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LETTURA PLENARIA 1
CLINICAL BIOCHEMISTRY IN ORGAN TRANSPLANTATION

Sala A

Mercoledì 12 Ottobre 2005, ore 9.00 - 10.00

LP1

CLINICAL BIOCHEMISTRY IN ORGAN TRANSPLANTATION

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Introduction: In the last years the frequency of transplantation of organs like kidney, pancreas, liver, heart and lung has dramatically increased. Aspects of immunological mechanisms involved in the graft versus host reaction are fundamental for new diagnostic and therapeutic concepts.

Immunology: Cellular and humoral mechanisms are involved in graft rejection and infections leading to release of cytokines, mediators, acute phase proteins, activation of the complement system and immune-competent cells. T-helper cells play the central role in cell-mediated immune response as they produce a variety of cytokines essential for activation and differentiation of other cells. Antibodies that bind to free antigen or antigens expressed on cells are the key component of humoral-mediated immunity. It can occur within hours after transplantation (hyperacute graft rejection) in recipients pre-sensitised against donor antigens. The cellular mechanisms involved in the rejection of a graft are a combination of specific actions of cytotoxic T-cells, pro-inflammatory cytokines and unspecific proteolysis.

The Clinical Laboratory: The clinical laboratory is strongly involved in the follow-up of patients after organ transplantation. Main diagnostic tasks are the diagnosis of transplant rejection, infection status, and the monitoring of immunosuppressive drug levels and of drug effects.

The diagnosis of graft rejection is based on two principles: 1) Monitoring of organ dysfunction and tissue damage: For this purpose so-called organ specific routine analytes for kidney, liver and pancreas function are used. 2) Monitoring of immunologic status.

For monitoring immunosuppressive drugs (cyclosporine A, FK 506, mycophenolic acid) mainly the measurement of blood target levels by means of HPLC or immunoassays is used. For interpretation of test results it must be considered that the results obtained are method dependent and the therapeutic regiments used are different for the various organs transplanted. In addition biological and toxic effects of the drugs can be monitored. Therapeutic drug monitoring in organ transplantation is a responsible and challenging task, since the clinicians use the blood drug levels and organ function tests reported for drug prescription and for estimation of the patient's compliance.

SESSIONE 1
LA GRANDE AUTOMAZIONE DI LABORATORIO

Sala A

Mercoledì 12 Ottobre 2005, ore 10.15 - 12.45

S1.1

INTERNAL QUALITY CONTROL AND ALIGNMENT OF CLINICAL CHEMISTRY ANALYSERS

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INTRODUCTION: In medium and large laboratories, despite the individual organisation, more than one platform is required in order to process a large number of general chemistry tests within a reasonable Turn Around Time. This is true independently of the adoption of "Total Laboratory Automation", of the Workcell approach or of single platforms. The use of several analysers, even if identical, requires a strict control of the alignment among the devices to guaranty unambiguous data. The control strategies are similar in laboratories with similar structural complexity and test volume.
AIM: evaluation of incidence of device un-alignment, identification of analytes out of control, their causes and possible solutions, assessment of effects of corrective actions.
MATERIALS & METHODS: Retrospective study upon 7 months (Nov. 2004 - May 2005) on Aeroset (Abbott) analysers in our lab. Data from Internal Quality Control (IQC), run by commercial 2-level control sera (Liquicheck, Biorad) and from parallel measurements of patient sera on the different devices were evaluated. Each result was compared with its analytical goal(1) for this analyte in our laboratory. Percent of unacceptable results was calculated.
RESULTS: 11400 IQCs (for each level) and 3300 alignment checks were performed. The percentage of monthly CVs higher than the specific analyte goal CV was 3-4% up to Feb. 2005 but on March and April it grew up (9%). Subsequent corrective actions led to 5%. The percentage of unacceptable alignment CVs was minimal in Dec. (0.4%), then grew up until March (3%). Corrective actions drove it down in May (0.6%). Analytes with more frequently unacceptable monthly CVs were Cholinesterase (6-7%) and Glucose (5-6%). Analytes with more frequently unacceptable daily alignment CVs were Magnesium (9.9%) and Uric Acid (5%).
CONCLUSIONS: the control of the device precision is a key problem in laboratories where the use of parallel devices is needed to achieve high productivity. The present approach allowed continuous control of most of the analytes. Few analytes yielded border-line results, and might require a different approach.
REFERENCES:
(1) Brooks Z. C. in "Performance-Driven Quality Control" AACC Press (2001)

S1.2

CHANGING FROM THREE HOSPITAL LABORATORIES TO AN OUTSOURCED CORE LAB IMPROVES QUALITY AND EFFICIENCY.

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Rationale: during the past years many changes in health organization and economics (population ageing, resources shortage, need to produce highly specialized tests) and in biotechnology have been occuring, leading to the activity consolidation.
Aim of the study: to describe the process benefits gained after consolidation of three hospital labs in a single, outsourced, paperless, highly automated work-cells laboratory.
Methodology: MultiMedica is a private health group currently consisting of 3 hospitals and 3 outpatient clinics . Before consolidation (BC) any hospital hosted a lab managing routine and STAT tests on different analyzers. Then, after consolidation (AC-1), all the activities were centralized in a pathology department out of the hospitals. The biochemistry workcell was served by an automated preanalytic system (Tecan FE500), 2 clinical chemistry (CC) stand alone (Aeroset), 1 modular (Architect i4000) and 2 stand alone (Axsym) immunoassay (IA) systems. One year later (AC-2) a consolidated platform Architect Ci8200 replaced 1 Aeroset. A middlewere informatic system (LabOnLine, OmniLab) was introduced to optimize data management and a remote survey (Abbott Link) was applied to all the analytical systems.
Results: changes of major indicators in different scenarios are shown in the following table:

PARAMETERS	BC	AC-1	AC-2
Tubes for serum panel	7	2	2
Tubes for biochemistry tests (all)	3	1	1
Major biochemistry analyzers	7	5	4
Biochemistry working places	5	2	1
Biochemistry FTEs	6	3	2
Internal TAT (CC+IA)	126’	87’	119’
Walkaway time	102’	88’	135’
Throughput chemistry (test/h)	946*	1410	807#
Throughput immunoassay (test/h)	68*	68	

*best performance of the three labs; #only the Ci8200
Automated preanalytical station minimized samples management time and variability, reduced the blood tubes and streamlined the workflow parallely processing aliquots and tubes on different platforms. In the BC configuration the backup was unconsistent, in AC-1 and AC-2 no trouble affected workflow. Saving FTEs on automated platforms let us develop new specialty tests.
Conclusions: consolidating the activity of three laboratories in a core lab, we avoided duplication of technologies and analyzers, increased lab performances without increasing the staff. We will move to full consolidation of platforms replacing soon Architect i4000 and Aeroset with an other Architect Ci8200.

SESSIONE 2
TOSSICODIPENDENZE
Sala C
Mercoledì 12 Ottobre 2005, ore 10.15 - 12.45

S2.1
DRUG ADDICTION

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Drug addiction is a chronic, relapsing disorder in which compulsive drug-seeking and drug-taking behavior persists despite serious adverse consequences. Addictive substances induce pleasant states (euphoria in the initiation phase) or relieve distress. Repeated use induces adaptive changes that lead to addiction (drug dependence). Drug addiction could include tolerance, physical dependence and withdrawal, sensitization, craving and relapse. Drugs of abuse are potent reinforcers that act in the neural circuitry of reward, the same system that use natural reinforcers (e.g. food, sex). This circuit includes the mesocorticolimbic dopamine system that originates in neurons in the ventral tegmental area and projects to limbic structures (nucleus accumbens), that are implicated in acute reinforcing, memory and motivation. Also projects to the prefrontal cortex, involved in control of behaviour and judgement. Drugs of abuse increase extracellular dopamine concentrations in the nucleus accumbens by direct (opioids, cannabinoids, ethanol) or indirect mechanisms (cocaine, amphetamines). The increase of dopamine is associated with the euphoria and hedonic effect of drugs. During drug withdrawal, dopamine levels in this area are decreased and seem associated to the dysphoria an anhedonia observed during this state.
The molecular mechanisms of tolerance and withdrawal of opioids have been studied extensively in animals. Up-regulation of cAMP in the locus ceruleus (noradrenergic neurons) seems a key mechanisms in opioid tolerance and withdrawal. When opioid levels fall, the firing rates of neurons in the locus are unopposed and lead to adrenergic overactivation. Stress systems are also activated, an increase in corticotrophin-releasing factor (CRF) in the amygdala have been related to stress and negative effects of abstinence. In addition, chronic activation of opioid receptors increases the phosphorylation of CREB and ?FosB, factors regulating gene transcription.These changes correlate with the manifestations of the withdrawal syndrome.
Long-term administration of addictivto relapse and facilitate craving even months or years after successful detoxification. Factors influencing vulnerability to drug abuse and dependence include genetic and environmental factors, personality traits (novelty seeking) and mental disorders. (dual diagnosis or co-morbidity). Genetic factors that influence the effects (e.g. receptors, membrane transporters) and pharmacokinetics of drugs (e.g. cytochrome P-450 polymorphisms) could contribute to the risk of addiction.

References
Cami J, Farre M. Drug Addiction. New Engl J Med 2003;349:10:975-86.

S2.2
STATE, REGIONS, SCIENTIFIC CORPORATIONS HAVING A ROLE IN THE CLINIC PHARMACTOXICOLOGY

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Clinic Pharmactoxilogy is getting in these days a critic engagement for implementing important news concerning its character and its technical scientific organization.Unfortunately in the last ten years we have noticed a progressive or even exponential increase of the abuse of various forbidden substances with a concerned increase of the number of inquiries for analitic parameters.
The decrease of resources distributed by the National Sanitary System, has created the necessity of a re-organization strategy able to conjugate efficiency, reliability and mostly the concepts of effectiveness with economy.
Clinic Pharmactoxicology is mostly engaged in the monitory of: medicines and dosages of abusive substances, with a limited field of action but essential not only for the clinic impact but also for the social impact. To this Service happen clinic-diagnostic inquiries (SerT, Diagnosis and care divisions) but also inquiries concerning forensic medicine like for example those arriving from Jail, Driving licence Commissions, Police-headquarters, Prefectures and Private or Public Organizations that have to deal with controls for workers with duties carrying safety risks.We have also to notice two specific needs: the first one is that in some cases the analyses required by SerT have a forensic medical character (see art, 75 T.U. 309/90, art. 94 or art. 90/91 T.U. 309/90, Minors custody), the second one is that a toxicolocical analysis made primarly with a clinic-diagnostic purpouse can get also a forensic medical value.
The most important characteristics of the parameters in Pharmactoxicology, i.e. : the strightly theurapetic index concerning drugs with optimal concentration (related to toxicity), the relapse of a wrong abstraction time, the uniqueness and the possible alteration of the sample that has to be submitted to dosage, the indispensable care chain including transport and preservation of such samples as well as the various purpouses of the concerned determinations, require the continuous presence and professionality of Operators with consolidated experience.
All this is the evidence of the impact and of the importance of the role of the Pharmactoxicology Laboratory and the subsequent problematics strightly connected to the honesty and professional responsability that therefore require preparation, continuous updating and audit of the concerned staff being this a safety factor of enormous importance in the determination of the complete outcome.
The actual tendency of utilizing the standard of the "clinic governance" also in the control of the general sanitary accounting is applicable in the section Pharmactoxicology through the acquisition of a new working philosophy, not related to the simple test execution but related to a

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S2.2

different methodology based on the appropriation of the request that has to intervene on:

·Pre-analytical phase: through the control of the access procedures, correctly disposing of the sample(it is important to dispose of at least two rates, one for the analysis and one for an eventual revision of the same analysis for comparison) avoiding in this way to process samples in which the result would not be suitable

·Analytical phase: in order to be able to supply scientifically correct and appropriate data

·Post-analytical phase: concerning the preservation and care of the sample as well as the compilation of a medical report easy explanable, as tests on abuse substances have to be utilized often by non specialistic staff such as advocacy or magistrature

The actual absence of a detailed regulations on this matter has created the use by each different laboratory of different operational modus with subsequent methological differences that may be found in the complete procedure. The result is a difference of treatment for the users who have to submit themselves to the same test in different Laboratories of the same corporation and same region.

State, Regions and Scientific Corporations must therefore, as soon as possible ,create and implement reglations that can help in the creation of "consensus" documents that have as unique object an univocal, standardized and unvarying method in order to give a full gurantee to the users and operators.

Scientific corporations have to be a guarantee for the various ideas and human experiences ripen in various study-groups.

The group SIBIOC " Clinic and Dopin Pharmactoxicology" must from its side implement and optimize a multi-disciplinary team, that through: the continuous involvement of different professional figures and the use and disposal of a guide and scientific material, must propose new and different models concerning organization and management of the Clinic pharmactoxicology. All this shared and with the "consensus" of other scientific corporations , so that once approved by the Superior Health Institute it will be applied in the various Regions.

The Regions from their side have to promote and guarantee the creation of a "network" of laboratories approved by law in order to optimize and rationalize this analitic methodology (screening and conferme).

The Superior Health Institute must be the referring Corporation for the crediting and the continuous updating of the complete staff with the result that it will become more and more specialistic in Pharmactoxicology.

This unpostponable regulation will be able to guarantee the necessary qualitative autonomy in the actual procedures of re-organizations based mostly only on the consolidation of more analytic lines on modular instruments, keeping in good consideration exclusively the economical savings.

In conclusion, whatever model will derive, it has always to respect and guarantee each service in the optics of a Total Quality.

S2.3

WHICH HEMATOCHEMICAL MARKER OF DAMAGE DUE TO CHRONIC ALCOHOL ABUSE FOR THE NEW "YOUTH DRINKING" PATTERN?

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In the last years "youth drinking" customs have changed from being events of occasional and non intentional abuse to becoming a from of cyclical "buzz", which involves both sexes.

We evaluated the hepatic damage deriving from chronic abuse using the following hematochemical markers: MCV, GGT, TGO and TGP, bilirubin, carbohydrate deficient transferrin and isoform, ialuronic acid. In considering the age of subjects (12 to 18 years) we identified the reference values which appeared to be generally lower in comparison to the general adult population.

The carbohydrate deficient transferrin turned out to be particularly sensible and specific in two thirds of the subjects and in all females, followed by MCV. Also the bilirubin was frequently found altered, while only occasionally alterations were found for the other indicators.

The study came to three conclusions: 1. Identifying specific age related reference values is a "sine qua non" condition 2. The carbohydrate deficient transferrin and MCV are the best markers to identify chronic alcohol abuse 3. Females are more exposed then their male peers due to a alcohol dehydrogenase defect. In general the new youth drinking models are particularly harmful and an increased social attention is recommended. (driving license problems?)

Reference

Occhineri E. et al. Indagine sull'uso dell'Alcol (abitudini alcologiche) nei giovani delle scuole medie inferiori e superiori del Comune di Mesagne (BR). European Journal of Alcohol Studies, 2003, 16 (1-2-3), 25-36

SESSIONE 2
TOSSICODIPENDENZE

Sala C

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S2.4

NEW MARKERS OF ALCOHOL ADDICTION
NUOVI MARKERS DI ABUSO ALCOLICO

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Various biochemical and metabolic abnormalities (biomarkers) are associated with acute or chronic exposure to ethyl alcohol. Alcohol biomarkers include markers related to a genetic vulnerability to alcoholism and tests indicative of acute or chronic alcohol consumption (diagnostic or state markers) (1,2) which actually represent valid diagnostic tools in clinical and forensic toxicology. In particular, state markers are related to recent and non recent alcohol drinking and also provide useful information on chronic alcohol abuse. As an alternative to the determination of ethanol in blood and breath, measurements of 5-hydroxytryptophol, ethyl glucuronide or ethylsulfate in biological fluids provide sensitive methods to disclose recent drinking, because their washout constants are much longer than that for ethanol. Among the diagnostic tools used to identify chronic alcohol exposure, Carbohydrate-Deficient Transferrin (CDT), which refers to changes in the carbohydrate composition of serum transferrin, proved to be a specific marker for identifying excessive alcohol consumption and monitoring abstinence during outpatient treatment, much more specific than liver function tests and mean corpuscular volume of erythrocytes.
Analytical procedures for the sensitive and specific determination of these biomarkers, based either on GC-MS, LC-MS/MS or CZE (3), will be discussed.

1. Musshoff F, Daldrup T.
Determination of biological markers for alcohol abuse.
J Chromatogr B. 1998; 713(1):245-64.
2.Lesch OM, Walter H.
New 'state' markers for the detection of alcoholism.
Alcohol Alcohol Suppl. 1996;1:59-62.
3. Musshoff F.
Chromatographic methods for the determination of markers of chronic and acute alcohol consumption.
J Chromatogr B. 2002;781(1-2):457-80.

SESSIONE 3
STANDARIZZAZIONE IN ENZIMOLOGIA CLINICA: LA TEORIA DELLA RIFERIBILITA' METROLOGICA ALLA VERIFICA PRATICA

Sala F

Mercoledì 12 Ottobre 2005, ore 10.15 - 12.45

S3.1

IFCC REFERENCE SYSTEMS FOR ENZYME STANDARDIZATION

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The primary goal of standardization for measurements of catalytic concentrations of enzymes is to achieve comparable results in human samples, independent of the reagent kits, instruments, and laboratory where the procedure is carried out. In order to pursue this objective, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) has established reference systems for the most important clinical enzymes. These systems are based on three hinges: a) reference measurement procedures, extensively evaluated and carefully described, b) certified reference materials (RMs), and c) a network of reference laboratories operating in a highly controlled manner. The original IFCC-recommended procedures for alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), g-glutamyltransferase (GGT), lactate dehydrogenase (LDH), a-amylase (AMY) and alkaline phosphatase (ALP) were modified to optimize them at 37 °C, with the definition of detailed operating procedures. A small group of laboratories, world-wide located perform these procedures manually, with self-made reagents on carefully calibrated instrumentations. In cooperation with the Institute for Reference Materials and Measurements (IRMM), five RMs, that were already available, have been re-certified by these laboratories for ALT, CK, GGT, LDH and AMY activities.In addition, recombinant human AST and ALP materials have been selected as candidate RMs and a certification campaign will begin very soon. Using these RMs and the manufacturer's standing procedures, industry can assign traceable values to commercial calibrators. Clinical laboratories, which will use routine procedures with these validated calibrators to measure human specimens, may finally obtain values which are traceable to reference procedures.In conclusion, these reference systems constitutes the structure of the traceability chain to which the routine procedures can be linked via an appropriate calibration process, provided that they have a comparable analytical specificity (i.e. they are measuring the same quantity).

S3.2

STATE OF STANDARDIZATION IN ENZYMO-LOGY: RESULTS FROM AN EUROPEAN PILOT PROJECT

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Main aim of this international study was to assess the state-of-the art in the measurement of 7 clinically useful serum enzyme activities, with particular reference to the traceability of results to the IFCC reference method values. The study design included the distribution of 1-mL aliquots of deep-frozen special serum preparation to 5 laboratories in 3 Countries (Germany, Italy and The Netherlands), each using one of 6 previously chosen, largely available analytical systems. Each participant was asked to assay the serum in 5 replicates, using his/her routine analytical system. The study included some special features. First, reference laboratories assigned the values of catalytic activity concentration (7 enzymes: ALT; AST; CK; LD; GGT; AMY; ALP) to the special serum with IFCC reference methods (with the exclusion of ALP, whose value was assigned by consensus). Second, the commutability of the test serum with patient sera was verified for a number of available analytical systems. In this way, measurement trueness could be evaluated by comparison with reference methods results, and the results were transferable to patient sera assay in "routine" condition. This report concerns essentially the analysis of the results obtained in or Country (Italy). Possible detection and elimination of outlier (or incongruous) results was a main problem in data evaluation. Although this operation may be always considered to be arbitrary, it was thought that data could be discarded when it appeared or it was known that: ALT and AST were assayed without P-5-P in the mixture; LD was measured in the Pyr®Lact instead of in the Lact®Pyr direction; DEA instead of AMP was the buffer in ALP assay. Following such criteria a total of 18 series of results (90 single results) were eliminated, considering a series the set of 5 replicate measurements performed in one laboratory. Due to the number of laboratories really participating, and to the elimination of outliers, the total number of "Italian" useful values was 835, a figure that still compares reasonably with the planned number (1050), and allows statistically robust conclusions. The within laboratory imprecision was from excellent to good: out of 42 mean CV% values, 37 were <1.5%, 4 were in the interval form 1.5% to 2.0%, 1 reached a peak value of 4.6%. The inter-lab/within system imprecision was higher: out of 42 CV% values 25 were <3.0%, 12 spanned the interval form 3.0% to 5.0%, 5 were >5%

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S3.2

(peak value: 7.8%).
The overall imprecision values were still higher, spanning the interval from 3.9% to 10.0%. The trueness was assessed by comparing the measured to the expected (reference or consensus) values, calculating the bias as: bias% = [(measured-expected)/ expected x100]. With the exception of amylase, the within-system bias values for each enzyme were, alternatively, negative or positive, spanning the interval from -19.0% to + 14.0%; for amylase the bias values were in the interval from -40.0% to -2.4%. Due to mutual compensation of negative and positive values, the overall bias values for 6 of the 7 seven enzymes were in the interval from -3.0% to +2.8%, for amylase the overall bias value was -16.0%. Concerning the inter-countries comparison, overall CV and mean values were made available from the project's organizer, Dr, Rob T. P. Jansen (The Netherlands). The number of useful results was: Germany, 833; Italy, 886; The Netherlands 460. Results from this elaboration are not fully comparable with our elaboration of the Italian results, because of different criteria in the elimination of incongruous results, as above outlined. However, Country-specific results are each other comparable because of the application of homogeneous criteria.. Country specific overall CV values (from 4.9% to 10.8%) showed to be substantially comparable, with the main exceptions of lactate dehydrogenase, were CV values as high as 43.5% and 48.1% were found, and amylase, with CV values of 13.0% and 19.3%, possibly as the result of averaging results obtained under different standardizations of measurement. This is also reflected in the overall bias values. Out of 21 bias values, 16 spanned the interval from -6.7% to +1.8%, but values as high as +22% and -32% were observed for lactate dehydrogenase and amylase, respectively. We can conclude that: 1) even within the same analytical system a few routine enzyme measurements are not traceable to the recommended IFCC standardization; 2) the high intrinsic repeatability of the analytical systems is not fully taken advantage of, possibly because of defects of calibration. The results of this project show that there is room for improvement and in which direction to address future efforts.

S3.3

HOMOGENEITY OF REFERENCE INTERVALS AND DECISION LEVELS IN CLINICAL ENZYMOLOGY: THE FINAL TARGET HAS BEEN MET?

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The goal of the IVD Directive, requiring traceability to higher metrological levels, is to allow the production of comparable results independently from the method used. Due to the standardization efforts of IFCC and JCTLM this goal could now be reached also in the field of clinical enzymology that, until now, was the reign of method dependent results (by definition catalytic activity depends upon the method or, better, is a property of the analytical systems itself). The definition of a reference system for the measurement of the concentration of catalytic activity of the most common enzymes and the publication of the list of reference measurement and materials on the JCTLM web site is driving towards the homogeneity the results, but what about reference ranges?
If we look at the present situation the picture is discomforting: on a database of about 590 Italian laboratories for ALT measurement more than 30 different upper reference limits are indicated just for males, spanning from 29 to 83 U/L. Forty two % of the laboratories claims identical intervals for males and females. If we restrict the field to the methods that claim to be "according to IFCC with pyridoxal phosphate" (only 47 labs), we still find 13 different upper reference limits just for males (from 31 to 70 U/L) with lower limits spanning from 0 to 30 U/L (lower and upper limits almost overlap!). The confusion of different analytical principles combined to laboratory dependent reference intervals creates a dangerous mix. And substituting the decision levels for the reference ranges, instead of normalizing the situation adds confusion to confusion. In fact usually, due to the difficulty of defining a unique decision level, the multiples of upper reference limits (URL) are adopted. It can be easily demonstrated that the combination of different methods with inappropriate reference ranges reduces the comparability of the clinical information provided instead of increasing it. To try to improve this unacceptable situation and to eventually complete the effort of standardization produced by the JCTLM and IFCC, IFCC itself has started a committee with the task of defining appropriate reference intervals for the most relevant mea surands, including enzymes.
These reference intervals will be obtained through a collaborative effort of an international network of laboratories and will probably be adopted by the manufactures that provide test kits traceable to the IFCC reference methods. The adoption of these common reference ranges should be the first step on which to develop appropriate decision levels for the most common clinical situations.

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S3.4

THE IVD INDUSTRY - AN IMPORTANT PARTNER IN THE PROCESS OF ENZYME STANDARDISATION

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Enzyme concentrations are usually not measured as mass concentrations but as catalytic concentrations, depending on the temperature, pH, buffer, ionic strength, presence of co-enzymes, activators, substrate etc. The peculiarity of these tests is that the analyte is defined by the measurement conditions. Therefore, contrary to the reference methods for substrates such as creatinine or glucose the reference methods for enzymes are consensus-based. Standardisation is in the interest of the IVD industry and has been pursued for many years, but most often at a national level, leading to non-comparable results and requiring manufacturers to provide different kits for different countries. The IVD industry supports IFCC's international enzyme standardisation approach.
Questions in this context are:
(1) Which enzymes?
To justify the financial engagement wide-spread use of the test is mandatory (cost / effort relationship).
(2) Which methods?
Manufacturers must be able to develop traceable routine methods or to adjust existing ones: Practical laboratory requirements, however, often mean that routine methods differ significantly from the reference procedures defining the analyte, causing problems with control materials and sometimes also patient samples. Examples are shorter pre-incubation and serum start instead of substrate start to increase the sample throughput. Sometimes liquid, ready to use reagents with a long shelf-life and calibration stability with some components added or changed are used.
(3) Acceptance of the clinical community?
It is important that the standardised - and thereby changed - analyte still reflects clinical requirements. And, unfortunately, standardisation of methods has not yet resulted in harmonisation of reference intervals even for equivalent reference populations. This is also required to make the standardisation clinically useful.
(4) How to standardise?
A good approach is to use a set of patient samples to trace the routine method back to the reference procedure in order to avoid commutability issues.
(5) Which reference laboratory to chose and how to organise the logistics?
Manufacturer's own laboratory or laboratory from IFCC reference laboratory network. (Long-term availability of the reference laboratory?) Cost considerations.
These questions need to be discussed between IFCC and manufacturers before the standardisation begins.

S3.5

REFERENCE INTERVALS OF ENZYMES: THE STATE OF THE ART FOCUSED THROUGH AN EXTERNAL QUALITY ASSESSMENT SCHEME (EQAS)

Secchiero S., Sciacovelli L., Zardo L., Plebani M.
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EQAS aim at improving the performance of laboratories by way of education, scientific recommendations and standardization taking into account clinical needs and quality specifications.
The participants to the EQAS managed by the Centre of Biomedical Research (CRB) submit their results on a medical report form with the indication of the Reference Intervals (RI) adopted, and, in order to stimulate the use of appropriate RI, we provide diagrams showing each laboratory the result obtained on a control sample against its own RI and with those of other Participants.
In 2004 we included the study of some clinical cases within the EQAS for Clinical Biochemistry on serum in order to stimulate participants to provide not only the analytical results but also a clinical interpretation and suggestions for additional laboratory investigations.
One of the clinical cases studied concerned a female, 35 years old, affected by a cholestatic syndrome recognisable by means of the results of the hepatic functionality indexes: Total Bilirubin = 26.8 mmol/L, Direct Bilirubin = 15.0 mmol/L, AST = 64 U/L, ALT = 61 U/L, GGT = 282 U/L, ALP (AMP) = 400 U/L, LDH (Piruvate à Lactate) = 776 U/L and ALP isoenzymes = presence of a biliary fraction further to the normal bone-liver isoenzyme.
We studied the RI of the 450 Laboratories that participated to this clinical case: 170 directly afferent to CRB and 280 to the EQA group of S. Orsola-Malpighi of Bologna with which we co-operate. The study pointed out a large variability in RI of hepatic enzymes, for example among the 170 CRB Participants 16, 10, 31, 16 and 10 gave for AST, ALT, GGT, ALP and LDH, respectively, RI detaching significantly from the other using the same diagnostic system.
The medians of the rate result/Max RI value of each laboratory showed that some diagnostic systems presented a significant statistically difference from the others. For example, for AST, the medians of the rates of Abbott (1.56), Beckman (1.53) and IL (1.58) were lower than Roche,Hit/Mod (1.92) and Dade-Behring (1.94); for ALT, the medians of the rates of Dade-Behring (1.25), Abbott (1.31) and Ortho (1.35) were lower than Roche,Integra (1.71) and Olympus (1.64); for GGT, the medians of the rates of Ortho (4.66) and Beckman (5.35) were lower than Roche Integra (8.75), IL (8.30) and Olympus (7.93).
The reason of these discrepancies probably comes out from the fact that the laboratories often adopt the RI issued on the package insert of diagnostic system utilized which are often inadequate to the results provided by the diagnostic system itself. In conclusion, a lot of work remains to do to standardize the IR of enzymes and information obtained from EQAS are useful both for the Laboratories and for the manufacturers.

LETTURA PLENARIA 2
The Evolution of Clinical Biochemistry Laboratory Iin the new century
Sala B
Mercoledì 12 Ottobre 2005, ore 9,00 - 10.00

LP2
THE EVOLUTION OF CLINICAL BIOCHEMISTRY LABORATORY IN THE NEW CENTURY

Giorgio Federici
Department of Laboratory Medicine, Tor Vergata University Hospital, Rome (Italy)

Even if it is difficult to predict the evolution of the clinical laboratory in our country, because of the variability of political and financial factors, a cultural trend is universally emerging: to extensively scan biological fluids and tissues with the aim to individualise the diagnosis and the treatment of diseases.
This new requirement of the clinical laboratory starts to be a real objective thanks to the advancements made in a number of fields like digital information technology, telecommunications, miniaturisation and to the development of new sciences of the "postgenomic era" like proteomics.
A first challenge for the laboratory will be "to focus on the patient" the diagnostic itinerary. This approach first needs of a global understanding of the relationships between factors involved in the physiopathologic processes and then of the knowledge of the heterogeneity in the population for each intervening factor. For many years, molecular biologists performed the analysis of one or a few genes at time. In the postgenomic era, the elucidation of the human genome together with the development of microarray technology have provided the means to perform global analysis of thousands of genes in a single assay. The applications of this powerful technology improved the knowledges about the relationship between genes potentially involved in disease susceptibility and in the physiopathologic processes as well as about the complex regulatory networks which control their expression. However the superficial semplicity of the microarray approach arises considerable problems in terms of experimental design, standardization, data confirmation and statistical interpretation. Another high throughput tool to study gene expression is represented by the Real Time PCR, a quantitative method with many possible clinical implications.
In parallel with the progress in molecular biology, a rapidly emerging set of new technologies have been realized to identify large number of proteins in a mixture, to map their interactions and their posttranslational modifications in the cell and to define protein expression profiles in health and disease leading to a new science: the "proteomics". It is now possible to examine the expression of more than 1000 proteins using mass spectrometry coupled with various separation methods. At the same time the microarray technology has been applied to study protein function and ration methods. At the same time the microarray technology has been applied to study protein function andbiochemical activities with relevant clinical implications. Methods are also being developed to directly scan tissues.
A second challenge for laboratory will be to provide new methods of dissecting the heterogeneity in the response to many drugs linked to inherited differences in drug metabolising enzymes, drug transporters antargets. This field of inquiry, traditionally termed "pharmacogenomics" provides an opportunity to identify biological predictors of drug response and may provide the means to determine the molecular substrates involved in drug efficacy. In this field also, genomic and proteomic scan methods have been applied.
Working with these powerful technologies, new tools are needed: a more friendly bioinformatics and the availability of integrated platforms to elaborate data obtained from genomic and proteomic analysis together with knowledges in biochemistry, cell and structural biology with the aim to translate each cultural advancement in clinical setting.
Examples of applications of these new technologies in the analysis of genes and their products in the clinical laboratory will be shown and discussed.

SESSIONE 4
CITOFLUROMETRIA: DALLA RICERCA AL CONSOLIDAMENTO DELLA ROUTINE
Sala B
Giovedì 13 Ottobre 2005, ore 10.15 - 12.45

S4.1
STANDARDIZATION IN FLOW CYTOMETRIC DIAGNOSIS OF HAEMATOLOGICAL MALIGNANCIES: A TYPICAL CASE OF WORK IN PROGRESS.

Claudio Ortolani, Maria Colomba Sanzari.

Flow cytometry has become the preferred method for the lineage assignment and maturational analysis of malignant cells in acute leukaemias and lymphomas. However, despite of several consensus conferences held in the past years, there are no common protocols to be used in conjunction with specific classifications of reference standards in order to provide unambiguous results. This uncertainty depends on several factors, namely the continuous evolution in the knowledge of haematological malignancies and the ruthless technical advances of flow cytometry. Nevertheless, the complexity of multiparameter analysis techniques and the multitude of available monoclonal antibodies demand a standardization of protocols for the use of flow cytometry in clinical laboratories in order to achieve interlaboratory reproducibility. Consequently, there is an increasing need of widely shared guidelines, which should regulate the three main points of the diagnostic procedure. The first point to be standardized is the diagnostic indication of the analysis, followed by the control of pre-analytical procedures (anticoagulants, lysing solutions etc.), the choice of antibodies to be used and the type of staining to be performed. The second point consists in the validation of analytical procedures, which should be comprehensive of the assessment of instrument sensitivity, set up, calibration, compensation, and standard list mode data analysis. The third point is represented by data analysis and interpretation, followed by data reporting. Although several guidelines exist, we believe there is the need of defining a new minimum of methodological procedures to be used as core components for the diagnosis of the haematological malignancies.
These procedures should be mainly based:
i) on the choice of a set of clones and dyes able to produce the most reproducible results;
ii) on the simultaneous exploration of different antigens able to delve either the lineage-associated maturation pathways or the presence of disease-associated phenotypes;
iii) on the unambiguous mathematical interpretation of the results produced by the analysis of weakly positive samples;
iv) on a new way of reporting the results, aimed to the description of the general framework, and not merely to the enumeration of cell populations.

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SESSIONE 4
CITOFLUROMETRIA: DALLA RICERCA AL CONSOLIDAMENTO DELLA ROUTINE
Sala B
Giovedì 13 Ottobre 2005, ore 10.15 - 12.45

S4.2
EXTERNAL QUALITY ASSESSMENTS IN CLINICAL FLOW CYTOMETRY

Bruno Brando
Transfusion Center and Haematology Laboratory, Legnano Hospital, Milan, Italy.

Clinical cell analysis is now routinely implemented in many laboratories. The major applications now include: Lymphocyte immunophenotyping for immunodeficiencies, CD34+ stem cell monitoring and transplantation, Leukemia/Lymphoma diagnosis and characterization, Low level leucocyte counting in blood products, Feto-maternal haemorrage analysis and Paroxysmal nocturnal haemoglobinuria assessment.
The analysis of monodispersed cell suspensions in the clinical laboratory poses several technical problems both in establishing universally applicable cell standards, reference materials, standardized procedures, and in designing appropriate internal and external quality assessment schemes (EQAS). Only lymphocyte immunophenotyping is amenable of fully automated processing, whereas the other cited applications still require laboratory skill and are highly operator-dependent.
The EuroStandards consortium is a multinational cooperative study group whose aim was the development and validation of stabilized cellular preparations to be used as reference standards in clinically relevant flow cytometric diagnostic procedures. During the last 4 years EuroStandards partners have been validating stabilized cellular standards that are now available on a worldwide basis by the National Institute for Biological Standard and Controls (NIBSC), like any other laboratory analyte.
UK NEQAS-LI offers now the widest and most credited EQAS support to laboratories involved in clinical cell analysis and leucocyte immunophenotyping. The aim of UK NEQAS is primarily educational, so participants can be assisted in optimizing their lab procedures and in solving the frequent technical problems occurring with clinical cell analysis.
Hundreds of labs are now involved and compared worldwide, and results can be also stratified on a single Country basis. Local liaison scientists operate as a reference to support participants in trouble and to assist new applicants to familiarize with the schemes. Result scores usually show a 'learning curve' morphology, indicating that the application to EQAS is of fundamental importance to reach and maintain performance excellence. UK NEQAS does not restrict participation to specific laboratory technologies, but may suggest participants to implement analysis strategies that have proven valid and reliable, as for instance has occurred with single-platform CD34+ cell counting ISHAGE protocol.
Flow cytometry thrived in Italy during the mid-eighties in many different clinical and research settings, and in some instances the involved operators were not properly educated in laboratory medicine or clinical pathology. This has generated a quite dishomogeneous attitude towards quality requirements and proficiency testings, which may be sometimes felt as an annoying burden instead of a fundamental educational tool for the continuous improvement of laboratory performance. Stabilized samples used in EQAS are more difficult to analyze than fresh ones, whereas terminology, complex scoring systems, the usage of a foreign language and a certain pressure towards some technical protocols can be sometimes additional obstacles for the average lab operator.
Flow cytometry clinical applications are now more and more complying to standardized protocols, and application to EQAS is encouraged thanks to the efforts of the Italian Society for Cytometry (GIC) and other scientific or volunteer associations with local or nationwide influence. The need to apply to an independent, internationally credited EQAS provider is stressed when clinical flow cytometry labs are under way for certification or accreditation. Much is still to be done to promote and establish quality in such a disorderly and complex national scenario.

SESSIONE 4
CITOFLUROMETRIA: DALLA RICERCA AL CONSOLIDAMENTO DELLA ROUTINE
Sala B
Giovedì 13 Ottobre 2005, ore 10.15 - 12.45

S4.3
COMPARISON OF CYTOMETER ASSAYS AND ELISA FOR CYTOKINE DETERMINATION

Di Carlo S., Bacosi A., Samoggia P., Rossi S., Pacifici R.
Dimartimento del farmaco, Istituto Superiore di Sanità, Roma

The aim of the study was the investigation of correlation between different ELISA and cytometric assays for cytokine (TNF alfa, IL-2, IL-4, IL-6, IL-5, IL-8, IL-10, IL-1 beta, TNF-beta, IFN gamma, TNF gamma) determination in 20 cell culture supernatant samples of stimulated lymphocytes.
In particolar, IL-2, TNF- alfa and IFN-gamma were analyzed by ELISA kit BioSource International Inc, and by Symplex and Multiplex Fluorescent Bead Immunoassay Bender Med Systems and Human Th1/Th2 Cytokine Bead Array (CBA) by Flow Cytometer. Other cytokines were analysed by the only latter methods employing Flow Cytometer.
The Elisa method is a solid phase sandwich Enzyme linked-immuno-Sorbent Assay with monoclonal antibody specific for cytokines and coated onto the wells of the microtiter strips. Cytometric (fluorescent beads immunoassay), similar in case of Multiplex Fluorescent Bead Immunoassay and Th1/Th2 Cytokine Bead Array (CBA) is based on microsphere classified by their size and by their spetral address, coated by cytokine specific antibodies. The coated beads are incubated with the standard and the samples and the cytokines present in the samples bind to the antibodies adsorbed to the fluorescent beads. A biotin-conjugated second antibody is added, the specific antibody binds to cytokines captured by the first antibody. Streptavidin-Phycoerythrin is added, binds to the biotin conjugate and emits fluorescent signals.
Correlations concerned: sensitivity, reproducibility and accuracy and were evaluated by ANOVA analysis of variance.
The results indicate that major correlation was obtained for IL-2 (r=0.999) and IFN gamma (r=0.996) with slope value near to unity, while TNF-alfa was over-evaluated by ELISA (slope major than 1) with respect to cytometric assays. In case of IL-10 a good correlation (r=0.998) was found between Symplex and Multiplex Fluorescent Bead Immunoassay Bender Med Systems, while an underestimation of IL-10 concentration was obtained with Human Th1/Th2 CBA. A correlation was obtained also in case of other cytokines, even if not always satisfactory, correlation.
Chen R, Lowe L, Wilson JD, Crowther E, Tzeggai K, Bishop JE, Varro R. Simultaneous Quantification of Six Human Cytokines in a Single Sample Using Microparticle-based Flow Cytometric Technology. : Clin Chem. 1999;45:1693-1694.

S4.4
CIRCULATING MONOCYTE-PLATELET AGGREGATES ALLOW EARLIER STRATIFICATION OF PATIENTS PRESENTING WITH SUSPECTED ACUTE MYOCARDIAL INFARCTION.

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Despite substantial advances in the diagnostic approach to patients presenting with suspected acute myocardial infarction (AMI), the stratification of patients is as yet challenging, as a substantial proportion of patients, up to 40-60%, may present with nondiagnostic concentrations of traditional markers at admission. Preliminary reports suggest that measurement of circulating monocyte-platelet aggregates (MPA) might improve the diagnostic approach to patients with suspected AMI. Therefore, MPA were serially measured, within two hrs from presentation, in 30 consecutive patients, admitted to the emergency department with acute chest pain, suggestive for AMI. A definitive diagnosis of AMI, based upon the established criteria issued by the World Health Organization (WHO), could be established in 9 out of 30 patients studied. Results of MPA measurement were further compared with those of 20 healthy controls, matched for age and sex, without any suggestive WHO criterion of AMI. Peripheral blood was collected by venipuncture without venous stasis by a 20 G needle, into siliconised vacuum tubes containing 15% K2-EDTA (Beckton Dickinson), after discarding the first 3 mL of blood. Samples for flow cytometry assay of circulating MPA were immediately fixed for 60 minutes with ThromboFix Platelet Stabilizer (Instrumentation Laboratory). Circulating MPA were quantified using dual-label flow cytometry employing anti-CD14-PC5 (lipopolysaccharide receptor) and anti-CD61-FITC (gpIIIa). Isotype-mathed mouse IgG FITC served as negative controls. Red cells were lysed in the dark for 10 min, by adding 1.5 ml of solution (gKHCO3, NH4Cl, H2O). All antibodies were purchased from Beckman Coulter. Flow cytometry was performed on a Coulter Elite (FACScan, Becton Dickinson) and data's elaborationon a software Expo 32 (Beckman Coulter).

SESSIONE 4
CITOFLUROMETRIA: DALLA RICERCA AL CONSOLIDAMENTO DELLA ROUTINE
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Giovedì 13 Ottobre 2005, ore 10.15 - 12.45

S4.4

A minimum of 2000 CD14+ events was acquired. Main results are synthesized in table 1; values are expressed as median and 0.025-0.975 percentile distribution. The median percentage of MPA was significantly higher in AMI patients than controls (p<0.001). ROC curve analysis showed excellent diagnostic performances; the area under the curve was 1.00, with 100 % sensitivity and specificity at diagnostic threshold of 31.6%. In conclusion, we confirm that MPA are a reliable an early marker of AMI, which may be useful for the stratification of patients admitted with chest pain.

1) Furman MI, Barnard MR, Krueger LA, et al. Circulating monocyte-platelet aggregates are an early marker of acute myocardial infarction. J Am Coll Cardiol 2001;38:1002-6.

	Controls	AMI	*P*
N.	20	9	
MPA (%)	26.9 (17.5-30.6)	45.9 (32.7-68.3)	<0.001

SESSIONE 5
LA FASE PRE-ANALITICA

Sala C

Giovedì 13 Ottobre 2005, ore 10.15 - 12.45

S5.1

INFORMATIC SYSTEM IN THE DEPARTMENT OF MEDICAL LABORATORY

Ilio giambini - dept of med.lab
University Hospital "Policlinico Tor Vergata"-Rome

Clinical Biochemistry is a biochemical science which studies the nature, structure and functional alteration of specimens of patients with chemical, physical and biological methods.
All these data, and clinical symptoms, are elaborated in order to prevent , diagnose, check or rehabilitate patients from any kind of illness. (A.Burlina)
Starting from the so above statement and considering that this branch of Medicine has developed enormously since last few years, the huge amount of data in a lab need to be managed through several controls and different processes (pre-analytical, analytical and post-analytical processes; supply of sources and their stockage, analysis of data, management of documents in different Quality System) and it seems to be evident that it's necessary to work out a complex, flexible and adaptable informatic system for each work-reality: reducing "Turn around Time"; checking "Point of Care Testing"; connecting any department terminal with the informatic system of lab.
ISO 15189 Standard Rule (Management of Quality in a Clinical Chemistry Lab) (see attached paper) gives some advices regarding how to use accurately an informatic system as well as how to behave ethically in a correct way.
8.1 Computerized System Laboratory
The mentioned paper advises how to organize correctly any system of collection, transmission, elaboration, printing and stocking of data and patient information.
Hereby we summarized all points treated:
1.environmental condition: how to install and care the hardware;
2.procedure handbook: management of documents both in hardware and in software way;
3.introduction of data and medical report;
4.recording files;
5.hardware and software: procedure how to use PC;
6.care system.
8.2 Ethics in Laboratory
The attached paper regards ethical rules to be adopted in a lab by professional figures. Such ethical code is highly recommended to be followed in compliance with national laws or instructions.
Hereby we summarized all points treated:
1.collection of information: patient identification;
2.reporting data;
3.recording and stocking of medical documents: saving against loss or legal responsibilities;
4.access to medical documents: controls of access and claims;

8.2 Ethics in Laboratory
The attached paper regards ethical rules to be adopted in a lab by professional figures. Such ethical code is highly recommended to be followed in compliance with national laws or instructions.
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1.collection of information: patient identification;
2.reporting data;
3.recording and stocking of medical documents: saving against loss or legal responsibilities;
4.access to medical documents: controls of access and claims;

Conclusion. A suitable informatic system of laboratory, connected with each in-patient department or day hospital, allows the structure to achieve fruitful benefits.
Indeed any test result or medical report can be easily displayed and printed in the department concerned, cutting any "turn around time" moreover, any kind of pathology could be outlined, any statistical result is likely to be given in time, and even a policy of full employment of human resources could be achieved and the correlation cost/benefit could develop relying on sources consumption and estimate budget.
At the very end any informatic system is a constant helpful aid to check and improve any sort of activity in hospital.

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D.Laterza, G.Trucco, O.Martinis e D.Lo Iacono

SESSIONE 5
LA FASE PRE-ANALITICA
Sala D
Giovedì 13 Ottobre 2005, ore 10.15 - 12.45

S5.2
INFLUENCE OF PHYSICAL EXERCISE ON THE PREANALYTICAL VARIABILITY OF PROBNP AND IMA.

Salvagno G.L.1, Lippi G.1, Montagnana M.1, Schena F.2, Ballestrieri F.2, Guidi G.C.1.
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The measurement of cardiac troponins is the gold standard for diagnosis and management of patients with acute chest pain. However, earlier markers and reliable prognostic indicators should support investigation strategies. The ischemia modified albumin (IMA) was recently proposed for early detection of myocardial ischemia, whereas the pro-Beta Natriuretic Peptide (proBNP) was reported as a prognostic marker of cardiac dysfunction. However, little is known on the influence of physical exercise on the preanalytical variability of proBNP and IMA. To establish the influence of a vigorous endurance training on the diagnostic approach of patients with suspected myocardial injury, cardiac Troponin T (cTnT), proBNP and IMA, along with lactate dehydrogenase (LDH), creatine kinase (CK), creatinine and albumin, were evaluated in 31 male professional or elite endurance athletes and compared with 24 sedentary healthy individuals, matched for age and sex. After collection, heparinized plasma was separated, aliquoted and stored at -70° C until measurement. cTnT and proBNP were assayed on Elecsys 2010 (Roche Diagnostics GmbH, Mannheim, Germany), whereas IMA, determined by a colorimetric assay (ACB, Ischemia Technologies, Denver, USA), LDH, CK, creatinine and albumin were measured on the Modular System P (Roche Diagnostics GmbH). Main results are synthesized in table 1; values are expressed as mean±standard deviation (median and 2.5%-97.5% distribution for IMA and proBNP). As expected, athletes displayed increased values of CK and LDH, but not of creatinine and albumin. The concentration of cTnT was always lower than the detection limit of the assay. IMA was significantly increased in athletes. Although athletes displayed a consistent trend towards lower proBNP values, the difference did not achieve statistical significance. In stepwise logistic regression, only LDH (<0.001) and IMA (p=0.007) were significantly associated with the vigorous aerobic training, The percentage of subjects displaying values exceeding the IMA diagnostic threshold (>100 Kunits/l) significantly differed between athletes and controls (52% vs 8%; p<0.001). No athlete or sedentary control exhibited values exceeding the proBNP diagnostic threshold (>14.8 pmol/l). These results indicate that IMA, but not cTnT and proBNP, may be influenced by a vigorous aerobic training.

	Sedentarians	Athletes	*P*
N.	24	31	
Creatinine (μmol/l)	86±11	88±10	0.523
Albumin (mg/l)	47.4±2.2	46.3±2.3	0.081
LDH (IU/l)	255±37	319±53	<0.001
CK (IU/l)	117±77	222±137	0.001
cTnT (ng/ml)	<0.01	<0.01	1.000
IMA (Kunits/l)	94 (83-106)	101 (87-112)	0.003
Pro-BNP (pmol/l)	2.9 (1.2-12.9)	2.4 (1.3-6.6)	0.056

S5.3
INFLUENCE OF THE PREANALYTICAL VARIABILITY ON COAGULATION TESTING.

Lippi G., Salvagno G.L., Montagnana M., Guidi G.L.
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There is consolidated evidence that nonadherence to rigorous and standardized preanalytical procedures is a major source of variation in laboratory testing (1). To verify to what extent preanalytical variables influence coagulation testing, we investigated the effects of the blood drawing technique (conventional straight needle versus a butterfly device), the final anticoagulant concentration (0.129 vs. 0.105 mol/l buffered sodium citrate), the composition of the tube (plastic vs. glass), haemolysis (from 0% to 12% lysis) and short-term venous stasis (no stasis vs. 3 min 60 mm Hg sphygmomanometer placing) on prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen and d-dimer. Coagulation assays were performed on a Behring Coagulation System (BCS, Dade Behring), employing proprietary reagents, whereas d-dimer was measured by the reference immunoassay on Vidas (bioMerieux). Blood, collected on 30 consecutive patients who gave an informed consent, was collected in the morning and immediately centrifuged. Plasma was separated and stored at -80° C until measurement. Results were analyzed by paired Student's t-test, Bland & Altman plots and compared to the current analytical specification for desirable bias. Specimen collection by a butterfly device produced no statistically, nor clinically significant variations, as compared to a conventional straight needle of the same Gauge size. Conversely, clinically meaningful variations were observed for each of the other preanalytical variables. In specimens collected in plastic tubes containing 0.129 mol/l buffered sodium citrate, PT and APTT values were significantly overestimated and fibrinogen lowered (all p<0.01), as compared to specimens collected in siliconized tubes containing 0.105 mol/l buffered sodium citrate, exceeding the current analytical specification for desirable bias for APTT and fibrinogen. Analogous results were observed for short-term standardized venous stasis, as induced by a conventional sphygmomanometer, which reliably mimics the tourniquet placing during blood collection. PT and APTT values appeared shortened (p<0.05 and p<0.01, respectively), whereas d-dimer and fibrinogen values were increased (p<0.01). Clinically significant variations were achieved for PT and fibrinogen. Haemolysis and blood cell lysis up to 1.2% significantly lowered APTT and fibrinogen, whereas PT and d-dimer were increased (all p<0.05), always exceeding the allowable desirable bias. Taken together, our results attest the a poor preanalytical standardization influences coagulation testing, generating misleading results, inaccurate diagnostic decisions and inappropriate therapeutic management of patients with haemostasis disturbances.

Reference.

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SESSIONE 6
RECENTI AVANZAMENTI NELLA CONOSCENZA E NEL MONITORAGGIO DELLA MALATTIA DIABETICA

Sala F

Mercoledì 12 Ottobre 2005, ore 10.15 - 12.45

S6.1
ON THE UNCOVERING OF THE PATHOGENESIS OF TYPE 1 DIABETES: ARE WE STUCK OR ARE WE WINNING?

Gian Franco Bottazzo
Roma

Type 1 diabetes is the results of the destruction of the insulin-producing cells (beta cells) in the pancreatic islets. The attack against the beta cells is autoimmune in origin and it occurs in genetically susceptible individuals (HLA DR3 and DR4, or both). The involvement of an environmental factor, responsible for initiating or precipitating the disease process, has often been postulated, but, for the moment, it remains elusive.
Despite type 1 diabetes is a disease with an acute onset, we know now that the onset is preceded by a long period of latency, where individuals, found to have detectable islet-related autoantibodies, do not go on to develop the disease quickly: sometimes it takes years before this happens and sometimes it never occurs. However, islet-related autoantibodies do not cause direct damage to the islet beta cells, they are simply serological markers of the ongoing disease process, what are instead pathogenetic are the cytotoxic T lymphocytes. When these cells are activated, the disease precipitates.
Until now, laboratories had problems in measuring autoreactive T cells in type 1 diabetes, but our laboratory has now solved the problem: evidence of that will be presented. But, how are autoreactive T cells generated? The thymus is the site in which all autoreactive T cells are eliminated, and this phenomenum occurs in the first three days of life. Autoreactive T cells recognise autoantigens expressed in the target cells, and, strangely enough, autoantigens are, most of the time, enzymes. The thymus expresses these autoantigens/enzymes and, as soon as they are expressed, the T cells, which recognise them, interact with them and, as a result of the contact, they are killed. Many amino acid are substrates for several enzymes (see, for example, glutamate and glutamic acid decarboxylase (GAD), the enzyme target autoantigen in type 1 diabetes). Well, what we have found is that in newborns, who subsequently developed type 1 diabetes, the content of amino acids in the blood spot, taken in the first 3 days of life, is low. Is it possible that this low level of amino acids at birth might represent a sort of lack of substrate for the enzyme/autoantigens, so these are not expressed. If the enzymes/autoantigens are not expressed, it is possible that the T cell, potentially autoreactive against them, are not eliminated, so finding easy to leave the thymus and harbour the peripheral lymphoid organs, e.g. lymph nodes. Following this new evidence, we propose a new hypothesis on the pathogenesis of type 1 diabetes, the so-called 'drifting mine hypothesis'. Evidence will also be produced on how to stop the 'drifting mines' to detonate. So, on "the unravelling of the pathogenesis of type 1 diabetes, are we stuck or are we winning?": the answer to the audience!

S6.2
THE IFCC GLOBAL CAMPAIGN ON DIABETES MELLITUS

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In 2003 the International Federation of Clinical Chmistry (IFCC) launched a global campaign on diabetes in order to improve laboratory diagnosis and management of this disease. The campaign was guided by two overall aims: a) to assist laboratories throughout the world in order to develop a patient-centered, evidence-based and collaborative approach to the diagnosis and management of diabetes by transferring what is "Best Practice" to all countries and maintaining that standard of practice; b) to closely associate IFCC with the diagnosis and management of diabetes for all people who have contact with this disease, including professional colleagues, patients and the general public.
In this presentation I will focus on some of the issues which have been debated more extensivly in the last year, essentially concerning the determination of blood glucose, the standardization of HbA1c, and microalbumin. With regards to glucose, the IFCC was active in promoting a questionnaire on self-monitoring blood glucose and its impact on patient outcome. Moreover, an approved IFCC recommendation on reporting results for blood glucose has been published. This document states that all glucose results have to be converted to plasma concentrations irrespective of sample type analyzed and method of measurement. Another questionnaire was circulated on the diagnosis of diabetes, HbA1c units, diabetes monitoring and analytical quality specification for different laboratory parameters.
With regards to HbA1c, the main news concern clinical studies (INTER-HEART, PURE, DREAM) on the value of HbA1c as a diagnostic tool and predictor of cardiovascular disease also in the non-diabetic subjects. The topic of HbA1c standardization is still under debate, and new units (mmol/mol) have been proposed for the identification of the IFCC aligned test measurements.
Finally, with regards to microalbumin, the IFCC has recently approved the establishment of a new working group, who will face the problem of the standardization of this test, also in the light of the possibility of measuring the non-immunoreactive form in urine which seems to be a better predictor for increased risk of renal, cardiovascular disease and death.
References. www.ifcc.org, www.ifcchba1c.net

SESSIONE 6
RECENTI AVANZAMENTI NELLA CONOSCENZA E NEL MONITORAGGIO DELLA MALATTIA DIABETICA

Sala F

Mercoledì 12 Ottobre 2005, ore 10.15 - 12.45

S6.3

PPARγ2 PRO12ALA POLYMORPHISM, INSULIN RESISTENCE, FATTY ACID METABOLISM IN OBESE CHILDREN.

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The relationship between Pro12Ala variant of peroxisome-proliferator-activated recptor -gamma2 (PPARγ2), insulin sensitivity and plasma fatty acid is unclear. To examine whether an association of PPARγ2 Pro12Ala with insulin sensitivity and plasma fatty acids may exist in obese children, 112 obese children (mean age 10.4 +/- 2.8 years) were included into this observational study. Obesity was defined in accordance with the International Obesity Task Force. Fasting blood samples were taken for measurements of glucose, insulin, total and HDL cholesterol, triglycerides levels, and fatty acid (FA) composition. Insuline resistance (IR) was estimated by the homeostatic model assesment (HOMA-IR). Genomic DNA was obtained from peripheral blood using standard methods, and Pro12Ala PPARγ2 variant was detected by DNA sequencing. Prevalence of Ala carriers was 19%. Multiple logistic-regression analysis disclosed that Pro12Ala genotype was independently associated with lower values of 1) fasting insulin levels ($p=0.032$), 2) HOMA-IR (HOMA-IR has been calculated as the product of fasting glucose an fastin insulin devided by 22.5) ($p=0.05$), 3) plasma levels of c20:3n-9 ($p=0.004$) and 4) n-6/n-3 PUFA ($p=0.063$) compared with Pro/Pro. Mean (SD) values of fasting insulin levels, HOMA, c20:3n-9 and n-6/n-3 PUFA in Pro/Pro and Pro12Ala groups were, respectively : 21.7(13.1) vs 13.1 (6.6) mU/ml; 4.7 (3.9) vs 3.0 (2.0); 0.17 (0.15) vs 0.09 (0.02) mg/dl and 14.2 (3.3) vs 12.7 (2.2). In this study Pro12Ala polimorphism was related to higher insulin sensitivity and to an healthy plasma fatty acids pattern. We speculate that obese children carriers of the Ala 12 allele might be protected from cardiovascular disease and type 2 diabetes by the phenotypic effect on insulin resistence and on plasma fatty acids metabolism.

S6.4

CORRELATION BETWEEN FOKI AND BSMI VITAMIN D-RECEPTOR POLYMORPHISM AND TYPE 1 DIABETES IN A ITALIAN COHORT.

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Type 1 diabetes results from an immune mediated destruction of islet beta-cells. 1-25-Dihydroxivitamin D3 seems to play pleiotropic roles as: a) immuno regulator[1-25(OH)2D3], inhibiting lymphocyte activation and affecting cytokine and immunoglobulin production; b) in NOD mice 1-25(OH)2D3 prevent the development of diabetes; c) some other non-calcemic activities. Vitamin D acts through the nuclear Vitamin D receptor (VDR), which presents different polymorphisms. Our study investigated the possible association of FokI and BsmI VDR genetic polymorphisms with type 1 diabetes (T1DM) in a homogeneous Italian cohort. 246 continental T1DM patients (not Sardinian) and 151 healthy subjects matched for age and sex collected as controlled blood donors have been assayed for the above mentioned VDR polymorphisms. Genomic DNA was utilized to amplify two fragments of VDR gene including the BsmI restriction site in intron 8 and the FokI restriction site at 5' region of the gene, respectively. PCR products were digested with specific enzymes (BsmI and FokI) and run on a 2% ethidium bromide-agarose gel. Each subject was classified as wild type (FF or BB), heterozygous (Ff or Bb) or homozygous (ff or bb). Significant differences ($p< 0.0001$) in FokI genotype polymorphism between T1DM patients and healthy donors was found. No significant difference was found for BsmI genotype polymorphism. A further analysis included the stratification of the patients for the combined genotypes of F and B mutations. Only a slight significant difference was found between the two groups. An interesting observation concerns the double homozygous patients that were always diabetic. The comparison of this subgroup versus all the others combined was statistically significant ($p<0.002$). Multiple logistic regression analysis confirmed these findings, highlighting the association between ff VDR polymorphism with T1DM. In this study we found a strict association between ff VDR genotype and T1DM. These results, in agreement with the in vitro studies which show a decreased transcription rate of VDR in ff genotype, may support the hypothesis of an influence on the immunoregulator activity of vitamin D in the pathogenesis of T1DM.

SESSIONE 7
APPLICAZIONE CLINICA DELLA SPETTROMETRIA DI MASSA E PROTEOMICA

Sala A

Venerdì 14 Ottobre 2005, ore 10.15 - 12.45

S7.1

EQUALIZER BEADS: THE QUEST FOR A "DEMOCRATIC PROTEOME"

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No proteome can be considered "democratic", but rather "oligarchic", since a few proteins dominate the landscape and often obliterate the signal of the rare ones. That is the reason why most scientist lament that, in proteome analysis, we keep seeing again and again the same set of proteins. A host of pre-fractionation techniques have been described (as reviewed in 1), but all of them, one way or another, are besieged by problems, in that they are based on a "depletion principle", i.e. getting rid of the unwanted species. Yet "democracy" calls not for killing the enemy, but for giving "equal rights" to all people. One way to achieve that would be to use "Protein Equalizer Beads". They consists in a library of combinatorial ligands coupled to 65 µm beads. Such a library comprises hexameric ligands composed of 20 natural amino acids, resulting in up to 64 million different structures. When these beads are impregnated with complex proteomes (e.g., human sera, egg white, any cell lysate, for that matter) of widely differing protein composition, they are able to "equalize" the protein population, by sharply cutting the peaks of the most abundant ones while simultaneously enhancing the concentration of the most dilute components. It is felt that this novel method could offer a strong step forward in "mining below the tip of the iceberg" for detecting the "unseen proteome" and, in the long run, might represent a big revolution in proteome analysis. Examples will be given of equalization of human urine and sera samples, resulting in the discovery of a host of proteins never reported before.

(1) Righetti, P.G. et al., Electrophoresis 26 (2005) 297-319.

S7.2

THE ROLE OF PROTEOMICS TO DEFINE MULTIVARIATE PROTEIN MARKERS IN CLINICAL BIOCHEMISTRY.

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One of the major task of the current biomedical research is the possibility to found combinations of markers that identify and stage a wide range of diseases with useful sensitivity and specificity. A general statistical argument is that a panel of independent multivariate protein markers should be less prone to the influence of the genetic and environmental "noise" than is the level of a single protein marker (1). So the search for multivariate protein markers is a real challenge for the next future in clinical biochemistry. Proteomics, defined from a biochemist's viewpoint as the study of more than one protein at a time (1), is now widely accepted as the complementary technology for genetic profiling. Proteomics consists of a combination of different technologies for high-throughput protein separation (2D electrophoresis, multidimensional chromatography, protein arrays, etc), protein characterization and identification (mass spectrometry) and bioinformatics. It is considered a powerful approach for the study of diseases and to identify and characterize multivariate protein markers that can characterize specific pathological states or drug effects much better than the analysis of individual or small numbers of proteins.

Different results obtained using proteomic approaches to define clusters of multivariate protein markers to characterize specific pathological states will be presented, such as the characterization of acute-phase protein markers in bacterial and viral infections (2), the definition of diagnostic protein markers in cancer (3) and the characterization of multivariate protein markers in lung diseases (4, 5, 6).

1-Anderson L. (2005), J. Physiol. 563.1, 23-60.
2-Bini L. et al (1996), Electrophoresis 17, 612-616.
3-Bini L. et al (1997), Electrophoresis 18, 2832-2841.
4-Magi et al. (2002), Electrophoresis 23, 3434-3444.
5-Rottoli P. et al. (2005), Proteomics 5, 1423-1430.
6-Rottoli P. et al. (2005), Proteomics 5, 2612-2618.

SESSIONE 7
APPLICAZIONE CLINICA DELLA SPETTROMETRIA DI MASSA E PROTEOMICA

Sala A

Venerdì 14 Ottobre 2005, ore 10.15 - 12.45

S7.3

BIOMARKER INVESTIGATIONS IN NEURODEGENERATIVE DISEASES

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Our Proteomics Group is mainly focused in the investigation of differential protein expression profiles in neurodegenerative diseases. In particular, we are currently focusing our efforts on Alzheimer Disease and Multiple Sclerosis.

We are actively establishing a set of novel mass spectrometry investigations in order develop potential assays for molecular biomarkers identification and characterisation .

2DE approach has been complemented by MALDI-TOF mass spectrometry protein profiles either in the screening of complete protein extracts and in direct analysis of tissue samples. Novel bioinformatics tools have been developed to specifically analysed MALDI-TOF-MS molecular profiles and possibly outline spectra differences. Signals annotation is further pursued both by nanoLC-MS/MS experiments and by LC-MALDI-TOF-MS.

We have investigated the cerebral spinal fluid (CSF) of Alzheimer Disease affected patients versus subjects without cognitive impairment. Our results indicate a differential distribution of TTR-cysteine and TTR-cysteinglycine adducts. In fact, both oxidised forms of TTR are significantly less abundant in the Alzheimer Disease group ($P=0.0001$). The investigated population has been diagnosed using the relative ratio of conjugated forms over the free TTR. A sensitivity > 90% and a specificity >70% was outlined in ROC curve analysis when the overall cohort is discriminated by the TTR-Cysteine signals.

A biomarker discovery investigation has been conducted both on the CSF and in sera of Multiple Sclerosis (MLSC) affected patients following the same experimental set-up. The outlined MS signals enable high sensitivity and specificity in the diagnosis (MLSC).

S7.4

PROTEOMIC ANALYSIS OF b2-MICROGLOBULIN AMYLOID FIBRILS

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Introduction Dialysis related amyloidosis (DRA) is a pathology associated with the persistent increase of monomeric b2-microglobulin (b2-m) caused by the dialytic replacement of renal failure. The high concentration that b2-m reaches in the plasma of DRA patients is necessary but not sufficient. It was speculated that some chemical modifications could be generated in b2-m during dialysis and facilitate protein aggregation and amyloid formation. A better understanding of the structural properties of the fibrillar b2-m might improve our knowledge on the molecular mechanisms of fibrillogenesis.

Methods Amyloid fibrils were extracted from amyloid deposits isolated from the femoral head. Proteins were extracted from samples of spleen and heart obtained postmortem from a patient affected by DRA and treated with regular hemodialysis for 20 years. A classical proteomic technology including 2D gel electrophoresis (2DE) and mass spectrometry (MALDI-TOF mass spectrometry) was used to recognise protein isoforms involved in the amyloid formation.

Results The proteomic of natural fibrils highlights the heterogeneity of b2-m and confirms the presence of the truncated species lacking of 6 N-terminal residues (1). In fibrillar b2-m Asn17 and Asn42 are partially deamidated and Met99 is oxidized. Other chemical modifications can not be excluded, but should involve less than 1-2% of the intact molecule. The truncation at the N-terminus is an event specifically observed in b2-m derived from fibrils but not from the insoluble non-fibrillar material deposited in heart and spleen of DRA patients. In these compartments b2-m can accumulate after long term haemodialysis, but rarely form amyloid deposits.

Conclusions The proteomic approach we have used allows us to confirm the presence of the truncated species of b2-m in the amyloid fibrils and to exclude their presence in other organs not belonging to the muscle-skeletal system. These data highlight the problem, still unsolved also for many other amyloid proteins, regarding the timing of proteolytic cleavage, before or after the fibrils formation.

(1) Stoppini M., Arcidiaco P., Mangione P., Giorgetti S., Brancaccio D., Bellotti V., Kidney Int. 57 (2000) 349-350.

SESSIONE 7
APPLICAZIONE CLINICA DELLA SPETTROMETRIA DI MASSA E PROTEOMICA
Sala A

Venerdì 14 Ottobre 2005, ore 10.15 - 12.45

S7.5
2-D ELECTROPHORESIS OF CEREBROSPINAL FLUID PROTEOME IN NORMAL PRESSURE HYDROCEPHALUS PATIENTS

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Normal Pressure Hydrocephalus (NPH), a neurological condition which occurs in adults 55-years and older, is an accumulation of cerebrospinal fluid (CSF) causing a triad of symptoms such as abnormal gait, urinary incontinence, and dementia. The treatment of choice for NPH patients is the placement of a CSF shunt, an implantable device designed to drain CSF fluid away from the brain thereby allowing the enlarged ventricles to return to a normal state. The aim of our study was to identify differences of CSF protein patterns by 2-D electrophoresis between patients in which the surgical insertion of a CSF shunt proved to be effective and patients in which the surgical intervention did not improve the troubling symptoms of NPH.
CSF samples from 12 subjects, of which 6 improved significantly after surgical treatment and 6 did not, were collected during surgery. Approximately 200 ug of total proteins were extracted according to Castagna et al (1) and loaded on the 11cm IPG strips. The strips were focused on the Protean IEF Cell (BioRad) for about 50000 Vh and the second dimension, performed on Criterion XT Precast Gels (Biorad) at 200 V for 45 min. Proteins were then visualized by Sypro Ruby staining and the analysis performed with PDQuestTM (version 6.2.0) 2-D gel analysis software (BioRad).
Using computer aided image analysis we found 131 matched spots; quantitation of the spots was performed and among these we identified four groups of protein isoforms and several single spot proteins in which the spot intnsity was significantly different between the two groups of patients. A tentative identification of the protein spots was performed by comparison with the Swiss 2-D page mastergel and resulted in Apo E, Apo J, haptoglobin and a-1B glycoprotein while the single proteins were not identified. By Mann Whitney test, Apo E resulted significantly overexpressed in not improved patients ($p=0.007$), Apo J on the contrary was overexpressed in improved subjects ($p=0.04$), haptoglobin and a-1B glycoprotein were not significantly different between the two groups ($p>0.05$).
These preliminary data seem to indicate a different protein pattern between patients who did benefit of a CSF shunt insertion and patients who did not. This could potentially allow the identification of patients who will benefit of surgical intervention. Further studies are in progress to confirm these data in a larger set of subjects and to identify differentially expressed proteins by MALDI-TOF analysis.

1.Castagna A. et al. Electrophoresis 2002, 23, 339-346.

SESSIONE 8
IMMUNOLOGIA E AUTOIMMUNITA'

Sala C

Venerdì 14 Ottobre 2005, ore 10.15 - 12.45

S8.1
INNATE AND ACQUIRED IMMUNITY

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The key function of the immune system is as a defender against pathogens. The few microbes that manage to cross the barriers of skin, mucus, cilia, and pH are usually eliminated by innate immune mechanisms. If phagocytosis cannot rapidly eliminate pathogen, inflammation is induced with the synthesis of cytokines and acute phase proteins. This early induced response is not antigen-specific and does not generate immune memory. Extracellular pathogens and their exotoxins are susceptible to phagocyte destruction and to antibody neutralization and opsonization. Intracellular pathogens must be eliminated by NK or by cytotoxic T cell lysis of the infected cell or macrophage activation by Th1 cells. The immune response can also damage the body by means of inflammation and cytotoxicity. Except in cases of wounds, injections, or insect bites, the initial barrier to infection is the skin and internal mucosal epithelial surfaces. Besides the physical barrier of the tightly opposed epithelial cells, mechanical barriers of beating cilia, air movement, mucus, and peristalsis remove the pathogen unless it can adhere to the epithelium. Chemical barriers to microbial colonization include low pH, hydrolytic enzymes, and antibacterial defensins. Normal flora also cover surfaces and compete with pathogens for physical space and nutrients. Once a pathogen penetrates the skin or mucosal epithelium, it usually establishes a local infection. Tissue damage and pathogen antigens signal tissue macrophages to secrete chemotactic cytokines called chemokines to attract additional phagocytes and allow more fluid and cells to enter the tissues at the infection site. Neutrophils and macrophages engulf pathogens and destroy them. On of the mechanism by which the immune system senses the invasion of pathogenic microorganisms is through the toll-like receptors (TLRs), which are evolutionary conserved to recognize pathogen-associated molecular patterns (PAMPs), that are present in microbial components (viruses, bacteria, fungi and parasites), but not in mammalian cells. TLRs show considerable target specificity and today many progresses have been done on TLR signalling pathways and their regulators (MyD88s, IRAKM, SOCS1, NOD2, PI3K, TOLLIP and A20). TLRs is one of the receptors able to recognise pathogens belonging to pattern recognition receptors (PRRs). The C reactive protein (CRP) is the first innate immunity receptor identified and together with serum amilyloid P component are classic short pentraxin (PTX) produced in the liver. Among long petraxin there is PTX3, that is produced in response to TLR engagement and inflammatory cytokines and acts as a functional ancestor of antibodies, recognizing microbes, activating complement, and facilitating pathogen recognition by phagocytes.

The alternative complement cascade is activated on pathogen surfaces to promote phagocytosis and pathogen lysis. Anaphylatoxins C3a and C5a attract more leukocytes and increase capillary leakiness at the infection site, allowing phagocytes and complement to enter the tissues. The liver responds to macrophage cytokines by secreting acute phase proteins that further promote complement activation and phagocytosis. If the innate immune response does not rapidly eliminate pathogens, adaptive immune responses are stimulated. The acquired immune system responds to peptide sequences called antigens. Antigens are sections of broken up proteins from cells that have been ingested by several different types of leukocytes. These leukocytes, known as antigen presenting cells (APC: macrophages and dendritic cells), then present these antigens to both B- and T-cells. Antigens are the way that the acquired immune system recognises invading bacteria, viruses and other harmful organisms (pathogens). Both B- and T-cells have surface receptors that recognise specific antigens. All the receptors on each individual lymphocyte are monoclonal, which means that they are all the same and so each lymphocyte is programmed only to recognise a specific antigen. To initiate adaptive immunity, soluble antigen and APC containing antigens are carried in the lymph to nearby lymphoid organs. There, antigen binds B cells and APC present antigen peptides on their membrane MHC to activate antigen-specific T cells. T helper (Th2) cells activate antigen-binding B cells to produce antibody and become effector plasma cells. These then shed their receptors in a soluble form known as antibodies and release them into the blood and lymph fluid. However, some of the activated B-cells do not become plasma cells, but become what are known as memory B-cells which continue to produce small amounts of the antibody long after the infection has been overcome. When an antibody binds to an antigen on a cell, it acts as a signal for neutrophils, eosinophils, basophils and macrophages to engulf and kill (phagocytose) it in a process known as opsinisation. T-cells are manufactured in the thymus where they are tested for their response to self-tissue. Any that respond to self-tissue are usually rejected. However, it is clear that in T-cell mediated autoimmune diseases (e.g. diabetes, multiple sclerosis) some T-cells with self-receptors somehow get manufactured. There are three main types of T-Cell - Helper T-cells, Cytotoxic T-cells and Memory T-cells and all three recognise specific antigens. Cytotoxic T cells (Tc) respond to presented antigen on virus-infected dendritic cells (DC) by becoming active cytotoxic T lymphocytes (CTL). T cells when recognise antigens, activate and migrate to the secondary lymphoid tissue, where they divide into multiple memory T-cells and killer T-cells. Cytotoxic T-cells destroy any cells they meet that express the antigen that its receptors match for. Adaptive immunity cannot be detected until 7-10 days following an initial exposure to antigen. However, the immune system is a network that involves many different players that interact with each other defending us against invaders.

SESSIONE 8
IMMUNOLOGIA E AUTOIMMUNITA'
Sala C
Venerdì 14 Ottobre 2005, ore 10.15 - 12.45

S8.2
FACTORS GOVERNINGTHE ONSET OF TYPE 1 DIABETES

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The development of Type 1 diabetes, like many autoimmune diseases, is controlled by many genes. It is also clearly influenced by environmental factors as the concordance rate for diabetes development in identical twins is around 40%.
It is possible to analyse genetic and environmental factors contributing to diabetes using animal models of the human autoimmune disease. The NOD mouse provides a useful good model of human Type 1 diabetes and studies with this rodent model have given insight into factors governing the onset of diabetes. Using this model it has been shown that CD4+T cells, CD8+T cells and macrophages are necessary for the development of diabetes. Induction of tolerance in T cells can be achieved by delivering antigen orally or as an aerosol. Administration of insulin orally or an insulin peptide using a tolerogenic protocol has been shown to prevent onset of diabetes in NOD mice. However, this approach is not effective once destruction of the pancreatic b cell has already commenced. Greater therapeutic efficacy when autoreactive T cells have been already primed has been achieved using monoclonal antibodies to target the T cells and induce tolerance.
There has been considerable interest in the role that infection might play as an environmental modifier. There have been suggestions that infection might precipitate diabetes but it has additionally been proposed that infection might have played a role in inhibiting diabetes development. This latter view has been linked to the Hygiene Hypothesis . There are now several experiments which demonstrate that helminth or bacterial infections will prevent onset of diabetes in NOD mice. By analysing how these infections inhibit Type 1 diabetes it might be possible to identify novel immunomodulators which may prove useful in the treatment and prevention of Type 1 diabetes.

S8.3
ANTI F-ACTINA IGA IN CELIAC DISEASE: EVALUATION OF NEW ELISA ASSAY AND CORRELATION WITH INTESTINAL DAMAGE

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Background. Recently has been described autoantibodies against the actin filaments(AAA) (1), whose presence seem to contribute to villus cytoskeleton damage and to the pathogenesis of intestinal damage in CD. The aims of the present study were to evaluate the relationship between the presence of serum IgA AAAs and severity of intestinal mucosa damage in celiac disease (CD) patients and to compare the IF with a new ELISA assay for IgA AAA determination. Materials and methods. IgA AAAs were assayed in 58 consecutive CD patients, 64 patients as "healthy" controls and 28 adults with autoimmune or gastrointestinal diseases other than CD as "disease" controls. None of "healthy" controls had symptoms suggesting CD, and all were negative for anti-endomysium and antitransglutaminase The IgA AAA enzyme immunoassay (ELISA) was performed with a commercially available method for anti-actin IgG determination (F-Actin Smooth Muscle; INOVA; ref. 708785) and an anti-serum anti-human IgA conjugate (INOVA; ref. 508549). The typical threshold value for AAA absorbance was arbitrarily fixed as equal to the mean value + 2 SD displayed by control sera (0.270). The AAAs have been determined in indirect immunofluorescence method using HEP II modified cells (HEP II actin, INOVA ref. 508090) and Anti-Human IgA (ref. 30240; Bio-Rad). Results and discussion. Serum IgA AAAs evaluated by IF were positive in 54 of the 58 (93%; 95% C.I, 88-98%) untreated CD patients; AAAs evaluated by ELISA were positive in samples from 51 of the 58 CD patients (86%; 95% C.I. 79-93%); The correlation between AAA results by IF and ELISA was high: r= 0.819 (Spearman correlation coefficient). The within assay imprecision (CV) of the ELISA method for IgA AAAs was 4.3%, and the between-assay CV was 9.2%. None of the healthy controls had AAA values, as assayed by ELISA, higher than the cutoff, and values were significantly higher in CD patients [mean (SD), 0.974 (0.924)] than in controls [0.157 (0.057)]; Mann-Whitney test, z= 6.4; P<0.0001. The AAA values of CD patients were significantly higher than those of the disease controls (Mann-Whitney test, P <0.001). There was a significant inverse correlation between AAA values by ELISA and the villus-height/crypt-depth ratio (r= -0.447; P <0.0001). Our results show that AAAs correlate with severity of intestinal villus damage in CD patients and are a reliable marker of severe intestinal mucosa damage. The ELISA technique offers a simple and accurate method for their determination.
(1) Clemente MG et al. Gut 2000; 47: 520-526

SESSIONE 8
IMMUNOLOGIA E AUTOIMMUNITA'

Sala C

Venerdì 14 Ottobre 2005, ore 10.15 - 12.45

S8.4

X-MAP® TECHNOLOGY IN THE DIAGNOSTIC APPROACH TO AUTOIMMUNE DISORDERS.

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Advances in biotechnologies applied to genomics and proteomics have recently permitted the development of analogous techniques designed for clinical laboratories, which have substantially revolutionized the whole diagnostic process. Microarray techniques, which are usually based on a solid phase, can be transferred in liquid phase by microbeads. Such an innovative technique, conjugated with the high multiparametric resolution power of flow cytometry, allows the simultaneous and rapid measurement of several analytes (Multy-Analyte Profile, MAP) within the same media, employing a rather limited amount of sample. Bead-based methodologies are being refined and developed for use as large-scale peptide and protein array. Such a simple and readily transferable approach, allow the simultaneous detection of interference in the proteic matrix, such as rheumatoid factors and heterophile antibodies. Analytical performances and results of the X-MAP technology were tested and compared with those of traditional autoimmune diagnostics (IFA and ELISA). Furthermore, the diagnostic performances of the X-MAP technology were evaluated in several clinical settings, including autoimmune disorders, thyroiditis and vasculitis. In particular, the diagnostic efficiency (sensitivity, specificity and ROC curve analysis) of the X-MAP technology was evaluated in a peculiar subset of patients with systemic sclerosis and results were related with clinical symptoms and disease progression. Reference:

1) Ruzzenente O. Tecnologia X-MAP: un nuovo approccio alla diagnosi delle malattie autoimmuni. Atti del 24° Congresso nazionale della Società Italiana di Allergologia ed Immunologia Clinica. Roma 4-7 maggio 2005; pag. 250.

SESSIONE 9
LA VALUTAZIONE DELLE EVIDENZE IN MEDICINA DI LABORATORIO DELLE PATOLOGIE CARDIOVASCOLARI PROPOSTO DALLA AGENZIA PER I SERVIZI SANITARI REGIONALI

Sala F

Venerdì 14 Ottobre 2005, ore 10.15 - 12.45

S9.1

CRITERIA FOR THE ASSESSMENT OF EVIDENCE ON THE ACCURACY OF DIAGNOSTIC AND PROGNOSTIC TEST

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Diagnostic and prognostic tests are very important tools used daily in clinical medicine. The production of evidence-based documents giving guidance on the use of these important tests is one of the aims of the Sistema Nazionale Linee Guida (SNLG), Italy's national guidelines programme. The methodological rules for the production of SNLG guidelines are summarised in the methods manual (www.pnlg.it). These include the assignment of a level of evidence (LoE) to each item, upon which the formulation of a recommendation is made and a strength (SoR) assigned to the recommended course of action. Both LoE and SoR are assigned on the basis of a formal system. SoR go from "A" (do under any circumstance) to E (do not do). LoE go from I (evidence from systematic reviews of randomised controlled trials, or more than one well conducted large trial) to level VI (experts' opinions). The assignment of LoE is thus based on the large body of randomised controlled trial (RCT) literature, mainly from pharmacoepidemiology, in which one or more drugs are compared among themselves or against a placebo. In RCTs the quality of randomisation, the robustness of blinding and bias minimisation are the main requisites of good methodology.

Despite increasing recent interest, the field of diagnostic and prognostic tests is not well developed as that of pharmacoepidemiology. Studies assessing the tests have unique methodological aspects such as the assessment of the presence and impact of spectrum and verification bias and ROC curve use. Biases, especially unknown ones, have a direct effect on the predictive value and on the daily use in the health service. There is no international agreement on the rules of good conduct, despite the recent publication of a study assessment tool (QUADAS) and a reporting standard statement (STARD statement). Given this situation, the SNLG must revise the rules of assignment of LoE and SoR in diagnostic and prognostic fields as part of the regular updating of the manual. Such a revision must be preceded by a thorough review of the main developments in the field of prognostic and diagnostic tests to produce a valid, practical and uptodate tool for users.

Until such a review is carried out, I propose the introduction of a simple interim classification in which LoE and SoR are expressed together as follows:

A - procedure or test for which there is evidence and/or a general consensus of usefulness and/or effectivness and/or safety for patient and operator.

B - procedure with uncertain effects

C - procedure or test for which there is evidence and/or a general consensus of a lack of usefulness and/or effectiveness and/or safety for patient and operator.

I propose that the interim classification should be used for no more than 12 months from its promulgation and should be replaced by an evidence-based classification published in the updated SNLG methods manual.

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Sala F

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S9.2
THE CARDIOVASCULAR RISK

MS Graziani

Cardiovascular disease is responsible for approximately 40% of deaths in Eastern Countries and is becoming increasingly important in the developing Countries. Prevention strategies are based on the evaluation of risk factors. In order to homogenise prevention strategies in different parts of Italy, the Agenzia per i Servizi Sanitari Regionali decided issuing national recommendations. These are based on the available evidence and graded as follows. Level A: evidence/opinion in favour of usefulness/efficacy of the procedure; Level B: conflicting evidence/opinion of usefulness/efficacy of the procedure, Level C: procedure should not be performed. The available guidelines are the Adult Treatment Panel III (1) and those published by the European Task Force (2). Both guidelines indicate that the rationale for a risk evaluation in primary and secondary prevention comes from intervention trials showing that decreasing LDL cholesterol reduces both cardiovascular and total mortality (Level A).

According to the available guidelines, the risk evaluation should be based on LDL cholesterol. However, it is common practice to perform the risk evaluation utilising a lipid profile including total and HDL cholesterol and triglycerides (Level A). The main reasons are: HDL cholesterol is a major risk factor, total cholesterol is included in some of the chart/algorithm used to calculate the total risk, HDL and triglycerides are part of the definition of the metabolic syndrome, it could be necessary to identify lipid disorders different from hypercholesterolemia

While both guidelines agree considering patients in secondary prevention and with diabetes as high risk subjects, they differ regarding the recommendations for primary prevention. The Adult Treatment Panel III suggests that every person aged > 20 years should have a risk evaluation, while the European Task Force prescribes precise priorities. First people with established coronary artery disease, second people at high risk because of the presence of multiple risk factors, third first grade relatives of subjects at high risk, and last any people encountered in clinical routine practice.

The risk evaluation should be global and estimated utilising the various algorithms or risk charts made available internationally or locally (Level A). The analytes which are not therapeutic targets (HDL cholesterol, triglycerides) should be evaluated according to the decisional levels established by the international guidelines (Level A).

Among the emerging risk factors, the Lp(a) measurement is not recommended in general population (Level C). Its measurement in individuals at increased risk of cardiovascular disease particularly in those with borderline or high LDL cholesterol or apolipoprotein B, could be useful in identifying subjects deserving particular attention because at very high risk (Level B).

C reactive protein has been also considered as risk indicator. In this case, the available evidence is the joint statement of the Centre for Disease Control and the American Heart Association (3). The recommendations are as follow: the test should not be used as screening test (Level C), but should be used in people at intermediate risk (10 to 20 % in 10 years) to direct further investigation or therapy (Level B). In patients with established coronary artery disease C reactive protein measurements may be useful as marker of prognosis for recurrent events (level B). Other acute phase reactant should not be measured for the determination of coronary risk (Level C). The diffusion of national recommendations will help to introduce the Evidence Based Laboratory Medicine into clinical practice allowing effective interventions and improving health outcomes.

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Sala F

Venerdì 14 Ottobre 2005, ore 10.15 - 12.45

S9.3

LABORATORY EVALUATION OF CARDIO-VASCULAR RISK FACTORS: DIFFERENCES BETWEEN HEALTHY AND HYPERTENSIVE PATIENTS.

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The high incidence, morbidity and mortality of cardiovascular disease, and the consequently sanitary cost imply the necessity to act at the right moment about risk factors "modifiable", promoting from age band more young an adequate life style and a regular check from the country doctor.
The cardiovascular risk expresses the probability that one subject has to became ill in the years to come, when the value of some risk factors is known.
These factors are divided in: "modifiable" (obesity, sedenterity, cigarette smoking, blood pressure, diabetes, alcool) and "unmodifiable" (age, sex, family history).
The objective of this study was to examine the epidemiology of the main risk factors "modifiable" and valuable in laboratory.
For this reason we have investigated according to the Adult Treatment Panel III guidelines (ATP-III), metabolism of lipids with the determination of: total-cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides and metabolism of glucose with the determination of glycemia.
We have investigated two kinds of subjects: the "healthy" and the "hypertensive". "Healthy" is a sample of patients stratified for age/sex and residence of people living in Zona Territoriale N°3 (Fano, PU); "hypertensive" is a sample of patients cared in "Centro Ipertesi" of Ospedale S. Croce.
In total we have analysed 2,300 serum of subjects "healthy" and 340 of subjects "hypertensive".
We found differences between "healthy" and "hypertensive", but also differences among "healthy" people living in different geographic area.
The differences find can be ascribed to different location of geographic area about municipal district (sea, town, hill) and consequently to different life style.
Although regard the "hypertensive" people we have found also differences in risk classes attribution between the Framingham risk function and Progetto CUORE risk function, in according to what are reporting in literature, and that is to say the cardiovascular risk estimated with Framingham risk function are sub-valuated when applied to European population.
With this study we have defined which laboratory tests are opportune and when to ask them in every clinical situation.
We have draft an algorithm easily consultable indicating which tests execute for a screening and when to do investigation of second level.

S9.4

TOWARDS AN EVIDENCE BASED CULTURE: A LABORATORY MEDICINE PROJECT

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The Azienda Ospedaliero Universitaria Careggi, Firenze Italy, underwent in 2005, a major organisational change. All the clinical activities were rethought according to a Department based model. Consequently, all local laboratories performing diagnostic tests contributed to the creation of the Department "Diagnostica di Laboratorio" (DAI 4). Although every single laboratory needed to maintain its specific expertise, it was considered of paramount importance to create a common cultural background, as well as a common long term goal. The latter was recognised as the dissemination of information about the rationale use of laboratory tests in the local health organisation, given the need of effective use of limited resources. The development of an Evidence Based culture in the field of diagnostic test was believed to be the first step in such direction. A specific project, as well as the event calendar was defined in collaboration with the Education and Quality Unit. The project is composed by several modules. The first module is devoted to teaching the basic concepts of Evidence Based Medicine oriented to Laboratory Medicine (EBLM). It is directed to clinical pathologists working in the Department and three editions are offered in order to make the course available to every health professional. The second module is directed to people already trained by the first module. It is based on work in small groups, focused on specific clinical issues. The groups are asked to find and answer clinical questions in the area of diagnosis, using the classical EBM method: ask-find-appraise-act. The third module's aim is to review and examine in details some specific methodological aspects, such as: meta-analyses and literature searching. The last element of the project is the construction of an intranet web site. The web site is meant to be a source for primary and secondary literature, guidelines, links to relevant internet sited devoted to EBLM. Furthermore it constitutes a space available for publication and, consequently, diffusion of reports originated by the second module. The project is presently in progress. The web site is already in use and is regularly updated; one edition of the first module has been completed and two groups are currently working on procalcitonin and brain natriuretic peptides. All other initiatives have been scheduled and will take place next year. At the end of 2006 we will evaluate the project results in terms of compliance to the modules and use of web site, in order to plan further strategies to implement the appropriate use of laboratory tests.