 2) The neurotropic viruses panel that have to be searched in CSF by PCR should include HHV-7; 3) There is a real need to reassess the pathogens that should be investigated in CSFs samples, also by microarray technology for screening. In effect, number of meningoencephalitis treated with empiric therapy is elevated for undetected pathogens, with recovery delay and increasing risk of possible complications due to ineffective treatment.

THERAPEUTIC DRUG MONITORING OF NEVIRAPINE IN PLASMA, CORD BLOOD AND BREAST MILK IN HIV-INFECTED WOMEN RECEIVING HIGHLY ACTIVE ANTIRETROVIRAL THERAPY DURING PREGNANCY AND BREAST FEEDING IN DODOMA, UNITED REPUBLIC OF TANZANIA

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BACKGROUND: HIV infected mothers can transmit HIV to the newborns transplacentally, at the time of delivery through the birth canal or during breastfeeding through the breast milk. The presence of ARVs in cord blood and in breast milk may reduces HIV mother-to-child transmission either through the direct inhibition of local HIV replication or by providing infant prophylaxis.
AIMS: This study was designed to determine serum, cord blood and breast-milk concentrations of nevirapine in pregnant and breast-feeding women attending an urban health center in Dodoma, Tanzania.
METHODS: Validated high performance liquid chromatography-UV method was used to measure the concentrations of nevirapine in serum, cord blood and whole breast milk from 69 HIV-1 infected Tanzanian women receiving ARV treatment.
RESULTS: The median maternal plasma nevirapine concentration at delivery and three months post-delivery was: 5199 ng/ml (IQR, 3187 to 7308 ng/ml) and 7145 ng/ml (IQR, 5462 to 9759 ng/ml), respectively. The median concentration in cord blood was 3865 ng/ml (IQR, 2261 to 4783 ng/ml) and the median percentage of the cord bloodto-maternal plasma nevirapine concentrations was 73% (IQR, 66 to 81%). Three months post- delivery the median breast milk nevirapine concentration, was 4416 ng/ ml (IQR, 3503 to 5908 ng/ml), and the median percentage of the breast milk-to-maternal plasma nevirapine concentrations was 62% (IQR, 54 to 73%). Preliminary laboratory data on liver function have not shown any relevant abnormality.
CONCLUSION: Our data clearly show that nevirapine reaches high concentrations in cord blood and in breast milk.
According to guidelines for the use of antiretroviral drugs in pediatric HIV infection, the minimum recommended level of nevirapine in the plasma is 3000 ng/ml. The concentrations we measured in breast milk and cord blood are higher than the required minimum. This concentration may provide protection against HIV infection with no major drug-related toxicity to the exposed newborn.

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MYELOID MALIGNANCIES: CLASSIFICATION AND DIAGNOSIS ACCORDING TO WHO 2008

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The revised (2008) WHO classification of Tumors of Haematopoietic and Lymphoid Tissues identifies five major subgroups within the myeloid neoplasms, that are the Myeloproliferative neoplasms (MPN), the Myeloid/Lymphoid neoplasms with eosinophilia and abnormalities of PDGFRA, PDGFRB or FGR1, the Myelodysplastic Syndromes (MDS), the Myelodysplastic/ Myeloproliferative neoplasms and the Acute Myeloid Leukemias.
Malignancies included into the group of Myeloproliferative neoplasms are:
- Chronic myelogenous leukemia. BCR-ABL1 positive
- Chronic neutrophilic leukemia
- Polycythaemia Vera
- Primary Myelofibrosis
- Essential thrombocythaemia
- Chronic eosinophilic leukemia not otherwise specified
- Mastocytosis
- Myeloproliferative neoplasm, unclassifiable

Myeloid/Lymphoid neoplasms with eosinophilia and abnormalities of PDGFRA, PDGFRB or FGR1
The identification of a new subgroup of diseases based on specific gene rearrangements irrespectively from the lineage(s) ratifies the modern approach to the classification of haematological malignacies, highlighting the relevance of gene analysis. In the previous classifications only the subgroup of Leukemias of ambiguous lineage, still existing in the present 2008 classification, shows a mixture of lineages in terms of CD immunophenotypic criteria.
Malignancies included into the group of Myelodysplastic Syndromes are:
- Refractory cytopenia with unilineage dysplasia
- Refractory anaemia with ring sideroblasts
- Refractory cytopenia with multilineage dysplasia
- Refractory anaemia with excess blasts
- Myelodysplastic syndrome with isolated del(5q)
- Myelodysplastic syndrome, unclassifiable
- Refractory cytopenia of childhood

Malignancies included into the group of Acute Myeloid Leukemias (AML) are:
- Acute myeloid leukemia with recurrent genetic abnormalities
- AML with myelodisplasia-related changes
- Therapy-related myeloid neoplasms
- AML NOS (Not otherwise specified)
- Myeloid sarcoma
- Myeloid proliferation related to Down syndrome
- Blastic plasmacytoid dendridic cell neoplasm

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DANCES WITH PROMYELOCYTES (AND PROMONOCYTES): OBSTACLES TO CLEAR IN LEUKEMIA CLASSIFICATION

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Identification and count of blast cells is a basic morphological task which is necessary to classify blood cell disorders. This concept has kept his importance over the last decades even in the era of molecular genetics, and through radical changes in classification criteria of acute leukemias and myelodysplastic syndromes from the 1976–1985 FAB approach to the new 2001–2008 WHO classification of myeloid neoplasm. The minimum threshold of blasts cells needed for the diagnosis of acute myeloid leukemia has been reduced from 30% to 20%, but the identification of a given cell as a blast still represents a decision which is demanded to a skillful recognition of morphological features. The CD34-positive cell count is not considered a reliable surrogate from a quantitative point of view, since some blast cells can be CD34-negative. The most difficult aspect of blast cell identification concerns the decision on which type of granular immature cells have to be considered blasts or normal versus abnormal or dysplastic promyelocytes. Moreover, some special promyelocytes have to be included in the blast cell count in the presence of the morphological, genetic and molecular picture of acute promyelocytic leukemia. Even in this area, however, not all promyelocytic blasts are equal: the hypergranular variant promyelocytes, in fact, have a characteristic shape with cytoplasm filled by granules and Auer rods and moderately condensed nuclear chromatin, while blast cells of the so-called micro-granular variant of promyelocytic leukemia apparently are devoid of distinct granulations and have a recognizable convoluted, mushroom- or sandglass-shaped nuclei with loose chromatin network. As far as other acute leukemias and myelodysplastic syndromes are concerned, morphologic criteria for the definition of normal and dysplastic promyelocytes and for their differentiation from granulated myeloblasts have been agreed by a Consensus Panel and published: number and orderly distribution of cytoplasmic granules, presence or absence of a clear paranuclear Golgi area, position of the nucleus in the cytoplasm are among the most important.
Similarly, the morphological identification of monoblasts, promonocytes, atypical and normal monocytes is fundamental for the correct differential diagnosis between acute monocytic and chronic myelomonocytic leukemias.

PILOT STUDY ASSESSING THE USE OF A SCORING SYSTEM IN BLOOD MORPHOLOGY EXTERNAL

QUALITY ASSESSMENT SCHEME (EQAS) IN THE AZIENDA OSPEDALIERO UNIVERSITARIA CAREGGI

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BACKGROUND: Examination of a blood smear is the first approach in the diagnosis of haematological disorders. In order to monitor the ability of the participants in interpreting morphologic abnormalities on blood smear, by 2011 the AOUC EQAS organizer has set up a blood smear scheme, following the EQALM guidelines.
METHODS: One sample of 4 different blood smears was sent to 179 EQAS participants for morphology evaluation.
Some patients details corresponding to the samples were disclosed. In 2012 were sent three exercises: a chronic lymphatic leukaemia (LLC), an haemolytic anemia and an acute lymphoblastic leukaemia (LLA). The participants were asked to select up to three significant morphology abnormalities using a coding list provided by the EQAS organizer. A diagnostic suggestion based on the WHO classification of disease(free text) was well accepted.
For each survey, individual results were assessed against the "correct" answers given by the manifacturer which provides a report of the definitive or likely diagnosis and of the observed morphological abnormalities. The assessment of individual results was calculated using a score system. Actually, do no exist predefined criteria for scoring "significant features" and "minor features" and for scoring the suggested diagnosis.
RESULTS: The evaluation of laboratory performance in blood smear interpretation showed: 1) 70% of the enrolled participants (119/179) average answered to the surveys; 2) different performance levels were detected relative to the laboratory category: higher score were shown by great laboratories, specialized laboratories and laboratories which provided services to hospitalized patients. No significant differences were found between private and public laboratories; 3) different performance levels were registered relative to pathological cells. The mean score of exercise n°1 (LLC) was 0.40, of exercise n°2 (haemolytic anemia) was 1.70 and of exercise n° 3 (LLA) was 0.70, indicating that pathological lymphoid cells were the most difficult to identify by the participants.
CONCLUSION: The results revealed substantial concordance with data of literature and they need a confirm with the next surveys. We are improving a user feedback questionnaire whom results will provide further directions for programme development.

USE OF THE NOVEL MONOCLONAL ASSAY FOR THE MEASUREMENT OF CIRCULATING FREE LIGHT CHAIN IN THE DIAGNOSIS, PROGNOSTICATION OF SURVIVAL AND ASSESSMENT OF RESPONSE TO THERAPY IN AL AMYLOIDOSIS

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Measurement of circulating free light chains (FLC) improved diagnosis, prognostication of survival and response assessment in AL amyloidosis. (Palladini, Clin Chem 2009) We evaluated a novel method for FLC quantitation based on monoclonal antibodies in 353 consecutive newly diagnosed patients. Serum FLC concentration was measured in duplicate on frozen sera by a polyclonal (Binding Site, BS) and a monoclonal (Siemens, S) immunoassay on a Siemens BN ProSpec nephelometer. Reference ranges are κ-FLC 3.3–19.4 mg/L, λ-FLC 5.7–26.3 mg/L, κ/λ ratio 0.26–1.65 for the BS assay, and κ-FLC 6.7–22.4 mg/L, λ-FLC 8.3–27.0 mg/L, κ/λ ratio 0.31–1.56 for the S test. The concordance correlation coefficient of the two assays was (0.92, 95% confidence interval [CI] 0.87–0.91) for κ and (0.78, 95%CI 0.73–0.82) for λ FLC. Diagnostic sensitivity was 82% (95%CI 78–86%) for the BS assay and 84% (95%CI 80–88%) for the S test. The combination of FLC measurement with serum and urine immunofixation increased sensitivity to 98% (95%CI 96–99%) with both assays. We evaluated the prognostic relevance of the difference between involved (amyloidogenic) and uninvolved FLC concentration (dFLC). Median values of dFLC were 180 mg/L by BS and 165 mg/L by S. Patients with dFLC above the median value had a worse outcome (41% vs. 65% surviving 2 years, P=0.001, with both methods).
These thresholds were incorporated into a staging system, including the median values of N terminal pro natriuretic peptide type B (1800 ng/L) and troponin I (0.07 ng/ mL). The resulting systems identified four groups with decreasing survivals. The discrimination between the groups with worse prognosis (stages 3 and 4) was not statistically significant with the BS test (P=0.134), while it reached statistical significance with the S test (P=0.022). We evaluated the applicability of the criteria for hematologic response validated with the BS test to the S assay, and observed relevant discrepancies. In particular, 26% of responders were classified as non-responders by S. The S assay has a diagnostic sensitivity comparable to that of the BS test and can be used for prognostic stratification. The discrepancies observed in the assessment of response indicate that different criteria may be needed when using the S assay.

VKORC1, CYP2C9 AND *CYP4F2* GENETIC BASED ALGORITHM FOR WARFARIN DOSING. PRELIMINARY RESULTS OF A PROSPECTIVE ITALIAN STUDY

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Background: We have recently developed and published a pharmacogenetic algorithm, based on *VKORC1*, *CYP2C9*, *CYP4F2* polymorphisms, age and body surface area (BSA). This algorithm could explain 55% of warfarin dose variability in Italian patients.
Aim: To verify in a prospective randomized study whether our algorithm (PGX arm) has any clinical advantage over standard warfarin dosing (STD arm).
Methods: We enrolled 103 patients with age >18 years, presenting atrial fibrillation as indication for ex-novo warfarin treatment initiation and with target INR of 2,5 (therapeutic INR range 2–3). At enrolment a peripheral blood sample was collected from each patient for genomic DNA extraction. V*KORC1* (-1639G>A SNP), *CYP2C9* (*1,*2,*3 alleles) (INFINITI™ Analyzer, Medical System) and *CYP4F2* (*1,*3 alleles) (Taqman chemistry) polymorphisms were analysed and INR was monitored on days 0,5,7,9,12,15 and 19 of treatment. Patients were randomized to receive initial warfarin doses either established according to the standard care (STD arm, n=46) or determined by the pharmacogenetic algorithm (PGX arm, n=46). Eleven patients didn't complete the observational period and resulted drop out.

Results: patients randomized to the PGX arm had a lower number of INR above 4 (F=6.381, p<0.05) and a lower time spent at INR>4 (0.2% vs 3.1%) (F=21.9; p<0.0001) than patients in the STD arm, implying a reduced Relative Risk of over-anticoagulation (RR=0.074; 95% C.I. 0.017–0.310).
We then subdivided patients in three classes: those expected to require low (<20mg/week), intermediate (20–40mg/week) and high (>40mg/week) warfarin maintenance doses. The lower risk of over-anticoagulation observed for patients overall was confirmed in patients requiring low doses of warfarin (RR=0.077; 95% C.I. 0.010–0.570). Moreover among these patients comparing the PGX arm to the STD arm a lower percentage of INR above 4 (0/30 vs 6/42) (F=4.675, p<0.05) and a reduced time spent at INR>4 (0% vs 13.2%) (F=13.88; p<0.001) were recorded.
Among patients expected to require high warfarin doses a lower risk of under-anticoagulation and a higher anticoagulation control were recorded in the PGX arm: the fraction of the study time spent at INR<1.5 in the PGX arm was lower than in the STD arm (20.3% vs 28.2%) (F=4.06; p<0.05) and the fraction of the study time (days) spent within the therapeutic range was higher than in the STD arm (54.1% vs 39.2%) (F=10.42; p=0.0016).
No significant difference were recorded considering time to stable anticoagulation in patients overall or subdivided in the three classes of warfarin dosing and when patients requiring intermediate warfarin doses were considered.
Conclusion: these preliminary findings suggest that the application of the PGX algorithm might significantly reduce the risk of bleeding events and increase the fraction of time spent at therapeutic INR values especially in patients requiring very low and high warfarin doses respectively.

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FAMILIAL COMBINED HYPERLIPIDEMIA: IDENTIFICATION OF SINGLE NUCLEOTIDE POLYMORPHISMS FOR EARLY IDENTIFICATION OF PATIENTS AT RISK

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Objectives: Familial Combined Hyperlipidemia (FCH) is the most frequent dislipidemia responsible for increased risk of premature cardiovascular diseases (1). To date, its diagnosis is based on clinical and biochemical data in the proband and in their relatives. The lipid profile of FCH patients includes an increase of total cholesterol, triglycerides and ApoB levels. The hallmark of this dislipidemia is the presence of small and dense LDL, closely associated with atherosclerosis development. Many genes involved in the lipid metabolism have been recognized as candidate genes for the association with this disease (2). We aim to identify Single Nucleotide Polymorphisms (SNPs) that could be used as a marker of the disease and improve early diagnosis.
Methods: We analyzed 20 SNP in 10 candidate genes in 165 patients suffering from FCH and in 142 healthy subjects. After DNA extraction from peripheral blood samples, genotyping was performed by TaqMan real time PCR. Genotype and allele frequencies were calculated and data were analyzed by comparing the frequencies through a chi-square test as well as linear multivariate regression or logistic multivariate regression. Statistical analysis was performed with PASW 18.0 and Haploview 4.2.
Results: We identified 3 SNPs whose genotype frequencies are different between FCH patients and controls: ApoA5 (S19W and -1131T>C) both with p<0.010, and USF1 (11235C>T) with p=0.002. The comparison of allele frequencies confirms the association of the minor allele of both SNPs in ApoA5 with FCH whereas the minor allele of USF1 to the healthy status (all with p<0.003). The presence of the minor allele at heterozygous or homozygous status of both ApoA5 SNPs is related to high levels of cholesterol (p=0.013 for S19W and p=0.043 for -1131T>C) and triglycerides (both p<0.005). The SNP S19W in ApoA5 is also associated with a small diameter of LDL (p=0.001). The presence of the minor allele at heterozygous or homozygous status of USF1 11235C>T is associated with low levels of cholesterol (p=0.005), triglycerides (p=0.013) and ApoB (p<0.001). All 3 SNPs are associated with the presence of FCH independently of all others: the presence of the minor allele of the 2 SNP in ApoA5 is associated with an increased risk of the disease (OR=2.65; 95% CI: 1.38–5.07; p=0.003 for S19W and OR=2.33; 95% CI: 1.32–4.12; p=0.003 for -1131T>C), whereas the minor allele of 11235C>T in USF1 is a protective factor (OR=0.44; 95% CI: 0.27–0.71; p=0.001). When total cholesterol and triglycerides are included in the model, the SNP S19W in ApoA5 remains associated with FCH (OR=5.50; 95% CI 1.13–26.68; p=0.034) independently of the levels of cholesterol and triglycerides.
Conclusions: Our data demonstrated an association of 3 SNPs with the presence of FCH. The combined study of different genetic variations could allow to better characterize FCH patients and to identify high-risk subjects. The variants are also associated with different levels of lipid variables, suggesting their active role in the development of the dyslipidemic phenotype. The prominent role of the SNP S19W in ApoA5 suggests its use as a marker in diagnostic protocols.

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FIRST DIAGNOSIS OF HEREDITARY FOLATE MALABSORPTION (HFM) IN A LITTLE ITALIAN COUNTRY: POSSIBLE GENETIC DRIFT OR INCIDENTALOMA?

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Background: HFM is an autosomal recessive disorder, recently shown to be due to mutations determining loss of function of the

proton-coupled folate transporter (PCFT-SLC46A1), resulting in impaired intestinal folate malabsorption. In the first months of life, infants with HFM usually present with significant growth retardation, pallor, and often diarrhea. In many cases, there are also neurological deficits such as developmental delay, mental retardation, due to very low levels of folate in the cerebrospinal fluid.

In this report, we describe a case of a one year old female whose symptoms started five months after birth with low body growth associated to pancitopenia, and who admitted at our hospital for active cytomegalovirus infection treated with specific therapy. In addition, baby developed megaloblastic anemia and hypoimmunoglobulinemia resulting in sepsis shock supported by Gram negative bacteriemia associated with lesions of soft tissues and oral recurrent aphthae. Serum folate levels were undetectable (<1ng/mL). After the oral administration of folinic acid, blood folate levels were still under cut-off. Continued treatment with intravenous folate restored the normal plasma levels and resulted in an increased folate levels in the cerebrospinal fluid. Based on these findings, the clinical disorder of the patient was suspected to be dependent on the hereditary failure of folates absorption.

Methods: Blood samples were obtained from the child and her parents. Genomic DNA was isolated from peripheral blood. All exons and flanking splice sites of the *SLC46A1* gene were amplified using new primers set, not reported in literature, and were DNA screened mutations by direct sequencing.

Results: Genetic analysis revealed that this patient have homozygous frameshift mutation (c.194dupG) of the proton-coupled folate transporter (*PCFT*) gene, resulting in a truncated protein (p.Cys66LeufsX99) lacking 296 amino acids from C-terminus. The same mutation was found in her parents who resulted heterozygotes.

Discussion: p.Cys66LeufsX99 was previously reported in the literature in HFM disease, an extremely rare disorder with less than 30 cases reported in the world. This is the first case described in Italy. The baby's parents were non-consaguineous. It is still unknown if this rare mutation is specific of a restricted geographic area or is homogenously distributed in the Italian population. In particular, this mutation was found in a very small town of Lazio Region, named Itri, a marshy region with history of endemic malaria. We cannot exclude a genetic drift on the Itri population due to possible environmental selection *SLC46A1* carriers by malaria infection. In this case, the restoring of normal blood folate levels only after intravenous treatment might suggest that two different way of absorption may exist: one at gastrointestinal level (via proton-coupled folate transporter) and another at peripheral levels, as also demonstrated by the increments in folate concentration in cerebrospinal fluid. As future perspective, we will organize a screening in Itri community in order to establish the prevalence of this mutation in this town and to organize program of health prevention regarding this disease.

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SALIVARY METABOLOME: A NEW APPROACH TO EVALUATION OF SPORT PERFORMANCE

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Many studies have been done to evaluate physical performance in team sports like soccer, basketball, etc all of them involving intermittent demanding physical exercises.

In these sports the ability to perform intensive exercise after short recovery periods appears to be decisive for the outcome of competition. Yo-Yo Intermittent Recovery test is a physical test able to evaluate athletes' ability to repeatedly perform high intensity exercise (Bangsbo J et al., 2008). Several studies demonstrated that this test may represent a measure of match-related physical performance (Krustrup P et al., 2003). Numerous papers have described the use of saliva for analytical purposes in clinical investigations and in physiological research, since it was shown to reflect endogenous biochemical changes (Bertram HC et al., 2009; Takeda I et al., 2009), while it can be easily sampled and thus promptly analyzed. The aim of this study was to examine salivary metabolic variations in professional sub-elite soccer players performing Yo-Yo level 1 intermittent recovery test, to investigate biochemical pathways involved in athletic performance and possible clusters of metabolites reflecting physical test results or the particular player's field role. Fourteen sub-elite professional soccer players from an Italian Lega Pro (C1) team participated to the study. The profiling of saliva metabolites concentration levels before and after the stressful physical activity was developed through Nuclear Magnetic Resonance spectroscopy, and the meaningful signal variations identified. NMR analysis allows a specific and simultaneous determination of a high number of metabolites while maximizing the chance for identifying important, but unexpected or previously unknown metabolites. The corresponding metabolites were identified and quantified, and further clustered by mean of the Principal Component Analysis (PCA) performed on the raw large data matrix collected. As a result, determinant metabolites able to differentiate pre and post exercise samples were urea, glucose, glycerol, lactate, citrate, acetate, glutamate, leucine, lysine, alanine. A specific metabolic cluster (comprising tyrosine, inositol, creatine, lysine, citrate, glucose, acetate, arginine, lactate, glutamate) was also able to discriminate best and worst performing athletes when NMR data of the pre/post were analyzed with respect to the distance outcome of the physical test. A further PCA analysis finally evidenced how the player's role on field could be identified by a different cluster of metabolites (namely glucose, citrate, acetate, leucine, ornithine, myo-inositol, lactate, glutamate). We thus demonstrated that saliva is a biofluid sensitive to the induced physical stress and as such may be of some utility to biochemical investigations in sport in general.

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BIOLOGICAL VARIATION OF NEUROENDOCRINE TUMOR MARKERS CHROMOGRANIN A AND NEURON-SPECIFIC ENOLASE

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Background. Chromogranin A (CgA) and neuron-specific enolase (NSE) are biomarkers for neuroendocrine tumors. Although the knowledge of their biological variation (BV) is critical, there is only one study on CgA BV (1), whereas no data are available for NSE. Therefore, we assessed BV components of these biomarkers in the same cohort of subjects by an accurately experimental protocol.
Methods. We collected five blood specimens from each of 22 healthy volunteers (10 men and 12 women, 23–54 years) on the same day every two weeks for two months. Particular attention was paid to pre-analytical sources of variability. Serum specimens were stored at –80°C until analysis and analyzed in a single run in duplicate. CgA and NSE were determined on ThermoFisher Kryptor® analyzer using Brahms immunoassay and Roche Modular Analytics EVO platform by an electrochemiluminescent immunoassay, respectively. Each serum aliquot was checked by estimating hemolysis index (HI) to exclude significant interference by erythrocyte NSE. Cochran's test was performed for outlier identification among observations and within-subject variances, whereas Reed's criterion was used for identification of outliers among mean values of subjects. A Shapiro-Wilk test was applied separately to the set of results from each individual to check data distribution. Data were analyzed by the ANOVA (2).
Results. No sample was found affected by visible hemolysis (mean HI ± SD = 9.8±4.9). After outlier exclusion, the suitable subjects for BV estimate were 21 (10 men, 11 women) for CgA and 21 (9 men, 12 women) for NSE, respectively. The Shapiro-Wilk test accepted the hypothesis of normality in a substantial proportion of subjects (86% for CgA and 91% for NSE), making the parametric statistical model appropriate for the estimation of variance components. Serum CgA concentrations were significantly higher for women than for men (P=0.01), whereas no difference was found for NSE. Intra-individual variance was not different between genders for both biomarkers. Within- and between-subject CVs were 16.3% and 33.5% for CgA and 13.6% and 11.5% for NSE, respectively. CgA showed marked individuality (index of individuality (II) = 0.24), suggesting that the use of population-based reference limits is inadequate for its interpretation. Conversely, the low individuality of NSE (II=1.44) allows the use of a single reference interval for this marker. Reference change values were 46% for CgA and 39% for NSE. Desirable analytical goals for imprecision, bias, and total error were <8.2%, ±9.3% and ±22.8% for CgA, and <6.8%, ±4.5% and ±15.7% for NSE, respectively.
Conclusions. We defined BV components of serum CgA and NSE and derived indices that may improve the clinical use of these biomarkers. The high individuality of CgA exposes the flaw in the argument that this marker should be utilized to diagnose neuroendrocrine tumors. CgA may be a useful adjunct only in monitoring neuroendrocrine tumors, where RCV and results of recent analyses for an individual are used as a pathology guide in that individual. On the other hand, for NSE, population-based reference intervals may be of value in assessing patients' results.

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PRE-ANALYTICAL STABILITY OF THE PLASMA PROTEOME

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Various pre-analytical conditions could have a different influence on the stability of the plasma proteomes and on the quality of the results in many proteomics analysis, especially in proteome research involving biomarkers discovery. Plasma protein profiles that are reported by various laboratories are often variable, reflecting different analytical methods and sample preparation processes [1,2,3,4]. In this context it is very important to establish a standard protocol to monitor the stability of human plasma samples upon storage. In this study we used 2D electrophoresis and MALDI-TOF MS analysis to evaluate the effect of storage temperature and of a few freeze-thaw cycles on the protein profile of the human plasma. The proteome comparisons was based on the difference in the spot intensity values between plasma samples stored at different storage conditions. The monitoring of the changes in protein spot intensity values gives a comprehensive image regarding the degradation processes taking place in plasma samples during storage. We investigated the plasma protein profile including medium and high molecular weight proteins (11 to 200KDa) using the modified TCA (trichloroacetic acid/acetone) proteins precipitation method [5]. Whole blood was collected from 6 healthy donors (3 male, 3 female, age 23 to 40 yr) by venipuncture. To evaluate the effects of different storage temperatures one aliquot of each plasma sample was immediately analyzed; the other aliquots were stored at –80°C, –20°C, +4°C and room temperature (20 to 25°C) and analyzed after 13 days. To evaluate the effects of a few freeze/thaw cycles one aliquot of each plasma sample was analyzed in the same day of which blood samples were collected; the remnants aliquots were immediately stored at -80°C for 12 and 28 days. All plasma samples were taken through zero, one and two cycles of freeze-thawing at -80°C, followed by proteome profiling. Statistical significance of spot intensity in 2-DE was determined using Wilcoxon paired sample test; values of $p < 0.05$ were considered significant. We observed that various plasma proteins were differently affected by proteolysis or other modifications, particularly in plasma stored at +4°C. Of the 24 plasma proteins identified, proteins as alpha-1-antitrypsin and haptoglobin seemed to be the labile at the storage temperature, while antithrombin-III seemed to be stabile at high temperature showing a change only at room temperature. We found that upon two freeze-thawing cycles the number of spots changed significantly, representing the 54% of the total significant spots. This study demonstrate modification or degradation of the plasma proteins as a result of a few freeze-thaw cycles, in particular upon just two cycles performed within 16 days. Our results suggest that when plasma samples can't be analyzed immediately they should be stored at -80°C for a short-time before proteomic analysis. These findings may assist in developing a standard protocol to monitor specimen storage conditions for newly identified plasma proteins.

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ELEVATED CEREBROSPINAL FLUID AND PLASMA HOMOCYSTEINE LEVELS IN ALS

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Background: Numerous recent evidence suggests that homocysteine (HC), a putative risk factor for stroke and coronary artery disease [1,2], could play a role in the physiopathology of several neurodegenerative disorders, such as Alzheimer's Parkinson's diseases and amyotrophic lateral sclerosis (ALS) [3,4,5]. HC, an aminoacid involved in the methionine metabolism, acts as a neurotoxin through several mechanisms, including free radicals and cytosolic accumulation, mitochondrial dysfunctions, activation of apoptotic pathways, and excitotoxic aminoacid-mediated damage [5]. A recent report showed that plasma HC levels were significantly elevated in ALS, and in particular in those patients with a faster progression of the disease, suggesting that this endogenous molecule might represent a marker of neurodegeneration in this devastating motor neuron disorder [5].
Objectives: Aim of the study was to assay the CSF and plasma levels of HC in ALS patients and controls, and to evaluate the relationship between HC levels and clinical variables of the disease.
Methods: Cerebrospinal fluid from sixty-nine (♂45, ♀24) and plasma from sixty-five ALS patients (♂42, ♀23) were taken and stored at -80° C until use. Controls (CSF = 55; plasma = 67) were patients admitted to our hospital for neurological disorders with no known relationship to HC changes. CSF and plasma from ALS patients and controls were obtained as a necessary step of the diagnostic workup. HC levels in CSF and plasma were assayed using a high performance liquid chromatograph (HPLC) and a fluorimetric detector.
Results: The median level of total HC in the CSF of ALS patients was 0.46 microM, significantly higher than that of the controls (0.24 microM, +91.6%, $P < 0.001$). A similar trend was observed when HC was assayed in plasma (ALS, 12.4 microM vs. controls, 7.26 lM, +70.8%, $P < 0.001$). The CSF and plasma HC levels showed no relationship with the disease progression, age at onset, and the site of onset.
Conclusions: CSF and plasma homocysteine levels were significantly increased in patients with ALS compared with controls. This enhancement seems to be independent of the vitamin levels. Our data suggest that homocysteine might represent a biochemical marker in ALS, and it might be related to the pathophysiology of the disease.

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EVALUATION OF THE EFFECTS OF A LONG-TERM COMBINED ANTIRETROVAL THERAPY ON THE PRODUCTION OF NEW T AND B LYMPHOCYTES IN HIV-1 INFECTED PATIENTS

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HIV-1 infection, besides causing a loss of $CD4^+$ lymphocytes, determines other alterations in the immune system, including an impairment of B lymphocytes and antibody production (1). Although the combined antiretroviral therapy (cART) is very effective in suppressing the viral load, it cannot completely restore the equilibrium of the immune system. Since an efficient immune reconstitution relies on the continuous generation of new T and B lymphocytes, it should not be monitored only by counting the total number of $CD4^+$ cells, also because this measure includes exhausted lymphocytes that cannot efficiently protect from future infections. Therefore, to find improved methods for a routine assessment of the immune system recovery, we employed a recently-developed assay (2) allowing the simultaneous quantification of both new T- and B-cell production, to analyse multiple samples obtained from 36 HIV-1 infected patients who were followed-up for 72 months after the start of cART. The assay is based on the absolute quantification, by Real Time PCR, of the T-cell receptor excision circles (TRECs) and of the K-deleting recombination excision circles (KRECs), which are episomes of DNA originating, respectively, during the T- and B-cell receptor gene rearrangements. As episomes, they cannot be replicated by the cell division machinery and therefore their number in the peripheral blood lymphocytes is considered a reliable marker of thymic and bone marrow output. The measures of TRECs and KRECs in long-term cART-treated patients were compared to those of 72 healthy donors and of 22 HIV-1 infected subjects not needing therapy. A cross-sectional six-color flow cytometry analysis was also performed at the end of the follow-up to quantify the effects of the long-term cART on the T- and B-cell subpopulations. We found that the two markers of T- and B-cell reconstitution followed opposite patterns: TREC production, which was impaired before therapy, quickly increased 6 months after the start of cART, particularly in patients with the lowest baseline CD4 counts, but did not reach that of untreated patients, who showed a thymic output comparable to that of healthy donors; on the contrary, KREC output, which was not reduced before treatment, significantly decreased after 12 months of cART. These alterations were mirrored by the reduced proportion of $CD31^+$ recent thymic emigrant T cells and of $CD10^+$ immature B cells, which are the cells likely to contain, respectively, more TRECs and KRECs. Multivariable regression demonstrated that the main clinical feature associated to these cART-induced changes was a longer pre-therapy disease duration, which predicted a minor TREC increase and a major KREC decrease. In conclusion, our Real-Time PCR assay for the simultaneous TREC/KRECs quantification is a new opportunity for a routine laboratory

setting to monitor the effects of current and future antiretroviral therapies on the composition and homeostasis of the immune system.

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USE AND MISUSE OF BASIC STATISTICAL METHODS

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Misuse of statistics in scientific publications is a major issue since it could deeply and negatively influence medical research and clinical practice. Errors may be due to lack of competence, negligence and in some cases intentional deception. In these last years, great efforts have been made by many journal editors to improve the quality of statistics of published manuscripts by adopting new guidelines for authors and reviewers or introducing a statistical Editor. Statistical pitfalls can affect all phases of a study, from design and data collection to data analysis, result interpretation and reporting. In particular, many common errors are mainly related to basic graphical and statistical methods. There are numerous ways in which a misleading graph may be constructed and presented: excessive usage of graphical elements, biased labelling, distorted effect of 3D perspective, improper scaling, truncated graph, no scale, data omission, improper extraction or categories not listed in the logical order. On the other hand, even descriptive statistics is often improperly used, such as when giving standard error instead of standard deviation or when using mean (and standard deviation) to describe highly skewed non-normal data. Among different statistical errors related to basic data analysis (such as the use of wrong statistical tests or inflation of Type I error), correlation is one of the most misused, misinterpreted and misreported method: such situations include attempting to correlate two variables where the former is a part and the latter represents the total, when correlation is used to study the relation between an initial measurement and the change in that measurement over time, when correlation is interpreted as causal relationship or when correlation is used to judge the agreement between two analytical methods. Additional problems also arise when results are misinterpreted ("non significant" interpreted as "no effect", confounding factors or potential bias not considered) or poorly reported (no confidence intervals given but only p-values). Moreover, despite clearly stated in the author guidelines of many journals, statistical methods are often not reported, preventing the reader from correctly interpreting the results. In conclusion, being aware of possible pitfalls in statistics will help in effectively presenting own data and detecting deliberate or unintentional errors in published works.

LABORATORY TESTING FOR HEPARIN INDUCED THROMBOCYTOPENIA

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HIT (Heparin Induced Thrombocytopenia) is an adverse immunomediated drug reaction that is associated with a high risk of venous and arterial thrombosis. Heparin exposure leads to the formation of IgG antibodies that recognize multimolecular complexes of platelet factor 4 (PF4) and heparin that form on the surface of platelets. These complexes bind to the FcγIIa receptors of platelets, resulting in platelet activation and release of procoagulant microparticles. The end result is increased generation of thrombin and the formation of venous and arterial thromboses that are the clinical hallmark of HIT. HIT is recognized as a clinicopathologic syndrome because diagnosis is based on the combination of a compatible clinical picture and the presence of platelet-activating anti-PF4 antibodies. Clinical prediction rules to assist physicians with determining the probability that a patient has HIT have been developed, the best studied of which is the "4Ts" score. Clinical assessment plays an essential role in the diagnosis of HIT because there is commonly a delay before the results of laboratory testing for HIT are available (management decisions must be made immediately), in addition isolated HIT antibodies are both frequent and not diagnostic of HIT. As clinical diagnosis of HIT is difficult, readily available laboratory confirmation of diagnosis is highly desirable, and sensitive and specific assays are needed. Unfortunately, no single laboratory test has 100% sensitivity and specificity; therefore, the results of laboratory tests should be interpreted in the appropriate clinical context. Functional and immunologic assays for PF4-heparin antibodies are available. Functional assays, including serotonin release assay (SRA) and platelet aggregometry (HIPA), evaluate aggregation activity of heparin-dependent antibodies after incubation of platelets of healthy donors with patient's serum and heparin. The highly sensitive and specific SRA is still considered to be the gold-standard. Unfortunately, it is laborious and it requires radioisotope and technical expertise. Most clinical centers use commercially available ELISAs because they do not have these limitations. The primary drawback of the ELISAs is their potential to overdiagnose HIT by detecting antibodies that are not pathogenic. ELISAs that only detect IgG antibodies appear to have better specificity for HIT. New commercial antigen assays that have a faster turnaround time than the ELISA (< 15 min) are now used. They include: a) ID-PaGIA Heparin/PF4 antibody test, a gel centrifugation assay; b) two new fully automated quantitative chemiluminescent immunoassays, the AcuStar HIT-IgG (PF4-H), specific for IgG anti-PF4/H antibodies and the AcuStar HIT-Ab(PF4-H), detecting IgG, IgM and IgA anti-PF4/H antibodies; c) a lateral-flow immunoassay (LFI-HIT) based on the capillary action; d) a fully automated immunoturbidimetric assay (HIT-Ab(PF4-H)) focused on ACL TOP family coagulometers. Further prospective clinical studies are recommended to evaluate if the high predictive negative value of these new assays for HIT can be improved when used in conjunction with the pretest probability.

UNITY ALERT: A USEFUL TOOL TO SUPERVISE ANALYTICAL QUALITY IN REAL-TIME

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Extensive use of automation in the clinical laboratory creates the potential for systematic errors that affect a large number of patient results before the error is discovered, and the laboratories use a portfolio of "controls" or "good laboratory practices" to prevent errors and ensure the integrity of laboratory processes and the accuracy of test results. These practices range from contemporaneous quality control testing to periodic employee competency assessment and from validation of new test methods to external proficiency testing and on-site laboratory inspection.

Therefore, even if the adoption of systems that automate most of the manual tasks characterizing routine activities, has significantly improved the quality of laboratory performance, a continuous monitoring of analytical quality seems to be mandatory in order to assure high level of performance as well as a timely identification of the analytical errors.
An interesting tool, Unity Alert (Bio-Rad Laboratories), that allow the remote monitoring of the internal quality control results as well as the opportunities to receive remote informations about the outliers for all o for selected tests, choosed on the basis of the analytical or clinical criticisms will de described. This support, part of the Unity Real Time, a software widely diffuse in clinical laboratory in order to manage the internal quality control procedures, seems to be particularly useful in a complex organizational setting. In our department, this software as well as the "third part" of the control materials has been implemented in the six laboratories, part of the department, allowing that acceptability rules, analytical goals and comparison of method performance should be shared between all personnel. Furthermore, the implemented system and particularly the Unity Alert sended automatically to the supervisor of each laboratory when an outlier has been produced in internal quality control for a selected critical test, such as troponin assay, provides an efficient tool to develop a trustworthy and timely supervised system for the analytical quality management.

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CLINICAL APPROACH TO INFLAMMATORY BOWEL DISEASE

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Crohn's disease (CD) and ulcerative colitis (UC) are chronic relapsing inflammatory bowel disease (IBD) whose aetiology is still not completely understood. Their pathogenesis lies in a dysregulation of the immune tolerance towards components of the intestinal microbiota in genetically susceptible individuals. Typical symptoms include abdominal pain, diarrhoea, rectal bleeding and weight loss, as well as extraintestinal manifestations. The diagnosis is based on a combination of clinical history and physical examination together with laboratory, endoscopic, histologic and radiographic investigations. The correct diagnosis is crucial for its implications in selecting both the appropriate medical treatment and the timing or type of surgery if required. Measurement of inflammatory or genetic biomarkers can help to differentiate IBD *versus* non-IBD and, moreover, CD *versus* UC in doubt cases, to predict disease course and its prognosis and treatment response. Several serologic markers have been investigated, such as anti-neutrophil cytoplasmic antibodies (ANCA) and anti-*Saccharomyces cerevisiae* antibodies (ASCA). ANCA are more frequently increased in UC, whereas elevated levels of ASCA are most likely found in CD. Three less applied further markers due to an unbalanced immune response to bacteria include antibodies to the *Escherichia coli* outer-membrane porin C (OmpC), *Pseudomonas fluorescens* CD-related protein (I2), and CBir1 flagellin. These latter are associated with an early onset of CD, fibro-stenosing and penetrating phenotype, and a premature bowel surgery. Additional biomarkers commonly used in clinical practice are faecal calprotectin and lactoferrin, which are very useful in the initial diagnostic work-up to determine whether a patient should undergo endoscopy. Moreover, increased levels of these faecal markers mirror mucosal inflammation in established IBD patients and are related to concurrent endoscopic active disease, as a consequence, they are now emerging as possible markers of mucosal healing. The S100A12 protein, a pro-inflammatory protein, not yet commercially available, may be considered a new marker of disease activity since it is strongly expressed in inflammed tissues of IBD patients. The acute phase C-reactive protein (CRP) is widely used to assess a patient's risk of relapse but it seems less accurate in UC than CD, except for acute severe colitis. Finally, genetic markers are used in clinical trials since no one is associated with disease risk to justify its routine use. NOD2 polymorphism has been associated with an early and more aggressive clinical course of CD and might be useful to predict anti-TNF responsiveness. Polymorphisms in multi-drug resistant 1 (*MDR1*), TNF and migration inhibitory factor genes are related to corticosteroid refractoriness in both CD and UC, and cyclosporine failure in those patients with steroid-resistant UC. In conclusion, it is arguable that in the next future, a combination of inflammatory and genetic biomarkers should help in the management of IBD and in tailoring the appropriate treatment to the single patient.

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THE PORTFOLIO OF ACTING OUT ROLES WITHIN THE HEALTHCARE

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The physician's and lab director's perspective on their role in managing the laboratory activity can be classified in four main behaviours: 1) clinically oriented, with focus on diagnostic value of laboratory information for the patients; 2) Super technologist, focused on the choose of analytical, instrumental and I.T. technology; 3) manager, that consider his main role the organization of personnel, logistic, and processes; 4) Clinical governance, oriented towards the integration of lab with the hospital and the health system, planning screening and clinical pathways to improve the quality of patients' care.

A CERSAS1 (Research Centre for Managing of Health and Social Care-Bocconi University of Milano) research showed that the clinically oriented behaviour is the less frequent role felt by Italian Lab Directors.
The portfolio of roles played by physicians in a clinically oriented lab was investigated by an interview study in Vicenza, San Bortolo Hospital. In the last years the main activities acted out to improve the clinical role of the lab were: to negotiate with clinics and wards new tests' panels for the hospital admission; to insert many of interpretative comments the results of the laboratory tests; to introduce reflex tests to achieve the diagnosis without further test requests; to improve the information given to the laboratory customers, family and clinical physicians; to improve the on line communication of lab reports and results. The common thread among these roles is communication. Thinking as clinicians for a lab physician or director seems to mean speak with your clinician colleagues. Not always this communication achieves the desired goals. The feedbacks from clinicians are rare; the biologists meet some difficulties in the relationship with ward's doctors about clinical problems; the hospital direction does not hamper this clinical approach, but in the same time does not care A clear and strength mission of the staff associated with the leadership of the director seem to be essential elements to maintain this clinical role, which requires more work but can really improve the patient care.

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FREELITE ASSAY: WHAT ORGANIZATIONAL REDESIGN IN THE LABORATORY MEDICINE?

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The International Myeloma Working Group has provided consensus guidelines for the use of immunoglobulin free light chains determination in the diagnosis and management of clonal plasma cell disorders and emphasises their important role in today's myeloma diagnostics (1).
In the Department of Laboratory Medicine of the University-Hospital of Padova, a nephelometric assay (Freelite™, The Binding Site Ltd) has been introduced in the clinical practice, after an accurate evaluation of the analytical performance, particularly lot to lot variation and patient case specific performance data, as well as the verification of the reference interval (2).
According to the clinicians requests, and the need to rationalize the biochemical evaluation in the diagnosis and monitoring of patients with suspected monoclonal gammopathies, an accurate and critical examination of the biochemical algorithm has been carried out in order to reassess the repertoire of tests included in the panel.
Traditionally, in our laboratory, protein electrophoresis, immunosubtraction/immunofixation electrophoresis of both serum and urine, as well as the quantitative evaluation of serum total immunoglobulins, total serum light chains (k and Lambda) as well as urinary free-light chains, represent the panel provided on the basis of the clinical needs and different plasma cell disorders. The incorporation of serum free light chains assays into the screening panels however, force us to evaluate the benefit of the various combinations of the proposed tests in our clinical setting (3), being well documented that no single clinical laboratory test has sufficient sensitivity for the spectrum of plasma cell proliferative disorders but some of the proposed tests, in our panel, may provide similar and/or redundant informations. An organizational and economical benefit without any reduction in the sensitivity and specificity provided by the biochemical informations for these specific diagnostic topics, have represented the aim of the revised algorithm.

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IMMUNOGLOBULIN FREE LIGHT CHAINS: EXPERIENCES AND ASSESSMENTS

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B cell clones, either malignant or benign, normally produce high amounts of monoclonal immunoglobulins (paraproteins). Paraproteins are made up of intact immunoglobulins, single light chains (Free Ig Light Chains – FLC), or more rarely, single heavy chains (1).
The Free Ig Light Chains, kappa or lambda, have been considered for a long time as a byproduct of plasma cells but recent published data indicate that FLCs may account for some specific functions during immune response (2). An abnormal free light chain ratio (FLCR) has proven to be predictive for progression of MGUS, solitary plasmacytoma of bone, amyloidosis, multiple myeloma (MM), Waldenstrom's macroglobulinemia, and smouldering MM (3). In the present study we combine different FLCs evaluation protocols: the Binding Site FLCs nephelometric assay, the Siemens N-latex nephelometric assay and "in gel" densitometric analysis.
We selected 13 patients (8 with High Sereum levels of Free Kappa Chains and 5 with High Serum levels of Free Lambda Chains). The serum absolute values of the involved paraproteins were more than 500mg/L. After nephelometric quantification, to separate the 25 kD bands, serum samples were processed for SDS-polyacrilammide gel electrophoresis under reducing conditions. Densitometric analysis was performed with the Gene Tools Program.
Our findings show that both nephelometric assays behave in the same way in 11 patients with comparable values. In one lambda patient we saw a N-latex overestimation and in one kappa patient we saw a FLCs Binding Site overestimation. We conclude that FLCs monomer quantification is feasible with nephelometric assays and is acknowledgeable with our rapid SDS-PAGE analysis.

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INTEGRATIVE MODELS BETWEEN CLINICAL BIOCHEMISTRY AND CLINICAL MOLECULAR BIOLOGY

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In the field of clinical molecular diagnostics and biomarker finding there are continuously advances in set-up of molecular test aimed to detect diseases in early stages or to define personal disease risk for several pathological conditions.The progress in biomarker technology avaliability, along with the pressure of market by companion producing molecuar tests, are stimulating not only the free access to genetic testing but also the emergence of new laboratories with high throughput technologies. In this regard, the future challenge of clinical diagnostics will be the represented by a) the safe translation into an individualized panel of trategies for diagnosis and treatment b) the administration to each individual personalized standard of care.
In this context, some efforts will be done from laboratory professionals and scientists in order to reduce the use of not effective tests and to armonize procedures for test administration, both at clinical and at technological levels. In fact, although the introduction in diagnostics of recent platforms based on Next Generation Sequencing (NGS) have introduced some novelties in the capability to offer genetic and molecular tests to consumers and patients, the modality of use of these platforms in clinical molecular diagnostics is still heterogeneous. In addition, the classical layout of laboratory of clinical molecular diagnostics is not longer adequate and it will rapidly change, since infrastructure aspects are really important when these NGS platforms are employed: this type of concerns regards not only the working areas of laboratory but also the personnel qualification and training, the latter being the main criticism when molecular tests are considered.
Regarding the integration models in molecular diagnostics, HLA typing for coeliac risk assesment may represent a perfect example of synergy between clinical biochemisty and clinical molecular diagnostics.
The present communication will cover the peculiar aspects of organization, technical equipment, staff training and integration of molecular diagnostics Units in the context of a integrated patient-oriented laboratory medicine.

THE KNOWLEDGE MANAGEMENT IN THE "MIULLI" HOSPITAL

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Introduction: Knowledge Management (KM) is used in the healthcare to facilitate the know-how administration and the circulation of information among the various professionals that make up the healthcare organizations. Since learning involves both assimilation and production of new knowledge, the training of the healthcare personnel, through new technologies (e learning), is essential for the KM.
In the "Miulli" Hospital the KM group has been founded for 2 years. It consists of professionals from different areas of operation, after a training course on "Learning Organization", it has created within the hospital, a space for reflection and discussion on the reality of the workplace and on the management of knowledge in various professional contexts. Objectives: circulating knowledge and making information and experience available, gathering and evaluating the feedback coming from the operators to foster networks of reflexivity and analysis tools; increasing forms of communication among the different stakeholders (management, departments, services, professional categories); identifying strategies to interpret reality and promote a culture focused on innovation and change.
Methods and Tools: out-door training, event management, creation of a corporate blog, news-letter, audit of knowledge and self-learning, focus groups, team building, motivational questionnaires and tests. Results: The study was conduct in 11 departments / services (45% of the personnel), the results highlight the need to acquire more knowledge and expertise concerning the organization of work (29%), corporate wellness (19%), ethics and care values (21%), technical knowledge (12%), work motivation (19%). Conclusions: the conclusions regard the needing of teachers with educational role as innovation strategy in healthcare organizations. The results of the analysis highlight the importance of a continuing education and the skills development specifically targeted to emotional-relational, technical, professional, organizational and procedural areas, which are able to develop and maintain the quality of the results.

ORGANIZATIONAL WELL-BEING IN A BLOOD BANK

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Introduction:
People are a valuable resource for a company, so it is important to pay attention to the nature and context of the job, relationship between different individuals and colleagues, highlighting the strengths that influence professional development and welfare of all workers.
It is well established in the literature that the collective productivity is strongly related to organizational welfare. It is therefore necessary to use instruments that can measure this aspect.
Methods:
The detection of the organizational climate has been completed in our unit in December 2011, using an instrument defined as "checklist on work-related stress for small businesses", by Nardella, Deitinger, Aiello. It allowed us to investigate the dimensions of organizational culture(?), workload, relationship and support of the Division. The questionnaire allows us to identify a score for each aspect, leading to an overall assessment of health status of the service. 34 questionnaires were distributed in a completely anonymous form and the same number has been collected later, correctly completed.
Results:
The statistical analysis of the raw data, with subsequent transformation into T points, gave the following results: organizational culture average 11.74 (51.99 T), relations and support average 3.35 (T 50.69) workload average 6.50 (T 57.88). The first two scores place our service in an intermediate situation while the result concerning the workload put the service at the beginning of a problematic situation.

Conclusions:
On the basis on the obtained results and to solve the emerging problems, medium and long term interventions were planned, such as the reduction of the active presence on service giving priority to ready availability, the improvement of communication and the introduction of a training schedule. In such a way the measure and analysis of the indoor climate can become a valuable tool for clinical governance.

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DIAGNOSING AMYLOIDOSIS: SENSITIVE TOOLS AND ALERT PHYSICIANS

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Plasma cell clones can give rise to disorders related to tumor burden, such as multiple myeloma and Waldenström macroglobulinemia, as well as to other more subtle and deceptive diseases, such as AL amyloidosis. In this disorder the clone is usually small in size and produces a monoclonal light chain that causes multiorgan damage (1). Here we report two cases of AL amyloidosis in which the diagnosis required advanced laboratory techniques.
A 68 year old man was admitted to the local cardiology unit due to the onset of heart failure. During the previous year he had developed xerostomia and bilateral carpal tunnel syndrome. The electrocardiogram showed no signs of ischemia. Troponin I (cTnI) was constantly elevated (0.07 ng/mL). Echocardiography showed left ventricular hypertrophy. A minor salivary gland biopsy, performed to rule out Sjögren syndrome, showed amyloid deposits. No monoclonal components were detected by serum and urine immunofixation electrophoresis (IFE). A ^{99m}Tc-DPD scintigraphy showed moderate tracer localization in the heart. These findings suggested transthyretin (TTR) amyloidosis (familial or senile) and the patient was referred to our center. By high-resolution IFE no monoclonal protein was detected in serum and a small band formed by k light chains was identified in urine. Circulating κ free light chain were 169 mg/L (κ/λ ratio 21.9), a bone marrow aspirate showed a 6% plasma cell infiltrate, N-terminal pro-natriuretic peptide type-B (NT-proBNP) was 6931 ng/L, cTnI 0.06 ng/mL, there were no signs of renal and liver involvement. The abdominal fat aspirate showed amyloid deposits which reacted with anti-κ light chain antibodies and did not react with anti-λ and anti-TTR antibodies at immuno electron microscopy. No mutations were found in the TTR gene.
A 53 year old woman had complained of dyspepsia and abdominal discomfort with weight loss (6 Kg) for 3 years before being admitted to the local hospital, where massive hepatomegaly was detected. A liver biopsy was performed that showed amyloid deposits. No monoclonal components were found by serum and urine IFE, and the bone marrow plasma cell infiltrate was 3%. The patient was referred to our center for further testing. By high-resolution IFE a faint κ band was detected in the urine. Circulating κ free light chain concentration was 612 mg/L (κ/λ ratio 77.3), albuminuria was 2.2 g/24h, alkaline phosphatase was 533 U/L (upper reference limit 150 U/L). There was no sign of heart involvement. The abdominal fat aspirate showed amyloid deposits which reacted with anti-κ light chain antibodies and did not react with anti-λ and anti-ApoAI antibodies at immuno electron microscopy.
In both cases a diagnosis of AL amyloidosis was made and chemotherapy was initiated. In these patients, only the combination of advanced laboratory techniques could identify the amyloidogenic monoclonal light chain and demonstrate its etiologic role. The increasing availability of the FLC measurement will facilitate the diagnosis, but difficult cases should be promptly referred to specialized centers for amyloid typing.

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MYOCARDIAL INJURY FOLLOWING CARBON MONOXIDE POISONING

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This report describes a 46-year-old white man who was sent to the emergency department with neurologic deficits and high suspicion of carbon monoxide poisoning. Serum carboxyhemoglobin (COHb) level was found to be 18%. Clinical symptoms of myocardial infarction were absent and his medical profile was negative for risk factors of coronary heart disease. The electrocardiogram and cardiac biochemical markers were consistent with an acute coronary syndrome and the echocardiogram showed hypokinesia of left ventricular apical lateral wall. The coronary angiogram performed one week after admission failed to reveal evidence of coronary obstructive lesions. It is hence assumed that COHb (following carbon monoxide exposure) may trigger myocardial infarction by severe generalized tissue hypoxia (impaired oxygen delivery) and a direct toxic effect on the myocardial mitochondria. Contributing factors that might also decrease myocardial oxygenation include inadequate myocardial perfusion and increased thrombotic tendency.

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THE ROLE OF INTERLEUKINS IN TOXIC EPIDERMAL NECROLYSIS OR LYELL (TEN)

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Lyell syndrome also known as toxic epidermal necrolysis (TEN) is defined as a pathological condition where there is a nearly total epidermal necrolysis in addition to the interest of the mucous membranes.

Stevens–Johnson syndrome (SJS) and TEN are severe adverse drug reactions, characterized by a low incidence but high mortality, initially described as separate entities, but today considered variants of the same pathologic process and differing only for severity. The majority of cases appear to be related to idiosyncratic drug reactions. The drugs most commonly involved are: antibiotics such as sulfonamides, b-lactam, tetracyclines and quinolones; anticonvulsants such as phenytoin, phenobarbital and carbamazapine; antiretroviral drugs; nonsteroidal anti-inflammatory drugs, allopurinol (1,2). There is common agreement to consider TEN as the manifestation of a disregulated immune reaction against epithelial cells. However, scientific literature reports also different suspected etiologies but these are very rare exceptions; mycoplasma pneumonia infection is a known cause of SJS, and a number of cases of TEN have been reported to complicate infection with this agent (3). There appears to be a genetic predisposition to the development of TEN. Hung et al. reported a strong association between allopurinol and carbamazepine induced SJS or TEN with respectively, HLA-B*5801 and HLA-B*1502 in a Han Chinese population from Taiwan and other Asian countries (4,5). During the first stages of TEN, apoptosis mediates keratinocyte death and the pivotal role of Fas–FasL pathway activation during TEN is undoubted. T cell cytotoxicity, demonstrated during TEN, has been shown to be mediated by the perforin–granzyme pathway. Recently, more attention has been given to the role ofcytokines in TEN pathogenesis. This clinical case refers to an adult female patient hospitalized at the Burns Unit of the SS Annunziata Sassari after taken allopurinol. During hospitalization has been practice therapeutic protocol encoded at the Burns Unit of the SS Annunziata Sassari involving IVIG treatment and plasmapheresis. At the end of therapy was observed a clinical improvement of both the local and global conditions. However, the patient had ocular complications. In this work we examined the 27 cytokines profiles on plasma samples during four different steps of therapy whit Intravenous immunoglobulin (IVIG) and plasmapheresis. Assessment of plasma cytokine concentrations was performed using commercially-available Multiplex bead-based sandwich immunoassays (BIorad). We founded that plasma levels of IL-5, IL-6, IL-7, IL-8, IP-10, IL-1b, IL-10, IFN-G, TNF-α, IL-13 varied significantly in the patient after the completion of the therapy. Our results, although preliminary, suggest that a peculiar cytokine pattern play a pivotal role in TEN pathogenesis; however, the conclusions will be more comprehensive when we have finished the studies still in progress. We can conclude that the IVIG treatment and plasmapheresis correlate to the cytokines plasma profile determination was built on the assumption pathogenesis of TEN increases survival.

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CAUSES OF *IN VIVO* HAEMOLYSIS

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Most haemolytic anaemias (HA) are congenital and are, therefore, diagnosed in infancy, although cases appearing in adulthood are not exceptional. It is usually relatively easy to identify haemolysis as the cause of anaemia: the patient has a low haptoglobin level and increased unconjugated bilirubin and lactase dehydrogenase. In cases of intravascular haemolysis, the onset is acute with haemoglobinuria and haemosiderinuria. Since HA are almost always regenerative, the acute forms are characterized by increased reticulocyte counts. In chronic, extravascular haemolysis, the anaemia may be microcytic (thalassaemias), normocytic (sickle cell disease), with moderate thrombocytopenia (from hypersplenism), and may be completely compensated. The causes of HA are classically divided into intrinsic and extrinsic to the red blood cells (RBC). With the exception of paroxysmal nocturnal haemoglobinuria, which is acquired, the former types of HA are congenital and are due to defects in the RBC membrane (hereditary spherocytosis being the most common), haemoglobin (sickle cell disease, thalassaemias) or RBC metabolism (deficiencies of G6PD and PK). The latter are all acquired disorders. In immune haemolytic anaemias (IHA), IgG and/or IgM antibodies bind to antigens on the RBC surface, triggering destruction of the RBC through activation of complement or the reticulo-endothelial system. IHA are classified as alloimmune, autoimmune or drug-induced and all give a positive direct Coombs' test (DAT). Mechanical haemolysis gives rise to HA with a negative DAT and schistocytes in a peripheral blood smear. Mechanical haemolysis can occur in congenital or acquired thrombotic microangiopathic syndromes, of which the two most important are haemolytic uraemic syndrome and thrombotic thrombocytopenic purpura. The most frequent infectious cause is malaria, although numerous bacteria can induce severe intravascular haemolysis. Haemolysis due to toxins such as copper sulphate and lead is rare. The history, clinical features and a few simple examinations, sometimes in a stepwise diagnostic process, are all essential to confirm or exclude a HA quickly and, if such an anaemia is present, to identify its cause. The clinical context, e.g., recent transfusions, exposure to toxic substances, a journey to an area in which malaria is endemic, thrombocytopenia in the 3rd trimester of pregnancy (HELLP syndrome), can provide useful information. A positive family history of anaemia with haemoglobinuria and bile stones is indicative of congenital RBC disorders. RBC indices are also important. Hypochromic, microcytic RBC suggest a thalassaemic syndrome, whereas an increase in MCHC should raise the suspicion of spherocytosis or IHA. It is essential to examine a peripheral blood smear, which may show the morphological alterations typical of some HA, and perform a DAT, which, if positive, is diagnostic of IHA. More specific tests may be necessary in complex cases.

SOURCES OF IN VITRO HEMOLYSIS

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Hemolysis is traditionally defined as the release of intracellular components of erythrocytes and other blood cells into the extracellular space of blood. The breakdown of red blood cells with subsequent release of hemoglobin and other intracellular contents into the plasma can occur either inside the blood vessels due to pathological

conditions (i.e., “in vivo” hemolysis) or during collection, handling and processing of specimens before the analytical measurements (i.e., “in vitro” hemolysis).
In vivo hemolysis is one of the leading challenges for clinical laboratories, since it is independent from the technique used for collecting blood and is therefore both virtually unavoidable and potentially insurmountable (1). Conversely, in vitro hemolysis depends mainly on the blood collection technique and can also arise from unsuitable inappropriate collection, handling, storage and processing of the specimens. As such, the leading factors that can trigger in vitro hemolysis include anatomical and physiological characteristics, as well as equipment, techniques and skinless used during phlebotomy (2). Nevertheless, the sources of in vitro hemolysis associated with the venipuncture are as yet prevailing (3). Blood forced through a very fine a needle or I.V. catheters frequently produces injury or even breakdown of blood cells, but also unusual location of venipuncture, specific antiseptics used before phlebotomy, long permanence of the tourniquet, both vigorous or no mixing of the primary tubes, tubes under-filled or filled from syringes are important causes (4).
After collection of the blood samples, at least other three preanalytical phases must be carefully managed to prevent deterioration: transport, centrifugation and storage. Transport by courier, especially for long time or under extreme temperature conditions, can damage the cells inside the tubes, up to their rupture. Pre-transport centrifugation also increases the percentage of hemolyzed specimens. For inpatients, the pneumatic tube transport system has also been implicated in vitro hemolysis (5). Critical conditions for centrifugation include the time between collection and processing, extreme conditions of temperatures and speed, poor separator barrier integrity and re-centrifugation. Finally, inappropriate conditions (i.e., time and temperature) of storage can negatively affect the integrity of specimens. As such, the huge number and complexity of all these possible causes of in vitro hemolysis highlight the importance of education and training of the staff with blood collection responsibility.

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ANABOLIC STEROIDS BY LC-MSMS

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Anabolic steroids or “steroids”, technically called anabolic-androgen steroids (AAS), are normally found in plants, animals and fungi. As steroids mimic effects of testosterone and dihydrotestosterone, they are used to increase muscle mass and strength in patients, affected by low levels of testosterone or loss of muscle mass, or as abuse in athletes and animals, altering sport performances. To hinder this relentlessly increasing illegal behavior, known as "doping", high speed, high sensitivity, fully reliable analytical methods are required. Mass spectrometry (MS) responds to these requirements.
In fact, MS has been extensively used in steroid analysis. Gas chromatography is commonly used in urinary or serum matrices in anti doping tests because its high robustness and sensitivity. However, hydrolysis and/or derivatization are adopted as preparation procedures, to enhance volatility and ionization efficiency, increasing sample preparation activities and time.
Faster and simpler preparation process results by coupling Liquid Chromatography (LC) with MS and tandem MS/MS, reducing also sources of errors.
The use of solid-phase extraction for sample preparation permits detection of a wide range of steroids in urine at concentrations, ranging from a few parts per billion (ppb) to a few parts per trillion (ppt).
Atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) are widely accepted ionization interfaces in doping analysis. Despite the introduction of atmospheric pressure photo ionization (APPI), atmospheric pressure laser ionization (APLI) or heated electrospray ionization (HESI), these ionization techniques have not yet found routine applications in doping analysis.
We present several applications of steroids analysis using liquid chromatography, discussing benefits and impacts on procedures in terms of time, effort and cost, and their respondence to speed and sensitiveness requirements of anti doping routines.

EFFECTS OF POLYTHERAPY ON PLASMA LEVELS OF CLOBAZAM AND N-DESMETHYLCLOBAZAM IN EPILEPTIC PATIENTS

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The Clobazam (CLO) is a 1,5-benzodiazepine mainly used in add-on with other anticonvulsant drugs in the treatment of refractory epilepsies. The oral absorption of CLO is rapid and complete; after oral administration the maximum concentration is reached between the first and the fourth hour. It is a highly lipophilic drug and it is mainly metabolized in N-desmethylclobazam or norclobazam (NORCLO), active metabolite, that is present in higher concentrations than parent drug but with an activity of the order of 20 to 40% of that of Clobazam. Adverse reactions are moderated, appearing more often for the highest concentrations; also the phenomenon of tolerance seems more frequent in high concentrations. However, due to the kinetics of interaction, monitoring of this drug is estimated useful. There is no validated therapeutic range, but the usual concentrations are in the range of 100–400 ng/ml for the CLO and about 10 times more for the NORCLO(1).
The aim of our study was to assess how the therapies associated with the CLO affect its metabolism in our patients.
We monitored 100 epileptic patients aged between 2 and 50 years receiving monotherapy or polytherapy with CLO. We estimated the relationship between plasma levels of NORCLO and plasma levels of CLO.
Patients on monotherapy (n.12) had a value of NORCLO/CLO (SD) = 3.1 (2). Other mainly frequent therapies were:
CLO + Valproic Acid (n.30) with NORCLO / CLO = 4.8 (3.9); CLO + Oxcarbazepine (n.12) with NORCLO / CLO = 13.7 (6.3); CLO + Carbamazepine (n.8) with NORCLO / CLO = 19.2 (7.1); CLO

+ Levetiracetam (n.7) with NORCLO / CLO = 4.25 (4.4); CLO+ Topiramate (n.7) with NORCLO / CLO = 4.6 (2.2);
CLO + Valproic Acid + Topiramate (n.12) with NORCLO / CLO = 4.6 (1.4).
The results obtained confirm the activity of metabolic inducer of Carbamazepine and Oxcarbazepine; slightly significant is the influence of Valproic Acid either alone or associated with Topiramate. The antiepileptic drugs recently introduced have an interference with the metabolism of CLO yet less significant. The NORCLO / CLO ratios exstimated in the treatments CLO + Oxcarbazepine and CLO + Carbamazepine show a lower metabolic induction of Oxcarbazepine than Carbamazepine.

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ORAL CONTRACEPTIVE USE INCREASES CHRONIC INFLAMMATION IN YOUNG FEMALE ATHLETES

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BACKGROUND— Chronic activation of innate immune system may play a crucial role in pathophysiology of several diseases including cardiovascular disease (CVD) and type 2 diabetes mellitus. Exercise training has been shown to have anti-inflammatory effects. However, the use of oral contraceptives (OCs) by fertile female athletes has the potential to increase low-grade chronic inflammation [1]. The increase of basal inflammatory status can hamper protective effects of training and can be potentially detrimental to athletic performance, which is commonly associated with inflammatory lesions. There are limited data exploring the effects OC use by athletes. Our aims were to evaluate the impact of OCs currently used by female athletes on levels of hsCRP, triglicerides and cholesterol in a population of young white female athletes.
METHODS AND RESULTS — We compared the association between OC use and hsCRP across 4 groups (OC user athletes, non-OC user athletes, OC user non-athletes, non-OC user non-athletes). A total of 277 young healthy Caucasian Italian women [mean age, 23 years (SD, 5 years); body mass index (BMI), 21 kg/m^2 (SD, 2 kg/m^2] were analyzed. Progressive cutoffs of hsCRP levels were evaluated in OC users (n=77, 27.8%) compared to non-OC users (n=200, 72.2%). Levels of hsCRP at high risk of future cardiovascular events from 3.0 to <10.0 mg/L were found in 27.3% (21/77) of OC users and in 8.5% (17/200) of non-OC users [odds ratio (OR)=4.0, P<0.001]. No differences were observed between athletes and non-athletes.
CONCLUSIONS—OC use markedly increases chronic low-grade inflammatory status in athletes as assessed by the increase of serum hsCRP. Our findings suggest that OC use may elevate CVD risk and predispose to a higher inflammatory response to physical stress and injury.

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CLASSIC IMMUNOASSAY METHODS APPLIED ON "NEW" MATRICES

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The new trend of toxicological analyses forced laboratories to extend drugs of abuse tests on several matrices like blood, hair and saliva. The high volume of tests, in particular as regard to hair analysis, led to apply immunochemical method to this new matrices.
Immunoassay (IA) tests were initially developed for detection of intoxications in clinical cases analyzing urine samples.
Due to different distribution of analytes, lower concentrations and forensic purposes, a complete validation of the immunoassay and an optimization of cutoff values is a must for each laboratory that have to shift from a matrix to another one. At this time we plan to underline, throughout the analysis of the whole validation process, the critical aspects, limits and capabilities of the most widespread methods.

BIOMARKERS OF ALCOHOL CONSUMPTION FOR CLINICAL AND MEDICO-LEGAL PURPOSES

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With the aim set a proper therapy, it is important to recognize the use / abuse of alcoholic beverages. In this concern, a pharmaco-toxicological laboratory should be involved in accurately discriminate a state of acute intoxication by ethyl alcohol from a condition of alcoholism, that is a condition of chronic alcohol abuse. In the first case, the breath analysis carried out by a breathalyzer and eventually followed by the analysis of a blood sample, are today the only analytical methods recognized by the scientific community as able to diagnose a state of psychophysical alteration due to ethyl alcohol consumption. If the use of the breathalyzer is quite simple, the detection of ethanol in blood is quite complex and requires the aid of analytical methods such as chromatography (gas or liquid) coupled to detectors such as mass spectrometry or the ionization flame. The headspace gas chromatography coupled to flame ionization detector is the method of choice to detect alcohol in a blood sample. These methods ensure high diagnostic sensitivity and specificity. Therefore, the analytical result can be subsequently used for administrative or medico-legal purposes.
The recent consumption of ethanol (up to 3–5 days before sampling) can be evaluated through the detection of ethanol itself in the urine and, more specifically, through the detection of its direct non oxidative metabolites: ethyl glucuronide and ethyl sulfate. Indeed both substances have shown to be highly sensitive and specific, discriminating a real alcohol consumption against accidental exposure to other products containing ethyl alcohol.
Finally, a chronic alcohol abuse can be identified in a blood sample through the evaluation of the traditional hepatic markers as well as through the detection of carbohydrate deficient transferrin (CDT, in particular of its desialylated form). However, the detection of ethyl glucuronide or fatty acid ethyl esters are promising biomarkers in disclosing information about alcohol use/abuse in the less recent past (months).

PRENATAL DIAGNOSIS DATA 2004–2009: THE CONTEST OF NATIONAL GUIDELINES

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In Italy since 1998 there is a law (1) about clinical investigation free of charge during pregnancy, also concerning invasive prenatal diagnosis (DPI): fetal karyotype (by chorionic villus or amniotic fluid or fetal blood sampling) is provided by National Health Service for:

1. women of 35 years or older,
2. parents with personal anamnestic findings of chromosomal defects, or a previous affected pregnancy
3. pregnancy with fetal structural anomalies detected by ultrasound examination,
4. pregnancy with a screening test result of risk higher than 1/250 for Down syndrome (DS) at mid trimester.

Italy didn't ever have a national registry of aneuploidies and so it's impossible to evaluate the impact of this law on chromosomal defects occurrence in 560.000 liveborn infants per year.
Italian women are very interested in prenatal testing because their age at pregnancy has shown a constant increase1 (2). In 2004 the median age of Italian mothers was 31,4 years and median age of foreign mothers was 27,6 while in 2009 maternal ages have respectively become 32,3 and 28,3 years; in the same period the group of oldest women (40 years or more) changed from 5,52% to 7,23%. Nevertheless, the DPI tests have shown a decreasing trend: they were 21,5% in 2004 and have become 18,8% in 2009. This is the impact of prenatal screening for DS: more and more women wish a risk evaluation before deciding to undergo a DPI test which can carry some degree of risk for miscarriage or other pregnancy complications.
In fact, even if in Italy there isn't yet a national program of prenatal screening for DS, since 1990 many laboratories can perform biochemical markers (both of first and second trimester of pregnancy) and risk evaluation, and since late '90 several Italian gynaecologists can evaluate fetal nuchal translucency thickness (NT), which is the main ultrasound marker of DS at 11–13 weeks. So, prenatal screening tests have had a large diffusion: along the years the "classic" triple test of the second trimester is going to be replaced by more modern and effective combined test and integrated test. Only some regions, as Tuscany (3) and Piedmont (4), have done efforts to make homogeneous a prenatal pathway free of charge to provide each woman her personal risk and DPI if the risk would result high.
Finally, in November 2010 Italian Ministry of Health published its Guide Lines for Healthy Pregnancy (5): in the chapter about prenatal diagnosis of DS recommendations are given to obtain that during the first contact with prenatal care unit each pregnant woman receives information about

- the main clinic features of DS
- the pathway for its prenatal diagnosis (risk evaluation and DPI if the risk is higher than fixed cut-off
- the possibility to terminate an affected pregnancy (law 194/1978).

Other information must be given about the different types of prenatal screening test (it's recommended the NT measurement at 11–13 weeks and the association of biochemical markers of the first trimester or of first and second ones) and of DPI tests.
[1]All demographic data referred here are collected by a form named CedAP (Certificato di assistenza al parto) which is written out by midwife for each delivery and send to Ministry of Health; the data are available in reports published about three years later. The last report has been diffused in January 2012 and relates to women who delivered in 2009.

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