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Riassunti Sessioni Scientifiche

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

LP1

THE PRESENT AND FUTURE OF LABORATORY MEDICINE

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In order to put the present and future of laboratory medicine in perspective, it is important to see how far we have progressed in the past few decades. There will be a brief discussion, therefore, of the past.

The present focuses on Point-of-Care testing, Evidence Based Laboratory Medicine, Molecular Diagnostics, sophisticated approaches such as Tandem Mass Spectrometry and consolidation of tests on to a single platform. Some of the present, and ever evolving challenges in the United States are short staffing and an aging workforce.

The future will clearly focus much more in Molecular Diagnostics, single cell analysis, such as is currently used for In Vitro Fertilization Laboratories, and multiplex testing using DNA chips. There is also the "omic" revolution which I will discuss, as well as nanotechnology, and the need for personalized and preventive Laboratory Medicine. The increasing use of information technology will have a major impact on our field. The necessity to reduce laboratory errors is and will be of utmost importance.

In addition to all of these factors the challenge for developing countries will be the improvement of analytical quality and transferability of results, as well as the problems of appropriate testing for prevalent diseases such as HIV, malaria, etc.

SS1

HOW TO MANAGE A PATIENT WITH A MONOCLONAL GAMMOPATHY. THE ROLE OF THE SPECIALIST IN INTERNAL MEDICINE

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The prevalence of a monoclonal component (MC) on serum protein electrophoresis increases with advancing age: less than 2% in subjects ≤ 40 years, it rises to 3.2% and 5.3% in those > 50 years and > 70 years respectively, and reaches 7.5% in individuals ≥ 85 years. The lack of signs and symptoms ascribable to immuno- or lymphoproliferative disorders allows to define these abnormalities as "monoclonal gammopathy of undetermined significance" (MGUS), characterized by: a) a serum concentration of the MC < 3 g/dL; b) bone marrow plasmacytosis $< 10\%$; c) the absence of bone lesions, anemia, renal failure and hypercalcemia. Thus, MGUS is mostly diagnosed in asymptomatic subjects.

On the contrary, a MC detected in the serum of a patient with clear, though variable clinical features can be found in: almost all patients with multiple myeloma (MM); patients with Waldenström's macroglobulinemia (WM); those with primary systemic amyloidosis (PSA) and a minority of patients with B-cell non-Hodgkin's lymphoma (NHL) or chronic lymphocytic leukaemia (CLL). The exact diagnosis and the ensuing therapy will obviously depend on clinical features, the possible skeletal involvement, the bone marrow biopsy, the histobiopic examination of lymph nodes or other tissues, the elective staining of amyloid deposition, and so on.

When a final diagnosis of MGUS is made, the patient should be informed that he/she needs no immediate

treatment, though periodic clinical and laboratory controls will be required in view of the potential risk of progression to a frank malignant condition. Among 100 patients with a serum and/or urine MC, MGUS is diagnosed in half of them, MM in 15-20% and a related disease (such as PSA, WM, CLL or NHL) in the remaining 30-35% of cases. The probability that a subject with MGUS can progress to MM or a related malignant condition is 1% per year, and is affected neither by age nor by the duration of the MGUS. A malignant evolution has been reported even after 30 years or more from the diagnosis of MGUS, and this explains why many subjects with MGUS eventually die as a consequence of diseases unrelated to the MG.

The absence of anemia, bone pain, weight loss or neurologic symptoms is a feature non only of the subjects with MGUS, but also of patients with "smoldering" (SMM) myeloma. In SMM, the serum concentration of the MC is ≥ 3 g/dL, bone marrow plasmacytosis is $\geq 10\%$ and the rate of progression to overt myeloma is strikingly higher than in MGUS. The cumulative probability of progression is 51% at 5 years, 66% at 10 years and 73% at 15 years. The extent of bone marrow plasmacytosis and the serum concentration of the MC at diagnosis are considered the most reliable criteria to assess the risk of progression to symptomatic MM. Similarly to the MGUS condition, patients with SMM should not be treated, although they should undergo a strict and closer follow-up because of their higher risk of progression to active MM.

SS2

THE ROLE OF THE CLINICAL CHEMISTRY LABORATORY IN THE DIAGNOSIS AND MANAGEMENT OF MONOCLONAL GAMMAPATHIES

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The clinic chemistry laboratory plays a central role in the diagnosis, in assessing the prognosis and in the follow-up of monoclonal gammopathies. These conditions are usually identified during routine laboratory controls, and in particular cases following clinical indication. The detection of a monoclonal immunoglobulin (MIg) relies on sensitive electrophoresis and immunofixation electrophoresis (IFE) performed on serum and appropriate urine samples. The quantification of serum free immunoglobulin light chains (FLC), by a nephelometric assay, and the kappa/lambda ratio is a useful complementary test in patients who have primary systemic amyloidosis (AL), light chain deposition disease, nonsecretory multiple myeloma, and light chain multiple myeloma. Evidence obtained in our laboratory indicates that the immunofixation of the urine remains necessary despite the availability of the FLC assay. Most of the individuals with a MIg detected by chance have a low-risk monoclonal gammopathy of undetermined significance (MGUS) which has a benign course. These individuals are monitored using few routine laboratory tests. In a small proportion of patients the MIg is the marker of a malignant condition, most frequently multiple myeloma. In these patients the laboratory contribution is essential in staging the disease, through the determination of serum albumin and beta-2-microglobulin, and in assessing the response to therapy. In AL amyloidosis the laboratory plays a fundamental role. The detection of the amyloidogenic light chains requires high resolution IFE of serum and urine associated with the quantification of FLC. The chemical characterization of the tissue amyloid deposits can be achieved using proteomics technology. The assessment of the systemic organ damage caused by amyloidosis is

based on the determination of markers of renal, cardiac, and liver function. In particular, the cardiac biomarkers natriuretic peptide type B (BNP) and troponins are essential for staging the amyloid disease and for assessing the organ response to chemotherapy. The hematologic response to chemotherapy is based on electrophoresis, IFE, and FLC assay. The combined use of FLC and BNP in patients with AL amyloidosis allows a fine titration of chemotherapy, optimizing the benefit/toxicity ratio, thus improving the care of this complex disease.

SS3

MONOCLONAL GAMMOPATHY AND THE KIDNEY

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Monoclonal gammopathy (MG) is a common hematologic entity characterized by abnormal synthesis of immunoglobulin molecules or subunits and frequently is complicated by renal involvement, so much so that it should be considered part of the disease.

The abnormal immunoglobulin molecules or subunits (most commonly free light chains) reach the glomerulus via the systemic circulation and are associated with the development of a variety of pathologic lesions. All the four components of renal parenchyma (glomeruli, tubules, interstitium and blood vessels) may be involved. Free light chain molecules may pass through the glomerular basement membrane and form casts within distal tubular lumina (myeloma cast nephropathy) or form crystals within the cytoplasm of proximal tubules (light chain Fanconi syndrome). Alternatively, immunoglobulin molecules or subunits may form paraprotein tissue deposits and produce an array of pathologic lesions, most commonly amyloidosis and monoclonal immunoglobulin deposition disease. The pattern of renal parenchymal disease is determined by the unique properties of the immunoglobulin molecule or subunit. Each of the patterns of renal disease is in turn associated with unique, but frequently overlapping, clinical features and outcomes.

Renal disease in MG most often presents as renal insufficiency and proteinuria. Occasionally, patients with MG present with renal tubular dysfunction. Renal insufficiency is found in almost 50% of patients and severe renal insufficiency is seen in 15-20% of cases. Although proteinuria is observed in >80% of cases, it most often consists of light chains, and light chains proteinuria can be massive (>10 g/die).

Some pathologic and clinical point should be clarified before considering the individual disease entities. First, the various forms of MG-associated renal diseases may be the initial manifestation of the plasma cell dyscrasias or may occur in the setting of known MG. Second, clinical features cannot distinguish among the various patterns of MG-associated renal diseases. Renal biopsy is necessary to establish the individual diagnoses and often provide important prognostic and therapeutic information. Third, multiple patterns of MG-associated renal diseases may coexist in the same biopsy sample.

If renal disease is present, the prognosis of the patients affected by MG is poorer. In these cases treatment strategy has to consider both plasma cell clone and renal manifestations. Combined nephrological and hematological management is therefore necessary.

Nephrologists' role is to reverse renal failure and preserve renal function. It is important to focus not only on prolonging life but also on improving quality of life by getting patients off dialysis. Preservation of renal function also keeps

therapeutic options open since many clinical trials exclude patients who are dialysis dependent.

Several therapeutic strategies for patients with MG and renal disease have been proposed and tested in studies with limited numbers of patients. However, controlled trials are rare and should be performed in the future. Factors justifying differentiated therapeutic strategies should be defined to improve the outcome of these patients.

Reference

1. Markowitz GS: Dysproteinemia and the kidney. *Adv Anat Pathol* 11: 49-63, 2004
2. Korbet SM and Schwartz MM: Multiple Myeloma. *J Am Soc Nephrol* 17: 2533-2545, 2006
3. Haubitz M and Peest D: Myeloma-new approaches to combined nephrological-hematological management. *Nephrol Dial Transplant* 21: 582-590, 2006

SS4

MONOCLONAL COMPONENTS AND CONTRAST MEDIA: A SOLVABLE PROBLEM?

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In the early literature there are a number of case reports of renal failure, including anuria, occurring after contrast administration for urography in patients with multiple myeloma. It was postulated that contrast medium and dehydration contributed to the precipitation of Bence - Jones proteins in the urine causing tubular obstruction. However, a retrospective review of 7 series of patients with multiple myeloma (N = 476) indicated that contrast medium was not a major risk factor for acute renal failure in this population.

Nevertheless, a document distributed by the Italian Health Ministry in 1975 considered Waldenström's paraproteinemia and multiple myeloma among the contraindications to injection of iodinated contrast media. Another document was distributed by the same Authority in 1997 and reported that even after administration of the safer non ionic compounds, some groups of patients are to be considered at risk for contrast media reactions, namely patients with severe hepatic or renal or cardiovascular impairment or patients with Waldenström's paraproteinemia or multiple myeloma. The opportunity of a contrast enhanced procedure is to be discussed in these cases by the radiologist and the referring physician. In the same document the clinical evaluation and the analysis of the patient history in cases referred for contrast medium injection is considered the main issue for prevention of reactions. Moreover, predefined sets of examinations/diagnostic procedures are not required to prevent contrast media reactions. Such tests are advisable to define in at risk patients the severity of the disease and myeloma patients are considered in this group. That is, by no means blood samples for protein electrophoresis neither urine analysis for Bence - Jones proteins are required in the general population.

However, a significant proportion of myeloma patients have a renal impairment and this is a well established risk factor for contrast induced nephropathy. If this is the case, measures for avoiding this adverse event are advisable. Guidelines were published by the Contrast Media Safety Committee of the European Society of Urogenital Radiology (ESUR) and recommended in this setting an adequate hydration, the use of low - or iso - osmolar contrast media, withdrawal of nephrotoxic drugs for at least 24 hours and the use of the lowest amount of contrast medium.

LP2

THE GLYCEMIC CONTROL IN DIABETES

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Glycemic control is fundamental to the management of diabetes. Optimal glycemic control can substantially reduce the risk and progression of microvascular (retinopathy and nephropathy) and cardiovascular diabetic complications.

The introduction of self-monitoring of blood glucose caused a shift in the focus of diabetes management from the physician's office into the hand of the patient. Regular self-monitoring of blood glucose by the patient, constitutes a key component of diabetes self-management and can improve the proportion of patients achieving their glycemic targets.

Continuous glucose monitoring (CGM) is in its infancy as a practical tool. At present there are several CGM product on the market and more are under development. These monitors measure glucose concentration in subcutaneous interstitial fluid, which can reflect changes in blood concentrations reasonably quickly.

Over the last 25 years HbA1c testing has come into common use serving as a convenient method for evaluating average glycemia over the previous several months.

HbA1c represents the gold-standard parameter to assess the success of the treatment regime.

The decades-long effort to educate the patients and the physicians about the importance of measuring HbA1c and the goal of maintaining HbA1c at less than 7%, has obtained a promising but not conclusive result.

In adult diabetic patients other metabolic risk factors must be tested and normalized and particularly lipid levels, such as LDL and HDL-cholesterol and triglycerides, in order to carry out an useful primary and secondary prevention of cardiovascular events.

SS5

SELF-MONITORING OF BLOOD GLUCOSE (SMBG) IN DIABETES PATIENTS, INSTRUMENT QUALITY AS WELL AS CLINICAL UTILITY

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Large amounts of money are used for SMBG instruments although systematic reviews have shown conflicting evidence when the effect of SMBG on long term complications of diabetes mellitus or decrease of HbA1c have been evaluated, especially concerning the effect in type II DM. A recent randomized trial for type II patients did not show any decrease in HbA1c during a 12 months trial.

There are some presuppositions that should be present for successful SMBG and which is lacking in most of earlier studies:

- Instruments should have good enough quality.
- Patients should be able to use the instruments.
- Patients should be able to interpret the results and take actions when necessary.

Quality specifications should be set for these instruments depending on their use. In theory such quality specifications can vary from situation to situation. Quality specifications, based on how results from the instruments are interpreted by the diabetic patients, showed that imprecision should be

less than 5% and bias less than 5%. The ISO standard 15197 states that 95% of the results should be within $\pm 20\%$ of the target value and results < 4.2 mmol/L should deviate less than 0.83 mmol/L. Although such criteria can be met when well-trained technicians are using the instruments, they are not fulfilled by patients for all instruments. And there are poor correlations between results from evaluations performed under "controlled" conditions and evaluation performed by patients themselves. We have therefore developed a standardized protocol where evaluations of glucometers were carried out both by a technicians and patients (with training and without training) and with three different lots of strips. Nine different instruments have been evaluated by this method and the results from these evaluations will be presented. Since the costs by using SMBG instruments are large and one of the fastest growing area in laboratory medicine, it should be carefully evaluated what patients that benefit from using the instruments. The instruments used should be of good enough quality and the patients should be trained in using them. In Norway, the Government has established a professional group that advice the Health Authorities on what instruments that should be used in Norway.

SS6

SCREENING AND DIAGNOSIS OF GESTATIONAL DIABETES AFTER THE HAPO STUDY

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Gestational diabetes is a pathology whose frequency has been constantly increasing in the latest years and which, if not treated, has adverse maternal and fetal outcome. For such reasons, this condition has to be diagnosed in time and properly treated. It has however to be stressed that, at this moment, the screening and diagnosis of gestational diabetes are not based upon univocal criteria. The HAPO study (Hyperglycemia and Adverse Pregnancy Outcomes) will be able to give a definite solution to the problem. This multicentre study, involving 25,000 pregnant women of different ethnic groups, will establish which level of maternal hyperglycemia is associated to a measurable risk for the fetus, at which level of maternal hyperglycemia it is necessary to intervene in order to reduce maternal and fetal morbidity, what is the range of normality for the 75 g oral glucose tolerance test, and the possibility of using the 75 g OGTT for a one-phase diagnosis of gestational diabetes. In the meanwhile, waiting for the results of the study, it is better not to change the already known criteria for the screening and diagnosis of gestational diabetes.

SS7

DIABETES AND COAGULATION

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Diabetes mellitus is a metabolic disorder principally characterized by hyperglycemia and complications related to micro and macroangiopathy. On 100 million persons worldwide affected, 5-10% have type I and 90-95% have type 2 diabetes mellitus. Type 2 diabetes is one of the principal risk factor for coronary, cerebrovascular and peripheral arterial disease and patients with diabetes are two to four times more likely to develop cardiovascular

disease compared with non diabetic patients. Cardiovascular disease is the principal cause of death and disability in people with diabetes. Diabetes with obesity, hypertension, elevated triglyceride and cholesterol levels, is considered in the definition of the individualized cardiovascular risk and its treatment significantly reduce the risk of cardiovascular events.

Numerous studies have demonstrated that the metabolic disorder in diabetic patients causes endothelial damage, platelet activation and imbalance of the coagulation cascade, with a relative prothrombotic state due to increased levels of von Willebrand factor, fibrinogen, D-dimer, thrombin and factor VII. In diabetic patients Tissue factor, factor VII and thrombin are increased, while natural anticoagulants such as thrombomodulin and protein C are decreased. Impaired fibrinolysis is strongly involved in the genesis of vascular complications in type 2 diabetes.

Endothelial damage, platelet activation and aggregation, coagulation factor activation, reduced inhibitory coagulation potential with impaired fibrinolysis increase the risk of thrombosis in diabetes, associated to cardiovascular complications.

Reference

1. Watkins K, Connell CM. Measurement of health-related QOL in diabetes mellitus. *Pharmacoeconomics* 2004;22:1109-26.
2. King H, Aubert RE, Herman WH. Global burden of diabetes 1995-2025: prevalence, numerical estimates, and projections. *Diabetes Care* 1998;21:1414-31.
3. Kannel WB, McGee DL. Diabetes and cardiovascular disease: the Framingham study. *JAMA* 1979;241:2035-8.
4. Kannel WB, D'Agostino RB, Wilson PW, Belanger AJ, Gagnon DR: Diabetes, fibrinogen and risk of cardiovascular disease the Framingham experience. *Am Heart J* 129:672-676,1990.
5. Nilsson B, Berne C, Korsgren O. The recent finding that tissue factor is produced by the pancreatic islets constitutes a possible link between insulin resistance and cardiovascular disease. *Am J Ther* 2005;12:551-4.
6. Esmon CT. Crosstalk between inflammation and thrombosis. *Maturitas* 2004;47:305-14.
7. R. Testa, S. Testa. Diabete e Coagulazione. *Biochimica Clinica* 2006; 30: 463-467

CO1

EFFECT OF PHYSICAL ACTIVITY ON HIGH-SENSITIVITY C-REACTIVE PROTEIN (HS-CRP), IL-6, IL10 AND TNF-ALFA SERUM LEVELS IN TYPE 2 DIABETES MELLITUS (T2DM) SUBJECTS WITH METABOLIC SYNDROME (MTS)

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Low-grade systemic inflammation and elevated CRP (>3mg/L) are associated in patients with T2DM. Exercise training has been shown to reduce hs-CRP level in sedentary subject with high initial hs-CRP. The aim of this study was to investigate the ability of different modalities of

exercise training to decrease hs-CRP levels on T2DM subjects with MTS.

In this study were enrolled 80 patients 35 F, 45 M, with T2DM and other MTS traits (IDF), and sedentary life style, age 62.4 + 4.3 years, diabetic from 8.3 + 4.3 years, BMI (30.5 + 5 kg/m²), waist circumference 101 + 13 cm, HbA1c 7.8 + 1.6, hs-CRP 3.5 + 2.4 mg/L. These patients were randomized in 4 groups of 20 subjects, group A sedentary (<10 Mets/h Wk), group B low dose/low intensity activity (walking >10<20 Mets/h Wk), group C high dose/moderate intensity (brisk walking > 20 Mets/h Wk), group D low dose (>10<20 Mets/h Wk), high intensity aerobic plus progressive resistance training (LD/HI-A+PRT), (40 min x 2 at Wk 70% Vomax) + (30 min x 2 x Wk to 80% 1 RM). Patients with hs-PCR >10mg/L and chronic inflammatory diseases were excluded from the study. Activity status was determined using a Minnesota LTPA questionnaire and fitness level (Vo2max) estimated by a graded submaximal exercise test. For each patient smoking, alcohol consume, pharmacological therapy, blood pressure, anthropometric measures and body composition were assessed. Lipidic and glycemic profile, HbA1c, insulin sensitivity (HOMA index), homocysteine, microalbuminuria, cytokines (IL-6, IL-10), tumor necrosis factor (TNF-alfa), and hs-PCR were measured at baseline, after 3, 6, 9 and 12 months of treatment.

Our results indicate that serum hs-CRP increases of 0.3 mg/L (8.1%) in group A, while decreased by 0.8 mg/L (-21%), 1.0 mg/L (-30%), 2.0 mg/L (62%) p<0.004 to B,C,D groups respectively. Preliminary results indicate that IL-6, IL10 and TNF-alfa significantly decrease between group A and D, showing a good accordance with hs-PCR behaviour. Indicating that physical exercise determine a decrement of hs-CRP, cytokines and TNF-alfa, emphasizing the anti-inflammatory nature of exercise. Furthermore the stronger reduction into high activity group D, shows that a good physical training produce the better anti-inflammatory effect on T2DM subject with MTS.

CO2

IS THE PLATELET SIZE ACTUALLY A RISK INDEX OF VASCULAR COMPLICATIONS IN PATIENTS WITH TYPE 2 DIABETES?

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Introduction. Patients with type 2 diabetes and vascular complications were described with a high mean platelet volume (MPV). However, MPV is influenced by pre-analytical phase (time from sampling, anticoagulant) and analytic technology (impedance or light scattering). This work evaluates size platelet (MPV) and percentage of large platelets (P-LCR) in a group of type 2 diabetic subjects in correlation to vascular complications.

Methods. We compared samples from 130 healthy subjects and 183 diabetic type 2 patients (subdivided in 4 groups: asymptomatic, with microvascular, with macrovascular and with both micro and macrovascular complications). All the samples were collected in fasting conditions, in K2EDTA evacuated tubes and analysed between 2 and 3 hours from collection. In diabetic patients the glucose concentration and the glycosilated haemoglobin were also measured.

The platelet size parameters were measured by the Sismex XE 2100 (DASIT, Milan) based on impedance methodology. The glucose concentration and glycosilated haemoglobin

were determined respectively by exokinase method (Glucoquant, Roche, Milan) and HPLC (Variant II, Biorad, Milan).

Results. The results are reported in Table 1. There is a direct, but small correlation between the size parameters MPV and P-LCR and both glucose concentrations and glycosylated haemoglobin.

Conclusions. MPV and P-LCR were not significantly different between controls and each diabetic type 2 group. However, they are not useful as markers of vascular complications or platelet activation on these patients.

Reference

1. Z. Hekimsoy, B. Payzin, T. Örnek et al. Mean platelet volume in type 2 diabetic patients. *J. Diab Complications* 2004; 18: 173-6

PARAMETER	CONTROLS			D. WITHOUT		D. MICRO		D. MACRO		D. BOTH	
	Mean	mean	F	mean	F	mean	F	mean	F	mean	F
PLT (10 ⁹ /L)	247	217	S	217	NS	225	S	221	NS		
MPV (fL)	10.8	11.0	NS	10.8	NS	11.0	NS	10.9	NS		
P-LCR (%)	31.9	33.0	NS	31.3	NS	33.5	NS	32.3	NS		

SS8

EFFECTOR CELLS AND MEDIATORS OF FOOD ALLERGY

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Allergic reactions to food represent a major clinical problem because of their increasing incidence worldwide and the potential severity of the clinical picture. Food allergy, in most instances, is an IgE-dependent disease and is generally considered as an early clinical manifestation of atopy. The pathogenesis of these reactions is similar to that of respiratory allergy but it is characterized by unique aspects related to the properties of effector cells resident in the gastrointestinal tract. Lymphoid tissue is particularly abundant in the intestinal mucosa. This makes the gastrointestinal tract an optimal environment for immune responses and, eventually, allergic sensitization. Th2 cells play a key role in the development of allergic sensitization through the production of IL-4 and IL-13, two cytokines that are crucial for the production of allergen-specific IgE. An important role is carried out by Th17 cells, a recently identified class of T cells capable of maintaining chronic inflammation in the gastrointestinal tract. Basophils and mast cells are the only immune cells that express the high affinity IgE receptor (FcεRI). The binding of allergen to the specific membrane IgE activates FcεRI+ cells to release chemical mediators involved in the early phase of the allergic reaction, such as histamine, cysteinyl leukotrienes and Th2 cytokines. Histamine is stored in the cytoplasmic granules of mast cells and is rapidly released upon immunological activation. Histamine and cysteinyl leukotrienes induce microvascular dilatation and increased vascular permeability, resulting in mucosal edema and enhanced mucus secretion. These effects are responsible for the main symptoms of allergic reactions to food such as nausea, vomiting, abdominal pain and diarrhoea. The gastrointestinal tract of patients with food allergy is largely infiltrated by eosinophils, which are recruited in the gastrointestinal mucosa by Th2- and mast cell-derived chemokines (IL-5, GM-CSF and RANTES). The eosinophils further amplify allergic inflammation by producing cytokines

(IL-2, IL-4, IL-6, IL-10 and IL-12) which are able to promote proliferation and polarization of Th2 cells. Thus, the clinical manifestations of food allergy are due to a complex network of cellular and molecular interactions resulting from the impact of environmental factors on a specific atopic genotype.

SS9

DIAGNOSTIC TESTS IN VITRO AND UNPROVEN DIAGNOSTIC PROCEDURES

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Diagnostic tests in vitro. The double blind placebo controlled food challenge (DBPCFC) is the gold standard for the diagnosis of adverse reactions to food, due to the high sensitivity and specificity of the test. The IgE mediated mechanism of an adverse reaction to food (ARF) is confirmed by the presence of specific IgE in the serum of the patient positive to the DBPCFC. This information is very important, because severe anaphylactic reactions to food, which may be fatal, are IgE mediated. The diagnostic accuracy of the measurement of serum specific IgE varies among foods, but generally is quite low; this is due to on the poor standardization of the food allergens and the cross-reactivity between inhalant and food allergens, mainly between pollens and vegetable foods. The DBPCFC is time consuming and potentially dangerous for the patient, therefore some authors have tried to identify a diagnostic decision point for the serum IgE concentration for specific food allergen, in order to avoid the DBPCFC. A decision point has been found for egg, milk, fish and peanut, however the amount of specific IgE for the same food varies among authors, depending on the age and of the symptoms of the studied population. Moreover, most of the patients with positive outcome of the DBPCFC have specific IgE below the decision point. Therefore, in clinical practice, these results should be extrapolated with caution.

Specific IgG to eaten foods are normally produced by all individuals: until now there is no evidence to support the diagnostic utility of specific IgG and their subclasses in the diagnosis of immunologically mediated ARF.

Unproven diagnostic procedures. "Alternative" approaches to diagnosis and treatment of food allergy and food intolerance are based on unproven diagnostic tests, such as cytotoxic test, electrodermal testing (VEGA), applied kinesiology, sublingual provocation and neutralization, and DRIA test. Some of these tests have been around decades and the scientific literature clearly shows their diagnostic inaccuracy. That is the case of the cytotoxic test, recently recycled as a "new" test in Italy a few years ago. Every so often, old unproven test are reintroduced as novelties by new companies.

SS10

FOOD ALLERGENS AND MOLECULAR DIAGNOSIS

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Food allergy is a pathological change of the physiologic immune response normally leading to tolerance to food proteins. A correct diagnosis of food allergy is needed to establish a causal relationship between food ingestion and the clinical symptoms reported by the patient, to identify the triggering allergen and the immune mechanism determining

the reaction. The current approach to food allergy diagnosis using well established tools include medical history, skin testing, IgE detection and oral challenge. Current availability of new immunological reagents, i.e. allergenic molecules (1), and in vitro assays from advanced biotechnology, i.e. microarray systems (2), is going to change this flow chart.

The application of biochemical and immunochemical tools and novel molecular biology methodology have lead to the identification of 438 food allergens (retrieved from the Allergome database, www.allergome.org, as of June 29, 2007). Allergenic molecules can be used as the most objective tool for the finest diagnosis and unequivocal identification of affected subjects. It has been reported the use of Bet v 1 as marker of a condition associating Fagales pollen allergy with OAS to apple and hazelnut in a population not exposed to birch pollen but to other Fagales pollen (3). The study of food allergenic molecules instead of extracts, is leading to overcoming the distinction between inhalant and food allergens in terms of primary sensitizers. Specific IgE production, induced by molecule sensitization via the respiratory mucosa, co-recognize the same epitope on food allergens (1). Examples are Bet v 1-, hevein-, and profilin- and tropomyosin-related allergens (4,5). The application of allergenic molecules in routine diagnosis may qualify them as markers of a condition.

To overcome the problem of multiple testing, the use of biochips or microarray to the proteomic field allows to quantitatively define multiple analytes in a single run using very low amount of all reagents (2). The chance to test hundred of allergens, could bring the IgE testing to replace the skin test as the first approach to the food allergy testing.

References

1. Ferreira F, Hawranek T, Gruber P, Wopfner N, Mari A. Allergic cross-reactivity: from gene to the clinic. *Allergy* 2004; 59(3):243-67.
2. Harwanegg C, Hiller R. Protein microarrays for the diagnosis of allergic diseases: state-of-the-art and future development. *Clin Chem Lab Med* 2005; 43(12):1321-6.
3. Mari A, Wallner M, Ferreira F. Fagales pollen sensitization in a birch-free area: a respiratory cohort survey using Fagales pollen extracts and birch recombinant allergens (rBet v 1, rBet v 2, rBet v 4). *Clin Exp Allergy* 2003; 33(10):1419-28.
4. Breiteneder H, Radauer C. A classification of plant food allergens. *J Allergy Clin Immunol* 2004; 113(5):821-30.
5. Ayuso R, Reese G, Leong-Kee S, Plante M, Lehrer SB. Molecular Basis of Arthropod Cross-Reactivity: IgE-Binding Cross- Reactive Epitopes of Shrimp, House Dust Mite and Cockroach Tropomyosins. *Int Arch Allergy Immunol* 2002; 129(1):38-48.

CO3

CLINICAL VALUE OF FOOD-SPECIFIC IgG4 ANTIBODIES

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Background. The appropriateness of serum antibodies to common food antigens (IgG4) determination was assessed in order to evaluate its impact on food intolerance management.

Methods. 128 patients (median age 36 years, 10-71 years; female 79%) attending our clinical service for symptoms referred to a suspected food intolerance and 22 asymptomatic healthy subjects (median age 39 years, range 25 – 55 years; female 81%) were studied. Serum

IgG4 antibody concentrations to 16 common foods were measured by an automated immunoassay.

Results. The area under the ROC curve was found to be 0.92 (SE 0.04) and, at a threshold value of 2.3 U/mL, the IgG4 determination has a sensitivity of 0.81 with a specificity of 0.87. With a positive test result, the post-test probability of having disease is 24 or 61% if the pre-test probability is 5 and 20%, respectively. If the result is negative, a pre-test probability of 5 or 20% corresponds to a post-test probability of 1.1 and 5%, respectively. Cohen's kappa value, an index of agreement between symptoms and IgG4 concentrations, was found to be 0.83.

Conclusions. The measurement of serum IgG4 shows a good diagnostic accuracy and it may play a role in ruling out food intolerance, sparing patients an unnecessarily restrictive diet and allowing a more appropriate treatment without any delay.

Reference

1. Atkinson W, Sheldon TA, Shaath N, Whorwell PJ. Food elimination based on IgG antibodies in irritable bowel syndrome: a randomised controlled trial. *GUT* 2004 Oct;53:1459-64

SS11

BIOCHEMISTRY AND PHYSIOPATHOLOGY OF BONE TISSUE

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This presentation calls attention on the fundamental role of collagen in determining bone quality and suggests how its alteration by overglycosylation may help to explain this relationship in three clinical models. This hypothesis may open the scene for a completely different and new point of view with respect to determinants of osteoporotic fractures and of the mechanism(s) by which agents used in this condition may be more or less effective. Even if it is well known that collagen is such an important component of bone structure, it is surprising that it has not been considered in the past in terms of studying directly or indirectly its precise role in determining resistance to mechanical stress or the effects of drugs, already used for a number of years, on this important bone constituent. The majority of studies done on mechanical properties of bone have concentrated on the role that mineral plays in bone strength and stiffness and their relation has been repeatedly exposed over decades, while the role of the organic component of bone has been much less emphasized. At the biochemical level, there is probably the best model of altered bone quality, that is, Osteogenesis Imperfecta, where a specific biochemical alteration of collagen molecules leads to bone altered quality and consequent fragility. Osteoporosis is one of the major world health concerns because of enormous health, social and economical effects and knowledge and perspectives of new approaches for this important disease should be made available to the medical general public.

SS12

POSTMENOPAUSAL OSTEOPOROSIS: DIAGNOSTIC E THERAPEUTIC CONCEPTS

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Menopause is the time in a woman's life when reproductive capacity ends. Natural menopause occurs between 45 and 55 years of age everywhere in the world. Ovarian activity decreases, and sex hormone production diminishes substantially. This period may be associated with a large variety of symptoms affecting the cardiovascular and urogenital systems, as well as skin, hair, and bone. Bone capital is accumulated by the end of the second decade, and remains more or less constant up to the time of menopause. Sex hormone deficiency leads to accelerated bone turnover, a negative balance between bone formation and bone resorption, and micro-architectural deterioration, which compromise bone strength and thereby increase bone frailty.

Osteoporosis is defined as a skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture occurring with low-energy trauma. The presence of a fracture is not, however, required for the diagnosis of osteoporosis. Bone strength primarily reflects the integration of bone density and bone quality. Bone density is expressed as grams of mineral per area or volume, and in any given individual is determined by peak bone mass and amount of bone loss. Bone quality refers to architecture, turnover, damage accumulation (eg, microfractures), and mineralization.

Currently there is no accurate measure of overall bone strength. Bone mineral density (BMD) is frequently used as a proxy measure and accounts for approximately 70% of bone strength. The World Health Organization (WHO) operationally defines osteoporosis as bone density 2.5 SDs below the mean for young white adult women by dual-energy x-ray absorptiometry. Based on this definition, approximately 30% of post-menopausal women have osteoporosis, with 17% of apparently healthy women in this age group having spinal osteoporosis, 16% having osteoporosis of the femoral neck, and 12% having total hip osteoporosis.

Osteoporotic fractures mainly include those of the vertebrae, proximal femur, forearm, and proximal humerus. The incidence of all fractures tends to start to rise at, or around, the menopause. There are a number of severe debilitating sequelae of osteoporotic fractures that can lead to permanent disability, and can even result in death.

Assessment of bone mass, identification of fracture risk, and determination of who should be treated are the optimal goals when evaluating patients for osteoporosis. Fracture prevention is the primary treatment goal for patients with osteoporosis. Several treatments have been shown to reduce the risk of osteoporotic fractures, including those that enhance bone mass and reduce the risk or consequences of falls. Adults with vertebral, rib, hip, or distal forearm fractures should be evaluated for osteoporosis and given appropriate therapy

SS13

GLUCOCORTICOID-INDUCED OSTEOPOROSIS: DIAGNOSTIC AND THERAPEUTICAL ASPECTS

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Glucocorticoid-induced osteoporosis (GIO) is the most common form of secondary osteoporosis. Fractures may occur in 30-50% of patients receiving chronic glucocorticoid therapy. They may occur early after exposure to glucocorticoids, since bone mineral density (BMD) declines rapidly. Glucocorticoids have direct and indirect effects on the skeleton. The primary effects are on osteoblasts and osteocytes. Glucocorticoids impair replication, differentiation and function of osteoblasts and induce apoptosis of mature osteoblasts and osteocytes. These effects, together with glucocorticoid-induced suppression of the GH/IGF-1 axis, lead to a suppression of bone formation, a central feature in the pathogenesis of GIO. Glucocorticoids initially also favour osteoclastogenesis and as a consequence increase bone resorption. A direct relationship between BMD and fracture risk in GIO has not been established. However, this is likely to be different from that found in postmenopausal osteoporosis because fractures in GIO occur at higher BMDs. Current guidelines recommend a T score of -1.5 or even -1.0 as threshold for intervention in GIO as opposed to the -2.5 threshold accepted for postmenopausal osteoporosis. Although biochemical markers of bone turnover can be useful measures of bone remodeling and can predict fracture risk, their value in GIO has not been established and their levels may depend on duration of glucocorticoid treatment. Preventive measures in patients exposed to glucocorticoids for longer than 3 months (5 mg prednisone equivalent, or higher, daily) should be recommended: these include lifestyle changes, such as tobacco cessation and reduction of alcohol consumption, an exercise program, restriction of sodium intake in the presence of hypercalciuria, sufficient calcium intake and adequate vitamin D supplementation. Bisphosphonates are the pharmacologic gold standard for the prevention and treatment of GIO. In fact, they are more effective than vitamin D in the prevention of bone loss and fractures in GIO, although they should be given in association with supplemental calcium and vitamin D. Bisphosphonates in several clinical trials have been shown to be able to significantly decrease the risk of fracture in GIO. In hypogonadal subjects with GIO, replacement of sex hormones may preserve bone mass and can be an additional therapeutic option. Parathyroid hormone (PTH) in perspective may be a more pathogenetic treatment of GIO since it induces skeletal IGF I and inhibits osteoblast apoptosis, increasing osteoblast number. Moreover, PTH administration induces an initial uncoupling of bone remodeling with an early increase in bone formation followed by a more gradual increase of bone resorption (concept of the "anabolic window"). Antifracture activity of PTH which has been demonstrated in postmenopausal osteoporosis is currently being tested in clinical trials in GIO.

SS14

TRADITIONAL AND NEW INFORMATIONS FROM THE BONE BIOCHEMICAL MARKERS ASSESSMENT

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With the ageing population, disorders of bone and mineral metabolism are becoming increasingly relevant. Consequently, the interest in, and the need for effective measures to be used in the screening, diagnosis and follow-up of such pathologies have markedly grown. Together with clinical and imaging techniques, biochemical tests play an important role in the assessment and differential diagnosis of metabolic bone disease being non-invasive, comparatively inexpensive, easy to perform, providing fast, sensitive and dynamic indices of bone remodelling.

The current available markers of bone turnover, include both enzymes and non-enzymes peptides derived from cellular and non-cellular compartments of bone, are usually classified according to the metabolic process related (bone resorption and formation markers). Changes in biochemical markers provide an integral measure reflecting alterations in the metabolism of the entire skeletal envelope.

The current evidence concerning the clinical use of biochemical markers of bone remodelling in the management of osteoporosis indicates that bone turnover markers are helpful tools in defining fracture risk and in the therapeutic assessment of metabolic bone disease.

Bone markers, in fact, are independent predictors of fracture risk: markers of bone resorption, in particular, seem to be stronger predictors of future bone loss than markers of bone formation. Moreover, being high bone marker concentrations at baseline associated with a better response to antiresorptive therapies, patients stratification by the measurement of bone markers concentrations before treatment may be useful from a pharmaco-economic point of view.

Furthermore, biochemical markers are an useful tool for quantifying and monitoring the effect of drug therapy. Significant changes, that can be observed from three to six months from the start of the therapy allow an early intervention to modulate treatment and to improve its effectiveness. However, even if most current guidelines on osteoporosis management do not recommend them for routine clinical use, this situation likely will change in the near future because the same available markers, when appropriately interpreted by combining formation and resorption indicators can also provide new informations.

Several markers, in fact, seem to provide insight into antiresorptive or anabolic action on bone of existing and novel drugs. They enable a deeper analysis of the changes in resorption process showing the parallel changes in function and number of the osteoclasts. The number of osteoclasts rather than their functional activity, in fact, mediates the coupling between bone resorption and formation, an essential mechanism for continuous bone remodelling and tissue reparation in order to maintain the high quality of bone.

Furthermore, the recently identified TNF superfamily cytokine, RANKL, which interacts with two receptor RANK and OPG, provides a new and informative link between bone matrix synthesis by osteoblasts and bone resorption by multinucleated osteoclasts. OPG-RANK-RANKL pathway seems to be a key regulator of bone metabolism, involved in osteoclast differentiation and activation, that provides a crucial signal for proper bone homeostasis.

At this moment, in fact, because of the enormous social and economic impacts of bone loss, it is of paramount importance to identify essential factors involved in osteoclast development and in bone remodelling.

SS15

THE BIOLOGICAL VARIABILITY IS CRUCIAL PART OF THE ANALYTICAL VARIABILITY

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The automation of the clinical laboratory improved the analytical precision, the accuracy increased due to industrial and basic research: the laboratory professionals are now appropriately skilled, having a specific cultural approach for interpreting the laboratory data.

A problem to be solved is the comparison between the observed values in individuals and the reference intervals obtained from the general population. The interlaboratory variability, the differences among analytical techniques and among healthy reference groups, induce some discrepancies between the high analytical efficiency and the patient and physician compliance and confidence.

It is necessary to personalize the laboratory data: it is now not sufficient and appropriate to compare individual data with general population intervals. The comparison should be made with previous data of the same individual or with data of group of individuals having same physiopathological characteristics. The biological variability has a crucial role in the correct interpretation of the laboratory result, not only for a individual, but also for epidemiology. In fact, the individuality index (ratio between intraindividual and interindividual variabilities) supplies a fundamental information about the usefulness of traditional reference limits. A value >1.4 means that the reference intervals are useful, whereas a value <0.6 means that reference intervals should not be used. The most important haematological parameters show an individuality index <0.6 : diagnosis and therapy should be done only by evaluating continuously and periodically the individual data.

It is evident that the importance of standardization, quality control schemes, data comparability and commutability is clearly growing for correctly interpreting the laboratory data. The biological variability is different among various parameters, considering biological rhythms, analytical sensitivity, analytes lability. The use of variability parameters could not be not universal and homogeneous, but it should be followed almost for the some common analytes, especially when used in some defined evidence-based clinical protocols for diagnosis and therapy.

The critical difference, which includes total variability, that is the sum of analytical and biological variability and also the statistical significance level, should be the correct and usual approach for judging the laboratory data.

The knowledge of laboratory professionals on this field can improve and strengthen their role in clinical medicine.

SS16

PREANALYTICAL VARIABILITY IN COAGULATION TESTING

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Pre-analytical variables are notoriously difficult to standardise in relation to coagulation testing. Under filling of blood collection tubes, haemolysis in the collection and processing of samples and altered sample characteristics for example as a consequence of high levels of lipids can significantly complicate analysis and data interpretation. Currently there has to be a series of manual checks on any blood sample for coagulation testing before analysis can be undertaken, to ensure there is an appropriate blood to

anticoagulant ratio and that there is not sufficient haemolysis to invalidate results. Automating such checks has occurred in relation to some chemistry testing and is beginning in relation to coagulation tests., allowing more consistency and reducing subjectivity arising out of operator variability.

One coagulation analyser (Sysmex CS2000i, Sysmex Japan) automatically checks test plasma for the presence of haemolysis, icterus and lipaemia (so called HIL check) and flags results to alert the operator. Haemolysis in particular is a problem for coagulation laboratories since analysis is normally possible but the level of haemolysis which causes interference and the nature of the changes in results obtained have not been well defined. More data are now required to allow informed decisions about which results can safely be used for patient management when haemolysis is present. We investigated the effect of haemolysis on routine coagulation tests with the CS2000i. We determined PT, APTT, Thrombin time (TT) and Clauss fibrinogen (Fib) (Dade Behring reagents). We used the method of Lippi et al (Arch Pathol Lab Med 2006;130: 181-4) to prepare 12 plasma samples from each of 6 different normal subjects which contained haemolysed red cells. The average plasma Haemoglobin (Hb) in the 6 sets of samples ranged from 0 to 21 g/l.

For 4/6 normal subjects there was no effect on TT and only a minor effect on PT. For the remaining 2 subjects PTs could not be measured in samples containing Hb of > 18 g/l and thrombin times were approximately doubled when Hb was >20 g/l. The mean Clauss fibrinogen results in the samples with different Hb levels were within 10% of each other.

The test most influenced by haemolysis was the APTT depending on reagent. Results with Actin FS showed a trend to shorter results. The mean baseline APTT was 28.7 and the mean result at Hb of 21 g/l was 27.3 sec. The effect of haemolysis on APTT was found to be reagent specific since when APTTs were determined with a second reagent there was a continuous trend of shorter APTTs as the plasma Hb increased, from an average of 33 sec at baseline (no haemolysis) to 29 sec (for samples with Hb of 21 g/l). Thus it could be predicted that a sample with a genuinely raised APTT could have a false normal result.

We conclude that haemolysis can have important effects on some clotting tests and automatic flagging is a useful tool in the standardisation of an important pre analytical variable.

SS17

TRANSFERABILITY OF LABORATORY DATA AND STANDARDIZATION OF PREANALYTICAL VARIABLES

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According to the traditional Lundberg's brain-to-brain loop, the total testing process begins from the physician's brain with formulation of a clinical hypothesis and selection of the most appropriate examinations, further develops through patient's preparation, sampling and handling of the biological specimens (preanalytical process), sample analysis (analytical process), and appropriate results reporting to the requesting physician (postanalytical process). While considerable attention has been focused on definition and enhancement of the analytical quality, laboratory diagnostics still struggles with some inherent problems, which endorse the potential to make the entire diagnostic reasoning vulnerable, enhancing costs and jeopardizing the patients' health. Such problems have a common background: the extra-analytical variability, a conventional definition that includes both pre and post-

analytical variables. Errors resulting from problems in the pre and post-analytical phases still occur with frequency, varying from 26 to 78% of identified medical errors in the primary care. Moreover, they are the most likely to result in major harm to the patient or precipitate hospital admission and are on overall less preventable. Although some of these steps tend to be placed outside the direct jurisdiction of the laboratory, they all contribute and influence the quality and efficiency of laboratory performances and should hence be targeted by specific and tailored interventions, which included risk analysis, benchmark policies, standardization, continuous monitoring by reliable extra-analytical quality indicators of performances and cutback of the processes most vulnerable to errors. Implementation of a wide set of extra-analytical performance indicators would provide meaningful information on the local testing processes that are more susceptible to errors or uncertainties for both in- and outpatient specimens, stat and routine requests. This is basically a four-step process, involving identification of accredited quality performances indicators, implementation of a reliable tracking system with systematic error identification and reporting, methodical data analysis and reporting, constructive audit and feedback policies with stakeholders. Deduction and implementation of common reference intervals to be shared by a regional network of clinical laboratories appear also a crucial step to increase efficiency and harmonization. Quality performances resulting from widespread implementation of common reference intervals and longitudinal comparison of patient's data, will allow clinical laboratories to accomplish with a major transferability, amplifying health benefits and meeting increasing health systems demand.

SS18

QUALITY INDICATORS IN THE EXTRA-ANALYTICAL PHASE

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The patient safety agenda is gaining momentum in the health care systems of all developed Countries. However, the adverse events detection systems and initiatives to reduce error rates in medicine are in their infancy. There is consolidated evidence that, today, most laboratory errors fall outside the analytic phase, and that pre- and post-analytical processes are more vulnerable to errors than analytical processes (1-3). As a consequence of this expected distribution of errors, clinical laboratories are urged to focus their attention on pre-analytic and post-analytic processes to improve patient safety (4), as these phases seem to present the greatest potential for quality improvement, once reliable strategies are identified and properly applied.

While laboratory activities have been traditionally classified as pre-, intra- and post-analytical, exploration at the beginning and end of the total testing process (TTP) reveals the existence of a pre-pre-analytical and a post-post-analytical phase (6). The first phase starts with test request, patient and specimen identification, blood drawing, sample collection and handling, and ends with the transportation of biological specimens to the laboratory. The final phase includes the interpretation and reaction of the clinician to laboratory data. The crucial question, therefore, is "have clinical laboratories to assume responsibility for the whole testing process, including appropriateness in test request and interpretation"? According to the ISO Technical Report 22367, a laboratory error is defined as "a defect occurring at any part of the laboratory cycle, from ordering

tests to reporting results and appropriately interpreting and reacting in these" (7). This broad definition presents several advantages and in particular it stimulates a patient-centered evaluation of errors in laboratory medicine. While quality indicators for the intra-analytical phase of laboratory activity have been well defined and their specifications internationally recognized, there is no consensus concerning types and acceptability limits for extra-analytical quality specifications. Comparing data collected on these indicators by different laboratories may allow a benchmark activity and the identification of realistic targets for quality in extra-analytical phases. If a "zero defect" represents the final goal for quality initiatives, the identification of valuable indicators for extra-analytical phases is a fundamental step for assessing and improving laboratory services. Laboratory medicine is a very dynamic sector of health care. With the constant development of more complex tests, remarkable advances in instrument technology, fully integrated laboratory information systems, the frequency and types of errors in laboratory medicine have been changed and are expected to change over time. While analytical quality still represents a major issues, the greatest impact for overall improvement would be to focus on pre- and post-analytical processes, in which most and "gross" errors occur. Moreover, these errors may translate into adverse events or risk of adverse events for patients. The total testing cycle represents the unique framework to identify and reduce errors in laboratory medicine and laboratory professionals must be leaders in ensuring patient safety both within and outside the walls of clinical laboratories.

References

1. Plebani M, Carraro P. Mistakes in a stat laboratory: types and frequency. *Clin Chem* 1997; 43: 1348-51.
2. Bonini P, Plebani M, Ceriotti F, Rubboli F. Errors in laboratory medicine. *Clin Chem* 2002; 48: 691-8.
3. Astion ML, Shojana KG, Hamil TR, Kim S, Ng VL. Classifying laboratory incidents reports to identify problems that jeopardize patient safety. *Am J Clin Pathol* 2003; 120: 18-26.
4. Howanitz PJ. Errors in laboratory medicine: practical lessons to improve patient safety. *Arch Pathol Lab Med* 2005; 129: 1252-61.
5. Westgard JO, Westgard SA. The quality of laboratory testing today. *Am J Clin Pathol* 2006; 125: 343-54
6. Plebani M. Errors in clinical laboratories or errors in laboratory medicine? *Clin Chem Lab Med* 2006;44:750-9.
7. ISO/PDTS 22367. Medical laboratories- Reducing of error through risk management and continual improvement- Complementary element.

CO4

RIORGANIZATION OF CLINICAL LABORATORY BY CENTRALIZATION: A REAL GOAL?

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The manifest unsuitableness of many requests due to the displacement of the production from clinical context has been previously highlighted (1). This aspect induces a vicious circle, leading to operating costs instead of the expected savings. After a two years settling-down period, the comparison between blood tests for in-patients required during the first quarter of the year 2007 and the same period of the previous year, shows that the routine tests, referring to the two centralized laboratories of Casalpusterlengo and Codogno, changed from 74797 to 70867 (decrease of nearly 5%), while the emergency tests

changed from 35974 to 49612 (increase of nearly 38%). Such consideration confirms the previous report and points out that hospital wards, to obtain their data beforehand, demand for several tests of little value for a differential diagnosis (they often count >70%). Regarding the various wards, the cardiology one, with which this laboratory had started up a working group to examine the suitability of the tests requested in the course of some cardiologic pathologies, shows, on the contrary, a decrease of both emergency (from 2521 to 2353, decrease of nearly 1%) and routine tests (from 5199 to 3338, decrease of nearly 36%). These data support the need to reach an agreement on protocols and diagnostic-therapeutic documents regarding different pathologies in various wards. Specific standards could be identified on a reduced number of routine tests in comparison with the current ones which include an excessive number of tests (occasionally >40), but of little relevance from a diagnostic point view, though more comfortable. In order to achieve this purpose, we have already started monthly meetings to finalize such protocols, whose results would be evaluated one year thereafter. This is probably the more practicable strategy to lower laboratory costs while reaching at the same time an effective clinical diagnosis through a limited but more suitable panel of tests.

References

1. A. Montani, *Biochim.Clin.* 30, 274, 2006

CO5

INFLUENCE OF STORAGE TEMPERATURE ON ROUTINE COAGULATION TESTING

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Background. Among preanalytical variables, the storage conditions prior to centrifugation might exert a strong influence on coagulation testing.

Aim. The present investigation was designed to evaluate the influence of temperature and time before centrifugation on routine coagulation tests.

Methods. Samples were collected from 26 volunteers in evacuated tubes containing 0.105 mmol/L buffered sodium citrate and divided in 7 aliquots. The first aliquot was immediately tested, whereas the others were stored for 3, 6 and 24 h at either room temperature (RT) or 4°C. aPTT, PT, Fibrinogen and D-dimer (DDHS) were assayed on an IL ACL TOP.

Results. Significant biases from the reference values were observed for all the aliquots but that stored 3h at 4° C for aPTT and those stored 3 to 6h at 4° C and 24h at RT for PT. Meaningful biases for fibrinogen were observed in both aliquots stored for 24h.

Conclusion. Results of our investigation evidence a clear influence of the storage conditions of the samples on aPTT and PT. Fibrinogen and D-dimer are more robust towards the sample storage. When delayed testing is necessary, we suggest sample storage at 4° C for less than 6h.

	aPTT	PT	Fibrinogen	D-Dimer
Baseline	38.3 (7.0)	28.4 (11.9)	401 (70)	248(503)
3 h at RT	39.4 (7.0)*	27.6 (11.1)*	401 (67)	255 (546)
3 h at 4°C	38.6 (6.8)	27.9 (11.2)	403 (63)	251 (526)
6 h at RT	39.5 (7.3)*	27.3 (10.9)*	403 (68)	250 (539)
6 h at 4°C	39.5 (6.9)*	28.1 (11.0)	402 (66)	244 (496)
24 h at RT	42.3 (7.8)*	28.4 (11.2)	378 (61) *	250 (516)
24 h at 4°C	42.3 (7.6) *	29.1 (11.0)*	376 (61)*	256 (522)

*p<0.001, data are expressed as mean ± standard deviation

SS19

CREATININE MEASUREMENT AND GLOMERULAR FILTRATION RATE ESTIMATION

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Reliable serum creatinine measurements in glomerular filtration rate (GFR) estimation are critical to ongoing global public health efforts to increase the diagnosis and treatment of chronic kidney disease. It is accepted that use of serum creatinine concentration alone as a GFR marker is inadequate. International recommendations favour the reporting of creatinine-based estimates of GFR using equation that was developed from the Modification of Diet in Renal Disease (MDRD) study, i.e. the "four-variable" MDRD equation that uses age, sex, race, and serum creatinine parameters. However, a limitation of this equation for general implementation in healthcare is related to the use of differently calibrated creatinine measurement procedures among laboratories. In particular, creatinine results which were used to generate the clinical basis for the MDRD equation estimating GFR were not traceable to high-order reference measurement procedures and reference materials. Consequently, the GFR calculation is very dependent on the accuracy of the creatinine method in use. The only way to achieve universal implementation of the GFR prediction equation, with the associated clinical benefits for the patients, is, therefore, to promote worldwide standardization of methods to determine creatinine together with the introduction of the revised GFR estimating equation appropriate for use with standardized creatinine methods. Standardization of calibration does not, however, correct for analytical interferences of methods (non-specificity bias). Establishing calibration traceability to the creatinine reference system will align the average performance of methods to each other, but will not substitute for improvement of suboptimal routine methods. To account for the sensitivity of alkaline picrate-based methods to non-creatinine chromogens, some manufacturers have adjusted the calibration to minimize the pseudo-creatinine contribution of plasma proteins, producing results more closely aligned to the reference method (isotope dilution-mass spectrometry), but this strategy makes an assumption that the non-creatinine chromogen interference is a constant among samples, which is an oversimplification. Analytical non-specificity for substances found in individual patient samples can affect the accuracy of GFR estimates computed from serum creatinine values for any picrate alkaline method including the so-called "compensated" Jaffe methods. The use of assays that are more specific for serum creatinine determination, such as those based on some enzymatic reactions, may provide more reliable estimated GFR values. Supporting the choice of more specific assays by clinical laboratories represents one of the main tasks of our profession in order to achieve the ultimate clinical goal, which is to routinely report an accurate estimate of GFR in all the pertinent clinical situations.

SS20

THE LABORATORY CONTRIBUTION: CYSTATIN C

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Low molecular mass plasma proteins play a key role in health and disease. Cystatin C is an endogenous cysteine

proteinase inhibitor belonging to type 2 cystatins superfamily. The mature, active form of human cystatin C is a single non-glycosylated polypeptide chain consisting of 120 amino acid residues, with a molecular mass ranging 13,343 - 13,359 Da, and containing four characteristic disulfide-paired cysteine residues. Human cystatin C is encoded by the CST3 gene, ubiquitously expressed at moderate levels. Cystatin C monomer is present in all the human body fluids; it is preferentially abundant in cerebrospinal fluid, seminal plasma, and milk. Cystatin C L68Q variant is an amyloid fibril-forming protein with high tendency to dimerize. It forms self-aggregates with massive amyloid deposits in brain arteries of young adults leading to lethal cerebral hemorrhage. The main catabolic site of cystatin C is the kidney: more than 99% of the protein is cleared from circulation by glomerular ultrafiltration and tubular reabsorption. The diagnostic value of cystatin C as a marker of kidney dysfunction has been extensively investigated in multiple clinical studies on adults, children, and in the elderly. In almost all the clinical studies, cystatin C demonstrated a better diagnostic accuracy than serum creatinine in discriminating normal from impaired kidney function, but controversial results have been obtained by comparing this protein with other indexes of kidney disease, especially serum creatinine based equations. Despite the multitude of clinical data in the literature, cystatin C has been not widely introduced in the clinical practice, perhaps because of a combination of factors, such as a general diffidence among clinicians, absence of definitive cut-off values, conflicting results in clinical studies, no clear evidence on when and how to request the test, poor commutability of results, and no accurate examination of costs and of the routine use in a stat laboratory.

SS21

THE LABORATORY CONTRIBUTION: MICROALBUMIN

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Albuminuria is considered a sensitive marker of renal derangement and is included in the definition of chronic kidney disease stage 1 and 2 by the National Kidney Foundation. Furthermore, elevated concentrations of albumin in urine are prevalent in several common disease states, such as hypertension, diabetes, obesity. It is also considered a marker of endothelial dysfunction and has been included in the 2006 guidelines of the National Academy Clinical Biochemistry among the emerging markers for cardiovascular disease and stroke. In spite of its clinical importance, the standardization of the laboratory measurement is lacking. Implementing the standardization of an analyte involves as a primary step an accurate definition of the analyte itself. Recent studies have, however, demonstrated that the nature of albumin in urine is more complex than previously thought. Albumin can be excreted as intact molecule or degraded as fragments of different molecular weights; furthermore the intact molecule can be immunoreactive or not due to chemical modifications of the molecule such as glycation. Methods based on different principles are able to detect different forms of albumin and give thus different results.

Current microalbuminuria assays use different kinds of immunochemical methods. These methods may lead to underestimation of albumin in urine samples containing non immunoreactive albumin or albumin fragments. To overcome this problem, different kinds of methods have been proposed. Among them it is worthy to mention: size exclusion HPLC, chip electrophoresis and liquid chromatography-tandem mass spectrometry. Before the

results of these new techniques could be validated for clinical utilization, a number of questions should be answered and in particular it should be demonstrated that different measurements lead to effective improvement in clinical outcomes.

Analytical quality specifications, and in particular sensitivity, are another critical issue. There is increasing evidence that a continuous relationship between albumin excretion and risk exists so that non lower bound between normal and increased albuminuria can be identified that segregates subjects at different risk. In this view it will become increasingly important to establish urinary albumin concentrations below which therapy is no longer beneficial. The sensitivity of the laboratory method is, in this context, crucial.

To assess the state of the art of urine albumin measurement in Italian Laboratories, a National survey was launched by the Intersociety Study Group on Diabetes Mellitus. The results show that there is a need for improvement of the analytical quality of the albumin measurement in urine and that it is of even more importance the standardization of the post analytical phase (reporting and interpreting the results).

CO6

ACCURACY IMPROVEMENT OF CREATININE MEASUREMENT BY USE OF AN ENZYMATIC ASSAY

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Background. The estimation of glomerular filtration rate (GFR) is considered the best index of kidney function. Laboratories may routinely report a GFR estimate from serum creatinine concentrations by applying the "four variables" Modification of Diet in Renal Disease study (MDRD) equation. The general implementation of this equation is, however, impaired by the use of creatinine assays having different performances (1). In this study, we evaluate the impact of the introduction of an enzymatic assay (E) on trueness and imprecision of creatinine measurements in comparison with a kinetic alkaline picrate "compensated" method (AP).

Methods. Trueness was evaluated by measuring creatinine concentrations of three commutable materials with target value assigned with gas chromatography-isotope dilution mass spectrometry, while the imprecision was estimated using a liquid-frozen control material during a eight-month working period for a total of approximately 300 measurements for each method. For creatinine measurements we used four platforms (all from Roche Diagnostics), the Integra 800 and Modular P (for trueness evaluation), and the Hitachi 917 and Modular P (for imprecision study).

Results. The trueness of creatinine measurements was significantly better using E, mainly at physiological concentrations (0.858 mg/dL) where the AP (experimental bias, +5.13%) was unable to fulfil the desirable bias ($\pm 3.4\%$). The imprecision of creatinine measurements also decreased when E replaced AP. In particular, Hitachi yielded overall mean CVs of 2.18% (AP) and 1.25% (E) ($P=0.015$) and Modular yielded CVs of 2.19% (AP) and 1.51% (E) ($P=0.96$), even if the difference between Modular's CVs was not statistically significant due to elevated random sampling variability. While only one out 16 monthly CVs by E was higher than the desirable goal ($<2.2\%$), six monthly CVs by AP (37.5%) surpassed this limit.

Conclusions. We conclude that, for suitable clinical usefulness of creatinine measurement, laboratories should consider to definitively replace AP with E.

References

1. Myers GL, Miller WG, Coresh J, et al. Recommendations for improving serum creatinine measurement: A report from the Laboratory Working Group of the National Kidney Disease Education Program. *Clin Chem* 2006;52:5-18.

CO7

PROGNOSTIC VALUE OF COMBINED USE OF B-TYPE NATRIURETIC PEPTIDES AND INTERLEUKIN-6 IN PREDICTING CARDIAC MORTALITY IN END-STAGE RENAL DISEASE PATIENTS

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Introduction. Cardiovascular risk prognostication is a main problem in end-stage renal disease (ESRD) patients. B-type natriuretic peptides (BNP, NT-proBNP) are currently widely used as a tool for diagnosis and prognostic stratification in heart failure (HF) patients. The aim of the present study is the measurement of plasma cardiac natriuretic hormones and their relation with prognosis in ESRD patients without clinical evidence of HF.

Methods. We investigated the relationship between plasma cardiac natriuretic hormones and outcome in 104 haemodialysis patients from four hemodialysis centers (65 males, age 64 ± 2 years, ejection fraction -EF, $56\pm 2\%$, mean \pm SE). All patients underwent a comprehensive clinical and biomarker evaluation including B-type natriuretic peptides interleukin-6 (IL-6), ferritin and C-reactive protein) between May 2004 and May 2006. Overall and cardiac mortality and cardiac events (cardiac deaths and non-fatal cardiac events) were considered as end-points of the analysis.

Results. After a 24-month follow-up (median 21 months), 18 all-cause deaths were recorded, as well as 10 cardiac deaths; 23 cardiac events. Plasma BNP/NT-proBNP concentration was significantly higher in patients with end-stage renal disease (BNP 430 ± 53 , NT-proBNP 11700 ± 1246 ng/L, mean \pm SEM) than in sex/age matched healthy subjects (BNP 11 ± 1 and NT-proBNP 50 ± 3 ng/L, $p<0.01$). At the multivariate analysis age and NT-proBNP (OR 1.04, $p<0.05$ and 1.80, $p<0.01$) were the only predictors of all-cause death; NT-proBNP and IL-6 of cardiac death (OR 1.88, $p<0.01$; 1.87, $p<0.05$, respectively) and of cardiac events (OR 1.76 and 1.82, respectively, $p<0.05$). NT-proBNP was more effective than BNP in discriminating cardiac mortality at ROC-analysis (AUC 0.745 and 0.634, respectively; cut-off: NT-proBNP: 11729 ng/L; BNP: 453 ng/L).

Conclusions. High plasma NT-proBNP level correlate with cardiac mortality: its predicting power increases if interleukin-6 is considered. The combined use of NT-proBNP and IL-6 might be a useful tool to predict cardiac mortality in ESRD.

SS22

ORGANISATION OF THE "PROTEIN LAB"

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Is the lab which handles the analysis of siero-proteins, situated half-way between the initial discovery of sieroproteins and development of proteomics, still valid? This question can be answered referring to the guidelines published for monitoring patients with monoclonal gammopathy. An important characteristic of the "protein lab" is the observation of changes regarding the infection of virus C, the presence of cryoglobulins and the reduced heterogenous characteristic of IgA Immunodeficiency. The organisation permitting these qualitative objectives, in the diagnosis of sieroproteins, to be reached are represented by capillary electrophoresis and the observation of capillary patterns as routine tests at the first level. This is followed by immunofixation at the second level. Other tests include carbohydrate deficiency transferrin (CDT) useful to identify the abuse of alcohol. It is also possible to identify the presence of Tau protein in nasal secretions and hence discover the presence of cerebrospinal fluid. The "protein lab" must guarantee the answers to diagnostic queries within twenty-four hours. This can be realised when at least 33,000 electrophoresis tests are performed per annum, using two capillary analysers, an agarose sector, two hydrazes and one instrument to analyse specific proteins. If the "protein lab" is well organised and follows the required guidelines it is able to offer the patient a complete response and shorten the time required for a correct diagnosis.

Bibliography

1. Petal A.H.M Wijnen and Maria P. van Dieijen-Visser: Capillary Electrophoresis of Serum Protein. *Eur J Clin Chem Clin Biochem* 1996;34:535-545
2. David F.Keren, Raymond Alexanian et Al.: Guideline for Clinical and Laboratory Evaluation of Patients with Monoclonal Gammopathies. *Arch Pathol Lab Med - Vol 123 Feb 1999*:106-107
3. Mlsiani R., Bellavista P.M., Fenili D. et Al.: Hepatitis C Virus Infection in Patients with Essential Mixed Cryoglobulinemia. *Annals of Internal Medicine* 1992;117:573-577
4. J. Bladè: Monoclonal Gammopathy of Undetermined Significance. *The New England Journal of Medicine* 2006;355:2765-2770
5. A.H.A Latiff and M.A. Ken: The clinical significance of Immunoglobulin A deficiency. *Ann Clin Biochem* 2007;44:131-139

SS23

INTEGRATION BETWEEN CORE-LAB AND WITHIN-HOSPITAL AND TERRITORIAL POINTS-OF-CARE IN THE DIAGNOSIS OF EMERGENCY CARDIOLOGY

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The changing needs of today's citizens, the steady lengthening of average life spans and the transformation of family models require a review of the primary treatments and a process of change aimed at the constitution of a socio-health integration area which focuses on the citizens and their needs. Within these changes, Laboratory

Medicine's importance is destined to increase as knowledge deepens and technological progress develops. To achieve these aims, its professionalism must be taken outside the laboratory by using closely interconnected tools: communication with clinicians and general practitioners in order to identify criticalities and needs, processing of all the information of the Citizen-Patient to produce integrated reports, consolidation and automation models (intra-laboratory), consolidation and Point of Care (POCT) models (within-hospital), assistance models on the territory (out-hospital). We identified Emergency cardiology as a critical area for which new strategies need to be developed. The laboratory had insufficient consolidation and automation processes to guarantee an improvement in the Therapeutic-Turnaround Time (TAT) and POCT emerged as a realistic solution. As patient outcomes are the goal, Total Quality Management principles must be incorporated into the management of POCT: in this setting and in compliance with the hospital-based POCT model described in the National Committee for Clinical Laboratory Standards guidelines, we have implemented POCT of cardiac markers in emergency/cardiology department (ECD): cardiac troponin I (cTnI) analytical performance (IFCC quality specifications), clinical evaluation and staff training were carried out in our ECD. The POCT system was bidirectionally interfaced to the laboratory information system, and automatic validation protocol was identified as the most suitable. Today 3 POCT cardiac markers are implemented in the various cardiology departments of our institution and our model was accredited by the Clinical Pathology Accreditation (CPA) and certificated according to the International Standard Organization (ISO) 9000-2001. As the management of patients with chest pain could be improved if therapeutic decision-making takes place before arrival at the hospital, cTnI POCT in the ambulance was implemented according to the processes described previously and experimental software was used: the central laboratory and the main Tele-Cardiology Unit both receive analytical data for troponin I online via an Internet connection. The central laboratory supervises the overall processes. Our POCT model may be exported to any clinical area right up to the houses of citizens-patients: this integration rationale may provide an improvement in the managerial efficiency and efficacy of patient outcome. Quality management and information communication technology (remote-diagnosis, remote-assistance, remote-consultancy) are the keys for the success of the change.

CO8

AN AUTOMATION EXPERIENCE IN MOLECULAR BIOLOGY: THE GENEXPERT DX SYSTEM FOR BCR-ABL MOLECULAR MONITORING IN CML PATIENTS

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The molecular signature of BCR-ABL fusion gene in chronic myeloid leukaemia (CML) provides a unique tool for diagnosis and monitoring of tumour burden during therapy. The introduction of imatinib mesylate, allowing the achievement of high clinical and cytogenetic remission rates, has revolutionized CML patients treatment and reinforced the fundamental role of BCR-ABL qPCR monitoring to assess minimal residual disease; nevertheless many procedural aspects of this complex technique require a strong inter-laboratory optimisation, so recommendations for harmonizing the different methods have recently been proposed. The GeneXpert (GX) Dx

System (Cepheid) is a fully automated platform that can complete, in less than 2 hours, the steps of nucleic acid isolation, reverse transcription and real-time qPCR into a single disposable multi-chamber cartridge, requiring only 200 µl of sample. A few In Vitro Diagnostic (IVD) assays have been recently developed to be used with GX: one of these (Xpert BCR-ABL Monitor™), properly designed for the molecular monitoring of p210 transcript in CML patients peripheral blood (PB), was used to analyse 20 samples (19 PB and 1 bone marrow, BM), corresponding to 19 patients submitted to imatinib therapy. The results were compared to those obtained with both a home-made SybrGreen qPCR method and a commercial Taqman IVD assay (M-bcr FusionQuant® Kit, Ipsogen). Sixteen samples (80%) produced a valid result with GX: 10 were positives with all the methods, 4 positives with GX but negatives with at least one qPCR method and 2 negatives with all; the 4 not valuable samples were correctly evaluated with the qPCR assays. The correlation of GX results with qPCR ones was good. The BM analysis was successful. This experience suggests that, even if more extensive studies are undoubtedly needed, the GX System could offer a valid alternative to traditional qPCR, allowing a better standardisation of the molecular techniques used for CML monitoring. Some technical issues, such as the use of other sample types (i.e. BM, isolated cells, RNA) or the analysis of samples with high WBC count and/or high transcripts level, could be improved in the near-future, not forgetting economical considerations (i.e. the costs of repeated samples).

CO9

POTENTIAL APPLICATION OF REAL-TIME QUANTITATIVE REVERSE TRANSCRIPTION-PCR FOR HER2 DETECTION IN PERIPHERAL BLOOD FROM BREAST CANCER PATIENTS

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Background. The HER-2/neu gene, that encodes for the human epidermal growth factor receptor type 2, is amplified in 25–30% of human breast cancers and its amplification is a well known indicator of poor prognosis. Immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH) are reliable ways to identify overexpression or amplification of the HER-2 neu gene, but these techniques are invasive. The implementation into the clinical setting of therapeutical strategies directly targeting the Her2neu gene product, has create the need for the development of non-invasive analytical techniques that could be used for monitoring minimal residual disease and response to the treatment.

In this study, we developed and evaluated real-time quantitative reverse transcription PCR (QPCR) test for detection the expression of HER2/neu mRNA in peripheral blood from breast cancer patients.

Methods. The QPCR analyses were performed on blood samples obtained from 30 breast cancer patients positive for Her2neu overexpression by immunohistochemical analysis (IHC), 10 breast cancer patients negative for Her2neu overexpression, and 24 healthy controls.

Results. Her2neu positive tumors showed a significant increase in mRNA transcripts as compared with both healthy controls (n=24) and Her2neu negative patients (n=10). After establishing a cutoff value, 18 out of the 30 Her2neu positive patients by IHC, scored positive for Her2neu expression, whereas only 1 out of the 10 Her2neu negative patients was weakly positive. All samples from patients with a more aggressive disease not immune-treated scored positive for the Her2neu transcript. The sensitivity of the QPCR as compared with immunohistochemical examination was 60%, while specificity was 91%. Overall the predictive value of the test was 95%.

Conclusions. Her2neu QPCR is a suitable method for Her2neu overexpression detection in peripheral blood from breast cancer patients. QPCR could be used to identify breast cancer patients with poor prognosis and for monitoring response to the therapy.

References

1. Savino M et al. Development Of Real-Time Quantitative Reverse Transcription-Pcr For Her2 Detection In Peripheral Blood From Patients With Breast Cancer. CCA, 2007. In press

LP3

TRAINING OF LABORATORY MEDICINE PROFESSIONALS

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Introduction. During the last few decades there have been periodic concerns about the future of laboratory medicine. These have included a shortage of positions for graduates, too many clinical pathologists or not enough clinical pathologists. There have been many articles and meetings about the need for new skills in clinical pathology and how to avoid creating more "dinosaurs".

Methods. Data about residency applications, job opportunities, laboratory budgets and the demographics of laboratory medicine professionals was obtained to help predict how training efforts should be focused in the future. **Results.** Data indicates that there is a shortage of pathologists and clinical laboratory scientists in the U.S. and other countries that is likely to get worse. The median age of pathologists is > 55, yet the number of residency and other training positions is down. Additional evidence for the shortage of pathologists is the current high average number of job offers for residents looking for positions. Simultaneously, the demands on laboratory medicine continue to increase. The increase in knowledge following the completion of the human genome project and related discoveries will challenge laboratory medicine with many more potential new tests and markers than ever before. The choice of subspecialization and where laboratory budgets are growing can be an important indicator of where new training efforts should be focused.

The purpose of postgraduate training in laboratory medicine for both M.D. and Ph.D. graduates is to produce a competent laboratory director. Skills needed to become a clinical laboratory director include a thorough understanding of pathophysiology, testing methods, test interferences and

the effects of drugs and disease on laboratory tests. Of equal importance is the ability to perform basic and/or clinical research and to be able to critically evaluate the scientific and clinical literature with an understanding of evidence-based laboratory practices. Next, the laboratory director must have an understanding of management and laboratory flow practices that focus on patient safety, cost-effectiveness and regulatory compliance. Finally, the laboratory director must be able to communicate effectively with clinicians in order to serve as a laboratory consultant. A model for accomplishing this in the short 3-4 year period for postdoctoral training will be presented that includes didactic teaching, research and hands on learning for management and consulting skills will be presented.

Conclusions: We must address recruitment of young medical students and scientists to our field and assure that training is appropriate for the new challenges facing us. Approaches to refocusing training efforts in laboratory medicine include mirroring the changes in areas of specialization of academic clinical pathologists and changes in laboratory budget spending over the last decade. These two indicators, area of specialization and where the money goes, should provide us with directions toward training future clinical laboratory pathologists and scientists – if we can recruit them!

SS24

REFERENCE INFORMATION MODEL FOR CLINICAL LABORATORIES: IN RETROSPECT REVIEWED

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A reference information model for clinical laboratories is defined as a branch specific, general model that contains information about all essential processes of that organization as well as all procedures used in that organization to function properly. Based upon the WHAT of the organization, the HOW is described and readily assessable archived.

In the mid nineties a reference information model was designed and implemented for hospital pharmacies by two of us. Thereafter we started an ambitious project to design a similar reference model for hospital laboratories. This model was described into great detail in a book titled 'Reference Information model for Clinical Laboratories', published by IOS press (ISBN 90 5199 414 1) in 1998. In the TRANSFORM project the same authors challenged the information model concept to describe the way primary care and hospital care work together (2005).

All three projects provided a huge insight in the way the various departments worked, how from a conceptual point of view the professional activities interact and, even more important, how they could be improved. A strict separation between organization independent (WHAT) and organization dependent (HOW) entities was made. The detailed description of standard operating procedures, the overall coherence of the model including job descriptions, including organizational layouts, instruments and all kind of registered documents, makes RILA into a universal but very practical description of a hospital laboratory operation. It is capable of describing large operations into great detail. The applied commercial software from Capgemini checks on any inconsistencies, generates all kind of professional as well as organizational information and provides 'real time' insight in the particular organization from any point of view. In retrospect reviewed: a play ground was constructed to evaluate and build on the organizational structures involved.

The application of RILA facilitated a steep learning curve. The implementation of total quality management systems was not yet common practice and the use of a reference information model was a great way to structure and to smooth complex activities. The level of automation combined with the lack of a single TQM model could all be beaten by the use of RILA.

RILA provides a process oriented, holistic description of any laboratory that enables the user to implement a procedure oriented, coherent description of any focus field within a particular laboratory, such as quality management, Human resource management and professional services produced. Conclusions: the tedious design and implementation of a RILA has become obsolete nowadays. However the writing of a laboratory handbook, the generation of management reviews including SMART goals, the maintenance of the TQM and to stay accredited by a notifying body using a formalized (but as simple as possible) system, process-oriented approach, are all issues currently to be fulfilled. The design, description and implementation of a reference information model in clinical laboratories have laid the foundation to do so.

SS25

INFORMATION TECHNOLOGY FOR PERSONALIZED MEDICINE

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The considerable growth of basic biomedical sciences in the last few years, together with the development of ICT (Information and Communication Technology), is providing laboratory medicine with potentially very useful new tools.

This results in an improvement of medical assistance, not only in the field of traditional therapeutic medicine but even as regards preventive and predictive medicine.

Nowadays, three objectives are more and more within our reach:

1. The integration of the laboratory report into the patient's clinical history through the EMR (Electronic Medical Record) / HER (Electronic Health Record)
2. The management of medical knowledge, mainly in the field of genetics, to define personalized profiles for the assessment of the risk of disease for every citizen
3. The application of communication tools (e.g. mobile phone, digital television) to update in real time the citizen/patient about his health status.

The onset of a clear disease is likely to be ruled out or at least postponed thanks to an appropriate change of life style, nutrition, habits, etc.

This will result in an improvement of the quality of life as well as in an increase of life expectation.

Last but not least, preventive / predictive medicine will reduce the incidence of severe complications in many chronic diseases (e.g. diabetes, hypertension).

This lecture will highlight the improvements in this field and the strong concern of the European Community towards these objectives.

SS26

INFORMATION TECHNOLOGY IN THE DIAGNOSTIC APPROPRIATENESS EVALUATION

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If Clinical Governance is a framework through which our organization is accountable to improve the quality of care, laboratory professionals should identify high quality standards and systematically and rigorously monitor against the outcomes that represent the diagnostic process. The problem of this approach is that there are many aspects that are impossible or difficult to measure, therefore proxies and measure process rather than outcomes are used. Laboratory Medicine is information-intensive, involves complex tasks of data creation, organization, presentation, transfer and archiving and consequently it fit well with computerisation. Most laboratories may manage their information by a Laboratory Information System LIS, to automate the routine step including test ordering, specimen labelling, reporting results, quality control, efficiency-productivity management, reference testing. As Information Technology deals with the performances, management and interpretation of laboratory test ordered offers great potential benefits for knowledge management to support and to evaluate clinical decision making. In the case of glycosylated haemoglobin (HBA1c) the test offers the possibility, by evaluating diabetic patient records, to assess the quality of care delivered. In this example, a common measurement of quality care in diabetes is the number of HBA1c tests undertaken during one year assuming that regular testing helps patients and doctors track blood sugar levels over time to reach target level of control. The detected levels of HBA1c are acceptable measure of diabetic patient clinical outcomes. Nevertheless multiple forces are now driving the adoption of IT in healthcare institutions to manage workflow and patient's pathway, IT gives causes for major concern. It is reported a mortality rate significant increased after IT implementation in a US hospital and the studied fraction of reports that were never accessed by clinicians in the setting of an Emergency Department in a English hospital suggested that 3% of the reports were never viewed. In conclusion to paraphrase Leonardo da Vinci, it is time for us to stop to discuss on the possibilities of using information technology to support Clinical Governance and healthcare quality in Laboratory Medicine, but it is time to do it in a culture of evaluation and continuous understanding.

SS27

INFORMATION TECHNOLOGY AND APPROPRIATE USE OF DIAGNOSTIC TEST: FROM INTRANET EDUCATION TO LABTEST ON LINE

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In developed countries the number of diagnostic tests ordered by both primary care and hospital based physicians, is growing and growing. Furthermore, patients themselves actively ask for tests and often their expectations on lab tests are not supported by evidences.

In recent years, the growth of Evidence Based Laboratory Medicine brought new emphasis on the appropriateness of diagnostic tests and on meta-analyses. The literature trend as well as the production of Evidence Based Guidelines, allowed the professionals to find a more general agreement

about the appropriate role of diagnostic investigation in the clinical decision-making process. However, the need of protecting patients by the potential risk of a cascade of unnecessary testing is still under debate. The attempt to modify the laboratory test ordering behaviour through education and feedback, achieved variable success, as pointed out in a 2003 Cochrane review. The implementation of computer-based decision-support systems for ordering test were reported to give better results and to decrease the overall number of both tests and venipunctures. Moreover, when the systems are based on evidence supported guidelines, the tests can be assumed to be ordered in an optimal way. Intranet is widely available in Hospitals and University and it is known to be a powerful tool for communication between health professionals. The experience of Oxford Radcliff Hospital, one of the largest teaching hospitals in the UK, showed the ability of intranet in disseminating high quality information on laboratory tests. The project includes immediate access by doctors to patient blood test results and guidelines on the tests available and intends to improve the access to evidence-based medicine knowledge. Several Australian hospitals are currently evaluating information systems aimed to develop transferable and sustainable, albeit evidence based, changes in test-ordering practices. Citizens and patients are more and more actively involved in health related decisions and, as a consequence, they need to be informed about risks and benefits.

The American Association for Clinical Chemistry- AACC developed a web site (LAB tests Online www.labtestonline.org) designed to help patients to better understand the clinical lab tests that are part of routine care as well as diagnosis and treatment of a broad range of conditions. The site can be used even by medical professionals as a quick reference tool to keep up with advances in laboratory science. SIbioc (Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica) is currently editing the Italian translation of the web site and the publication is expected soon. All the approaches we have considered so far (physicians education and training, decision-support information technology tools, patients empowerment and education) must support a more appropriate behavior in test ordering.

CO10

WEBCOMMUNITY PILOT PROJECT

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The phenomenon of the "digital divide" is one of the main causes of social exclusion and isolation. There are some categories where it is more extreme: the handicapped (3 million in Italy) and the elderly (20% of the Italian population is over 65 and 80% of these do not use a computer). The WebCommunity pilot project offers online information, booking, remote consultancy and referencing services. In its initial phase the project is implemented in a partnership with Associazione Nazionale Famiglie Fanciulli e Adulti Subnormali/ANFFAS and Associazione Nazionale Tumori/ANT della Regione Puglia and is served by the SAN-X web portal (FIMESAN S.p.A.). The aim is to assess new information paths also for the disadvantaged categories (elderly and/or handicapped), through Eye-Tracking technology, in order to reduce the distance between the hospital and the surrounding area and place the Citizen-Patient at the centre of an Integrated Social Welfare System. Eye-Tracking technology was proposed to make sure that anyone with a difficulty in using a mouse may in any case use a Personal Computer (PC) and surf the net, by relying on technology that tracks eye

movements. SAN-X does not alter the organizational methods of the laboratory since it can be integrated with the Laboratory Information System (LIS). Disadvantaged users interact with the laboratory by using an Eye-Tracking PC. The report is treated according to the same logic of an online bank account as if it were a "wire transfer". Therefore, the laboratory data feed the "health account" of the Citizen and can be viewed by other authorized medical staff. The "transaction" of the report occurs in a protected web area, through a telematic communication channel that complies with legislation on privacy. All the analytical data integrate with the Electronic Health File and the Clinical Folder of the GP and/or Specialist. The aims to achieve are: a better Service for the Citizen-Patient, with special focus on the disadvantaged groups, reduced waiting times to provide services, the availability of online reports and the optimization of laboratory human resources.

CO11

EUROPEAN NETWORK FOR RARE AND CONGENITAL ANAEMIAS (ENERCA) THE SECOND PHASE

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This is an European co-financed Project by the Rare Disease Programme. Its primary goal is to contribute to the immediate detection of these, and other uncommon diseases in Europe by improving awareness, knowledge, diagnosis and treatment and by the development of quality provisions for their prevention. There was a first phase for the project that ran from October 2002 to April 2004 and, in September 2005; ENERCA has entered a second phase, following a Grant Approval from the European Commission's DG SANCO Health Programme (contract number 2004110). The second phase of ENERCA runs for 36 months and twelve experts from seven European countries consolidated international cooperation at the kick-off meeting that took place in Barcelona in November 2006. To meet its objectives an ENERCA website (www.enerca.org) employing two distinct features was created to provide relevant and proven information covering:

- Detailed list of centres specializing in these disorders
- Definitions of most of the uncommon and congenital anaemias.
- Information on patients' support organisations listed by countries
- On-line forum (conversation platform) to exchange experiences with other people in the same situation.

One of the most important purposes of ENERCA is to promote cross-border cooperation among experts in Europe in order to establish the basis for implementing the following objectives:

1. The establishment of referral laboratories and/or experts in order to provide the best professional assistance throughout the European Community
2. The creation and availability of easy-to-understand information for patients
3. The promotion of a Medical Alert Card (MAC) for patients

In terms of epidemiological surveillance, available data on RA in Europe will be evaluated and systematic neonatal screening for haemoglobinopathies will be encouraged in member countries without existing databases.

SS28

SMART DRUGS: ANALYTICAL, PHARMATOXICOLOGICAL AND LEGISLATIVE ASPECTS

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Smart-drugs are a series of compounds of vegetal and synthetic origin which contain vitamins, psychoactive principles (the most diffused of which are ephedrine, caffeine, synephrine) which can show hallucinogenic properties. The smart drugs can be found under shape of energetic or alcoholic drinks, smokeable grass, dietary products, (mainly dried vegetal extracts). They are "smart" since they cannot be prosecuted or prosecutable by the law since many of them are not present (as plants or as contained active principles or as synthetic molecules) in the Law Tables of the corresponding laws which prohibit the use of psychotropic substances.

For many of these drugs, reference standards of the active principles are lacking and consequently there are no analytical methods for quali-quantitative determination. Information on pharmacotoxicological properties and related side-effects or consequences on CNS of smart-drugs are scarce. Since 2003, within the "Smart Drugs Monitoring" project we developed analytical methods for determination of several active principles contained in the preparations sold as "smart-drugs"; revised and investigated the pharmacological properties of psychoactive smart-drugs and related side-effects and the studies of the effects on the CNS with the aim of inclusion in the Law Tables (DPR 309 – 1990).

The strategy of analysis adopted in our laboratory, for the analysis of more than 150 products, has been following: extraction at basic, neutral and acid pH and subsequent screening analysis by gas chromatography-mass spectrometry. Once identified, the active principles were then quantified by specific methodologies from the international literature or developed in house. This latter was the case of methylxanthines in energetic drinks and the dietary supplements from plants such as Guaranà, Cola nitida, Ilex paraguayensis, Yerba mate; of ephedrine and related alkaloids in dietary products containing Ma-Huang or Sida Cordifolia; of synephrine in Citrus Aurantium dietary supplements; of Salvinorin A in Salvia Divinorum leaves and in biological matrices from consumers; cannabinoids in hemp food.

Based on scientific evidences collected in our study and following the favourable indication of Istituto Superiore di sanità, Salvinorin A and Salvia Divinorum plant have been included in the list of illegal drugs included in the new law (21/02/06) following DPR 309/90.

An extensive technical report on the pharmacotoxicological properties and side effects of 25 psychoactive smart drugs has been prepared, presented to the Ministry of health and available for Emergency Departments of Hospitals facing with intoxications eventually due to these substances and as an information for health professionals dealing with the issue of illicit drugs.

Two new research lines have been recently set up regarding:

- "Research Chemicals": new substances of synthetic origin with mixed psychostimulant and hallucinogenic like psychoactive effects with a use pattern of small domestic settings sold over internet web sites a sort of "smart drugs" available on illicit market and on internet web pages;
- Prenatal exposure to "Smart Drugs" and birth outcomes dealing with determination of "smart drugs" and eventual

metabolites in neonatal biological matrices in association with clinical effects during perinatal period.

SS29

ON SITE DRUG TESTING: USE, ABUSE AND MISUSE

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The consumption of drugs is in continuous increase above all in the juvenile population, consequently is assisted to an increased demand of toxicological analyses. In this context the expansion is placed on the market of devices on site that without the instrumentation aid, permits to find, on conventional biological matrices and not, of the substances of abuse in prearranged base to a cut-off. The devices of screening on site are simple to use, rapid, accurate, sensitive with acceptable global analytical performance if they come uses correctly, from qualified staff and to the inside of a standardized process and of quality. The problematic connected to their use can be reassumed in some points:

- the qualitative dosage does not allow to an appraisal of the samples having borderline that is a concentration of the substance dosed next to the cut off, information that could contribute to discriminate a poisoning acute from a not recent assumption and to estimate if the positivity is imputable to interferences of various substances regarding that one searched.
- cross reactivity and interfering: interfering substances and/or molecules pertaining to the same class come recognized from antibodies and generate falsely positive results, an example is the assumption of the codeine to therapeutic scope that is cause of the positivity to the test of screening opiate
- their use also outside analytical laboratory from part of not competent staff. As an example they can be used for the control of the workers with duties at risk for the emergency, the safety and the public health (test execute as an example on the workplace), for the therapeutic control of the rehabilitation programs (test execute as an example in the outpatients' departments of the Ser.T), for the controls in the circle of street emergency (art. 186 and 187 of the C.d.S.); In these cases in order to supply a reliable and sure result, the single technology is not sufficient, the interpretation of results cannot be based on the single reading of the test but they are indispensable acquaintances on the pharmacokinetic and the metabolism of the single substances, therefore also in this case the tests on site must be inserted in a rigorous, effective protocol, in a position to guaranteeing the identification of the subject subordinate to analysis, the authenticity and integrity of the used biological matrix, the sampling for the test of confirmation and eventual contra-analysis, adapted conservation of the samples, interpretation of the result and formation of the staff used through the introduction of a corrected guard chain.
- The necessity of periodic verification of the reliability of the analytical data by means of adhesion to programs of external appraisal of quality (VEQ).

For being able to supply useful analytical data and reliable is indispensable, the confirmation with a chromatographic method of samples with positive results at screening, adapted professional competence for the interpretation of the data in the light of the acquaintance of the potentialities and the limits of the method used to the aim to avoid misleading interpretations, in a standardized process of Quality that leaves from the demand for the dosage and finishes with the report.

SS30

LEGAL-MEDICAL COMMISSIONS: PROPOSAL OF METHODOLOGICAL GUIDELINES SHARED BY PROFESSIONALS

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Legal-medical Commissions are devoted to assess psycho-physical conditions in case of any disease, which can lead to impairment when driving motor vehicles. The commissions work following the Rules of Street Code included in the article 330 of the Republic President Decree 495/1992.

Up to now, the choice of methodologies and evaluation criteria for any assessment, performed through clinical and laboratory tests, is at discretion of the single Commission, which operates following local organization models and using scientific background of participant structures.

On the basis the various documents prepared by professionals from Legal Medicine and Forensic Toxicology Institutes, the National Institute of Health set up a proposal of Guidelines for Laboratory tests of legal-medical Commissions as a tool to be shared between experts of Laboratory Medicine and as a contribution towards harmonization in the establishments of local medical Commissions.

The Guidelines have the intention to standardize each different step of the assessment process: from collection of double samples for counter-analysis to the accurate toxicological analysis by GC-MS and LC-MS, to the use of structured forms for registration of all the different operative steps applied to samples, to the custody chain, to the intra and inter laboratory quality control, to the final documentation storage.

SS31

PROFESSIONAL LIABILITIES OF THE CLINICAL AND FORENSIC TOXICOLOGIST IN ADMINISTRATIVE AND CIVIL CERTIFICATION

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The increasing duties of toxicological laboratories carrying out analytical tests with a medico-legal bearing are obliging laboratory specialists to face new responsibilities both towards the facility where they operate and towards the patient, as well as new legal liabilities.

In fact, they are responsible for the biological sample, from receipt to preservation; for the analytical processes; for management of the devices; for the methods of transcription of the results; for drawing up the analytical report.

In the present work, the duties of clinical and forensic toxicologists are examined, as well as the various types of liability they face: civil liability as the technical consultant for one party (CTP); penal liability as the technical consultant for one party (false evidence; other false testimony to the CTP; misleading communications to the judicial authorities; slander and libel); civil liability and deontological responsibility as a consultant; administrative liability (damages and heavy liability of the employee; damages to the public facility; causal link between the negligent conduct and the ensuing damages); erroneous certification of analyses. The ethics code and deontological code are also examined.

Only full compliance by analysts and the facilities where they operate with the norms and regulations dictated not only by the Civil and Penal Code but also the Ethics and Deontological Codes and Guidelines for correct performance of the duties, can bring about a reduction in lawsuits for unprofessional conduct and/or negligence filed by dissatisfied clients.

CO12
SUGGESTIONS CARBOHYDRATE-DEFICIENT TRANSFERRIN (CDT) MEASUREMENT IN FORENSIC USE AND A TRAFFIC SAFETY CONTEXT

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Background. Alcohol-mediated changes in the glycoform pattern of serum transferrin, known as carbohydrate-deficient transferrin (CDT), is used as a biomarker for identifying chronic excessive alcohol use and for follow-up of abstinence. A recent Italian research project pointed out that CDT measurement in a traffic safety context is not a homogenous test. The aim of this work is to make recommendations for CDT testing based on the most important recent international reviews, turn them into guidelines, and list common procedures and routines for CDT determination and reporting.

Methods. Most references have been identified through a MEDLINE bibliographical research (April 2000-April 2007), using "carbohydrate-deficient transferrin" as the keyword. Articles published in *Biochimica Clinica* have also been examined.

Results. The suggestions are as follows: CHARACTERISTICS OF THE LABORATORY: A public body, if private well know in the field of scientific research, The appropriate laboratory technology, Staff being experts in biochemistry and forensic toxicology, A consolidated quality management system (like UNI EN ISO 9001:2000), A reliable internal quality system (CQI) and use of external quality controls (VEQ). PRE-ANALYSIS: Sample: serum collected from fasting people who should be always identified, and in full observance of the chain of custody. Storage: better if frozen, although transferrin is stable in the cold for days and in the freezer for months. ANALYSIS Glycoform target: disialotransferrin.

Standard. CDT reference materials from purified human serum when available.

Method. HPLC after complete iron saturation of transferrin as reference method, until a mass spectrometric method becomes available. DATA AND INTERPRETATION CDT values: expressed as a relative amount to total transferrin (%CDT), Cut-off: measured by each laboratory with reference to moderate drinkers, Transferrin variants: if interfering with the determination of CDT, the result should not be reported.

Conclusions. These suggestions about routines for CDT determination should improve collaboration and agreement between laboratories, help medical staff in the diagnosis and forensic work, and last, but not least, strengthen the general public trust in this tests and the institutions

CO13
DRUG MONITORING IN BREAST MILK: AN INDIVIDUALIZED APPROACH FOR BREAST-FEEDING MOTHERS

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There is an increasing percentage of pregnant women with antiepileptic or psychotropic drugs (AED,SSRI) who plan to breastfeed. Data from breast milk studies are of primary importance during defining the risk-benefit assessment of AED and SSRI therapy in postpartum women in lactation. The physical and emotional benefits of breastfeeding have to be balanced with the potential risk of neonatal medication exposure. The transfer of drugs to breast milk depends on a concentration gradient that allows passive diffusion of nonionized, non protein-bound drugs. Medications that are poorly protein bound, that have small molecular weights or that are highly lipid-soluble tend to enter the breast milk in clinically important quantities.

Analysis: milk is a notoriously difficult matrix for drug analysis because of its high protein and lipid concentrations. We have developed an original system to quantify the concentration of multiple SSRI and AED in breast milk and in maternal and neonate serum involving HPLC-UV after liq/liq or SPE extractions. Breast milk samples (2 for each mother at the beginning and at the end of lactation) were collected in sterile tubes stored at -20°C. For the assay they were thawed at 25°C and mixed.

Results. Mean milk/maternal plasma concentration ratio (range) tab1.

The infants' levels of AED and SSRI drugs in milk in our case series is substantially comparable with that described in available case reports.

By monitoring the milk concentration we are now able to establish the exposure level of individual infants.

References

1. Hoestetter AL et al. A novel system for the determination of antidepressant concentrations in human breast milk. *Ther Drug Monit* 2004,26:47-52

Valproate (n=3)	0.07 (0.05-0.10)
Levetiracetam (n=2)	1.07 (1.00-1.12)
Carbamazepine (n=3)	0.25 (0.20-0.38)
Clobazam (n=2)	0.20 (0.18-0.22)
10-OH-carbamazepine (n=2)	0.83 (0.78-0.88)
Citalopram (n=1)	0.45 (0.40-0.47)
Phenobarbital (n=3)	0.39 (0.30-0.51)
Lamotrigine (n=2)	0.44 (0.42-0.46)
Carbamazepine-Epoxyde	0.34 (0.28-0.42)
Nor-Clobazam (n=2)	0.23 (0.19-0.25)
Venlafaxine (n=1)	1.25 (1.20-1.33)
Olanzapine (n=1)	1.49 (1.45-1.60)