**2° Evento Nazionale Congiunto SIBioC-SIMeL**

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## *Riassunti Sessioni Scientifiche*

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| • **SS01-SS03 CO01-CO02** | Il carcinoma della mammella: un esempio emblematico del ruolo della Medicina di Laboratorio in oncologia |
| • **SS04-SS06 CO03-CO04** | Il laboratorio nel percorso diagnostico-terapeutico del paziente diabetico (1° parte) |
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Legenda:

SP Sessione Plenaria
SS Sessione Scientifica
CO Comunicazione Orale

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

SP1
**RENAL FAILURE**

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Significant loss of renal function is becoming an epidemic in the Western world. Main causes are hypertension and diabetic nephropathy. It is essential to standardize methods and measures of laboratory assays in order to follow-up the increasing population of individuals with chronic renal failure (CRF). Presenting symptoms include loss of appetite, nausea and vomiting. These features can be associated with advanced renal failure often needing the beginning of chronic dialysis treatment (HD). Severe renal failure with mental obtundation, myoclonic twitching and coma need instead prompt HD. Initial signs of renal failure are usually proteinuria and/hematuria. Incidental finding of raised serum creatinine can be detected as part of "routine" biochemical screen in patients without symptoms. The first question to answer when we first see a patient with reduced renal function is: Acute or chronic? Pre-renal acute renal failure (ARF) need to be excluded. A simple and effective assay is to measure urine Na and K in extemporaneous urine samples. Higher levels of urine K compared to urine Na is a reliable indicator of pre-renal ARF. Management of CHR rely mainly on laboratory findings. Underlying diseases as glomerulonephritis need frequent proteinuria measures in order to identify disease progression or treatment effectiveness. Complications of chronic renal failure instead require accurate assessment of hyperparathyroidism, anaemia, acidosis, hyper lipidemia, and drug doses. Hyperparathyroidism is a major complication of CRF and is associated with bone disease, vascular and extra-articular calcification. Serum levels of calcium, phosphate and parathyroid hormone are essential laboratory measurements for Nephrologists. It is for treatment of CRF patients before and under HD, particularly those eligible for kidney transplantation. Anaemia can be treated with erythropoietin, vitamin B12, folate and iron supplements. Levels of serum vitamin B12, folate, iron, ferritin, transferrin and total iron binding capacity (TIBC) are essential for proper treatment and follow-up of anaemia in the course of CRF. Acidosis should be corrected in order to control bone disease and muscle wasting. Blood gas and bicarbonate levels need to be analyzed at monthly intervals particularly in HD patients. Hyperlipidemia is associated with the risk cardiovascular risk in renal failure. In our days cardiovascular disease accounts for approximately half of all deaths in HD patients regardless of age, gender, or primary renal disease. It is progressively worsening when we consider the increasing age of our HD patients and that diabetes is the major cause of CRF worldwide. Drug doses have to be adjusted in CRF or HD patients to avoid accumulation when they are cleared mainly by kidneys. Kidney transplantation is an emerging challenge for clinical and laboratory workers. Levels and dose adjustment of immunosuppressant as cyclosporine, tacrolimus, sirolimus and everolimus need increasing accuracy as they are associated to graft toxicity and increased infection susceptibility.

SP2
**STANDARDIZATION OF THE MEASUREMENTS OF CREATININE AND NEW MARKERS OF RENAL INSUFFICIENCY: AN UPDATE**

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The publication, in 2003, of the National Kidney Foundation Practice Guidelines for Chronic Kidney Disease, recommending the use of the MDRD formula to estimate GFR put an enormous relevance on the need for standardization of serum creatinine measurement. In fact the eGFR is highly dependent on the accuracy of the creatinine method in use. Relevant progresses occurred in the last years and a reference measurement system for creatinine was clearly defined by the Joint Committee on Traceability in Laboratory Medicine (JCTLM). Nonetheless the work published by Delanghe in 2008 (but performed in autumn 2005) to compare the performances of several analytical systems throughout Europe, demonstrated a high dispersion of the results. The problems were of two types: incorrect calibration, not traceable to the IDMS reference measurement procedure and non specificity of the alkaline picrate based methods. Correct calibration is an important issue: still in 2007 only 22% of the laboratories in USA were using IDMS traceable calibrated systems. To allow traceable calibration for Jaffe methods the positive interference due to proteins was corrected by some manufactures introducing a "compensation" factor. It is now clear from recent publications (Panteghini, Cobbaert, Schwartz) that this correction is able to improve the average performance, but cannot work on specific patients and, especially with children, can introduce an overcorrection. Only the enzymatic methods, being more precise and specific, allow really to obtain results traceable to the IDMS based primary reference measurement procedure. An IFCC Working Group on the standardization of GFR assessment is performing a study on the specificity of creatinine assays that will provide soon useful information on this matter. The availability of a frozen certified reference material from NIST (SRM 967), that proved to be commutable with a large number of field methods, gives to the manufactures a substantial help to guarantee traceability. Moreover EQA providers are launching programs using materials with values assigned by the reference method, giving to the clinical laboratories a real accuracy reference.
Several authors are proposing cystatin C as an alternative to creatinine in formulae to estimate GFR. The main problem of this measurement is lack of a

reference measurement system that makes difficult a wide application. The IFCC working group on cystatin C standardization has produced a candidate primary recombinant reference material. The pure cystatin C has been used to prepare a secondary reference preparation spiking with the primary preparation a stabilized serum matrix. The work is in progress, stability, value assignment and commutability trials are ongoing. The availability of such a preparation will provide a sound basis for standardization and will probably allow the use of cystatin C on a worldwide scale.

SP3

## GLOMERULAR FILTRATION RATE MEASUREMENT: FROM EQUATIONS TO REPORT CONSENSUS

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In the clinical assessment of kidney function a simple measure of solute concentration or solute excretion or urine output does not describe the real "function" of the organ, since urinary flow, body size and solute concentration in blood could be very different between individuals. The Glomerular Filtration Rate (GFR), i.e. flow rate of filtered fluid through the kidney, is considered the best overall index of kidney function both in health and disease. The GFR cannot be measure easily in clinical practice. It takes an integration of many parameters, in a balance between capillary and interstitial hydrostatic and oncotic pressure. In practice, it is not possible to directly identify the needed values for this equation. The "clearance" of an ideal molecule, fully filtered by the glomerular membrane, without reabsorpion or secretion by renal tubuli, could be a way to compare renal function among different individuals. But the "ideal" marker does not exist and many exogenous molecules currently used are expensive, complex or lead to error of 5-20% in different measurements. Since seventies, a number of formulas have been devised to estimate GFR values on the basis of serum creatinine levels. The Cockcroft-Gault equation is one of the most widely recommended and used. In 1999 a new prediction equation, derived from 1628 subjects with renal insufficiency enrolled in the Modification of Diet in Renal Disease study (MDRD), was published and a simplified equation, that used serum creatinine as the only serum assay, was published in 2000. One of the main advantages of this equation is to avoid the anthropometric measurement. The formula has been widely applied in both clinical care and research since its publication. Later studies demonstrated the MDRD equation to be accurate at least as much as the other formulas. Moreover, this formula was the only studied and reformulate in regard to the standardization of creatinine measurement and it is the only corrected for the isotope dilution mass spectrometry (IDMS) calibrated creatinine. All creatinine-based equations, other than the IDMS-traceable MDRD Study equation will give values that, in most cases, are higher than the values obtained using traditionally calibrated creatinine methods.
However, MDRD formula not solves every problem and cannot be applied in all subjects. First of all, it is expected that the in vitro diagnostics (IVD) industry will complete the recalibration of routine blood, serum, or plasma creatinine methods during 2009. During this interim phase, clinical laboratories need to choose between IDMS-original MDRD or IDMS-traceable MDRD, according to the used method to measure creatinine.
Secondly, this formula was derived by adult individuals. Subjects under 18 or over 75 years cannot be evaluated in their renal function. For children, other formulas could be applied, and the Schwartz formula, known since 1970, was recently improved. This new equation is based on an enzymatic creatinine method, IDMS traceable. Thirdly, the MDRD was derived by subjects with chronic kidney disease. It is known that the MDRD formula systematically underestimates the renal function. Especially for this reason, the National Kidney Disease Education program and many other guidelines recommend reporting eGFR values greater than or equal to 60 mL/min/1.73 $m^2$ simply as ≥ 60 mL/min/1.73 $m^2$, and not as an exact number. It could reinforce the erroneous belief that renal function is normal in all such situation.
Given these limitations, Levey and colleagues few months ago published their new formula on behalf of the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI):

$$GFR = a \times (\text{serum creatinine}/b)^{c} \times (0.993)^{age}$$

where *a* takes on race and sex, *b* sex and *c* sex and creatinine measurement. Obtained from a dataset of 5504 subjects, the new equation seems to be more accurate than the MDRD study equation, yielding a lower estimated prevalence of kidney disease, and could replace it for routine clinical use.

SP4

## URINARY ALBUMIN EXCRETION IN CHRONIC KIDNEY DISEASE: CRITICAL ISSUES IN MEASUREMENT AND REPORTING

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Urinary excretion of albumin is a cardinal sign of kidney disease and it is recognized as a risk factor for progression of kidney disease and cardiovascular disease. Because of its clinical importance there is an urgent need for an accurate measurement of the protein and for clearly reported results. The National Kidney Disease Education Program and the IFCC established a Working Group on "Microalbumin" with the aim to identify specific areas for improvement.
At the moment there is a consensus opinion on the following issues

- the term “microalbumin” is to be discouraged
- first morning void is the preferable sample since provides a lower variability than other types of samples
- urinary albumin should not be measured in frozen sample (unless they have been stored at –70°C)
- an albumin/creatinine ratio (ACR) should be reported with all measurements
- albumin concentration in milligrams per litre should not be the only value reported

Many areas require further investigation; among these

- albumin adsorbs on plastic surfaces, so the influence of the container type on albumin concentrations should be carefully evaluated
- the nature of albumin in urine is more complex than previously thought, so there is an absolute need of an accurate definition of the measurand
- development of a reference measurement procedure: primary and secondary materials and reference method
- development of urine creatinine reference measurement procedure
- identification of appropriate EQAS materials in order to be able to compare the analytical performances of different methods
- definition of the reporting units (g albumin/mol creatinine; mg albumin/g creatinine; ug albumin/mg creatinine

Regarding the post analytical phase and the reporting issues, it should be noted that the existing threshold limits have been established for people with diabetes. If these limits could be used for people without diabetes is still matter of debate. ACR varies with age, sex and ethnicity: the decision limits for these subgroups need further studies and investigations. However, there is increasing evidence that a continuous relationship between urinary albumin excretion and risk of chronic kidney disease or cardiovascular risk exists, so that no 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. Some epidemiological studies have already demonstrated that the amount of albumin which can be considered “negligible” is much lower than the threshold limits established for diabetic nephropathy. The sensitivity of the laboratory method is, in this context, crucial and should be carefully evaluated when examining the analytical performances of a method.

SS1

## TAMOXIFEN: THE FIRST TARGETED-THERAPY TO MANAGE PATIENTS AFFECTED BY EARLY BREAST CANCER: IS THERE STILL A ROLE FOR IT?

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While several large trials have now established that aromatase inhibitors are more effective than tamoxifen when used as adjuvant endocrine therapy, the optimal adjuvant strategy, in particular whether AI should replace tamoxifen from the beginning or after a few years of anti-oestrogen treatment, remains a matter of debate.

For some Authors, upfront use of an AI offers the best opportunity to reduce the risk of early relapse, whitin the first 2-2.5 years after surgery, for postmenopausal patients likely to harbour tumours primarily resistant to tamoxifen. In the more recent analysis of the ATAC trial, at a median follow-up time of 100 months, and in the BIG 1-98 trial, at a median follow-up of 76 months, there was no convincing effect of such an approach on breast cancer and overall mortality, in spite of the fact that both ATAC and BIG 1-98 trials are large and data quite mature.

No effect on mortality comes also from the metanalysis of the two trials, which includes more than 9.000 patients. In contrast to this findings, a small, but statistically significant, breast cancer- and overall mortality advantage comes out from two of the four trials on switching and from the different metanalysis available, which confirms the positive effect of switching on both breast-cancer and overall mortality. As cure still remains the major goal of adjuvant treatment of breast cancer, it is questionable at this point wether replacing tamoxifen with an AI since the beginning should really represent the gold standard for all women with endocrine-sensitive tumors, also in view of the side effects that so prolonged estrogen-deprivation might exert on bone resorption or ischemic disease and of the increased costs of treatment.

Nowadays there is no doubt that switching to aromatase inhibitors is not only strongly advisable for patients already on treatment with tamoxifen from 2 or 3 years, but might represent a reasonable initial choice for all women with endocrine sensitive tumors, unless it might be reasonably argued that they might be intolerant to or anyway unsuitable for receiving tamoxifen. The up-front use of aromatase inhibitors represent a reasonable choice for the patients at higher risk of relapse or for whom a suboptimal response to tamoxifen might be predicted, as well as cost/benefit to be defined in the individual patient.

SS2

## THE SHIFT FROM BIOCHEMICAL TO IMMUNOHISTOCHEMICAL METHODS FOR THE DETERMINATION OF OESTROGEN RECEPTORS IN BREAST CANCER: AN EVIDENCE BASED DECISIONAL PROCESS?

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The relation between estrogens and breast cancer was based on empirical data since the seventies, when specific oestrogen receptors (ER) were identified. Thus,

the determination of ER in tumor tissue became the first "biological target" used in the clinical practice for therapeutic decision. ERs were measured with a Ligand Binding Assay (LBA) that was progressively standardized and thus became the reference method in Europe. The prediction of the responsiveness on the basis of ER positivity was approximately 55-65% while 10% of ER negative tumors were responsive as well. To increase the predictive power of ER, the biochemical characteristics of ER were studied and active and inactive forms of the receptor were identified on the basis of the different sedimentation coefficients on sucrose density gradient centrifugation. The determination of progesterone receptor (PR), a parameter of ER function, further increased the predictive accuracy when associated to ER. Most importantly, the real amount of ER was shown to be positively associated with the probability of response to endocrine manipulations.

In the late eighties monoclonal antibodies specific for ER were identified. They provided information on the tissutal, cellular and ultrastructral localization of the receptor and permitted the development of new immunometric methods (EIA, IHC). In the same time period, mammographic screening resulted in such a dramatic decrease in the size of the average breast cancer that it became difficult to collect tissue for ER determination by LBA in many cases. Therefore, IHC has progressively substituted LBA as the routine method for ER determination. A paper published in 1999 in the J. Clin. Oncol. by the San Antonio Group stated that ER status by IHC is superior to the LBA assay for predicting response to adjuvant endocrine therapy in breast cancer, thus advocating the systematic substitution of LBA with IHC. Further on, the investigators of the International Breast Cancer Study Group (IBCSG) showed that the ability of ER status as determined by IHC to predict responses to endocrine therapy is superior to that of ER status as determined by LBA. So, should IHC be considered the established gold standard method for routine determination of ER? Two recent studies (more than 7,000 evaluated cases) found that the distribution of ER values using IHC was essentially bimodal, with more than 90% of tumors being either completely ER negative or unequivocally and strongly ER positive confirming that IHC is not an intrinsically quantitative method and there is not a direct, linear relationship between the intensity and distribution of the chromogenic reaction product as determined by IHC and the amount of ER protein in breast cancer cell nuclei. On the contrary, LBA is based on the direct binding of estrogen and ER in an essentially 1:1 ratio, thus providing a quantitative determination of ER content of the tumor. Using LBA, breast cancers were found to exhibit a broad range of values for ER and the magnitude of the benefit from endocrine therapy was related to the quantity of ER. However, was the "quantitative" information potentially provided by LBA exhaustively investigated and applied in clinical practice? To answer this question we examined studies published between 1996 and 1999 in which: (1) the objective of the study was prognosis and/or prediction of responsiveness to endocrine manipulations; (2) LBA was used; (3) at least 100 cases were investigated; and (4) multivariate analysis was applied. Sixty papers (with 51,515 examined patients) fulfilled the criteria. Surprisingly, dichotomic criteria based on a cut-off point were used in 54/60, whereas ER were examined on continuous scale in 3 studies only. The "qualitative" use of biochemical quantitative results justified a very quick transition from LBA to IHC. On the other hand, NEQUAS results of interlaboratory reliability and reproducibility of estrogen and progesterone receptor assays in Europe documented reliable HIC assays in 24/66 (36%) participating laboratories. Likewise, four IHC methods approved by the Japanese Ministry of Health were shown to provide significantly different results, so that it might be difficult to generalize the results and compare results of different institutions. Recently, the interlaboratory comparison performed by NEQUAS (EU) and in USA have convincingly confirmed meaningful interlaboratory variability, so that currently, there are legitimate concerns worldwide that IHC testing are insufficiently standardized.

It can therefore be concluded that the shift from LBA to IHC has been encouraged by practical reasons rather than supported by an evidence based decisional process.

SS3

## MOLECULAR APPROACH FOR SELECTION OF ENDOCRINE-BASED THERAPY IN BREAST CANCER TREATMENT

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Most breast cancers are initially hormone-dependent and estrogens play a pivotal role in their development and progression. In postmenopausal women, estrogens are generated from androgens through aromatase-dependent enzymatic conversion. The estrogen receptor (ER) plays an important role in the clinical care of breast cancer patients both as a prognostic factor as well as a therapeutic target and its expression is found in 60% of primary breast cancer. Aromatase inhibitors (AI) such as letrozole, by eradicating estrogens, suppress both genomic and non-genomic action of ER. Increasing evidence has shown that aromatase inhibitors are superior to the conventional anti-estrogen tamoxifen in the treatment of hormone-dependent breast cancer in postmenopausal women. While aromatase inhibitors have resulted in significant clinical progress in the endocrine treatment of postmenopausal women with ERve+ breast cancer, initial (*de novo*) or subsequent (acquired) resistance still limits their benefit for many patients. Laboratory studies using various models to investigate these mechanisms of resistance

have demonstrated that both peptide growth factor pathways and oncogenes involved in the signal transduction cascade become activated in breast cancer cells during long-term estrogen deprivation. To the classical genomic pathway, estrogens exert rapid non-genomic actions mediated by a subpopulation of ERs that is located in the plasma membrane. This membrane-bound ER can directly interact with membrane kinase receptors such as Insulin Growth Factor Receptor 1 (IGF-IR), Epidermal Growth Factor Receptor (EGFR), and HER2 and rapidly activate various signalling cascades including the Ras/Raf/mitogenactivated protein kinase (MAPK) pathway and the phosphatidylinositol 3-kinase (PI3K)/Akt/mTOR pathway Conversely, ER can be directly activated in the absence of estrogens through phosphorylation by various kinases, including MAPK and PI3K/AKT. Combining endocrine therapies for breast cancer with various targeted biological therapies has become a very active area of clinical research aimed at overcoming or preventing endocrine resistance. There are evidences that Erb-B2 and MAPK pathways are up-regulated in cells resistant to letrozole. Current clinical trials have investigated three approaches to overcoming endocrine resistance, including maximal blockade of ER signalling, combinations of endocrine therapy with novel therapies that target the HER family or other relevant downstream signalling pathways such as PI3K/Akt/mTOR or MAPK related pathways. Not all approaches have been successful to date, despite often very encouraging preclinical data. Signaling and cell survival pathways are also abrogated in hormone-resistant breast cancer, in particular the PI3K/Akt pathway. Akt is a serine/threonine kinase that promotes cell survival and is activated in response to many different growth factors including IGF-1, bFGF, EGF, VEGF). Further progress critically depends on understanding mechanisms of resistance to AIs, and utilizing novel approaches such as STIs to combat resistance pathways and block cross-talk.

CO1

## L'INTERLEUCHINA 8 E LE METALLOPROTEASI DELLA MATRICE 2 E 9 SONO FATTORI PREDITTIVI DI RISPOSTA ALLA SOMMINISTRAZIONE METRONOMICA DI ACIDO ZOLEDRONICO E TAXOTERE IN PAZIENTI CON CARCINOMA PROSTATICO ORMONE REFRATTARIO (CPOR)

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Coorti di 3-6 pazienti (22 pazienti totali età mediana 73 anni; range 43-80) hanno ricevuto una delle tre dosi scalari di DTX
(30, 40 e 50 mg/m2) in combinazione con una dose fissa di ZOL (2 mg), entrambi somministrati ogni 14 giorni in due diverse sequenze. Sequenza A: DTX al giorno 1 seguito da ZOL al giorno 2. Sequenza B: ZOL al giorno 1 seguito da DTX al giorno 2. L'MTD non è stata raggiunta con la sequenza A. Due pazienti al terzo livello della sequenza B hanno sviluppato DLT con G3 tromboflebiti e ischemia cardiaca. In 4 pazienti (livelli 2A e 3A) si è riscontrata una riduzione # 50% dei livelli di PSA.
In tutti i pazienti abbiamo valutato 18 citochine diverse su sieri prelevati al tempo 0 ed ogni 14 giorni al riciclo. Dopo 2 cicli di terapia abbiamo rilevato variazioni significative di interleuchina 7 (IL7), 8 (IL8), metallo-proteasi della matrice 2 (MMP-2) e 9 (MMP-9. In particolare vi era una riduzione mediana di 1.5% di IL8 e di 15 e 42 % di MMP-2 e 9 nella sequenza A (9 pazienti valutati) mentre era rilevabile una riduzione mediana di 1% di IL8 ed un aumento del 9% e del 26 % di MMP-2 e 9 nella sequenza B (9 pazienti valutati). Stratificando i pazienti per la risposta clinica [risposte parziali (PR) e stabilizzazioni (SD) in 6 pazienti valutati] vs. progressioni di malattia (SD in 11 pazienti valutati) sono stati ottenuti i seguenti risultati: si riscontrava una riduzione mediana del 41.5, 24.5 e 44.5% di IL8, MMP-2 ed MMP-9 nei pazienti responsivi mentre si rilevava un incremento mediano del 64%, 13% e 10% di IL8, MMP-2 ed MMP-9 nei pazienti non responsivi. Stratificando i pazienti per la risposta sierologica (declino di PSA > 10% in 7 pazienti o assenza di declino del PSA >10% in 10 pazienti) sono stati ottenuti i seguenti risultati: si riscontrava una riduzione mediana del 17.5, 15 e 26% di IL8, MMP-2 ed MMP-9 nei pazienti responsivi mentre si rilevava un incremento mediano del 64%, 6% e 1.5% di IL8, MMP-2 ed MMP-9 nei pazienti non responsivi. Questi dati suggeriscono che la riduzione dei livelli di IL8, MMP-2 e MMP-9 possono essere considerati fattori predittivi di risposta alla combinazione metronomica di DTX e ZOL nel CPOR.

CO2

## THE SIMULTANEOUS ASSESSMENT OF CIRCULATING LEVELS OF FREE CA15.3 AND CA15.3-IgM FOR IMPROVING BREAST CANCER DETECTION

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Aim. Biomarkers of cancer may be detected in the bloodstream associated with immunoglobulin M (IgM)

in the form of immune complexes (biomarkers-IgM) and their measurement by tailored immunoassays has led to improved cancer detection when compared to gold standard serological tests1,2. We evaluated the diagnostic accuracy of CA15.3-IgM immune complexes in sera of primary breast cancer patients and healthy controls and compared it to the accuracy of free CA15.3.
Methods. A total of 189 serum samples were collected (prior to treatment) from 103 women with stage I and II breast cancer and from 86 age-matched healthy women. To evaluate the presence of CA15.3-IgM we developed and validated a novel ELISA using a polyclonal rabbit anti-human CA15.3 antibody as the catcher antibody. The CA15.3-IgM was detected with peroxidase-conjugated anti-human IgM using ABTS and hydrogen peroxide as substrate. The levels of CA15.3-IgM were expressed in arbitrary units per ml (AU/ml) by interpolation on a calibration curve obtained by serial dilution of a reference calibrator purified by gel filtration chromatography from a pool of serum samples of patients with breast cancer.
The linear range of the CA15.3-IgM assay was from 3.12 to 100 AU/ml. Intra and inter-assay coefficients of variation ranged from 2% at 100 AU/ml to 12% at 3.12 AU/ml. Mean analytical recovery was about 80%. Free CA15.3 levels (U/ml) were assessed on each sample using an automated immunoassay system.
Results. We used the clinical cut-off value of 31.5 U/ml for free CA15.3 and 755 AU/ml for CA15.3-IgM (95°percentile of CA15.3-IgM levels serum distribution in healthy controls). CA15.3-IgM had a sensibility of 9% and a specificity of 95% compared to free CA15.3 test that obtained a sensitivity of 8% and specificity of 100%. By combining serum levels of CA15.3-IgM and free CA15.3, the sensitivity for breast cancer detection reached 17% with 95% specificity over healthy subjects.
Conclusion. These results demostrate the presence of circulating CA15.3-IgM in breast cancer patients. The combination of CA15.3-IgM and free CA15.3 measurement is the best possible approach for breast cancer diagnosis.

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SS4

## DIAGNOSTIC-THERAPEUTIC "COMPARED"

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Diagnostic-therapeutic patient care pathways represent the contextualization of guidelines relating to a given disease or clinical problem in a given organizational reality of the public healthcare facilities, in the light of the available resources. When we design a diagnostic-therapeutic patient care pathway it is of fundamental importance to state how far it extends, qualifying it as pertaining to the hospital and/or to territorial services. When the diagnostic-therapeutic patient care pathway describes the process relating to a health problem in its management both outside and inside the hospital, we can speak of an integrated treatment profile, a path that focuses on continuity, integration and completeness of patient care. The care of diabetic patients includes different diagnostic-therapeutic pathways, depending on the stage of their disease and an presence or absence of acute and chronic complications, so distinct pathways are identified, though they may be interconnected.

SS5

## THE "WEIGHT" OF EVIDENCE BASED MEDICINE

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The International Federation of Clinical Chmistry (IFCC) launched a global campaign on diabetes mellitus (DM) in order to improve laboratory diagnosis and management of the disease (1). The campaign was devoted to assist laboratories to develop a patient-centered, evidence-based approach to the diagnosis and management of DM by transferring the Best Practice to clinical practice. It is in the terms of references the review of the current use of laboratory test to educate doctors and patients in the interpretation of laboratory tests used in diagnosing and monitoring of DM. The main issues were the determination of blood glucose, the standardization of HbA1c, and microalbumin. Practice guidelines (GLs) are statements that help healthcare professionals to make a decision about the care of patients, GLs may be a tool to improve the managing of DM. As little it is known regarding the quality of GLs that make diagnostic recommendations, it is an open question if GLs for the Diagnosis and Monitoring of DM fulfill the criteria of Evidence-Based Guideline. A pilot assessment of the most well-known guidelines for the diagnosis and monitoring of DM was performed (2) to appraise the methodological quality of GLs on DM published in English between 1999 and 2005 identified by systematic searching in Medline and international guideline databases. The study reported that the selected four guidelines on DM have significant shortcomings in demonstrating and/or reporting multidisciplinary stakeholder involvement in the guideline development process, evidence-based methodology for formulating recommendations, applicability of statements, and disclosing any conflicts of interest or reporting editorial independence. Further, a recent NACB GL on POCT diagnostic test evaluated the following issues on EBM basis (3). Does blood glucose self-testing (i.e., primary care setting) lead to an improved clinical patient (outcome in diabetes mellitus? Does blood glucose self-testing (i.e., primary

care setting) lead to an economic benefit in diabetes mellitus? Does blood glucose point-of-care testing (POCT) in the hospital (i.e., secondary care setting) lead to an improved clinical patient outcome in diabetes mellitus compared with central laboratory testing? Does the provision of the HbA1c result at the POC lead to an improved clinical patient outcome when compared with central laboratory testing? The poor quality and lack of explicitness of recommendations in DM call for methodological standards of guideline development and reporting to increase the internal and external validity of recommendations in laboratory practice (2).

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SS6

## PRE-ANALYTICAL AND ANALYTICAL PHASE

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Out of the parameters measured for the diagnosis and monitoring of diabetes, the determination of plasma glucose, urinary albumin and glycated hemoglobin are those of primary importance. Goal of this presentation is to focus on the recommendations recently issued in Italy by the Italian Diabetes Study Group promoted by the two major Laboratory Societies (SIBioC and SIMeL). Some of the major points highlighted by these recommendations are mentioned as follows. With regard to the measurement of plasma glucose, in the past year a document on the use of glucometers has been prepared and is actually under publication (1). Maximum allowable error should be <10 % for glucose concentrations between 1.7 and 22.1 mmol/L (30-400 mg/dL). A guideline on the correct execution of the oral glucose tolerance test for the diagnosis of diabetes has been issued in the past years (2), and a new version is actually under revision in order to enlarge the set of the interfering substances and to reduce the threshold for the diagnosis of the subjects with impaired fasting glucose, from 6.1 to 5.6 mmol/L (from 110 to 100 mg/dL). With regard to the quantitative determination of albumin in urine for the diagnosis of diabetic nephropathy (microalbuminuria) the Study Group is going to produce a recommendation, in order to limit the number of samples and to harmonize the units, as previously remarked from a pilot survey (3). Cut-off values for normoalbuminuria for fresh spot samples have been recommended (<30 μg/mg creatinine). Finally, with regard to glycated hemoglobin, a group of experts, nominated by a number of Italian associations and scientific societies, recently published a document in order to promote a well coordinated plan to implement the standardization of glycated hemoglobin in Italy, according to the reference system promoted by the International Federation of Clinical Chemistry (IFCC) (4). Goals for the total errror (6.7 %) and imprecision ($CV_a \leq 2.0$ %) have been defined, together with the new S.I. units (mmol/mol). How to relate old and new units, and timeline for the change have been indicated. Out of the pre-analytical phase a position has still to be formulated with regard to the interference of hemoglobin variants.

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CO3

## BIOLOGICAL VARIABILITY OF GLYCATED HEMOGLOBIN: A SYSTEMATIC REVIEW OF THE LITERATURE

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Glycated hemoglobin (HbA1c) is the most important marker for monitoring of the glycemic state in diabetic patients. Different analytical techniques are available for HbA1c determination. The lack of comparability between results from different methods has been overcome by the development of the IFCC reference system; however, a clear definition of the clinically allowable error of measurements is still lacking. Information from biological variation of the analyte can be used to derive these goals. We systematically reviewed the published studies on the biological variability of HbA1c to check consistency of available data in order to accurately define analytical goals. We searched literature in PubMed, without limitation of period, for "Biological variation & HbA1c", "CVI% & HbA1c" e "CVG% & HbA1c" keywords. Only studies designed toexperimentally derive data on HbA1c biological variation were included. The critical review of the papers focused on the conformity of the applied protocol to the recommendations addressed by Fraser and Harris in 1989. All the 8 recruited studies showed major limitations on preanalytical variables (selected population, frequency of sampling, sample storage), on the employed analytical methodology (different analytical specificity) and in statistical evaluation of data. With regard to evaluated subjects, four studies

enrolled diabetic patients instead of healthy subjects and two studies, performed on healthy individuals, evaluated persons from only one gender. With regard to the length of the study, some protocols were performed over 6 months, considered the maximal clinically relevant time-span for HbA1c use. From the analytical point of view, considering the measurand as defined by the IFCC, the only suitable method was a turbidimetric immunoassay used in one study. In many studies, subject samples were neither assayed in duplicate nor in a single run after completing sample collection, making difficult to correctly assess the contribution of analytical variation to the total variation of results. In conclusion, there is still an urgent need of an accurately designed study to determine biological variability of HbA1c using a traceable and specific assay, appropriate protocol and right statistical derivation of data.

CO4

## SCREENING AND DIAGNOSIS OF GDM: A NEW APPROACH

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No international guidelines are untill now available on the screening and diagnosis of gestational diabetes mellitus (GDM), a pathology characterized by high fetal and maternal morbidity. The HAPO study (Hyperglycemia and Adverse Pregnancy Outcomes) has recently been conducted in order to clarify the risk of adverse outcome associated with various degrees of maternal glucose intolerance less severe than in overt diabetes mellitus.This multicenter study has evaluated 25.000 pregnant women at 15 centers in nine countries that have been subjected to a 75 g. oral glucose tolerance test(OGTT), with measurement of plasma glucose at time 0', 60' and 120' of the test, at 24 –32 weeks of gestation. The results of the study, recently published, indicate a strong, continuous association of maternal glucose levels, below those diagnostic of diabetes, with increased birth weight and increased frequency of fetal morbidity.On the basis of these results an International Consensus Panel Members has been extabilished with the aim to evaluate the HAPO study data in order to extabilish new screening and diagnostic tests for GDM. The preliminary conclusion reached by the Panel are that any recommendations for the use of an oral glucose tolerance test will be based on a 75 gm glucose load; the glucose challenge test for the screening of GDM must be avoided and substituted with the 75 gm glucose load to be done in pregnancy at 24-28 gestational weeks; the suggested threshold values are: fasting plasma glucose 92 mg/dl, 1 hour plasma glucose 180 mg/dl, 2 hour plasma glucose 153 mg/dl. The final document that will give the new recommendations on the screening and diagnosis of GDM is under approval by the Panel.

SP5

## BIOMARKERS: STATE OF THE ART AND PROSPECTIVES

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Recent years saw an explosive increase of biochemical markers in medicine, mainly due to the translational research on the molecular basis of human diseases and to technological research that resulted in efficient procedures for the analysis of such molecules. For example, thanks to high throughput gene sequencing, we can analyze a complete genome in a few days, and microchip based technologies permit to study hundreds of gene variants in a single run in large series of subjects.
Every day a new gene responsible for a human disease is identified and it is becoming clear that "modifier" genes, inherited independently of the disease-gene, influence the phenotype of each patient and may help to predict the outcome. A large group of diseases (diabetes, cardiovascular diseases and obesity) are associated with complex interactions between several genes (some still unknown), and are influenced by environmental factors. This complex interaction, which seems to vary from patient to patient, has led to the concept of "personalized medicine". Studies on xenobiotics metabolism revealed a myriad of gene and gene variants that modulate the individual's sensitivity to drugs, thus leading to the concepts of pharmacogenomics and pharmacogenetics. Similarly, genetic variants may influence the nutrient's absorption, pathway and nutritional effects (nutrigenomics). All these genetic variants are biomarkers that may be easily analyzed.
The Human Genome Project showed that the human genome is constituted by 20,000-30,000 genes (a surprisingly low number when compared to other species). Therefore, a number of mechanisms that regulate gene expression may be active in humans.

Some of these mechanisms have been studied and have already been related to human diseases. For example, the altered methylation of some genes is related to various human neoplasia. Similarly, microRNAs are involved in the regulation of the expression of a myriad of human genes, and mounting evidence indicates that also this mechanism may be impaired in human diseases.
mRNA sequences are also currently analyzed by RT-PCR technology. These molecules may be markers of specific retroviral infection (i.e., HCV, HIV), or may represent a signal of neoplastic cells (minimal residual disease), or may help to predict micrometastases in neoplastic patients. In addition to specific mRNA molecules, the chip technology permits today to assess the expression profile of cells and tissue testing hundreds of mRNA molecules together.
And again, proteins. After the era of "genomics", the interest of researchers focused again on proteins and more specifically on "protein-protein" interactions, a complex network that is another mechanism to regulate the differential expression and activity of gene products. Such complex interactions may be impaired in human diseases and can be analyzed by "proteomics" methodologies.
Finally, the relationships between genetics and human behaviour are starting to be elucidated. Aggressive or depressive behaviour is under the control of a genetic network; suicide may be related to alterations of the epigenetic regulation of specific genes. Also partner selection may be related to DNA, and in some species the specific genes have been identified – another example of "personalized biomarkers".
To conclude: the technology and the study of biomarkers is becoming more easy, but the deep understanding of clinical biochemistry and molecular genetics confirmed the real "uniqueness" of each individual, This may be an excellent opportunity for laboratory medicine to reposition the patient at the heart of the medical process.

SP6
## CAN WE PERSONALIZE DRUG THERAPY IN COLORECTAL CANCER PATIENTS?

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For the treatment of metastatic colorectal cancer, the practicing physicians have available a large armamentarium of therapeutic options. The last decade has seen significant advances in survival of metastatic cancer patients compared to the era of single-agent therapy with 5-fluorouracil, mainly due to the approval of novel therapies. Today, clinicians can rely on the use of 4 classical cytotoxic agents (5-fluorouracil, capecitabine, irinotecan, and oxaliplatin) and 3 targeted therapies (bevacizumab, cetuximab, panitumumab). These drugs have a wide variety of antitumor activity and toxicity, and are usually given in combination. For any given patient, the selection of the best therapy is based upon the analysis of risk/benefit for that patient, and takes into account several factors, including tumor histology, pathological features, stage, comorbidities, age, performance status, and other features. This evaluation is the traditional way of choosing cancer therapy, but is far from optimal. Many (and in most cases the majority) of treated patients do not have significant benefits from the treatment while they often experience moderate to severe toxicities. The outcome of colorectal cancer patients needs to be improved by using strategies aiming to minimize the risk of toxicity and maximizing the efficacy of the treatment. Finding markers that can guide the selection of the best therapy for each patient is a step forward towards the application of personalized medicine. As the cost of the newer therapies is high, the use of molecular markers can improve the affordability of expensive therapies that are indicated for certain patients.
By having access to the germline DNA of patients and from the primary tumor, we can now use markers to select drug therapy. For example, immunochemical analysis of tissue slides of the primary tumor is performed to screen for the expression of EGFR in tumor cells; cetuximab, a monoclonal antibody against EGFR, is indicated for patients who are EGFR positive by staining. However, recent data indicate that tumors positive for the K-ras mutation do not benefit for EGFR blockade with cetuximab and panitumumab, and wild-type K-ras colorectal cancer patients have better clinical response in terms of prolonged progression-free survival and overall response rates when compared to mutant K-ras. Germline DNA information is now used to predict the patients who are at high risk of severe neutropenia from irinotecan; the UGT1A1 test for the *28 polymorphism is now included in the "black box" warning of the package insert of irinotecan in the USA.

SP7
## HIGH SENSITIVITY C-REACTIVE PROTEIN AND OTHER MARKERS OF CARDIOVASCULAR INFLAMMATION: FROM BANCH TO PROVE OF EFFICACY

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The interest of CRP in CHD arise from its long half life and the robustness and reliability of its assessment.To date, CRP is the inflammatory marker most extensively assessed in prognostic studies, both in primary and in secondary prevention, and the only one recommended in guidelines. More than 25 different prospective studies have reported a significant and independent association between increased concentrations of hs-CRP and future cardiovascular events in apparently healthy subjects. Increasing quartiles of hs-CRP are associated with an increasing risk of future CHD at up to 10 years follow-up in apparently healthy men and

women, in elderly subjects (and, even more interestingly, hs-CRP may add important information to the Framingham risk score and to the family history in the population at intermediate risk, thus allowing an improved reclassification of these subjects in either high or low risk. Lastly, in the JUPITER trial, the statin-mediated reduction of the CRP levels in subjects with normal LDL-cholesterol levels (<130 mg/dl), not candidates for statin therapy on the basis of NCEP III guidelines, was associated with a lower incidence of cardiovacular events at two years follow-up.
In patients with NSTEACS, many large studies have confirmed an independent strong value of CRP in predicting the recurrence of cardiac events, such as death, myocardial infarction and need for coronary revascularization procedures. Strong data are available about the prognostic value of CRP in the mid to long term: they clearly show that CRP levels predict recurrence of cardiac events, especially death, for up to five years, either in medically and in surgically or invasively treated patients, in which pre-procedural CRP levels correlate with incidence of restenosis after stent implantation. Of note, CRP gives incremental information on top of the TIMI risk score and of other biomarkers as NT-proBNP and troponin. To date, CRP is the only inflammatory biomarker currently recommended by NACB guidelines for risk assessment of patients with ACS (class IIa, level of evidence A). Growing evidence suggests a role of CRP as therapeutic guide, both in patients with ACS and in healthy subjects, and, intriguingly, as direct therapeutic target. In patients with ACS, treatments associated with reduced mortality, such as aspirin, clopidogrel, Gp IIb/IIIa inhibitors, ACE inhibitors, ARBS and especially statins, are also associated with lowering of CRP and are most effective in patients with high CRP levels. So far, it has been clearly shown that the presence of elevated CRP levels (> 3 mg/L) at admission or their persistence after optimal revascularization demands an aggressive medical treatment, and the therapeutic goal of very low CRP levels seems very likely. Also in apparently healthy subjects, data from the AFCAPS/ TexCAPS (30) and JUPITER (14) trials suggest that CRP screening might be an effective method to identify subjects who are more or less likely to benefit from statin therapy for cardiovascular risk reduction, regardless of cholesterol levels.

SP8
**NON INVASIVE DIAGNOSIS OF GASTROINTESTINAL DISORDERS: GASTROPANEL AND FIBROTEST**

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Clinical biochemistry of gastritis: Gold standard for the diagnosis of type and etiology of gastritis is the histological examination of gastric mucosal biopsies obtained from the antrum, the corpus and the angulus. A non invasive screening procedure able to identify subjects at high or low risk for gastritis should allow to limit un-necessary endoscopies and histological examinations especially among dyspeptics. A serological panel combining pepsinogen I and II (PGA and PGC), gastrin-17 (G17), and anti-Helicobacter pylori antibodies (anti-Hp)(GastroPanel) is actually the most reliable non invasive test to screen patients for gastritis: it has a very high negative predictive value (about 95%) and a positive predictive value of about 65%. A classification algorithm including the four biochemical parameters measured in fasting sera, allows to classify patients as having or not non atrophic or atrophic gastritis and to ascertain whether gastritis is associated or not with H. pylori infection. GastroPanel was demonstrated to be of utility in screening subjects at high risk for gastric cancer. This test, in fact, is highly sensitive (80-90%) and specific (90-100%) in identifying the precancerous chronic atrophic gastritis. Gastric corpus mucosal atrophy associates with reduced PGA levels (<25 ug/L), reduced PGA/PGC ratio (<3) and increased G17 (>10 pmol/L). On the basis of anti-Hp values, GastroPanel allows also to determine whether or not gastric atrophy is secondary to H. pylori infection. Its utility, however, seems limited by cases of gastric carcinoma that arise in stomachs without atrophic mucosa.
Clinical biochemistry of hepatic fibrosis: Fibrosis is a frequent, life-threatening complication of most chronic liver diseases. Non-invasive and reliable (serum-) biomarkers indicating the activity of fibrogenesis may be classified as Class I (serum components having a direct relation to the mechanism of fibrogenesis) or Class II (simple standard laboratory tests grouped into panels). Class I biomarkers comprise either secreted matrix-related components of activated hepatic stellate cells and fibroblasts and mediators of extracellular matrix synthesis or turnover. They suffer both in sensitivity and specificity. Class II biomarkers fulfil most criteria for detection and staging of fibrosis and to a lesser extent grading of fibrogenic activity. More than 20 scores are currently available, among which Fibrotest is the most popular one. Fibrotest allows to predict the presence and grade of hepatic fibrosis by employing a classification algorithm based on the serum determination of five biochemical parameters: alpha-2-macroglobulin, apolipoprotein A1, haptoglobin, g-glutamyltranspeptidase, and bilirubin. Fibrotest is highly sensitive and specific in predicting significant fibrosis (area under the ROC curve: 0.81; 95% CI: 0.78-0.84; data point from 8 studies) and liver cirrhosis (area under the ROC curve: 0.90; 95% CI: not calculable due to 2 available data point). This test has lesser accuracy in detecting early stages (mild) liver fibrosis.

SS7
**CLINICAL RISK: IS IT A PROBLEM OR AN OPPORTUNITY?**

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Every human action involves an element of risk: “Risk

is the possibility that a negative event will occur and it can be measured as the relationship between the probability that a specific event will occur and the extent to which this event's consequences will be negative"[1].
Nowadays, risk is considered to be a social issue because it is thought to be "socially unacceptable" and therefore it is placed among the major bioethical issues of our time.
Medically speaking, doctor errors is almost never tolerated or accepted because it regards an individual's health, or, in extreme cases, life or death.
Any type of structure which offers healthcare, whether it be a hospital, clinic or home nursing scheme implies a high degree of risk. This is because the medical profession involves situations with a high degree of changeableness (also biological) and uncertainty which persist despite all recent technological and scientific advances and make the healthcare system more complex and vulnerable. For this reason, when we look for human error we often discover that this error was not committed by an individual but by an organization. In 2002, Cineas reported that organizational problems influences 70% of hospital error.
We can identify different risk categories in healthcare: occupational (physical, biological and chemical), financial (these are "non clinical" risks which regard security and eventual economic risks) and, finally, a clinical risk which regard the possibility that a patient might be harmed by some types of treatment. This last type implies both a structural and organizational risk and includes elements which involve physician error.
The three types of risk are closely correlated and the same risk can belong to more than one category.
Particular attention should be paid to clinical risk since the well being and safety of patients should represent the utmost priority for any health care organization.
The Ania (National Insurer's Association) estimates that in 2004, three hundred and twenty thousand patients were victims of accidents. Furthermore, insurance claims for malpractice went up by 148% between 1994 and 2002 (source: Sole 24 ore).
Clinical risk is clearly that which is most specific to medical treatments performed on patients and inherently involves the behaviour of medical personnel and the way in which their organization functions.
It's vital to be aware of and analyse these facets of the healthcare system so as to improve security and quality control systems. Clinical risk, therefore, involves a systematic and multidisciplinary approach involving specific analytical instruments.
Healthcare and patient safety should be viewed from a global view point which considers different facets of quality, not just from one which only considers risk. A proactive stance must be taken before errors are committed: all healthcare providers must implement a patient oriented strategic plan in order to reduce risk and error.
Therefore, identifying and managing risk are all important activities when one wishes to make safety a priority; this holds true in all areas: managerial, administrative and clinical.
Specific instruments must be adopted in order to identify, evaluate and reduce any and all elements which pose a risk to patient safety. However it's important to accept that risk can never be completely eliminates in the healthcare field, even though better patient care and quality control serve to significantly reduce its occurrence.
The validity of a clinical risk management system depends above all on the creation of a positive "culture" surrounding the idea of risk, based on knowledge that error is human and "to cover up your mistakes is the most serious of intellectual crimes"[2].
In Italy, unlike other countries, risk management is still not taken seriously throughout the healthcare industry. Few medical facilities are equipped for the development and maintenance of security and risk management systems. "Overall, the Italian healthcare system is still trying to quantify the extent to which safety and risk are problems. Consequently, limited action has be taken up till now, at least in terms of global organizational strategies"[3].

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SS8

## FMEA-FMECA: A RISK MANAGEMENT'S STRUMENT CRITICAL PROCESSES

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Introduction. The clinical risk unit of ASL Napoli1 Center takes care about the implementation of procedures to enforce ministerial recommendations, concerning sentinel events reduction and prevention.
Great attention has been paid to recommendation N° 5: "prevention of transfusional reaction by AB0 incompatibility". Thanks to both procedures adopted by "Immunohaematology and Transfusion Medicine Service" to services and patient safety and law criterias application, new improvement procedures were developed by using "clinical governance" tools and methodologies, such as the FMEA and FMECA (Risk management)
Methods. The selected strategy turned out by the use of different methods.
In order to satisfy the aim to prevent rather than to react, it has been chosen to adopt an approach through proactive analysis and a step by step process as follows:
- Different management systems integration and its recording tools: Quality management system in accordance with ISO 9001:2008 regulation and Risk management;
- Analysis about processes related to transfusion risk

and detection of critical process that has to be analyzed through FMEA / FMECA
- checking and recording of nonconformity, in acceptance sector of SIMT
- multidisciplinary group identification to FMEA / FMECA analysis execution;
- Identification of critical process (unique identification sample / patient)
- FMEA / FMECA Analysis
- Processes validation
- monitoring

Results. The analysis showed that the rules established by SIMT allow to block the greatest parts of beginning process mistakes, but many troubles still exist in patient identification during the blood sample.
If the evaluation of analytic determinations referred to the two samples of the same person show a difference, so SIMT can reveal the patient exchange, but if an error occurs on both samples, then it will be impossible to note it;
By the evaluation of priority risk index carried out after the FMEA/FMECA analysis, middle and long terms corrective actions have been selected intrevention priority- corrective actions selected:
- Introduced a three new procedures conforming to Recommendation No. 5 of Ministry of Health and (AB0 Incompatibility) the DM of 3 March 2005 (transfusional safety)
- Introduced registration forms for non-conformity and monitoring of indicators
- the personnel has been formed about the right procedure to follow
- measurament and monitoring

Conclusions. The application of the methodology FMEA/FMECA was a aggregation multi-professionality that allowed the assessment of various issues in a broader vision.
The barrier posed by the procedures selected, allows to prevent the error.

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SS9

## PRE-ANALYTICAL PHASE: RISK MANAGEMENT AND RISK ASSESSMENT PRODUCTIONS OF A HOSPITAL LABORATORY

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The importance of the pre-analytical phase in the context of laboratory medicine activities is well known. However, little has been done with proactive functions in managing this phase: one must pass from error gathering to an activity linked to problem solving, in a risk management per- spective. The very analysis of errors and their frequency is the basis of the solution proposed and implemented at the Tradate hospital facilities (Busto Arsizio Hospital Corporation) medical analysis laboratory. Starting from risk analysis and assessment centered on three aspects of the pre-analytical phase (inadequate patient preparation, wrong tube/non-compliant container, off-schedule sample delivery/reception errors) managed as non-compliances, graded on a scale (probability, detectability and seriousness) in order to generate a risk priority index. This risk assessment model has given the starting point to analyze the territorial wards and structure stakeholder needs. The model made it possible to develop a sw in which proactivity is a prerequisite. The SW was developed keeping within the lab (work group) often outsourced competences (analysis and programming). This choice is reinforced by the reasons of the fulfillments provided for by Financial Law 2007 (Art. 1, comma 796, letter O of Law 296/2006 - Guidelines on the Contents of the Plan on the Laboratory Network: Updating of the Organizing and personnel standards consistent with the efficiency increase processes made possible by the availability of automated methods.) The solution is a document with hypertext links (non-mod. HTLM) made of tables, each containing: a) container image and relevant identification, b) list of exams related to said container, c) Analysis Facilities/Area, d) information for sample gathering and preservation by Facilities/Laboratory, and, e) possible preservation material safety specifications. Facilities/Area identification is linked to the division in wards of the Hospital Corp., thus making lab analysis addressing easier both in the area and in the facilities which actually perform the exam. The SW is also a preventive control system which supports the efficiency of the pre-analytical phase in all the organizational units pertaining to the lab. Area facilities identification also makes it possible to further expand to other hospital offices/facilities, thus making effective division in wards easier. It is integrated to the corporation ethical code, thus becoming part of one of the behavioral organizing models, an incentive to further improve the activities performed by the corporations through their collaborators. Decision Making, developed in 2006, led to a decrease of pre-analytical errors (on the three variables) from 7.8% in

2005 to 2.2% in 2007 and 1.3% in 2008. More specifically, on the first year, 2007, inadequate patient prep. was 0.2%, wrong tube/non-compl. container 1.1%, off-schedule sample delivery 0.9%, for a total of 2.2%. In 2008, total data further decreased: 1.03 (0.2%; 0,03%, 0.8%). Besides stakeholder collaboration, this success is also linked to the innovative implementation of risk assessment activities as per ISO 27001. This is the ethical model: the lab, well aware of container characteristics, realizing (data from 2005) that the latter's choice makes up one of the sources of error, solves the problem by choosing to implement a decision making activity in letting the stakeholders choose lab exams and containers. The new integrated procedures, which follows step by step the choices of the physician, also develops the model's principle of efficiency: developing the SW product is made possible by the very product's simplicity of use and updating.

SS10

## RISK MANAGEMENT TRIGGERS IN POSTANALYTICAL PHASE

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Background. The Laboratory routinely monitors TAT at each step of the testing process to maintain stated quality goals.
Aim. Assessing the compliance with the meeting criteria (TAT <1 h) for pivot test (HGB, glucose, potassium, cTnI) ordered in the Emergency Department (ED); mapping the risk and assessing the impact of a prolonged TAT (> 2h) on the outcome of the patient.
Methods. The TATs (analytical, preanalytical, postanalytical) of 5000 patients were retrospectively collected and the causes of delays were analyzed with Root Cause Analysis (RCA). Moreover, clinical data of 245 consecutive patients were collected to map the patient's risk associated with delays in TAT. By a questionnaire ED professionals (14 physicians, 32 nurses) evaluated the risk induced by a delayed TAT (> 2 h).
Results. The 90th percentile of TAT are 43 min for HGB, 70 for glucose, 67 for potassium, 79 for cTnI. The mean and median of the monthly TATs are: preanalytical 30 and 27 min; first analytical result 27 and 13; complete analytical results 49 and 38; postanalytical 125 and 46; Vein-to-brain 146 and 80. The RCA identified outliers in Drawn-to-receip by incomplete demographic data entry in CPOE, delay in specimen collection, delay in transport the specimens to laboratory; in Receipt-to-Report by influence of different processing and testing of the specimens, verification and availability of results (abnormal results: unsuitable sample, critical results, reviewing and repeats/rerunning); in Result-to-brain, by delayed night/morning staff change.
Risk mapping has shown correlation between the attention of the ED staff and patient risk. The questionnaire showed that the customize order test profiles and imaging test orders are related with the Emergency Severity Index level (ESI), and that specialist's referral are required for complex patients or for rule out purposes. According to respondents to the questionnaire, a delay of more than 2 hours can produce serious consequences in 14 clinical suggested situations, but it has not same impact in 13 other proposed cases. In 8% of cases all ordered tests' results are not available at the expected time.
Conclusions. The preanalytical TAT, mainly the transport to the laboratory, is the predominant outliers' factor and actions were considered for improvement, such as POCT, automatic transport of samples to laboratory, communication of the patient ESI to the laboratory.
The monitoring of TATs and analysis of the causes of outliers, discussion over the findings, and elaboration of possible solutions with the ED's "clients" were powerful tools for team interaction and collaborative decision-making, and real opportunities for improving care path for critically ill patients. Therefore, the monitoring of TAT is not only a control of the total testing process, but an indicator of the quality of the communication and the risk management.
Key words: risk management, TAT, risk assesment.

CO5

## MODEL OF QUALITY INDICATORS: UN PROGETTO DELL'IFCC-WG "LABORATORY ERRORS AND PATIENT SAFETY"

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Il monitoraggio degli errori è diventato un'attività indispensabile per i laboratori al fine di garantire la sicurezza del Paziente.
Il progetto "Modello di Indicatori di Qualità" sviluppato del Gruppo dell'IFCC "Laboratory Errors and Patient Safety" ha l'obiettivo di stimolare i laboratori a valutare e monitorare ogni fase processo di analisi, conoscere e diminuire il tasso di errore, migliorare le prestazioni. Il

progetto, al quale è possibile aderire collegandosi al sito web www3.centroricercabiomedica.it, si articola in una fase sperimentale, in corso, ed una fase applicativa, nella quale il modello sarà proposto a tutti i laboratori come un Programma di Assicurazione della Qualità. Il modello comprende 16 indicatori per la fase pre-analitica, 4 per la intra-analitica e 5 per la post-analitica. Vengono riportati i dati preliminari dei laboratori (valore percentuale minimo e massimo) che volontariamente hanno aderito al progetto. Richieste: 9,5-87,4 (con quesito clinico), 69,2-97,3 (con test appropriati), 0-21,1 (senza identificazione del medico), 0-21,4 (incomprensibili); Errori di accettazione: 0-10,5 (identificazione paziente), 0-6,21 (identificazione del richiedente), 0,1-14,5 (test omessi), 0-53,3 (test aggiunti), 0-17 (test misinterpretati); Irregolarità di campione: 0,01-0,61 (non ricevuti), 0-8,8 (errato contenitore:), 0,3-0,95 (emolizzati in ematologia), 0,3-3,4 (emolizzati in chimica clinica), 0,01-1,7 (coagulati in ematologia), 0,03-1,5 (coagulati in chimica clinica), 0,01-1,14 (volume insufficiente), 0-52,5 (errato rapporto volume/ anticoagulante), 0-0,1 (danneggiati nel trasporto:), 0,002-48,4 (impropriamente etichettati), 0-0,11 (errata conservazione); Prestazioni non accettabili nella VEQ: 1,4-4,94 (totali), 0-45,8 (per causa già trattata); CV% più alti del target selezionato: 5,6-11,1; Ritardi di refertazione: 0,02-17,4 (totali), 0-0,01 (per guasti strumentali); Valori critici: 53,7-100 (comunicati), 8,9-22,3 (tempo medio di comunicazione); Commenti interpretativi con impatto positivo sull'outcome: 1,55; Linee-guida preparate con clinici: 1-11. I dati riportati dimostrano la necessità di definire lo stato dell'arte per ogni indicatore e gli obiettivi di miglioramento per permettere un'efficace attività di benchmarking.

SS11
## ANALYTICAL QUALITY CONTROL

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To develop an Internal Quality Control (IQC) system means to plan, to activate and to use procedures ensuring that methods/instruments performances are stable over time and achieve pre-determined quality (analytical goals), providing alarms when changes in method performance may cause patient samples to exceed defined quality specification[1, 2].
From a practical perspective, this activity includes the implementation of a non-statistical component (process control), which is needed to eliminate or reduce the variability sources in laboratory workflow ("Materials, Methods, Men and Manuals"), and the implementation of a statistical strategy, which is necessary to provide real alarms when our methods/instruments don't meet quality specifications. The recently published SIBioC guidelines about management of internal quality control [3] suggests an operational path for the correct application of the theory behind this process.

In agreement with the theoretical assumptions described in these guidelines, our approach is based on breakdown the process in some steps of simple implementation, using tools provided by quality management systems (e.g. the PDCA or Deming cycle):
PLAN
- Creation of a Microsoft Access database for collecting and managing analytical goals derived from various sources of quality requirements (Biological variability, Italian providers of External Quality Assurance (EQA) programs, State of the Art, historical data of our methods/instruments).
- Selection of appropriate quality control materials and standardization of treatment procedures.
- Planning and application a total process control for elimination/reduction of laboratory variability sources.
- Determination of method/instrument performance (in terms of Coefficient of Variation and Bias) using a Microsoft Excel spreadsheet specifically created.
- Choice of analytical goals by using proper algorithm that takes into account the current method/instrument performance.
- Selection of appropriate Westgard's rules and numbers of control measurements by using sigma-metrics chart.

DO
- Preparation of operating instructions and training laboratory staff to use informatic tools.
- Implementation of the system by a dedicated QC monitoring software.
- Resolution and treatment of out-of-control situations according to specific algorithms for each method/instrument.

CHECK
- Long-term monitoring of the system through a panel of indicators.

ACT
- Periodic reassessment of the system.

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SS12
## THE USE OF PORTABLE GLUCOSE METERS

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Rapid blood glucose measurement, first routinely used in the sixties, is employed to meet the clinical need for the frequent control of insulin dosage in diabetic patients. Currently the treatment of these patients involves blood glucose tests using POCT instruments, while the diagnosis of diabetes is made in the laboratory on the

basis of plasma glucose determination. Yet, despite their long history, the use of portable meters can be problematic.
Pre-analytical phase. The choice of the type of sample (capillary, venous or arterial blood) and also the site of puncture can cause significant variability in results, particularly in the hospital setting. Peripheral perfusion and oxygen saturation can cause variations in concentrations in critically ill patients.
Analytical phase. As yet there is no consensus on whether blood or plasma glucose calibration should be used. In Italy, both are used without there being a clear awareness of the real entity of differences, which systematically amount to 11%. Moreover, as pointed out by several authors, the analytical performances of these meters appear to be insufficient for their specific clinical use in insulin dosage determination. Other possible causes are the manual choice of calibration code and an insufficient sample amount.
Post-analytical phase. Currently the transfer and recording of data is insufficient, both in patient self-testing and in hospital use. In one study conducted by our team, it was found that 3% of manually reported results were incorrect and 10% of registrations were inaccurate.
Appropriateness. Portable glucose meters have been demonstrated to improve the clinical outcome of subjects with type-1 diabetes, in particular if access to the self-test is easy, whereas appropriateness has not been well demonstrated for “tight glucose control” in intensive care units and other conditions, such as type-2 diabetes or in emergency medicine.
In conclusion, the laboratory must address the issue of the correct use of glucose meters, provide guidelines, control analytical quality and offer support to all users.

SS13
**HOW TO BUILD A QUALITY PROCESS**

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Nowadays it is recognized that the application and improvement of a quality management system is a method distributed at every level of the organization.
One pivotal point of the ISO 9000:2005 principles is the *Focus on Customer*, that is to say that the organization depends on their customers and therefore should understand current and future customer needs, should meet customer requirements and strive to exceed customer expectations.
The same principle is also emphatized by the requirements of Joint Commission International, that is focused on the patient demands, his rights and those of his family.
In particular, the diabetic outpatient represents a model for the building of the quality process, because of the chronicity of the disease and also due to the participation of a lot of specialists in its management: in fact, patients and their families are involved in repeated visits at the hospital site in order to check the evolution of the disease, to define its treatment, to monitor the clinical status, and to prevent all possible complications.
Indeed, our organization has implemented a method of process analysis to draw the process *as is* and to define the process *to be* in order to develop improvement actions enabling the passage from the first to the second step.
This above mentioned analysis starts from the so called SWOT Analysis, where SWOT stands for: Strengths and Weakness (internal factors), Opportunities and Threats (external factors).
SWOT analysis is a tool for auditing the internal organization and its environment. It is aimed at analyzing the relations between internal and external factors, at defining key performances resulting from the conjugations between these factors: development, internal and external adaptation, survival. Next step is the selection of strategical options on which we decide to focus the process revision actions. Based on this analysis method, in our hospital the building of the Quality Process has been performed by an inter-departmental working group (WG), represented by physicians, nurses, technicians and quality specialists.
The WG has identified the following key actions related to the key performances:
- development: reinforcement of scientific cooperations, protocols revisions (EBM) to improve treatment, new technologies implementation, care-givers education
- internal adaptation: informatic system improvement, telemedicine, adaptation to resources
- external adaptation: participation to technical sessions Hospital-ASL-Region, marketing activities
- survival: resources and logistic reorganization, protocol definition to improve clinical risk management and user’s safety.

Starting from these strategical guidelines we defined the flow chart *as is* to check the critical aspects and we decided the improvement action plan to reach the flow chart *to be*.
Two of the main actions decided are the following:
We are now working on the reorganization of acceptance and reservation process for blood collection to reduce the waiting time and to ameliorate the entire work flow, to improve patient’s satisfaction.
We are also studying the implementation of telemedicine, to get a better organization efficiency.

CO6

## ARE THE LABORATORY PLASMA LIPID REPORTS OF HELP FOR THE DIABETIC PATIENT MANAGEMENT? RESULTS FROM A SURVEY AMONG ITALIAN LABORATORIES

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Background. It is very well known that plasma lipid and lipoprotein measurements should be evaluated according to the values established by the different epidemiological and intervention studies in order to establish cardiovascular risk (CV) or the efficacy of the treatment. This is more true for diabetic patients which require specific therapeutic goals. Aim of this work is twofold: to verify the state of the art in Italian laboratories when reporting lipid measurements and to check if special reports are in use for diabetic patients.
Methods. Participants to Italian VEQs have been asked to send the details of their reports.
Results. Up to now 109 reports have been examined, mainly from the Veneto and Emilia-Romagna Regions. 40% of laboratory reports contain indications concerning decisional limits or therapeutic targets; 25% of these suggests the need to include a number of parameters (blood pressure and weight, smoking habits, secondary prevention, etc) when evaluating the data. These percentages are higher when LDL cholesterol is concerned and much lower when triglycerides are reported. Only in 4 reports (out of 106) we found specific notes for diabetic patients.
Discussion. After more than 20 years of robust scientific evidence regarding the inadequacy of reference ranges for the evaluation of plasma lipid concentrations, 60% of the Italian laboratories still ignore these recommendations. Since the correlation between plasma lipids and CV risk is continuous, a significant amount of risk is included in concentrations within the reference ranges. Diabetic patients which are considered at high risk regardless the plasma lipid levels, could be even more endangered by this kind of reports. On the other hand, reference ranges could be more appropriate when lipids are requested for reasons different from risk classification, as it is the case for in-patients. In the era of “personalised medicine”, it is perhaps time to think of “personalised” laboratory reports, taking into account specific characteristics of the single patient.

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CO7

## CHILDREN’S OBESITY ATHEROSCLEROTICS RISK: THE ROLE OF INFLAMMATORY MARKERS (hs-CRP), OXIDANT STATUS (PGF-2alfa,MDA), ANTI-OXIDANT STATUS (VITAMIN E) AND INSULIN SENSITIVITY

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Purpose. The aim of our study has been to estimate, in obese and lean children, the role of some metabolic parameters (inflammatory, oxidants, anti-oxidants and insulin-resistance) in development of atherosclerosis in adulthood.
Methods. We studied 120 pre-pubertal children who had been referred to Paediatrics Department. All subjects have been recruited estimating BMI and subdivided in three groups: 40obese, 40 lean e 40 control subjects. A complete physical examination was performed, including anthropometric (heigh and weight), ecografic (carotid intima-media thickeness cIMT) and biochemical parameters such as inflammatory markers (high sensitive C-reactive protein hs-CRP), lipid profile (total cholesterol, low density lipoprotein (LDL)-cholesterol, and TGs) oxidant (urinary isoprostanes (PGF-2a) and malondialdehyde (MDA) and antioxidant status (vitamin E), and insulin sensitivity.
Results. hs-CRP was not different between lean and control subjects (P=0.45), while higher values were found in obese compared with lean and control children (P<0.001 and P<0.001 respectively). PGF-2a and MDA were higher while lag phase shorter in lean and obese subjects compared with controls (lean P<0.001; P<0.001; P<0.001 and obese P<0.001; P<0.001; P<0.001 respectively), while no differences were documented between lean and obese subjects (P=0.78, P=0.019, and P=0.53 respectively). Compared with controls, cIMT was increased in lean and in obese subjects (P=0.001; P=0.004), while no differences were documented between obese and lean subjects (P=0.1). In a multiple stepwise linear regression analysis, cIMT was related with PGF-2a (beta=0.641, P<0.001) and HOMA-IR (beta=0.307; P<0.001).
Conclusions. Pre-pubertal lean and obese children present increased oxidative stress and impaired inflammation and insulin sensitivity, which in turn seem to result in a similar impaired endothelial dysfunction and early signs of atherosclerosis, already in childhood.

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SS14

## THE STATE OF THE ART OF WORKPLACE DRUG TESTING IN EUROPE

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Workplace Drug Testing (WDT) is well established in the United States, where it is strictly controlled. Many US multinationals now wish to test their European employees also. Alongside this, health and safety issues are driving the creation of WDT legislation in different European countries, particularly in safety critical areas. The EWDTS was formed in 1998 with two main aims: to ensure that workplace drug testing in Europe is performed to a defined quality standard and in a legally secured way, and to provide an independent forum for all aspects of workplace drug testing. It is the leading body dealing with the topic. The EWDTS guidelines for WDT in urine have been adopted by EA, the European co-operation for Accreditation. Further draft guidelines for the collection of urine and oral fluid samples and for hair collection and analysis have been published on its website www.ewdts.org). The different legislation around Europe and some of the pitfalls of dealing with the varied laws, customs and languages of the 27 EU member states will be discussed. The need for a unified approach within the EU will be addressed.

SS15

## PROCEDURES FOR WORKPLACE DRUG TESTING IN ITALY

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With the coming into force of the law concerning the procedures for the workplace drug testing (defined as the procedures for the investigation of absence of drug addiction or sporadic drug consumption in workers with risky jobs), operating procedures have been identified to be followed by the companies, the physicians, the local health services and the hospitals involved in the measures described above. These procedures will be reviewed in details, in order to enable the timely and harmonized implementation by the concerned personnel. Some key-points of the law, such as those related to the cut-offs to be used in screening and confirmatory tests, or those related to urinary metabolites of the major drugs of abuse and finally the eventual possibility of sample adulterations will be also enlightened, with particular attention to differences in different Country regions.

SS16

## WORKPLACE ALCOHOL ABUSE TESTING: COMPARISON OF ETHYL GLUCURONIDE IN HAIR AND CDT IN

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Background. Ethyl glucuronide in hair (HEtG) recently emerged as a sensitive and specific marker in the diagnosis of excessive chronic alcohol consumption. The aim of this study was to evaluate the reliability of this marker in a workplace testing, in comparison with carbohydrate deficient transferrin (CDT) in serum, the most popular biomarkers in the diagnosis of a chronic alcohol abuse.
Methods. Ninety eight volunteers were recruited among teetotallers, social drinkers, heavy drinkers and alcoholics at the beginning of an in-patient alcohol detoxification program. A self-administered anonymous questionnaire enabled to estimate their ethanol daily intake (EDI) within the last 2-week and 3-month time period. HEtG determination (0-3 cm proximal segment) was carried out using a fully validated LC-MS-MS procedure and ranged from <LOD (2 pg/mg) to 890.5 pg/mg. CDT was measured in serum either by immunonephelometry or by HPLC.
Results. HEtG cut-off level providing the best performance in terms of sensitivity (0.92) and specificity (0.96) at detecting an EDI≥60 g during the last 3-month period was 27 pg/mg. It was observed that HEtG is not significantly influenced by factors known to affect ethanol metabolism and/or the diagnostic power of other markers of ethanol use, or hair analyses, including age, gender, body mass index, tobacco smoke, prevalent beverage (wine or beer), hair colour, cosmetic treatments and hygienic habits. However, slight differences in HEtG performance were observed for some factors (e.g. body mass index, smoke and hair treatments). Sensitivity and specificity of HEtG (27 pg/mg cut-off) and CDT (2.5% cut-off for either immunonephelometry or HPLC, as recommended by the respective manufacturers) as indicators of and EDI≥60 g during the last 2-week and 3-month period were measured. Considering the 2-week EDI, HEtG showed equal selectivity (0.93 for both HEtG and CDT-immunonephelometry; 0.70 for both HEtG and CDT-HPLC) and 2 times the sensitivity of either of the two CDT methods (1.00 vs. 0.44 for CDT-immunonephelometry; 0.96 vs. 0.50 for CDT-HPLC). The same difference in performance but with higher absolute sensitivity and selectivity were observed for the 3-month EDI (selectivity: 1.00 for both HEtG and CDT-immunonephelometry, 0.89 and 0.78 for HEtG and CDT-HPLC, respectively; sensitivity: 1.00 vs. 0.47 for CDT-immunonephelometry; 0.98 vs.0.51 for CDT-HPLC).
Conclusions. Our results confirm that HEtG is a

sensitive and specific marker of chronic heavy drinking. HEtG provides as much selectivity as CDT at considerably higher sensitivity. In addition, because of the not invasive hair sampling and the larger time-window of detection HEtG appears to be a more suitable marker for the diagnosis of an excessive chronic alcohol consumption in workplace testing.

CO8

## VKORC1 AND CYP2C9 PHARMACOGENETICS: RELEVANCE IN WARFARIN MAINTENANCE DOSING PREDICTION

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Background. Warfarin is a widely used anticoagulant with narrow therapeutic index and relevant inter-individual variability in dosing requirements. We studied the influence of pharmacogenetics on warfarin maintenance dose.
Methods. CYP2C9 (*1,*2,*3 alleles) and VKORC1 (-1639G>A SNP) gene polymorphisms were retrospectively analyzed in 437 Italian patients under stable warfarin therapy (target INR=2.5). Clinical-demographic features, the use of interfering drugs or substances were also considered.
Results. Mean weekly warfarin dose, ranging from 3.75 to 80 mg, was higher in males (c2=9.99; p=0.0016), correlated inversely with age (c2=22.53; p<0.0001) and directly with Body Surface Area (BSA) (c2=22.83; p<0.0001) or coffee consumption (c2=6.1334; p=0.0133). It was associated with CYP2C9 and VKORC1 polymorphisms (c2=80.15; p=<0.0001 and c2=170.17; p=<0.0001 respectively) being lower in mutated compared to wild-type homozygotes (CYP2C9*1*1 or VKORC1 G/G). In heterozygotes warfarin dosing was intermediate. At multivariate logistic regression analysis only CYP2C9 and VKORC1 polymorphisms, BSA and age were statistically significant, being VKORC1 the main dosing predictor. These predictors were independently selected (Biomarker Pattern Software) as the main discriminants to construct a classification algorithm on the basis of which the need of low (<26.25mg/week), intermediate (26.25-43.75mg/week) or high (>43.75mg/week) maintenance dose could be correctly predicted in 70.2% of the cases.
Conclusions. Warfarin dosing is predictable mainly by VKORC1 and secondly by CYP2C9 pharmacogenetics. Age and BSA have only minor effects compared to the genetic background. An algorithm based on these predictors was constructed and demonstrated to allow a correct classification of the therapeutic regimen in almost two thirds of the patients.

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CO9

## CYP2D6 GENOTYPING PANEL ANALYZED ON THE INFINITI TM ANALYZER WORKSTATION

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Background. Polymorphisms of the cytochrome P450 2D6 (CYP2D6) gene affecting enzyme activity are involved in interindividual variability in drug efficiency/toxicity.
Four phenotypic groups are found in the general population: ultra rapid (UM), extensive (EM), intermediate (IM) and poor (PM) metabolizers. The main aim of this study was to evaluate the performance of CYP2D6 genotyping assay with the INFINITI TM Analyzer Workstation in CYP2D6 phenotype prediction.
Methods. We genotyped 70 human DNA samples using the Infiniti CYP4502D6 Assay. We compared CYP2D6 genotypes with the Infiniti CYP4502D6 Assay and with polymerase chain reaction (PCR) restriction fragment-length polymorphism analysis (RFLP). For the Infiniti CYP4502D6 Assay, we performed multipad addressing and duplicate runs to test the intraand intercartridge precision, within- and between-run precision, and reproducibility of the defined genotypes.
Results. We used the Infiniti platform to genotype DNA samples for CYP2D6 *2, *3, *4, *5, *6, *7, *8, *9, *10, *12, *14, *17, *29, *41, *41A and gene duplication *XN and the RFLP analysis to analyze CYP2D6*41. The 2 methods showed 94.3% concordance in the genotyping results; we found only 4 discrepant genotypes among 70 DNA analyses. Confirmatory molecular analysis of the discrepant genotypes revealed that the Infiniti CYP4502D6 Assay showed better agreement.
Conclusions. We compared CYP2D6 genotyping using

the Infiniti CYP450 test to our routine methods and found 94,3% concordance. In summary, the Infiniti assay is an innovative technology, rapid, reliable, accurate and very easy to perform.

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SP9

## PHARMACOGENETICS: PROMISES OR REALITY?

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A common problem in medicine is that there are differences in how patients repond to drugs. Whereas most individuals will tolerate the standard dosages well, certain patients may either experience severe toxicity on the same dose, or may eventually show to be subtherapeutically treated. Since the 1960s, we are aware that individuals may differ in metabolizing capacity, due to genetic polymorphisms encosing drug metabolizing enzymes. Examples are NAT2 polymorphisms and isoniazide (used to treat tuberculosis), TPMT and azathioprine/6-mercaptopurine (Crohns disease, Acute Lymphatic Leukemia) and cytochrome P450 2D6 (CYP2D6). Yet, this knowledge has not resulted at that time in a large implementation for patient care. In the last 5 years, however, important improvements have been made, both in availability of genotyping techniques as in knowledge about translating genotypes to phenotypes. Interesting new applications are CYP2D6 testing for tamoxifen therapy (breast cancer), CYP2C9/VKORC1 analysis for anticoagulation, HLA-B5701 testing for abacavir (HIV treatment) and CYP2C19 analysis for clopidogrel (anticoagulation). Because of these examples, there seems to be a growing acceptance of pharmacogenetic testing.
How far are we at this moment? Can we integrate pharmacogenetic testing already in routine diagnostics, or are we still too early?

SP10

## PHARMACOGENETICS AND DIABETES

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Pharmacogenetics is an emerging discipline involving the research for genetic polymorphisms, commonly observed among the general population, which are able to influence drug response. This rising importance has been highlighted also in chronic illnesses such as diabetes mellitus. This illness is characterised by two major forms: type 1 and type 2 diabetes mellitus. From a genetic point of view it is possible to divide diabetes in monogenic and polygenic forms. Monogenic diabetes derives from one or more mutations in a single gene. It should be hypothesized and evaluated in diabetic patients with features inconsistent with their current diagnosis (unspecified neonatal diabetes, type 1 or type 2 diabetes) and clinical features of a specific subtype of monogenic diabetes (such as neonatal diabetes, familial diabetes, mild hyperglycaemia, syndromes). The list of these monogenic forms of diabetes includes MODY, mitochondrial diabetes, permanent neonatal diabetes (PNDM) and transient neonatal diabetes, familial lipodystrophies and some others. The knowledge of the molecular background of these specific forms of diabetes allows to point out the underlying aetiology and optimize therapy and the clinical follow-up. For example, patients with MODY2, caused by glucokinase mutations, show very mild diabetes characterized by modest fasting hyperglycaemia. Diet is frequently sufficient to control their glycol-metabolic balance. Some other forms of monogenic diabetes associated with impaired function of the beta-cell, such as MODY3 and PNDM linked to mutations in Kir6.2 and SUR1 genes, can be successfully managed by sulphonylurea agents. In these cases treatment could be successfully switched from insulin injection to oral sulphonylurea therapy. These are some examples of pharmacogenetics those patients can also benefit from genetic testing. The challenge for diabetologists is to recognise these monogenic forms whose care will be greatly helped by the treatment changes that follow molecular genetic testing. The list of new putative monogenic forms of diabetes mellitus is increasing in a exponential manner, showing a high interest of the scientific community. Regarding polygenic diabetes, the knowledge obtained from recent genome-wide association studies has been demonstrated to be useful in understanding the pathogenesis and development of type 2 diabetes, but its usefulness is still now uncertain.

SP11

## PHARMACOGENETICS OF ORAL ANTICOAGULANT THERAPY

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Oral anticoagulant therapy (OAT) based on coumarins (warfarin, Coumadin) and other vitamin K antagonists (acenocoumarol, Syntrom) has been the cornerstone of prevention and treatment of several thrombotic disorders, and these compounds are still the most used

anticoagulant medications worldwide. Warfarin is a racemic mixture of two optically active enantiomers; S-warfarin is prevalently metabolized by the CYP2C9 enzyme of the cytochrome P450 system, whereas R-warfarin is cleared by the two cytochrome enzymes 1A2 and 3A4 (CYP1A2 and CYP3A4). Warfarin and other coumarins produce their anticoagulant effect by contrasting the cyclic inter-conversion of vitamin K to its 2,3 epoxide. The enzymes vitamin K epoxide reductase (VKOR) and vitamin K reductase are essential in this process, because they reduce the vitamin K 2,3-epoxide to the active vitamin K quinole cofactor.
The management of OAT is difficult, due to considerable variability in the dose-response which can be ascribed to environmental, demographical, clinical and genetic variables. Predicting individual responses to the therapy represents a major challenge, and patients may be exposed to adverse heath outcomes from bleeding or thrombosis due to over- and undercoagulation. However, several lines of evidence indicates that up to 60% of the individual pharmacological response might be genetically regulated and influenced by single nucleotide polymorphisms in the genes encoding VKOR and cytochrome P450 CYP2C9. Therefore, genetic testing is currently regarded as a promising tool to help predict dose response during initial anticoagulation, assess dose maintenance variability, and identify warfarin resistance. Nevertheless, pharmacogenetics of OAT can not be considered as yet the “magic bullet”, current limitations including a suitable organization of genetic panels, a limited amount of information about inter-individual variability, a lack of analytical and quality specifications, a partial availability of outcome analyses that unequivocally confirm cost-effectiveness, a lack of universal agreement related to reliable dosing algorithms and other ethical and social issues. Therefore, it seems reasonable to conclude that it is premature to introduce routine OAT pharmacogenetics in the daily practice, though the development and clinical validation of simple but comprehensive algorithms integrating the most informative gene polymorphisms, along with demographical (age, race, body mass index) and clinical variables (comorbidities, drugs interference) as well as a standardized dietary intake of vitamin K, may provide a valuable tool in the individually managed care of patients on OAT. It is also to consider that the forthcoming commercialization of new anticoagulant drugs targeting thrombin and factor X will introduce a paradigm shift in long-term anticoagulation therapy, where consideration could be given to demise pharmacogenetics testing for OAT.

SS17
## NEONATAL SCREENING OF INBORN ERRORS OF METABOLISM

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Inborn errors of metabolism are genetic disorders that affect the capabilities of the organism to convert nutrients into one another or to use them for energy production. They are due to impaired activity of enzymes, transporters, or cofactors resulting in accumulation of abnormal metabolites (substrates) proximal to the metabolic block or lack of necessary products. The measurement of some of these metabolites or their byproducts is the basis of expanded newborn screening. Due to the critical consequences of rare metabolic disorders, in fact, an early diagnosis based on newborn-screening method is the appropriate strategy to consider. Innovative sample preparation methods together with accurate and robust analytical systems make tandem mass spectrometry ideal for newborn-screening programs, being able to monitor more than 30 metabolic disorders in a single analysis (1). Mass spectrometry (MS) is a technique that identifies and quantifies molecules based on molecular mass or weight. Since thousands of molecules may be present in complex mixtures, such as biological fluids, and the intact ionized molecule (molecular ion) may share the same mass value as another, especially at low molecular weights (<1000), two mass spectrometers in tandem (MS/MS) can be used enabling the control of the formation of molecular and fragment ions and allowing their separation and identification. In fact in a typical MS/MS experiment, the instrument measures the mass of intact molecules in the first mass spectrometer, fragments them in a subsequent chamber known as a collision cell, and then measures the mass of these fragments in the second mass spectrometer. Fragments in the second mass spectrometer can be correlated with the intact molecules produced in the first mass spectrometer. This process enables unique mass spectrometry acquisition modes, such as precursor ion scan and neutral loss scan, performed in series on a single sample injection. Since certain compound classes share common fragment ions of neutral fragment molecules, a special analysis can be set up to only detect precursor ions with a particular fragment. This results in an ability to measure only a particular chemical class or subset of molecules without detecting the hundreds of molecules that are not of interest. The net result is selectivity and speed while maintaining a comprehensive set of related metabolites in a very fast assay. Depending on the type of scan function (precursor ion scan or neutral loss scan), only acylcarnitines or amino acids are detected.

Due to the speed of the scan processes, both molecular types can be obtained in one analysis, producing a comprehensive set of multiple mass spectra. With the addition of internal standards that are chemically the same as the molecules of interest but different by 3 or more mass units, the concentration of individual metabolites can be measured and quantified. A calculation of the concentrations of more than 65 metabolites and metabolite concentration ratios (molar ratios) can be routinely performed analyzing both acylcarnitines and amino acids. This lecture will focus on the description of characterization of inborn errors of metabolism. Examples of aminoacidopathies and organic acidemias will be presented. Finally new analytical strategies will be described for the identification of new markers of metabolic diseases.

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SS18

## NEWBORN SCREENING FOR GENETIC DISORDERS: LIGHT AND SHADOW. EXPERT OPINION

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As the model for public health-based population genetic screening, newborn screening is recognized as an essential program that aims to ensure the best outcome for the nation's newborn population.
To be included as a primary target condition in a newborn screening program, a condition should meet the following minimum criteria:

1. It can be identified at a phase (24 to 48 hours after birth) at which it would not ordinarily be clinically detected
2. A test with appropriate sensitivity and specificity is available for it
3. There are demonstrated benefits of early detection, timely intervention and efficacious treatment of the condition being tested.

Light**.** The public health system faces many challenges as newborn screening capabilities continue to evolve.
In addition, new technologies have brought major challenges to newborn screening as the expanding knowledge base of the etiology and treatment or potential treatment of genetic diseases, the rapid expansion of diverse technologies such as multiplex platforms that may be used in screening, the increased use of testing strategies to enhance the positive predictive value of an initial abnormal result.
Shadow. The lack of newborn screening program uniformity for infants, the changing dynamics of emerging technology, and the complexity of genetics require an assessment of the state of the art in newborn screening and a perspective on the future directions such programs could take.
In response to this need, a process of standardization of outcomes and guidelines for state newborn screening programs has to be continuously outlined to define responsibilities for collecting and evaluating outcome data, including a recommended uniform panel of conditions to include in state newborn screening programs, based on the best scientific evidence and analysis of that evidence.
Recommendations. Universal newborn screening is an essential public health responsibility that is critical to improve the health outcome of affected children.
Newborn screening is more than testing. It is a coordinated and comprehensive system consisting of education, screening, follow-up, diagnosis, treatment and management, and program evaluation.

- Newborn screening policy development should be primarily driven by what is in the best interest of the affected newborn, with secondary consideration given to the interests of unaffected newborns, families, health professionals, and the public.
- The medical home and the public and private components of the screening programs should be in close communication to ensure confirmation of test results and the appropriate follow-up and care of identified newborns.
- Recommendations about the appropriateness of conditions for newborn screening should be based on the evaluation of scientific evidence and expert opinion.
- Total quality management should be applied to newborn screening programs.

Public awareness coupled with professional training and family education is a significant program responsibility that must be part of the complete newborn screening system.
Centralized health information data collection is needed for longitudinal assessment of disease-specific screening programs.

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SS19

## HPV SCREENING

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Human Papillomavirus (HPV) makes a fundamental role in the cancerogenesis of most cancers (lung, oral tract, prostate), besides the cervical cancer. At the present time are known 120th genotypes of HPV, and about 40th of these genotipes are classified as high oncogenic types (HR-HPVs). Viral proteins E6 and E7 are capable to start the oncogenic cellular pathway, interferring with the most important check-points of cellular cicle: p53 and pRB. Molecular screening for

HPV undertakes greater importance for cervical cancer prevention, after the HPV vaccine achievement. These diagnostic techniques agree to try viral genome and/or to fix HR-HPV genotypes (PCR, Real-Time PCR, inverseDot-blot). NASBA diagnostic molecular method allows to search the E6/E7 mRNA of five HR-HPV types. To make HPV genome screening we also use immunoistochemistry techniques (IHC) and *in situ* hybridization (ISH). With IHC it is possible to search capsidic viral proteins, E6 and E7 proteins, and p16 protein by using monoclonal antibodies. At last, ISH takes over HPV positive cells into the epithelial tissues; moreover with ISH it is possible verify the integration of HPV genome in the cells.

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SS20

## THE FUNCTION OF GYNAECOLOGIST IN THE SCREENING OF HPV

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Papillomavirus (HPV) infection is very frequent among the population: more than 75% of women who have a sexual life infect themselves with an HPV virus, especially young women until 25 years.
Most of HPV infections are temporary because of the elimination of the virus by the immune system.
A persistent infection in the cervical cells is the necessary condition for the evolution to the cancer (1). The acquisition of high risk viral genotype increases the probability of the persistent infection: in this case precanceroses injuries can be developed and it can progress to cervix cancer. High risk viral genotypes more frequently implicated in the cervical cancer are 16 and 18: 16 in the 60% of the cases and 18 in the 10% of the cases.(2). Generally the time between infection and the onset of precanceroses lesions is about 5 years while the latency for the onset of the cervical cancer is able to be about decades.
This is the reason for what the prevention of the cancer is based on programs of screening that agree to identify the precanceroses injuries and to behave before the evolution of them in cancer.
It is well recognized that cytology-based programs reduce the burden of cervical cancer in developed countries. The incidence of cervical cancer has fallen by 50% or more since the introduction of Pap test smear screening for cervical cancer detection in developed countries.(3)
Based on the central role of persistent, carcinogenic human papillomavirus (HPV) in cervical carcinogenesis, testing for carcinogenic HPV has been introduced recently into cervical cancer screening. Compared with cytology, carcinogenic HPV testing has proven to be more reliable than cytology and to have greater sensitivity for the detection of cervical precancer.(4)
According to international guidelines, pap test in Italy is recommended every three years for women between 25 and 64 aged. In some Italian ASL pap test is associated with high risk HPV-DNA screening. Infact is shown that women aged 30 years and older who test negative for carcinogenic HPV and are cytologically normal are at an extremely low risk for incipient precancer and cancer for the subsequent 10 years or more.(5). A positive high risk HPV-DNA screening test associated with a negative pap test requests a colposcopic control and a repetition of pap test after a year but it can't alarm because a repeated cytology with a repeated colposcopic control represents a good way to control the evolution of precanceroses injuries HPV-dependent.

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SS21

## THE COLORECTAL CANCER SCREENING

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To be a suitable target for population-based screening efforts, a disease should represent a significant public health burden. Accordingly, colorectal cancer (CRC) is

the third most common cancer worldwide and the second leading cause of death from cancer (1). When CRC is detected at an early, localized stage, the 5-year survival rate is near 90%. However, only 37% of CRC are discovered at this early stage (2). Therefore, the goal of cancer screening is to reduce mortality through a reduction in incidence of advanced disease.
Several conventional methods for CRC screening are available, but most of them is invasive or lacks accuracy. In the laboratory setting, Fecal Occult Blood Testing (FOBT) is the most widely prescribed screening test for CRC because it is simple, non-invasive, and it has been associated with reduced mortality from CRC. All FOBT screening tests are based on the concept that colonic neoplasms and large adenomatous polyps may bleed intermittently, so that stool would contain a combination of hemoglobin, intact heme, and heme-derived porphyrins in amounts that depend on the site and amount of bleeding, and the transit time through the gut (3). Four types of FOBT tests are available, based on guaiac based fecal blood (gFOBT), colon albumin, heme-porphyrin, and immunological detection of hemoglobin (iFOBT). gFOBT is characterized by poor sensitivity, particularly with respect to detecting early stages. Colon albumin tests are no longer used, due to lower sensitivity and specificity as compared with iFOBT. Heme-porphyrin testing increases the sensitivity of the screening. Hemoglobin shed into gastrointestinal tract undergoes bacterial degradation to heme-derived porphyrins, which are not detectable by the pseudoperoxidase reaction. The immunological tests thereby demonstrate a significantly higher sensitivity and specificity. iFOBTs use antibodies specific to human hemoglobulin and do not require specific dietary and drug restrictions that otherwise limit the use of gFOBTs. At present iFOBTs seem to be the most cost-effective approach for non-invasive CRC screening. However they are more expensive than chemical tests. The analysis of fecal DNA represents an emerging field for early detection of colorectal neoplasia (4). Carcinogenesis is associated with several genetic abnormalities which presumptively accompany the pathological shift from normal to neoplastic cell. Stool-based molecular screening has a higher positive predictive value than the currently used FOBT, and offers a noninvasive option to patients (5).

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CO10

## BIOCHEMICAL SCREENING FOR TRAINING EVALUATION IN PROFESSIONAL SOCCER PLAYERS

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The present study evaluated the behaviour of more of 50 biochemical parameters in blood samples collected from 24 professional soccer players of an Italian First Division team during the agonistic season and after a 4 week rest (summer holidays).
The blood tests included: cell counts, reticolocytes, immature fractions, serum electrophoresis, thyroid hormones, electrolytes (Na+, K+, Cl—, Ca++, Mg++), iron status (iron, ferritin, transferrin, vit B12, Folate), enzymes (GOT, GPT, ALP, GGT, CK, pCHE), oxidative stress markers (Vitamin A and E, total serum thiols: RSH, Total Antioxidant Capacity: TAC, Hydroperoxides: ROM's), hormones (Testosterone, Cortisol, Prolactin, DHEA), metabolic panel (Glucose, urea, urate, creatinine, cholesterol, tryglicerides, HDL-C, Total protein, Bilirubin, mioglobin, glutamine:GLN, glutamate: GLU) and markers of inflammation (VES, PCR).
The two groups (intensive training vs after-rest samples) showed significant differences for: Uric Acid ($p = 0.03$, -25%), CK ($p = 0.04$,+75%), VitE ($p = 0.003$,-24%), ROMs ($p = 0.05$,+12%), RSH ($p = 0.01$,-27%), GLN/GLU ($p < 0.001$,-44%), cortisol/testosterone ($p = 0.02$, +25%).
In conclusion, the study seems to show that Glutamine/glutamate and Cortisol/testosterone ratios are the most specific biomarkers of intense training, i.e the inhability to fully recover after acute exercise.

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CO11

## THIRTY-FIVE YEARS OF THALASSEMIA SCREENING IN LATIUM REGION: ACTIVITIES, RESULTS AND PERSPECTIVES

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After the scientific discovery of thal trait as genetic reason for Mediterranean Anemia, Silvestroni and

Bianco started, in the 50ies, a wide action to screen Italian population, pointing out thalassemia epidemiological severity.
Since 1975, Centro Studi Microcitemie Roma (CSMR) takes place a prevention program in Latium region by means of two different activities: the first performed on all intermediate school students; the second, on young-adult population, sent by family doctors, gynecologists and National Health Prevention Services.
After an informating course and obtaining parents' consent, school screening protocol provides blood samples collection. In CSMR laboratories a step-by-step protocol is performed: RBC morphology on sharp smear and Simmel test; Blood count (GenS, Beckman Coulter Inc.); Hb A2 dosage (Hb electrophoresis or HPLC).
A letter with both diagnosis and short remark is sent to student parents. Positive student families are invited to CSMR for extended exams and genetic advice.
The screening protocol for young-adult population takes place in CSMR laboratories and provides: Simmel test, RBC morphology, blood count, Hbs HPLC, bilirubin, iron, UIBC and ferritin.
In both protocols, globinosynthesis and DNA analysis are performed in doubtful cases.
Since 1975, 1,400,346 students complied with our school screening (mean adhesion 69.37%). In the same period, 352,171 young-adult subjects went to CSMR.
Among the students, 25,478 subjects were thal trait carriers and 35,630 among the young-adult population, too.
Besides, many hemoglobinopathies carriers and several subjects affected by different kinds of thalassemia and Hb pathologies have been discovered as well.
1,096 couples at risk have been found out and performed genetic counselling; 843 pregnancies obtained prenatal diagnosis and 208 out of them have been interrupted.
The significant results of our thal prevention program are: increasing number of couples at risk informed of their conditions, attending genetic counselling; gradual tendency from retrospective to prospective prevention; decreasing of thalassemic births: in fact, since 1993, not one thalassemia affected child is born in the autochthonous population, from couples that were informed about their risk.

SS22

## MONOCLONAL B LYMPHOCYTOSIS; DIAGNOSIS AND NATURAL S.M.Y.

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The term Monoclonal B Lymphocytosis (MBL) defines the presence of monoclonal B lymphocytes circulating in the blood of otherwise healthy individuals (1). Though this phenomenon was known for long, only recently, thanks to the use of multi-color flow cytometric analysis, it became evident how frequent is in the general population, being detected in at least 3.5% of all adult individuals (2, 3). This percentage increases when more sensitive analyses are performed (4).
MBL can be distinguished based on the presence or the absence of the CD5 molecule on the cell surface of the B cells (3). Some cases do not express CD5 and are then defined *CD5$^{-}$ MBL*. The majority of cases are CD5$^{+}$ and can be subdivided into those expressing normal levels of CD20 (CD20$^{bright}$), defined as *atypical-CLL-like* and those with lower levels of CD20 (CD20$^{dim}$) named *CLL-like MBL*. This subtype is the most frequent overall and it is especially detected in the elderly being present in >7% of the individuals >75 years of age (4). The name *CLL-like MBL* originates from the close resemblance, in terms of phenotype, with Chronic Lymphocytic Leukemia (CLL) lymphocytes, expressing low levels of CD79 and Immunoglobulins (Ig) and co-expressing CD23, in the absence of FMC7 (1).
At present it is not known whether MBL may be considered direct precursors of CLL, and which molecular mechanism, if any, may control the evolution of MBL into CLL. MBL cells can be considered a pre-leukemic phase of CLL based on the phenotypic resemblance and the possibility of evolution into overt CLL in 1.1% of the cases per year (5). In contrast, if one considers that MBL is at least 100 times more common than CLL and it is more frequent among the elderly, it might simply reflect a phenomenon of lymphoid senescence. Accordingly, it has been shown that also polyclonal CLL-like MBL cases exist, suggesting that the acquisition of a CLL-like surface phenotype does not imply *per se* monoclonality of the cell population. This may just reflect a functional state following e.g. stimulation and activation by persistent/chronic exposition to particular antigens, either infectious or self. Several studies are ongoing aiming at identifying the molecular and functional features that can distinguish MBL from overt CLL and may help to discriminate the individuals at risk of leukemic evolution.
The distinction between CLL-like MBL and CLL is at the moment based on a numerical cut-off. When the cells are present at a concentration of $<5x10^{9}$/L then they are considered MBL, if no lymphoadepathies, organomegalies or other signs of disease are found (1). The absolute B-cell count has been reported to be an independent prognostic factor associated with progression when MBL cases were studied in the context of a lymphocytosis brought to clinical attention (5). In contrast, most MBL are found in the general population in the absence of any alterations of the lymphocyte count. In these cases, they account for a very minor cell population in the PB, representing <10% of all B lymphocytes (4). Molecular and biological

features can help to distinguish these two forms of MBL suggesting that one day we may utilize molecular characteristics of the leukemic clone to predict those cases that are more prone to progress into a frank leukaemia (4).

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SS23

## BIOLOGICAL PROGNOSTIC MARKERS IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA


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B-cell chronic lymphocytic leukemia (B-CLL) is a neoplastic disease where quiescent B cells accumulate in the peripheral blood and it has long been known to have a variable clinical course. The clinical staging systems of Rai and Binet, developed in the early 80s, are useful for assessing prognosis but do not allow identification of patients in early stage who are likely to progress and those in whom the disease will remain stable and the management of this heterogeneity has to be based on the individual risk for each patient. Therefore it is very important to identify factors that can predict poor prognostic and also identify patients who will benefit from intense therapy in an early stage of the disease. The continuous effort to search new prognostic markers of B-CLL that would help to define risks and treatment strategies has provided several laboratory tests that are particularly promising but need systematic investigation in prospective clinical trials.

In this scenario, recent studies have shown that cytogenetic abnormalities are important prognostic factors; using interphase fluorescence *in situ* hybridization (FISH), cytogenetic lesions can be identified in > 80% of B-CLL cases (1). We now know that the common chromosomal abnormalities in CLL are: deletions in the long arm of chromosome 13 del13q14, deletion and/or trisomy of chromosome 12, del 11q23 and del17p13. There is increasing evidence that some of these chromosomal abnormalities in B-CLL have prognostic significance, therefore it is recommended that cytogenetics be performed before treating a patient and the repetition of FISH seems justified prior to second and third-line treatment.

Gene expression profile of B-CLL samples using DNA microarrays has shown differences in the expression patterns of hundreds of genes (2-3) and the presence or absence of somatic mutations in the immunoglobulin variable (heavy-chain) region genes (IgHV) is currently referred to as the 'gold standard' in predicting survival in B-CLL. The outcome of CLL patients with unmutated IgHV gene is inferior to that of patients with mutated IgHV gene indicating that IgHV mutational status is critical in determing the prognosis and may identify a distinct subset of CLL. Unfortunately performing IgHV mutational status is too difficult for clinical laboratories and other biological parameters has been employed as prognostic factors.

Interestingly, among these, expression levels of ZAP-70 (zeta associated protein of 70kD) (4) and CD38 have been associated with IgHV mutational status but different studies (and methods) gave discordant results and the association between ZAP-70 and CD38 and IgHV is not absolute.

More recently a set of 13 microRNA genes has been found to correlate with the expression of ZAP-70 and unmutated IgHV genes (5) and other factors such as the expression of the telomerase reverse trancriptase (hTERT) and the telomere length have been proposed as new prognostic markers in B-CLL.

In conclusion all these new biological markers in B-CLL must be standardized and validated in large prospective clinical trials, in a combination of clinical and biological features.

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SS24
## DATA MINING IN FLOW CYTOMETRY DATA ANALYSIS

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Data mining is the extraction of implicit and unknown information from analytical results, in order to recognize the patterns and the structures underlying the data. Data mining is based on artificial intelligence techniques, such as cluster analysis, neural networks, and decisional trees, and in an biologic environment is usually applied to data bases produced by gene expression experiments. In the phenotypic analyses of large groups of patients, flow cytometry allows us to capture and store vast quantities of data, whose meaning is largely lost with the conventional interpretative approach, which tends to ignore features such as data distribution. In data sets produced by flow cytometry, data mining is able to find otherwise unrecognized patterns, identifying and characterizing subsets of patients according to the similarity of the antigen expression profiles. This is a new and powerful way of exploring the meaning of the biological heterogeneity of neoplastic blood diseases.

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## A NEW METHOD FOR THE SCREENING OF CHRONIC LYMPHATIC LEUKEMIA

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Background. The VCS technology of Beckman Coulter differentiates white blood cells based on measures of their volume, conductivity and light scatter. This study investigate the predictive value of index measures, known as research population data, compared to morphologic and cytometric analysis for the detection of chronic lymphatic leukemia (CLL).
Methods. Morphology, blood cell counts and flow cytometry analysis were performed in samples from 28 patients with CLL and 84 healthy using the Beckman Coulter LH780 and the cytometer FACSCanto II TM (BD Biosciences, San Jose, CA, USA), respectively. Means and standard deviation of volume, conductivity and scatter, and immunophenotyping antigen distribution patterns of lymphocytes were evaluated as predictors of disease.
Results. Research population data and flow cytometry immunophenotype patterns were different between two groups. For CLL immunophenotype antigen distribution patterns showed CD5+CD23+ spread/spread and CD5+CD23+ homogeneous/ spread, FMC7 homogeneous weak, CD79b homogeneous bright, CD20+CD200+ weak homogeneous/weak homogeneous and mean of lymphocytes volume showed most relevant difference in comparison to healthy samples, area under the ROC curve (AUC) was 0,897.
Conclusion. Research population data and immunophenotype pattern could be routinely used to screen for CLL.

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## PAROXYMAL NOCTURNAL HEMOGLOBINURIA: TWO CASES REPORT

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Introduction. Paroxymal nocturnal hemoglobinuria (PNH) is a rare acquired hematopoietic stem-cell disorder caused by a mutation in gene PIG-A that results in cellular inability to sinthesize the glycosyl phosphatidylinositol-anchored proteins (GPI-APs) that is needed to anchorer various proteins to the cell membrane. Deficiency of the GPI-anchored complement regulatory proteins CD55 and CD59 accounts for the intravascular hemolysis that is the primary clinical manifestation of the disease. PNH diagnosis is esthablished by demonstration of defective GPI-APs CD59/CD55 expression on RBC and/ or WBC. This report describes two clinical cases: 1 male of 50 years and 1 female of 16 years both admitted in Clinical Medicine of Padova Hospital with diagnosis of anaemia and leukopenia.
Methods. By flow cytometric analysis we evaluated the expression of cell surface markers CD16 on granulocytes and CD14 on monocytes. The expression of GPI-AP CD59 was performed using a monoclonal antibody CD59 with stain-no lyse-no wash direct immunofluorescent technique for RBCs and with lyse-wash-and-then-stain direct immunofluorescent technique for WBCs. In parallel two peripheral blood from healthy individuals were also analyzed and used as control.
Results. In both cases we rilevated an important riduction of CD16 expression on PMN (27% vs 98% e 12,5% vs 100%) and of CD14 on monocytes (29% vs 89% e 11% vs 94%).These markers presented a bimodal pattern. Both patients were affected by deficit of GPI-AP CD59 on myelomonocyte component (CD59 PMN: 46% and16%; CD59 monocytes: 35% e17%).Only in male there was a reduction in CD59 expression in red cells (82% vs 100%).
Conclusion. Our data confirm the necessity to investigate CD16 and CD14 expression in PNH diagnosis, other than GPI-APs expression. Infact, a bimodal expression of these markers permit to identify a PNH clone.