ABSTRACT SESSIONI

LETTURA PLENARIA
Sala A
Mercoledì 13 settembre, ore 9.15-10.00

LP1

Zuccaro P.

HEALTH PROMOTION IN SPORT AND ANTIDOPING CONTROLS

Istituto Superiore di Sanità, Roma, Italy

The misuse of drugs and medicaments in sports has been evidenced as an increasing problem in the last 30 years. According to International Olympic Committee (IOC), Doping is defined as the administration of or use by competing athletes of any substance foreign to the body or of any physiological substance taken in abnormal quantity or taken by an abnormal route of entry into the body with the sole intention of increasing in an artificial and unfair manner his/her performance in competition.

List of prohibited substances was firstly prepared in 1968, continuously revised and includes:

1) Prohibited classes of substances as: stimulants, narcotics, anabolic agents, diuretics, peptide and glycoprotein hormones and analogues;

2) Prohibited methods as: blood doping, and pharmacological, chemical, and physical manipulation;

3) Classes of drugs subject to certain restrictions as: alcohol, marijuana, local anesthetics, corticosteroids, beta-blockers.

The extent of sport doping presents an ethical aspect of competing under non-equal opportunities. Furthermore, the misuse of drugs for sport enhancement is a strong potential health risk because of kinds of drugs abused, the large doses usually given and the stress present in the situation of competition state.

The prevalence of doping has been recently estimated by rigorous methods (Laure P. J Sports Med Phys Fitness, ITALY, 1997, 37: 218-24). Among youngsters, doping prevalence resulted around 3-5%. Among adults, in self-reported use studies, doping prevalence may be estimated at 5-15%, where projected use studies report a mean-prevalence near 15-25%. Moreover, studies provide few data about the sports that maximally produce drugs misuse.

It can be said that he only two dopings of established efficacy are: anabolic steroids for resistive performance, and blood doping for endurance performance. Although medical control of athletes is reputably poor, reported accidents attributable to doping have been until now very rare. However Doping is unfair, and must as such be banned from competitions and medicalized doping is unethical.

Deterrent measures such as dope testing and legal sanctions are of importance. Indeed, doping control has become one of the strongest measure to reduce the impact of doping in sport. Analyses are usually conducted on urine samples by Laboratories accredited by IOC.

Initial screening procedures are designed with a sensitivity adequate to eliminate true negative samples from further consideration. Eventual identification of the presence of a specific drug or metabolite is accomplished with liquid or gas chromatography. Confirmation is always performed by gas-chromatography mass spectrometry for low-molecular weight compounds, while confirmatory immunoassays may be used for some peptide hormones. New detection techniques as tandem mass spectrometry, high-resolution mass spectrometry and isotope ratio mass spectrometry have been introduced to be coupled with gas chromatography in case of substances in very low concentration or in case of synthetic androgenic steroids. Analytical reports of quality are of importance for resolution of any suspected doping case. An adequate interpretation of the laboratory results is also needed and therefore their revision by panels of experts or medical commission is foreseen.

The "win at all costs" mentality is becoming increasingly common in youth sports, and young athletes are often looking for methods to improve sports performance and avoid injury. More studies and interventions are required to improve knowledge of doping as a public health issue. Health education based interventions can be effective in preventing drug use among adolescent athletes because sports medicine is nowadays in need of scientific and moral revalorization.

NUOVE FRONTIERE DEL LABORATORIO: BIOCHIMICA *IN VIVO* CON TECNICHE DIAGNOSTICHE NON INVASIVE

Sala A

Mercoledì 13 settembre, ore 10.30-12.00

S1.1

Lodi R.

MAGNETIC RESONANCE SPECTROSCOPY

Dipartimento di Medicina Clinica e Biotecnologia Applicata "D. Campanacci", Università di Bologna, Bologna, Italy

Magnetic resonance (MR) is a completely non-invasive modality able to investigate biochemistry, physiology and morphology in living systems. Magnetic resonance spectroscopy (MRS) enables to measure *in vivo* the concentration of metabolites by utilising the spin of a number of atomic nuclei (^{31}P, ^{1}H, ^{13}C, ^{23}Na etc.). Nuclei of an atom, placed in a strong magnetic field (typically from 1.5 to 2.0 Tesla for clinical studies), resonates at slightly different frequency, depending on what chemical they are bound to. This property enables independent measurement of the concentrations of different molecules containing the same nucleus. In particular two nuclei, phosphorus (^{31}P) and proton (^{1}H), provide important biochemical information that have been shown to enhance diagnostic capabilities and improve patient outcomes. ^{31}P-MRS detects phosphorus-containing compounds, magnesium and cytosolic pH. The major phosphorylated compounds detectable are ATP, phosphocreatine (PCr) and inorganic phosphate (Pi). The regulatory free ADP concentration can be calculated from the MRS data using the creatine kinase equilibrium reaction. Clinical ^{31}P-MRS has been largely used to investigate the patho-physiology and in the diagnosis of vascular, inflammatory, degenerative and metabolic diseases of skeletal muscle, heart and brain (1-4). ^{31}P-MRS invaluable information on tissue bioenergetics can be also used to determine the efficacy or citotoxicity (anti-cancer agents, for example) of therapy.

Reliable insights on brain biochemistry are also provided by ^{1}H-MRS that can reach a spatial resolution of a few cm^3 and thus gives metabolic information from definite brain areas and systems. The most relevant compounds detectable *in vivo* by ^{1}H-MRS are N-acetyl-aspartate (NAA), lactate, choline (Cho), glutamate-glutamine (Glx), myo-inositol (mI), and creatine-phosphocreatine (Cr). NAA is the most important resonance signal being a neuronal marker present only in the neuronal bodies and axons. Reduced NAA concentration can be typically seen in neurodegenerative disorders (Alzheimer Disease for example, 5), in areas of brain ischaemia or in tumours. Recent studies have shown that the reduction in NAA signal is not only an index of neuronal loss, but can also be the result of neuronal dysfunction. This explains the partial reversibility of NAA deficit after therapy (6) or in a recovering acute brain pathology (7). The concentration of lactate is too low to be detected in normal adult brain. It accumulates in several pathological processes such as reduced substrate delivery in hypoxia, ischaemia etc., increased energy demand (fast growing tumors), or deficits of oxidative phosphorylation (mitochondrial encephalomyopathies). Glx signal is characteristically increased in acute anoxo-ischaemia and in liver failure (5). The Cho signal, manly due to membrane phopsholipids, tends to increase in acute anoxo-ischaemic events as a consequence of cell membrane damage (5) but also characteristically increases in tumours. mI is a compound of central importance in cellular osmotic regulation and is specifically reduced in liver failure and increased in Alzheimer Disease (5).

MRS represents a new way to study tissue biochemistry in pathological conditions. The use of MRS in the clinical practice has been proven to be cost effective in diagnosis, staging diseases, planning treatment and monitoring therapy by giving additional information and avoiding more invasive procedures.

References

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S1.2

Pietrini P.

NEW FRONTIERS IN THE LABORATORY: IN VIVO BIOCHEMISTRY WITH NON-INVASIVE METHODOLOGIES. POSITRON EMISSION TOMOGRAPHY (PET)

Dipartimento di Scienze dell'Uomo e dell'Ambiente, Università degli Studi di Pisa.

In the last decades, the development of PET methodologies has made it possible the in vivo study of body organ metabolism in a non-invasive fashion in animals and in human subjects. By using short-lived positron emitting radionuclides to label a variety of biological compounds, including glucose analogs, protein and neurotransmitter metabolites, it is now possible to determine several metabolic processes in different organs, both in physiological conditions and in the presence of pathological manifestations. The research conducted by our group over the last ten years has focused on the neurometabolic bases of brain function in humans. We have used PET methodologies to determine regional cerebral glucose metabolism (rCMRglc) and regional cerebral blood flow (rCBF) in relation to cognitive skills, emotion and behavior in healthy human subjects and in patients with neuropsychiatric disorders, including Alzheimer's disease and frontal lobe dementia.

Regional cerebral metabolic rates for glucose (rCMRglc), as determined using PET with 18F-fluoro-2-deoxy-D-glucose (18FDG), represents a reliable index of functional synaptic activity in the human nervous system. Adenosine triphosphate (ATP), the main energy carrier in the cell, is produced in the brain almost exclusively through the oxidative metabolism of glucose, to which rCBF is coupled. More than 60% of the consumption of energy in the form of ATP is for maintenance and restoration of ionic gradients and membrane potentials due to electrical activity associated with action potentials and transmission of impulses from neuron to neuron. Therefore, increases in functional electrical activity increase the demand for ATP and, in turn, for glucose utilization and are associated with parallel changes in capillary blood flow in the same brain regions. Indeed, the frequency of action potentials and the rate of glucose utilization show a direct linear correlation. Furthermore, increments in glucose utilization, over a baseline resting state, observed when neurons are being stimulated reflect almost exclusively increased energy demands required to restore membrane ionic gradients following depolarization and are localized to the regions with dense synaptic contacts between axons and dendrites and not where the cell bodies are. Therefore, changes in glucose utilization and blood flow during brain stimulation represent a valuable index of regional changes in functional synaptic activity. In this paper we will review and discuss some of the main findings originated from these in vivo PET studies in the field of brain function and metabolism. Specifically, we will focus on the metabolic abnormalities that affect the brain in patients with Alzheimer's disease at different stages of dementia severity. Several studies indicate that metabolic abnormalities appear early in the course of the disease and involve mostly the neocortical association areas of the parietal and temporal lobes; in patients with isolated memory loss in the initial phase of the disease, these metabolic abnormalities may precede and predict the development of subsequent cognitive impairment and parallel the distribution of neuropathological lesions observed at post-mortem autoptic examinations (for a review see Pietrini et al., Int. J. Psychophysiology, 37:87-98, 2000). Moreover, brain metabolic abnormalities similar to those seen in demented patients with Alzheimer's disease may be revealed in the preclinical phases in subjects at risk for developing Alzheimer's disease by using experimental paradigms that increase the brain metabolic demand (Pietrini et al., Am. J. Psychiatry, 154:1063-1069, 1997) or by examining cerebral interregional metabolic/functional correlation patterns (Pietrini et al., Dementia, 4:94-101, 1993; Azari and Pietrini, J. Neurol., 242:112-114, 1995). These findings suggest that in vivo neurometabolic measurements, along with genetic and neuropsychological studies, may contribute to the early diagnosis of neurodegenerative disorders, such as Alzheimer's disease.

NUOVE FRONTIERE DEL LABORATORIO: BIOCHIMICA *IN VIVO* CON TECNICHE DIAGNOSTICHE NON INVASIVE

Sala A

Mercoledì 13 settembre, ore 10.30-12.00

S1.3

Nichelli P.

FUNCTIONAL RESONANCE IMAGING (FMRI) AS A TOOL FOR STUDYING BIOCHEMICAL CHANGES IN VIVO

Clinica Neurologica, Università di Modena e Reggio Emilia

Functional resonance imaging (fMRI) has rapidly emerged as a powerful technique for studying local changes in the concentration of deoxyhemoglobin in the brain. When a neural event occurs in a region of the brain, there is a subsequent increase in local blood flow. This increase is hypothesised to result in a decrease in the concentration of the deoxygenated haemoglobin in the local microvasculature of the activated region. Since deoxyhemoglobin is paramagnetic, it causes local inhomogenity in the magnetic field that can be detected as decreased magnetic resonance signal.

The advantage of fMRI over PET include the following: (1) It does not require injection of a radioisotope and is otherwise non-invasive, (2) It has better spatial resolution, and (3) It has better spatial resolution. For all this reasons, despite some limitations, fMRI is considered the method of choice for studying the neural bases of the behavior.

I will present two examples of application of fMRI to clinical neuroscience: (1) in vivo localisation of speech areas both in normal volunteers and in patients with a left hemisphere stroke, and (2) localisation of areas involved in processing facial emotions.

I will conclude that fMRI has the potential to address a number of cognitive neuroscience questions with a degree of inferential and statistical power not previously available. Furthermore, it can offer us a non-invasive tool for studying patients' local changes in blood oxygenation related to brain functioning.

S1.4

Ferrari M., Quaresima V.

NEAR INFRARED SPECTROSCOPY

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Point-of-care or near-patient testing is the term for any analytical process performed for, or by, a patient outside the traditional clinical laboratory. Advances in technology have led to the development of instruments and kits designed for use in this role, which are able to provide an increasing repertoire of tests. Analytical tests/instruments are now available for use in operating rooms, hospital emergency departments and in the homes of patients in primary care. Several optical methods are under consideration as bedside equipment to measure vital functions. Near infrared (NIR) spectroscopy (NIRS) with its intrinsic features meets these objectives[1]. Due to the relative transparency of the biological tissues (brain, skull, muscle) to the light in the region 700-1100 nm, NIRS can be used for monitoring non invasively, at a depth of about 2 cm, the concentration changes of the compounds (deoxyhemoglobin, HHb; oxyhemoglobin, O_2Hb) which have absorption characteristic in this spectral region. The conventional single channel continuous wave clinical NIR instrumentation, in combination with a pre-determined value of the optical pathlength, have been utilised to measure intracerebral oxygenation changes in foetus, newborn, and adult during intensive care and surgery[1,2]. In addition, also muscle oxygenation has been investigated at rest and during exercise in physiology and pathology[3]. Indirect NIRS methods have been developed to measure O_2 delivery, O2 consumption, blood flow, O2 resaturation, and recovery times in diverse muscle groups at rest and during exercise3. The recent introduction of NIR instrumentation, based on frequency domain and spatially resolved spectroscopy (SRS) has filled the lack to measure directly tissue hemoglobin O2 saturation (tissue O2 index, TOI). This new generation of NIR photometers is called tissue oximeters (NIRO-300 Hamamatsu Photonics, Japan; Invos 4100, Somanetics, USA). TOI, reflecting predominantly the tissue (muscle and intra-cranial) saturation of venous compartment of circulation, is a good indicator of the balance between O2 supply and O2 demand/consumption in the volume of tissue under examination. The tissue oximeters are also equipped with two channels in order to measure TOI of two different regions concomitantly. We recently evaluated the reliability of the simultaneous use of the two channels of the NIRO-300 in two head regions4. We found that, the distance between the optodes being equal, the two NIRO-300 channels provide no significantly different TOI values measured on the same forehead side. This result is extremely important in clinics because it makes possible to use SRS instruments for cerebral mapping (i.e. to evaluate the cerebrovascular reactivity and to follow the recovery of TOI after stroke). We have recently evaluated the dynamic of TOI during carotid compressions in cerebrovascular patients. In addition, we found no significant differences between right and left forehead TOI values in healthy subjects. Due to the lack of gold standards for tissue saturation measured by NIRS, it is not still clear if the provided values from each instrument are really representative, therefore if they might be considered clinically relevant for medical decisions. In our study, performed on 16 subjects breathing room air, we found that TOI values do not differ significantly from the calculated venous O2 saturation (SvO2) values measured by partial occlusion of the jugular veins. A good agreement was also found between the two NIRS methods and the bias (1.6 %) reflects the tendency of mixed tissue saturation (TOI) to be higher than SvO2. In conclusion, these results confirm that cerebral cortex hemoglobin O2 saturation, measured directly by the SRS method, reflects mainly the saturation of the intra-cranial venous compartment of circulation.

The vascular response to functional cortical activation has largely investigated by functional magnetic resonance imaging and positron emission tomography. Recently, functional NIRS also has become a widespread tool to non invasively map HHb and O2Hb cortical changes in response to motor, visual, and cognitive stimuli5. Functional NIRS, with its biochemical specificity, high sampling time (up to 50 Hz) and low spatial resolution (1 cm), offers the advantage of being less restrictive, more comfortable and suitable for brain oxygenation monitoring than the well established brain mapping techniques. Other NIR instruments, presently under pre-clinical testing, operate in the time as well as in the frequency domain. Measuring the absorption and the reduced scattering coefficients (μa and μ's), they offer the advantage of obtaining information about the tissue optical properties as well as the absolute value of O2Hb, HHb, and total blood volume (O2Hb+HHb). Our experience and the literature (on average one clinical NIRS article is included every day in the MEDLINE) confirm the potential utility of the new generation NIR devices as a non-invasive, low cost technology for point-of-care testing of brain/muscle oxygenation in newborn and adult clinical settings.

Acknowledgements: Research supported in part by the EC DG XII, and the EC Fifth Framework Program Project MEDPHOT.

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ORGANIZZAZIONE DI LABORATORI DI BIOLOGIA MOLECOLARE

Sala C

Mercoledì 13 settembre, ore 11.45-12.45

S2.1

Rosatelli M.C.

MOLECULAR GENETICS LABORATORY IN MODERN MEDICINE

Dipartimento di Scienze Applicate ai Biosistemi, Università degli Studi di Cagliari, Via Jenner 091248 Cagliari

With the progress of biotechnologies, an increasing number of genetic diseases are detectable using a molecular approach. Molecular tests allow the analysis of human DNA, RNA, chromosomes, proteins in order to diagnose genetic disorders. Such technologies are particularly useful for large scale newborn and carrier screening, high-risk family's studies, prenatal diagnosis as well as for clinical diagnosis or prognosis. A complex interaction between the patients/individuals/families to be tested and the clinical practitioner o genetic counselor, including several interviews before and after the genetic test, to explain the risks and benefits of the test, is required. For these reasons is important that a molecular genetic laboratory works in close collaboration with the referring clinical and counseling staffs as well as obstetric staff in case of prenatal diagnosis. For each disease to be studied it is necessary to have knowledge on:

- The molecular pathology of the disease
- The frequency of disease-related alleles in the different ethnic groups under study
- Genotype-phenotype correlation
- How to detect molecular defects (available protocols etc)

In developing new methodologies molecular genetic laboratories must conduct a pilot phase in which they verifies that all steps in the testing process are operating appropriately ensuring the analytic validity of the test. Clinical sensitivity, to avoid false negative, together with clinical specificity, to avoid false positive must be assessed, as well as the predictive value of the test. Validation studies should be conducted in a group representative of the one in which the test is intended for clinical use.

Many methodologies require the availability of particular facilities such as radioactivity room, pre and post PCR separated rooms. Routine calibration of all instruments must be carried out. Internal quality controls, i.e. positive control, negative control, molecular weight marker, must be used routinely.

Finally as genetic test are poorly automated , it's very important that personnel involved in molecular genetic laboratories serving as directors or technical supervisors, proves to have a great expertise in the field. Such personnel must have formal annual training in human and medical genetics. Moreover participation in voluntary proficiency testing programs could be promoted to ensure high quality of the tests and national and international standardization

In our laboratories we are carring out molecular, pre- and post-natal, diagnosis for a large number of both frequent and rare genetic disorders: 21-hydroxylase deficiency, Achondroplasia, Alagille syndrome, Alpha thalassemia, Angelman Syndrome, Autoimmune polyendocrinopathy Type 1 (APECED), Beta thalassemias and hemoglobinopathies, Crigler-Najjar type 1-2, Cystic fibrosis, Di George Syndrome, Duchenne/Becker muscular Dystrophy, FacioScapuloHumeral Dystrophy, Fanconi anemia A, Fragile X syndrome, Friedreich ataxia, G6PD deficiency, Hemochromatosis, Hemophilia A, Hypochondroplasia, Lissencephaly, Myotonic Dystrophy, Nephrogenic Diabetes Insipidus, Neurofibromatosis type I, Phenylketonuria, Prader-Willi Syndrome, Simpson-Golabi-Behmel syndrome, Spinal Muscular Atrophy I, Williams Syndrome, Wilson disease. Every year we perform an average of 2000 postnatal molecular diagnoses and 400 prenatal diagnosis.

S2.2

Orlando C., Casini Raggi C., Pazzagli M.

MOLECULAR DIAGNOSIS AND QUALITY CONTROL: A PROGRAM FOR DNA ANALYSIS WITH PCR AMPLIFICATION

Clinical Biochemistry Unit, Department of Clinical Physiopathology, University of Florence.

In the last years molecular biology techniques have found large diffusion in clinical laboratories thanks to the great versatility of the PCR technique. In contrast to the large variety of possible applications of this technique, external quality control programs (EQC) are until now limited to only few tests with certain clinical value. Considering this restriction, EQC programs should be directed towards the evaluation of analytical aspects which are common to the great majority of tests based on PCR. We have developed an EQC program for the evaluation of DNA extraction, its amplification and the analysis of products after PCR. In a first phase, we estimated the stability of reference materials (samples of whole blood and aliquots of extracted human DNA). DNA samples have been prepared in order to contain two sequences of different dimensions but that can be amplified with the same pair of primers. The program presents three control levels: 1. DNA extraction (quality and quantity); 2. PCR performances (specificity and efficiency); 3. interpretation of the results after electrophoresis.

1. Program layout:

Each lot of control materials contains:

- one reference DNA sample (*Control*) obtained from normal human leukocytes. Control can contain, besides the natural target of amplification for each primer pair, additional targets of different sizes which are amplified by the same primers;
- one sample of 5 ml whole blood with a conventional anticoagulant (*Sample*).
- three pairs of primers.
- instructions for PCR amplification protocol.

2. Actions:

- DNA extraction from blood Sample with procedure in use ;
- DNA quality and quantity estimation with conventional procedures;
- amplification of Sample and Control with different primer pairs in duplicate;
- use one of each duplicate to determine number and size of PCR products, after conventional gel electrophoresis;
- mail the other duplicate sample to the organizers of the program for the global and simultaneous evaluation of all amplified samples.

3. Data Analysis:

All samples are checked simultaneously after gel electrophoresis to verify the number and size of bands and to measure, by image analysis systems, the intensity of each PCR products.

On May 1999 the first lot of reagents was sent to 35 Italian laboratories. Of these, 16 have completed the tests required. The analysis of the data collected shows a fair homogeneity with regard to DNA extraction, but a great variability in efficiency and specificity of the amplification procedures and results analysis. These preliminary results seem to confirm the validity of the approach of this EQC program. The program is carried out under the auspices of the Italian Society of Clinical Biochemistry and Clinical Molecular Biology (SIBioC).

LETTURA PLENARIA
Sala A
Giovedì 14 settembre, ore 9.00-9.45

LP2

Santi L.

BIOTECNOLOGIE E BIOSICUREZZA IN ITALIA

CBA-Centro Biotecnologie Avanzate, Genova, Italy

Il rapido progresso scientifico delle biotecnologie e la loro applicazione in settori che coinvolgono tutti gli aspetti della vita umana, animale e vegetale con evidenti ricadute economiche scientifiche, ambientali e comportamentali sulla società, impongono considerazioni e valutazioni indispensabili per scelte politiche e programmatiche. Occorre, a questo scopo, poter valutare consapevolmente rischi e benefici derivanti dalle applicazioni biotecnologiche, per garantire una programmazione coordinata e per applicazioni biotecnologiche finalizzate alla salvaguardia e al miglioramento della qualità della vita, della salute dell'uomo e dell'ambiente. L'attuale rilevanza nazionale ed internazionale delle biotecnologie nel quadro dello sviluppo delle innovazioni e delle tecnologie sostenibili impone, altresì, la ricerca di un rapporto tra Ministeri ed Enti pubblici e privati, per una politica armonica, efficace e durevole, anche al fine di coordinare la partecipazione italiana e quindi "la posizione italiana" in organismi internazionali e comunitari presso cui i problemi della biotecnologie assumono un rilievo di primaria importanza. Nell'ambito della Presidenza del Consiglio dei Ministri svolge la sua attività il "Comitato Nazionale per la Biosicurezza e le Biotecnologie" (CNBB), costituito in base a quanto stabilito dalla Legge 19 Febbraio 1992, n.142.

Questo Comitato, tenuto conto dello sviluppo scientifico di questi anni e quindi della continua estensione applicativa di prodotti biotecnologici in varie attività produttive, ha ampliato le sue competenze e in particolare sono stati maggiormente sottolineati i suoi compiti di coordinamento. In particolare, i criteri di valutazione dei rischi da agenti biologici sono oggetto di attenta considerazione e di notevole impegno da parte del Gruppo costituito *ad hoc* e dal Comitato nel suo insieme per:

- definire la validità di metodiche e test indispensabili per valutare i rischi da OGM nelle varie situazioni in cui tali organismi sono utilizzati, sia nel breve che nel lungo periodo;
- elaborare linee guida o protocolli finalizzati alla prevenzione, alle metodiche e alla salvaguardia dall'uso di OGM e dal loro rilascio nell'ambiente, tenuto conto di quanto già considerato e validato da organismi tecnici nazionali, europei e internazionali;
- fornire quindi una panoramica delle attività o delle iniziative, unitamente ad una loro relativa e comparativa valutazione a livello nazionale, comunitario e internazionale, con particolare riguardo agli Stati Uniti d'America e al Giappone;
- formulare proposte per un programma di ricerca per la messa a punto di tecniche o metodologie innovative per la valutazione e monitoraggio di rischi da lavorazione, uso e diffusione ambientale di prodotti biotecnologici.

La valutazione e il costante monitoraggio dei rischi per la produzione e l'uso dei prodotti transgenici deve peraltro essere oggetto di un intervento normativo da parte dell'Unione Europea che, per quanto riguarda l'alimentazione, ha in fasi di costituzione una Agenzia per la sicurezza dei cibi, Agenzia che dovrà occuparsi in modo preminente della contaminazione delle derrate alimentari ma anche dei cibi transgenici. L'eventuale costituzione di un organismo europeo per i rischi da OGM non deve però far trascurare il ruolo dei Comitati nazionali, sia perché devono poter essere considerate in modo più specifico talune situazioni che maggiormente interessano questa o quella Nazione, sia per l'esigenza di fornire, da parte dei singoli Stati, un continuo contributo alla elaborazione e all'aggiornamento dei protocolli che dovranno essere predisposti in sede comunitaria. L'attenzione da porre ai problemi della biosicurezza è peraltro giustificata dallo sviluppo incessante delle ricerche biotecnologiche e quindi dalla necessità di dover mettere a punto sistemi di verifica per i nuovi prodotti che sono così frequentemente introdotti nel mercato, ma anche dall'esigenza di stabilire norme più precise rispetto a quelle a cui sinora ci si è riferiti che in molti casi appaiono troppo generiche, come ad esempio quelle relative al concetto di "equivalenza sostanziale".

Un altro aspetto che deve essere meglio definito riguarda il principio di precauzione attualmente utilizzato in modo troppo restrittivo o in modo troppo superficiale. Il principio di precauzione è invece valido per la gestione dei rischi in situazioni di incertezza scientifica per assumere decisioni senza dover attendere i risultati definitivi della ricerca, considerando situazioni potenzialmente pericolose e irreversibili (assumendo decisioni provvisorie) e i rischi non solo acuti, ma anche quelli a lungo termine. Il principio di precauzione dovrà peraltro rispondere a sei criteri:

- valutazione oggettiva del rischio, verificando ad ogni fase della preparazione del prodotto il grado di incertezza scientifica, identificando l'agente biologico che potrebbe avere effetto sulla salute, determinando in modo quantitativo e/o qualitativo la natura e gli eventuali effetti dannosi alla salute, stimando la relazione dose – risposta con metodi clinici, sperimentali o matematici, tenuto conto della variabilità naturale dell'agente patogeno e la suscettibilità individuale e, infine, valutando l'esposizione in base alle probabilità di contaminazione, al consumo e alle modalità di utilizzazione;
- allorché i risultati della valutazione dei rischi saranno conosciuti, le decisioni dovranno coinvolgere tutte le parti interessate, nella massima trasparenza e con un'ampia consultazione di tutti i soggetti coinvolti;
- le misure basate sul principio di precauzione devono essere proporzionate al rischio che si vuole limitare o eliminare;
- valutazione dei costi/benefici per garantire l'accettabilità del rischio;
- le misure basate sul principio di precauzione devono stabilire chiaramente chi ha la responsabilità di produrre le prove scientifiche per una valutazione dei rischi. A chi

LA LOGICA DIPARTIMENTALE NELLA ORGANIZZAZIONE DEI SERVIZI DI LABORATORIO

Sala A

Giovedì 14 settembre, ore 10.00-12.00

segue abstract Prof. L. Santi da pag. 238

l'onere della prova? Non è infatti condivisibile la procedura per cui sono le stesse ditte produttrici che devono effettuare le prove che sono poi soggette ad esame di organismi autorizzatori. Occorre invece sviluppare un piano di ricerca affinché istituzioni accreditate possano svolgere gli accertamenti necessari;

- le misure adottate in base al principio di precauzione dovrebbero avere sempre un carattere provvisorio in attesa di risultati scientifici certi.

Sono questi i concetti che dovranno essere meglio precisati e approfonditi per assumere decisioni non superficiali né dettate da fattori emotivi.

Lo sviluppo delle biotecnologie nel nostro Paese dovrà essere corrispondente a nostre esigenze che sono naturalmente espresse dalle nostre caratteristiche socio-economiche e quindi da specificità di mercato. La tutela della salute e dell'ambiente, il sostegno alla ricerca scientifica orientata alle priorità identificate, il miglioramento della tipicità dei nostri prodotti alimentari e la messa a punto di diagnostici per le malattie delle piante sono aspetti che dovrebbero essere la risultante di consensi convergenti.

Elemento centrale resta peraltro il coinvolgimento dell'opinione pubblica con una informazione adeguata che non vuol dire informazione pilotata ma frutto di un continuo confronto di opinioni con verifica scrupolosa degli interrogativi che potranno essere formulati in base a ipotesi scientificamente sostenibili.

S4.1

Cavina A.

DIPARTIMENTALIZZAZIONE DELLE AZIENDE OSPEDALIERE MISTE

Direttore Generale Azienda Ospedaliera integrata con l'Università Policlinico di Modena

L'ultimo decennio del XX secolo è stato caratterizzato da una grande trasformazione delle organizzazioni produttive e di servizi che ha contraddistinto il passaggio dalla centralità della struttura produttiva alla centralità del cliente. Una simile evoluzione non poteva non coinvolgere anche il mondo della sanità, caratterizzato da una elevata complessità organizzativa, elevato contenuto professionale-scientifico, ed in cui la qualità delle relazioni interne ed esterne condiziona fortemente la qualità della prestazione e la soddisfazione dei "clienti interni ed esterni".

L'organizzazione dipartimentale delle strutture ospedaliere è un tentativo di adeguare i "processi produttivi" alle nuove esigenze. Nelle Aziende Ospedaliere Miste riguarda le funzioni di assistenza, didattica e ricerca clinica.

L'organizzazione dipartimentale può costituire una efficace evoluzione organizzativa solo se è in grado di promuovere un adeguamento dei comportamenti professionali e delle relazionali funzionali.

Nella relazione l'autore riporta i principi normativi nazionali e regionali presi a riferimento; i presupposti culturali ed organizzativi alla base del modello di Dipartimentalizzazione identificato nell'Azienda Ospedaliera Integrata con l'Università Policlinico di Modena; lo stato di realizzazione del progetto.

LA LOGICA DIPARTIMENTALE NELLA ORGANIZZAZIONE DEI SERVIZI DI LABORATORIO

Sala A

Giovedì 14 settembre, ore 10.00-12.00

S4.2

Fiorucci G.C.

A CLINICAL-PATHOLOGY DEPARTMENT: FOUR YEARS OF EXPERIENCE

Definition of department:

The department is made up of uniform operating units, related or complementary, which follow commune events and are therefore interdependent between themselves, maintaining their own autonomy and professional responsibility.

The aim required in departmentalization is that of improving the efficiency and if possible the quality of the professional collaboration. To obtain this results it is necessary to change the organization, the working method and, most of all, the staff way of thinking.

The clinic pathology department of the hospital Maternal Infantile OIRM S. Anna of Turin, which became an Institute in 1996, is formed by 12 laboratories:

- 2 laboratories of Clinical Chemistry and Microbiology
- 2 pathologic anatomy
- 1 immunoematology and trasfusion,
- 1 cytogenetics
- 1 cytoimmunodiagnostic
- 1 haematology
- 1 oncology
- 1 facilitated procreation
- 11 immunology
- 1 gynaecologic

The hospital laboratories work in a structural form with the departments, regarding the diagnostic assistance activities. The Universities participate in a structural form whereas the hospital technician staff and the executive staff also work in both forms.

At the beginning the institution had a statute that can provide: an assembly, an elected and by rights council; board of directors part elected and in part chosen by the head of the department.

As foreseen, this organisation has proved to be non functional and has been particularly replaced by an uniformed organigram more agile, with a staff support of the head department formed on this way:

- a department secretary, supervisor of all the administrative and nursing staff;
- a technician staff supervisor;
- other four supervisors: for management control, for quality assurance, for security (DL 626) and one for the reorganising project.

The initial main problems involved the clinical chemistry and the microbiology labs, which were part of the two separated hospitals (infant and ostetric-gynaecologic): as such had a typology of mixed laboratories varing from basic routines to ultraspecialistic exams and all the analytical areas where twice, as much competence, instrumentation and staff.

The reorganisation project: problems and solutions:

Problems:

- fragmenting and duplicating the activities
- fragmenting the areas
- activity routines together with highly specialized activities
- two separated urgency areas with two equip 24 hour service

Solutions:

The main aim for the clinical labs reorganization was to achieve the presupposition of modern medical labs:

- excluding mixed and omnicomprensive megalaboratories with straightfoward specialistics
- organizing ultraspecialistic labs of regional relationship

With these presuppositions the reorganization of the labs is set up by dividing the two mixed labs in this way:

- Clinical Pathology (routine basis and simple specialistic)
- Microbiology and virology (routine basis and specialistic)
- Clinical chemistry (ultra specialistic, hormonology, prenatal diagnostics)

The achievements of the reorganisation project has taken about 2 years with subsequent stages:

- Stat labs unification;
- Unification of the various and secondary analytical lines;
- Organigrams redefinition;
- Staff unification;
- data management unification
- Increase of the areas of high specialisation such as the genetic molecular and the pre and neonatal screenings

Conclusions:

A forming departmentalization project cannot have a probability of success without these three fundamental steps:

1. Unified personnel management, with specific supervisors for technical, clerical, nursing and auxiliary staff
2. Nomination of Responsibles for underprojects and relative working groups:
    - management control
    - informatics and data management
    - operating module review
    - updating and training
    - security
3. Implementation of the quality system
4. Nomination of a quality assurance manager inside the clinic pathology department there are ISO 9002 CERTIFICATES PLUS European Professional Standards EC4 Four laboratories.

LA DIAGNOSTICA MOLECOLARE DEI TUMORI
Sala C

Giovedì 14 settembre, ore 11.45-13.15

S5.1

Radice P.

MOLECULAR ANALYSIS OF GENES PREDISPOSING TO FAMILIAL BREAST AND OVARIAN CANCER

Department of Experimental Oncology, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan

Two major genes responsible for hereditary predisposition to breast cancer, namely *BRCA1* and *BRCA2*, have been isolated so far. Although the physiological role of these genes has not been fully elucidated, their products have been shown to interact, directly or indirectly, with a variety of cellular proteins involved in the control of genome integrity and in the regulation of gene expression and cell cycle. Women who carry germline mutations in either the *BRCA1* or the *BRCA2* genes exhibit a strong tendency to the development of breast carcinoma, although variations in risk levels among different populations have been reported. Mutations in *BRCA1* are also associated with an elevated risk for ovarian carcinoma, whereas families with cases of male breast cancer have been found to be frequently associated with alterations in *BRCA2*. In principle, the presence of a mutation in a BRCA gene is always to be speculated in the presence of a family history of breast and/or ovarian cancer. However, since no phenotypic marker specific for hereditary cases has been identified, the occurrence of a predisposing genetic lesion can be assessed only through DNA analyses. The identification of mutation carriers is of much relevance in the management of at risk families. Patients who developed a cancer as a consequence of a genetic susceptibility may, in fact, require specific treatments and follow-up. On the other hand, unaffected mutation carriers can be addressed to the adequate programs of prevention and surveillance, when still asymptomatic. In addition, relatives of mutation carriers who are found negative to the test may benefit from molecular analysis both in terms of psychological relief and avoidance of unnecessary clinical examinations. Unfortunately, molecular testing in BRCA genes is a complex task, mainly because of the high heterogeneity of the associated mutational spectra. Several hundreds different *BRCA1* and *BRCA2* mutations have been reported, a large fraction of which have been detected in no more than a single family. These mutations are distributed almost uniformly along the entire coding sequences of both genes. As a consequence, screening for specific gene alterations is not a feasible procedure in the case of BRCA genes, except for some populations, where strong founding effects are present. Although the complete sequencing of a gene, including the entire coding region, exon-intron boundaries and regulatory sequences, is considered to be the most sensitive methods for mutation detection, this approach is particularly laborious and highly expensive in the case of BRCA genes, whose coding exons altogether span slightly less than 16 Kb. Therefore, several cheaper and less time-consuming PCR-based mutation screening methods have been applied to the analysis of *BRCA1* and *BRCA2*. Furthermore, to limit the cost of the analysis, statistical models have been developed for the selection of individuals to be tested. These models estimate the prior probability for a woman to have a mutation in either the *BRCA1* or the *BRCA2* genes, on the basis of her personal and familial history of cancer. More than 200 apparently unrelated cancer patients, selected for having a prior probability of mutation higher than 10%, have been tested in *BRCA1* and *BRCA2* in the Laboratory of Molecular Diagnostics at the National Cancer Institute of Milan. Approximately, one third of cases was found positive to the test. Additional mutations were identified through the complete sequencing of both genes in another set of cases. Of the more than 80 different mutations that were identified, only 16 (<20%) recurred in more than one family and only one half had been previously reported by other laboratories worldwide. Most identified mutations were predicted to lead to the formation of premature termination signals of protein translation, and, therefore, are considered as truly pathogenic. However, in a few cases missense mutations were detected as the sole alterations in the examined genes. In the absence of functional tests capable of revealing possible protein malfunctioning due to the amino acid changes introduced by the detected mutations, the significance of such genetic alterations remains uncertain. Approximately one third of the cases who developed a breast cancer before 36 years of age, selected irrespective of family history of cancer, was mutation positive. This frequency is on average twice as high as those observed in other countries. This finding suggests that among Italian BRCA-linked families the penetrance of mutations at younger ages is higher than in other populations. Alternatively, a reduced exposure of the general population to environmental risk factors, as compared to other Western populations, or the protective effect of agents such as nutrients present in the Mediterranean diet, can be speculated. It is likely that at least a fraction of negative cases are attributable to an insensitivity of the methods employed in detecting certain nucleotide changes, or to the occurrence of alterations that cannot be recognised by PCR-based mutation detection techniques. The families in which these possibilities can be ruled out, for example through the haplotyping of polymorphic markers associated with the BRCA genes in affected members, may be useful for the identification of still unknown breast cancer susceptibility genes. Finally, approximately 60 healthy relatives., mainly women, have been found to carry the mutations originally detected in the probands DNA. Questionnaires collecting information on medical history and on life-style and environmental risk factors are being submitted to these individuals, and periodically updated.

The data collected are expected to provide useful information on the effect of non genetic factors in modifying the penetrance of *BRCA1* and *BRCA2* mutations, thus providing the rationale for intervention strategies, aimed to the reduction of cancer risk in genetically predisposed individuals.

S5.2

Parziale A., Volpe G., De Micheli D., Serra A., Scaravaglio P., Giugliano E., Gottardi E., Saglio G.

CLINICAL RELEVANCE OF MOLECULAR DIAGNOSTICS AT DIAGNOSIS AND DURING FOLLOW-UP IN HEMATOLOGIC MALIGNANCIES

Dept. of Clinical and Biological Sciences, University of Turin, Osp. San Luigi, 10043 Orbassano-Torino

In the last decade a number of specific genetic lesions present in leukemia and lymphoma have been characterized. These molecular abnormalities provide unique disease markers, that may be useful: i) for a better characterization of leukemias and lymphomas at diagnosis; ii) as prognostic markers; iii) to detect minimal amount of residual disease at the end of therapies aimed at complete eradication of the neoplastic clone. At present and with some remarkable exceptions, acute myeloid leukemias are still treated as a single disease, although they embody different clinical entities, characterized by specific genetic lesions, which would probably benefit of distinct therapeutic approaches. In acute lymphoblastic leukemia (ALL), the presence of a Philadelphia chromosome and of the associated BCR/ABL gene rearrangements certainly represents a major negative prognostic factor, whereas in pediatric ALLs other abnormalities, as the recently discovered Tel/AML1 rearrangement, seem to play a major role in determining the prognosis. At the moment, cytogenetic/molecular abnormalities may be estimated to be detectable in approximately 50-60% of all AML cases, 35-40% of the adult ALL cases and 40-45% of the ALL pediatric cases. In addition to their prognostic relevance, these molecular markers represent useful tools for minimal residual disease detection. Generally this consists in a PCR-based approach, able to reach a very high degree of sensitivity. However, as in certain types od leukemias the persistence of some amount of MRD is still consistent with a prolonged complete remission, approaches of Real Time Quantitative PCR are needed to predict the risk of relapse in individual cases.

LA DIAGNOSTICA MOLECOLARE DEI TUMORI
Sala C
Giovedì 14 settembre, ore 11.45-13.15

S5.3

Biondi M.L., Turri O., Leviti S., Seminati R., Guagnellini E.

MATRIX METALLOPROTEINASE POLYMORPHISMS IN PROMOTER REGION AND CANCER

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Matrix metalloproteinases (MMPs) facilitate cellular invasion by degrading the extracellular matrix, and their regulation is partially dependent on transcription. Binding sites for members of the Ets family of transcription factors are present within MMP promoters and are potent positive regulators. Overexpression of MMPs is associated with tumor invasion and metastasis. Two polymorphism are described on promoter region of MMP1 and MMP3. Both influence the transcriptional responsiveness of human MMPs promoter. Recently, some authors, have suggested that the MMP1 one might represent a risk factor for development and progression of ovarian cancer.[1] To investigate the role of this two polymorphism in the pathogenesis of different type of cancer we determine the allelic frequency in 176 subjects and 164 control. 45 were affected of breast, 56 of colorectal, 30 of pulmonary, 24 of ovarian and 21 of melanoma cancer. Whole blood was collected, DNA extracted with a commercial kit (Istagene Matrix, Bio-Rad Laboratories) and amplificated with two copy of primers. Amplification was confirmed before direct sequencing by an automated capillary electrophoresis DNA sequencer (ABI Prism 310, PE Applied Biosystem). In MMP3 the 6A allele has a lower promoter activity than the 5A allele. In cancer patients 6A allele is less represented (5A/5A+5A/6A Vs 6A/6A ; $p<0.05$) then in control. In particularly in breast cancer (15+24 Vs 6 in patients, 42+74 Vs 48 in controls, $p<0,05$). No differences were observed in colorectal cancer. In MMP1 the 1G polymorphism may provide a mechanism for less aggressive matrix degradation. No significant differences were observed in 1G/2G polymorphism in all cancer patients together, but in colorectal cancer the 1G allele was significantly less represented (1G/1G+1G/2G Vs 2G/2G, $p<0.05$) than in control group. No significant distribution of both MMPs genotype was observed in ovarian, pulmonary and melanoma cancer. These results demonstrate the importance of determining the MMPs genotype in relation of cancer. More studies are necessary to correlate the MMPs polymorphism and the progression of cancer and to explain the difference between breast and colorectal cancer.

[1] Cancer Research 1999: 59, 4225-4227.

S5.4

Zanella I.@¤, Rossi G.*, Finazzi D.@¤, Capucci A.*, Pavia P.@, Pollonini G.@, Cariani E.@, Albertini A.@¤

A REAL-TIME RT-PCR METOD FOR THE QUANTIFICATION OF BCR/ABL EXPRESSION

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The bcr/abl rearrangement corresponds to t(9;22), observed in 95% of patients with chronic myeloid leukemia (CML). The detection of bcr/abl mRNA by RT-PCR does not predict disease progression, but the kinetics of bcr/abl expression might be useful for the monitoring of residual disease. A real-time PCR assay was developed to quantify bcr/abl mRNA in blood or bone marrow samples of CML patients. Total RNA from the K562 cell line, diluted in total RNA from HeLa cells, was used as a standard. cDNA synthesis was performed using random hexamers with 1 ug of RNA. The mRNA of the housekeeping gene GAPDH was used to normalize RNA quantity and quality and to monitor the efficiency of cDNA synthesis. Real-time PCR was carried out in duplicate using the GeneAmp^{TM} 5700 SDS (PE Biosystems).

RNA from K562 cells was positive until 10^{-4} dilution (1 K562 cell in 10^{4} HeLa cells, 5 log dynamic range). Intra-assay CV% was 7% and inter-assay CV% was 12%.

The real-time PCR assay was used to analyze 19 RNA samples from 10 patients. Results were expressed as ng of K562 total RNA with the same level of bcr/abl expression and were normalized to GAPDH mRNA (bcr/abl normalized Dose, nD). The bcr/abl nD ranged from 23.2 to 1880 in samples collected at diagnosis and from 0.214 to 186 in follow-up samples. A significant correlation was observed between the % of Ph' chromosome and the level of bcr/abl mRNA ($p<0.001$, Spearman coefficient).

Conclusions: 1. real-time PCR is a rapid and reliable method to quantify bcr/abl mRNA expression; 2. the correlation between cytogenetic and quantitative PCR data indicates that it may represent a clinically useful method to monitor residual disease.

DIAGNOSTICA DI PATOLOGIE NEUROLOGICHE
Sala A
Giovedì 14 settembre, ore 17.30-19.00

S6.1

Di Donato S.

GENETIC TESTING IN NEUROLOGY

Division of Biochemistry and Genetic, National Neurological Institute C. Besta, Milan, Italy

Molecular genetics has had a profound impact into the nosology, diagnostic procedures and the management of patients with neurological disease. This is due to the fact that many "classical" neurological disease are genetic disorders exhibiting either mendelian or mitochondrial inheritance, and to the very high phenotipic expression of genetic disorders in the nervous system. Muscular dystrophies, ion channel disorders, diseases of the peripheral nerves, dementia and mental retardation, ataxias, and other movement disorders, epilepsies, can be associated with autosomal dominant, autosomal recessive, or X-linked mutations in human DNA. Complex, multisystem neurological diseases are also associated with mutations in the mitochondrial genome.

All mendelian neurological diseases can be classified on the basis of the locus involved as well as the particular mutant allele at that locus: however, we see both genetic heterogeneity of neurological disorders, i.e. a unique phenotype can be due to mutations in different genes and phenotypic heterogeneity, i.e. mutations in the very same gene that give rise to different clinical expression. Information about the gene involved in a given patient or family with suspected neurogenetic disorders is the goal of current genetic testing. In principle, genetic testing requires full information to the patient and his/her relatives about risks, reliability, and private use of the results of such molecular approach to diagnosis. Depending on whether a specific gene has been cloned and its sequence is known and readily available, or the gene has been mapped in the human genome but not yet identified, DNA-based diagnosis can be made in two essentially different ways:

a) *Direct testing* of the proband's DNA for a specific pathogenic mutation

b) *Indirect testing* of both the proband's DNA and of critical relatives' DNA to detect by the use of linked markers whether the patient inherited the disease-carrying chromosome from the parents.

Direct Mutation Detection for Neurological Diseases

Once a disease gene has been isolated and the molecular structure of the normal and the mutant(s) form(s) have been delineated, a direct molecular detection of the mutation can be designed. This type of testing is straightforward and particularly useful when a single type of mutation is present and easily detectable in the diseased population, such as in the case of CAG repeat expansion in Huntington Chorea, the GAA repeat in Friedreich's ataxia, or the 1.5 megabase duplication at locus 17p in Charcot-Marie-Tooth disease. Another example is seen in some of the metabolic diseases where a prevalent mutation is present in affected individuals, as in medium-chain acylCoA dehydrogenase deficiency, where the A985G mutation accounts for more than 90% of all mutant alleles. In genetic diseases where patients exhibit a great diversity of pathogenic mutations or the gene has a very large size, such as in Duchenne/Becker types of muscular dystrophy or Neurofibromatosis type 1(NF 1), genetic testing could be more laborious: is these diseases, unless the pathogenic mutation is already known in a particular family, it is convenient to track the mutant allele through indirect testing.

Methods for direct detection of mutations

Direct DNA analysis is almost always done by polymerase chain reaction (PCR) in a wide range of samples including venous blood, pathological specimens such as muscle biopsies, cultivated cells such as fibroblasts, chorionic villuses or amniotic cells for fetus diagnoses. In principle direct DNA testing involves two different procedures:

a) methods for scanning a gene for a unknown mutation: (generally a point mutation) widely used when there is circumstantial evidence that a single mutation whithin a known gene is responsible for a disease;

b) methods for indentifying a known mutation:

Trinucleotide repeats diseases are the best candidates for molecular diagnosis since any given patient holds the same type of mutation, so that a single PCR reaction makes the diagnosis. For CAG expansions such as in Huntington Chorea, the repeat can be amplified by PCR and run on a polyacriamide gel in order to better discriminate the number of repeats in the probands' DNA.