S6.2

Govoni S.

BIOCHEMICAL MARKERS OF ALZHEIMER'S DISEASE (AD): THE BALANCE BETWEEN PHYSIOPATHOLOGY AND DIAGNOSTIC INFORMATION

Department of Experimental and Applied Pharmacology, University of Pavia

The finding of early biochemical markers of onset of AD could be of clinical relevance allowing the treatment at early stages of the disease and, togheter with the identification of genetic risk factors, can direct new efforts in studying molecular targets for prevention as recently suggested by a study on the interaction of the estrogen receptor polymorphism and ApoE4 (Brandi et al. BBRC 265:3351999).
In view of these facts, there has been an intensive search, particularly in Alzheimer's disease, for the identification of accessible tissues or body fluids suitable for exploring pathophysiological hypotheses and for providing a biological marker apt to confirm the diagnosis. Within this context we contributed studies on fibroblasts derived from AD patients showing that non amyloidogenic metabolism of the amyloid precursor protein (APP) is defective in sporadic AD patients, is more sensitive to oxidative stress (Gasparini et al. Neuroscience Letters. 263:197, 1999), but still responsive to receptor-mediated, calcium dependent stimulation (Racchi et al. Biochemical Journal. 330:1271, 1998). These studies on cultured cells led to the characterization of APP secretion pharmacology (Racchi & Govoni TIPS. 20:418, 1999) which may be altered in AD and in conditions in which APP expression is modified (Racchi et al Biochem. Journal 338: 777, 1999). Moreover these studies provided the background for further investigation of APP metabolism regulation. As an example, originally guided by the strong dependence of sAPP secretory patwhay to energy, observed in AD fibroblasts, we recently discovered (Solano et al. FASEB J, 14, 1015-1022, 2000) a new insulin and PI3 kinase-dependent sAPPα release patway.
Even if the search for a biological marker of the disease using fibroblasts has not led to a clear identification of a clinically useful diagnostic marker, has helped building a biological profile characteristic of AD cells (see for example Etcheberrigaray et al. Neurobiology of Disease. 5:37-45, 1998). In fact, the association of different parameters identifies a biological pattern of AD cells and sets the ground for more clinically oriented experimental studies carefully aimed at detecting the diagnostic or informative potential of the observed biological differences.

S6.3

°Rizzo V., *Montalbetti L., Moratti R.

GLUTAMATE AND GABA LEVELS IN CSF FROM PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

°Dipartimento di Biochimica, Sez. Analisi Chimico-Cliniche, IRCCS Policlinico San Matteo; *Dipartimento di Scienze Neurologiche, Università degli Studi di Pavia

Typical amyotrophic lateral sclerosis (ALS) is an idiopathic neurodegenerative disorder characterized by a selective athrophy of the motor neurons in the spinal cord and by a degeneration of the corticospinal tracts. Glutamate, a neuroexcitotoxic compound, has been implicated in neurodegenerative processes; furthermore, an unbalance between excitatory and inhibithory neurotransmitter amino acids has been consistently demonstrated in chronic neurodegenerative disorders such as ALS.
In this study, we investigated GABA and Glutamate concentrations in CSF from patients with middle-stage prymary ALS (n=14), compared with those from control subjects with disk erniation (n=40).
The amino acid assay was carried out using a Chrompack 3-C_{18} with a potassium phosphate buffer system (0.05 M, ph 6.0, 18% acetonitril) after precolumn derivatization with *o*-phthalaldehyde. Detection was effected by fluorescence measurement (RF-551, Shimadzu Italia; Ex:330 nm, Em:450 nm) (1)
Statistical analysis (by means of Mann Withney U test) showed, on one hand, a marked and significant reduction in Glutamate levels in ALS patients, [median (IQ) 0.11 M/l (0.088-0.27 M/l)], compared to controls [median (IQ) 0.68 M/l (0.5-0.83 M/l)]; on the other hand, GABA levels were significantly more elevated in ALS patients [median (IQ) 0.47 M/l (0.37-0.73 M/l)] than in controls [median (IQ) 0.13 M/l (0.08-0.17 M/l)].
This findings confirm that a biochemical change in aminoacid neurotrasmission is an hallmark of neuronal degeneration in ALS. Since Glutamate is cleared by glial cells, while neuronal cells are deputed to GABA clearance, our data seem to be in agreement with the hypothesis that degeneration of neurons having N-methyl-D-aspartate (NMDA) receptors may be accompained by a reaction of surrounding glial cells (mostly astrocytes), which, in many animal models and human diseases, have been found to replace the degenerate neurons.

1) Rizzo V., Anesi A., Montalbetti L., Bellantoni G., Trotti R., Melzi d'Eril G:V: *J. Chromatogr. A 729 (1996) 229-235*

S6.4

Ciusani E., Di Benedetto M., Di Tecco A., Croci D., Bernardi G.

ISOELECTRIC FOCOUSING SEPARATION OF GLYCOFORMS OF TRANSFERRIN: A POLYVALENT MARKER IN VARIOUS NEUROLOGICAL DISEASES

Istituto Nazionale Neurologico IRRCS "C.Besta"
via Celoria 11, 20133 MILANO

Transferrin (Tf), a 85 KDa iron-binding glycoprotein, is extensively distributed in body fluids. Due to structural variations in the glycan chains in the C-terminal domain, transferrin may be separated in seven different fractions, named consecutively from asialo- to hexasialo-Tf, based on the number of sialic acid residues bound to the glycan chains. Normal serum Tf is mainly composed of tetrasialo-Tf with lower amounts of penta- and hexasialo-Tf and very small amounts of mono-, di- and trisialo-Tf.

In clinical practice, glycoforms of Tf are used for screening and diagnosis of carbohhydrate-deficent glycoconjugate (CDG) syndromes, a series of autosomal recessive carbohydrate metabolism disorders, for the detection of alcohol abuse and for the analysis of nasal secretions in case of suspected CSF rhinorrea. Despite brings to neurological damage, alcoholic abuse is usually monitored in general labs with chromatographic methodologies, while, in neurochemical labs, IEF or electrophoresis of Tf are useful tools for the diagnosis of CDG and CSF rhinorrhea.

In or lab, IEF on agarose gel followed by immunoblottig of Tf on PVDF membrane and chemiluminescent detection, has been set up two years ago. In this period, we analysed the sera of 142 patients with suspect CDG from different Italian hospitals and, in 5 cases, it has been possible to clearly identify the presence of low sialificated Tf isoforms, that well correlated with other criteria of CDG syndrome. In fact, the partial deficiency of sialic acid in CDG patients causes a cathodal shift of Tf resulting in a marked increase mainly of asialo- and/or disialo-Tf with a consequent decrease in terta-, penta- and hexasialo-Tf.

In addition, the detection of CSF rhinorrea by IEF of Tf, is possible since intrathecally synthesised Tf is mainly asialificated, and the presence of asialo-Tf in nasal secretion may be used as marker of CSF contamination.

In our experience, IEF of Tf was the most sensitive and specific method for the detection of CSF contamination in nasal secretion of 17 patients in whom diagnosis was confirmed by endoscopic examination and clinical course.

IEF separation, followed by immunoblotting and chemiluminescence, is a sensitive and reliable method to detect all Tf isoforms, useful in diagnosis of various and heterogeneous neurological diseases.

G.Keir, BG Winchester, P Clayton. Carbohydrate-deficient glycoprotein syndromes: inborn errors of protein glycosylation. Ann Clin Biochem 1999 36:20-36

S7.1

Fargion S., Fracanzani A.L., Sampietro M., Fiorelli G.

HEREDITARY IRON OVERLOAD

Dipartimento di Medicina Interna, Ospedale Maggiore IRCCS, Università di Milano

Hereditary hemochromatosis (HHC) , the most frequent recessive disease in Caucasians (3-5/1000 homozygotes and 10-15/100 heterozygotes) is characterized by a progressive iron overload for unregulated iron absorption responsible for life threatening complications, including cirrhosis, hepatocellular carcinoma, cardiopathy... In the last few years knowledge on the genetic of HHC is extraordinarly increased. In 1996 the gene responsible for HHC has been identified on chromosome 6 and two mutations (the C282Y, characterized by severe iron overload, and the H63D, by a more subtle disorder of iron metabolism) associated with the disease. The protein encoded by HFE gene has been demonstrated to bind transferrin receptor and modulate transferrin binding to the receptor. The C282Y mutated protein is unable to bind the receptor while the H63D binds but it is unable to modulate the affinity between transferrin and its receptor. Although 65-95% of the patients with HHC are homozygous for the C282Y mutation, in a recent epidemiological study it was shown that more than 20% of subjects homozygotes for this mutation do not have iron overload. Moreover in Italy the prevalence of homozygosis for the C282Y mutation in patients with a phenotype compatible with HHC varies between 35 and 70% depending on the Northern or Southern Italy origin of the patients. Thus, increasing interest has been addressed to the detection of new mutations and/or new genes responsible for HHC. Besides the recent identification of a new gene located on chromosome 1 (HFE2 gene), associated with juvenile hemochromatosis, mutations in a recently identified trensferrin receptor 2 gene have been identified in patients with HHC from two Sicilian families. Several other recently discovered genes involved in iron transport have been suggested to play a role in HHC but no conclusive data are available. Thus, although several aspects of HHC have been cleared, many others remain to be defined.

S7.2

Cappellini M.D., Marelli S., Zatelli S., Fiorelli G.

SECONDARY IRON OVERLOAD

Centro Anemie Congenite, Dip. Medicina Interna, Ospedale Maggiore Policlinico IRCCS, Milano

Excessive iron absorption and progressive iron loading are characteristic of congenital anemias due to ineffective erythropoiesis. Life-threatening iron overload are common in non-transfused patients with thalassemia intermedia, congenital dyserythropoietic anemia type II (CDA II) and X-linked congenital sideroblastic anemia. The erythroid marrow activity is the major factor conditioning the entity of iron overload in these conditions. Although the mechanisms by which the erythroid marrow expansion induces a positive iron balance are still undefined, it is well known that increased iron requirement by erythron has important effects on the reticuloendothelial cells and on increasing the gastrointestinal iron absortion. Secondary iron overload is also the major complication in transfusion-dependent thalassemia patients (thalassemia major). Transfusional iron overload in parenchymal cells is fatal in the second decade of life usually from cardiac complications, unless a regular chelation therapy is administered. In transfused-thalassemia patients, iron overload also causes pituitary damage with consequent endocrine complications. Liver fibrosis and eventually cirrhosis, particularly if concomitant chronic hepatitis is present, is also a serious complication. However not all patients who undergo chronic transfusion therapy accumulate iron at the same rate and iron chelation is not equally efficient. Recently it has been speculate that mutations in HFE gene, responsible for hereditary hemochromatosis, might account for the differences in the severity of secondary iron overload, nevertheless, several studies in thalassemia patients conclude that HFE mutations do not influence the rate of iron loading in thalassemia following a regular transfusion and chelation program.
Regular iron chelation remains the major therapeutic approach of secondary iron overload. The only iron chelator currently widely available is deferioxamine which is subcutaneously administered. New oral chelators are under development and deferiprone is the most extensively studied today.

S7.3

Belloli S., Ruggeri G., Rossini A.*, Facchinetti D.*, Arosio P.**, Albertini A.

PREVALENCE OF C282Y AND H63D MUTATIONS OF HFE IN IRON OVERLOADED SUBJECTS IN BRESCIA

3 Laboratorio Analisi -*1, 3 Medicina Generale - Azienda Spedali Civili Brescia;**Istituto di Chimica Università degli Studi di Brescia.

Genotyping of HFE is a promising tool for the differential diagnosis of iron overload, which is often associated to hereditary hemochromatosis (HH). The prevalence of C282Y in HH is lower in Italy (67%) than in Northern Europe.

Aim: The aim of the study was to investigate the prevalence of HFE mutations in patients with serological signs of iron overload, both in presence and absence of liver disease.

Patients and Methods: The study included 39 unrelated patients referring to us with transferrin saturation >50% and/or serum ferritin >400 µg/L. Patients were divided into group A (no concomitant liver disease; n=13) and group B (viral or alcoholic chronic hepatitis; cirrhosis; porphyria; n=26). HFE genotyping was performed by PCR analysis using Rsa I and Dpn II to detect C282Y and H63D mutations, respectively.

Results: In group A, 7 out of 13 patients (54%) were C282Y or H63D homozygotes or compound heterozygotes. Excluding five patients with ferritin <1000 µg/L or with causes of secondary iron overload, a homozygous C282Y or compound heterozygous was present in 6 out 8 pts (75%). In group A 8 patients underwent liver biopsy: hepatic iron overload was shown in all C282Y and H63D homozygotes and compound heterozygotes. In group B 25 patients (96%) had normal genotype or heterozygous mutations, mainly H63D (46%). Among the 11 patients with cirrhosis none had HFE homozygous or compound heterozygous. Normal genotype was detected in 2/3 of patients with chronic liver disease HCV related, regardless of the stage of liver damage. In group B 10 patients underwent liver biopsy; all but one had liver iron overload.

Conclusion: Our preliminary results show that iron overload appears to be related to HFE mutation in the asymptomatic subjects; in contrast it is unrelated in patients with concomitant liver disease. We suggest that the HFE genotyping might be used instead of biopsy in subjects with biochemical signs of iron overload in the absence of associated liver disease. Furthermore, it might be the only test capable to identify HH in patients with liver disease and iron overload.

Adams PC et al. Genotypic/phenotypic correlation in genetic haemochromatosis: evolution of diagnostic criteria. Gastroenterology

S7.4

Chiarugi P.[1], Fanelli A.[1], Del Genovese A.[1], Alessandri F.[2], Boddi V.[3], Messeri G.[1]

INDIRECT DETECTION OF RECOMBINANT HUMAN ERYTHROPOIETIN BY MULTIVARIATE ANALYSIS

1) Laboratorio Analisi Chimico-Cliniche Careggi, Firenze
2) Federazione Ciclistica Italiana
3) Patologia Generale, Università di Firenze

The aim of our study was to evaluate by a multivariate approach the hematological profile of 23 top level cyclists, subjected to the health checks of the Italian Cycling Federation, in order to recognize significant changes that could be explained by recombinant human erythropoietin (rhEPO) intake. This group (group 1) was compared with 22 rhEPO non-abusers athletes (group 2) and 38 non-athletes healthy men (group 3). Discriminant function analysis was performed including in the model the following four variables: ferritin, hematocrit, reticulocytes and serum transferrin receptor. Two discriminant functions, that combined informations from these four parameters and allowed to reduce the model into a bidimensional space, was obtained. The first discriminant function accounted for almost 84% of the variability among the groups and the predictor variables showed, on the basis of their standardized coefficients, this order of importance: ferritin, serum transferrin receptor, reticulocytes and hematocrit. A good discrimination among the three groups was observed (Wilks' lambda=0.345, $p<0.0001$). Squared Mahalanobis distances (D^2) between group centroids indicated a clear separation between the group 1 and each of the others groups (1 vs 2 $D^2=5.783$, 1 vs 3 $D^2=7.352$), whereas the centroids of thr groups 2 and 3 were quite close ($D^2=1.690$). The D^2 and the discriminant scores could also quantify the distance of each case under examination from both the non-rhEPO abuser groups. We conclude that such a multivariate analysis could identify hematological profiles consistent with a suspect rhEPO misuse.

OM.1

Ceriotti F.[1], Panteghini M.[2]
(for the SIBioC Committee on Enzymes)

ESTABLISHING A REFERENCE SYSTEM IN CLINICAL ENZYMOLOGY

[1]Laboratorio Analisi, Istituto Scientifico H. San Raffaele, Milano and [2]Laboratorio Analisi 1, Azienda Ospedaliera 'Spedali Civili', Brescia, Italy

Even if the "recommended method" approach led to significant improvements in clinical enzymology, it is now clear that the goal of a single, universally used method for measuring the catalytic concentration of a given enzyme will never be achieved. In fact, over the last 25 years, the recommended standard procedures have been increasingly corrupted to be adapted to the use in clinical laboratories. This fact makes impossible the definition of common reference intervals, creates confusion among patients and practitioners, and causes problems to the EQAS organisers. For these reasons, the IFCC, by means of its Working Group "Calibration in clinical enzymology", launched a project for the establishment of a reference system (RS) in clinical enzymology. The RS is based on three hinges: a) reference methods (RM), extensively evaluated and carefully described, b) certified reference materials (CRM), and c) a network of reference laboratories (RLN) operating in a highly controlled manner. In RM resides the accuracy of the RS. The original IFCC recommended procedures have some characteristics that are impractical to be transferred in routine: temperature (30°C), need of sample blank, excessively prolonged reaction times, limited linearity. Moreover, they are not described in a detailed manner leaving some room for interpretation. The procedures have been therefore slightly modified to optimise them at 37°C and avoid sample blanking, also re-evaluating incubation times and linearity. This has led to the definition of very detailed standard operating procedures (SOP) for ALT, AMY, CK, GGT, and LDH (ALP and AST under development). CRM are used to control the operation of the NRL and, if commutable, they can be used directly (at least by the manufacturers) to transfer the accuracy of the RM to the field procedures. Partially purified materials prepared years ago by the Community Bureau of Reference (BCR) have been recertified by the NRL for ALT, CK, GGT, and LDH activities. NRL consists of a small group of laboratories (n=13, world-wide located) trained to perform manual procedures, with self-made reagents, on carefully calibrated instrumentation and following the above-mentioned SOP. The NRL is also able to perform split sample comparisons on fresh patient samples between RM and routine methods, thus directly certifying commercial systems. In conclusion, this RS 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 specificity (i.e. they are measuring the same quantity).

OM.2

Mosca A.
(for the SIBioC Committee on Enzymes)

MULTICENTER EVALUATION OF SERUM CHOLINESTERASE METHODS

Dept. of Sciences & Biomedical Technologies, L.I.T.A. of Segrate, University of Milan, Italy

Introduction. Seven commercially available methods measuring serum cholinesterase (CHE; EC 3.1.1.8) activity by different substrates and indicator reactions (benzoylcholine / choline oxidase - peroxidase, butyrylthiocholine / DTNB, butyrylthiocholine / hexacyanoferrate(III), p-hydroxybenzoylcholine / NADP hydroxylase, propionylthiocholine / DTNB, and succynildithiocholine / DTNB) have been compared. Seventy serum samples from healthy blood donors with normal to low CHE catalytic activities (<7 kU/L, butyrylthiocholine / DTNB method at 37 °C) were collected during a three-month period and screened for dibucaine and fluoride numbers by the method of Kalow and Genest. Among them, 48 samples (25 with normal and 23 with low CHE activity) were genotyped. In addition, a set of 82 serum samples collected from laboratory routine were analyzed in order to assess inter-method bias.
Results. 1) Method reproducibility, assessed by the mean of the differences between duplicates and expressed as CV, ranged from 0.9% (propionylthiocholine and succynildithiocholine methods) to 2.5% (p-hydroxybenzoylcholine). 2) The different methods were all correlated each other. The correlation coefficients (n=152) of each method with the butyrylcholine / DTNB method ranged from 0.9835 (succynildithiocholine) to 0.9970 (butyrylthiocholine / hexacyanoferrate(III)). 3) When dibucaine number was used, the inter-individual variability (CV 8.8%) was found smaller than using the succynildithiocholine method (CV 17.7%). 4) With regard to the 19 carriers of the Asp70Gly mutation, the best correlation among CHE activity and dibucaine number was found when the enzymatic activity was measured by the succynildithiocholine method.
Conclusions. In the case of CHE, the correlation between enzymatic activity and genotype is complex because more than one mutation is frequently inherited. With regards to the more frequent mutation found in our population ('atypical' enzyme), we may conclude that dibucaine and fluoride numbers are still useful to predict this CHE genotype.

OM.3

Panteghini M.

PANCREATIC AMYLASE: ESOTERIC TEST OR PRIMARY STEP IN THE EVALUATION OF PATIENTS WITH ABDOMINAL PAIN?

Laboratorio Analisi Chimico Cliniche 1, Azienda Ospedaliera 'Spedali Civili', Brescia, Italy

Increased activity of total amylase in serum has long served as a marker of pancreatic inflammation. Many experiences have made clear, however, that this marker has distinct limitations, especially a very poor specificity for acute pancreatitis. A foregone way in which the nonspecificity of amylase determination may be overcome is by direct measurement of its pancreatic isoenzyme (P-AMY). Since Rauscher and Gerber (1) developed the homogeneous immunoassay employing the synergistic action of two different monoclonal antibodies to selectively inhibit the salivary form, a simple and economic approach to direct quantitation of P-AMY has been made available.
The test can actually be performed inexpensively and rapidly using instrumentation available in most hospital laboratories. Its routine use since ten years in our clinical laboratory, after the deletion of the obsolete total amylase test from the pancreatic panel, has led to more accurate diagnosis and avoidance of further laboratory and costly radiographic investigations in about 12% of patients studied, those showing hyperamylasemia of extrapancreatic origin. Other practical experiences conducted in recent years confirm our results.
It is therefore very surprising that P-AMY determination is not used more widely. In fact, a wide acceptance of the simple immunochemical method for P-AMY determination would also lead to a better comparability of results between laboratories in different hospitals and countries, by eliminating the standardization problems that still exist for total amylase assays. Although these facts strongly suggest that P-AMY determination should supplant and replace total amylase assay as primary test for the diagnosis of acute pancreatitis, total amylase measurement continues to be a widely used test in many clinical laboratories, remaining among one of the most frequently requested clinical assay and still being a component of the test menu of several emergency services. The main reason is the cultural one. With regard to this point, increased cooperation and discussions between clinicians and laboratorians are required to obtain more cost-effective protocols using better biochemical tests in the differential diagnosis of acute abdominal diseases.

References

1. Rauscher E, Gerber M. Pancreatic alpha-amylase assay employing the synergism of two monoclonal antibodies. Clin Chim Acta 1989;183:41-4

ANALISI DELLE PROTEINE IN BIOCHIMICA CLINICA: STATO DELL'ARTE E PROSPETTIVE

Sala A

Venerdì 15 settembre, ore 9.00-10.30

S8.1

Whicher J.

STATE OF THE ART IN PROTEIN ANALYSIS: THE IMPACT ON CLINICAL PRACTICE

Division of Clinical Sciences, The Medical School, University of Leeds, Leeds, UK

The value of plasma protein investigation lies in the use of profiles of investigations comprised of quantitative measurements and qualitative tests using electrophoretic separation and characterisation. Integrated reporting of results using appropriate reference ranges and laboratory data bases allows useful diagnostic, prognostic and therapeutic information to be provided by the laboratory to the clinician. This is a more effective process than the provision of uninterpreted numerical data. Unfortunately this information-led approach is under threat due to the inexorable developments in medicine. In the past, new technologies and biomedical advances were integrated into laboratory practice by the development of skills, both in techniques and interpretation, by education and training. Unfortunately the overriding pressures of cost reduction have given rise to a culture of increasing automation and reduction of cost per test, increased numbers of simple tests with a yield of fewer pathological results. This is causing laboratory integration, loss of staff and crucially, loss of skills in techniques and interpretation. Health care managers are happy, clinicians are generally unaware that the process is occurring. Skills lost will be very difficult to replace. A more desirable alternative to this destructive strategy is a culture of knowledge-based tests. In this approach research and clinical experience are translated into the use of more specific, accurate and advanced tests, reduction in the use of uninformative tests with a consequent increase in the proportion of pathological results. It is here that real cost savings can be made. In 1997 the world wide pharmaceuticals bill was $120 billion, the expenditure on plasma protein analysis was only $4,000 million.

Serum protein testing is essential to the rational management of immunocytomas, immunological, allergic and chronic inflammatory diseases. However we now know that the full potential lies in the prevention or mitigation of disease due to the ability to detect subclinical illness of an inflammatory or immunological nature not easily detected by other means. At the turn of the millennium the field of plasma protein analysis is at its most exciting for 20 years with the introduction of new protein measurements such as cytokines, adhesion molecules and Cystatin C. Much more sensitive and accurate methods are appearing allowing 'old' proteins such as C-reactive protein and Serum amyloid A protein to have new roles. Proteins are being used for triaging and risk assessment already in cardiovascular disease, lung disease, rheumatic diseases and some cancers.

S8.2

Bini L., Allegria M., Liberatori S., Magi B., Raggiaschi R., Pallini V.

PROTEOME APPROACH TO THE CLINICAL ANALYSIS

Dip. Biologia Molecolare, Sez. Chimica Biologica, Università degli Studi di Siena

Proteome technologies comprehend: a) large-display of protein components (primary and post-translationally modified gene products) by two-dimensional electrophoresis. Present technology allows separation of up to 3000 spots in a single gel; b) spots assignment by automated Edman degradation, mass spectrometric procedures, immunoblotting and computer-aided matching to reference 2D maps in accessible internet databases; c) computer-aided analysis of electrophoretogram images, in order to describe spots and to define their association to health or pathology.

These procedures allow the large-scale search for new protein markers. With this approach we have characterised acute phase proteins in human sera (1,2), proteins of the human pathogen *Chlamydia trachomatis* (3), and protein markers in human ductal breast cancer (4).

All samples were denatured in 8M urea, 4% CHAPS, 40 mM Trizma-base, 65 mM DTE and subjected to 2D electrophoresis as described (5). First dimension (isoelectric focusing) was carried out on a non linear immobilised pH gradient gel (pH 3-10) and the second dimension (SDS-PAGE) was performed on a 9-16 % linear gradient polyacrylamide gel. Gels were stained with silver nitrate. 2D immunoblotting was obtained as described by Magi et al. (6). Spot identification was performed by N-terminal microsequencing, MALDI-TOF MS, and by computer-aided matching with 2D reference maps present in internet, such as Swiss-2DPAGE (http://www.expasy.ch/ch2d/), Danish Human and mouse 2-D PAGE databases (http://biobase.dk/cgi-bin/celis), Siena-2DPAGE (http://www.bio-mol.unisi.it/2d/2d.html). Computer analysis was performed by Melanie 3 system (GeneBio).

In this presentation we will describe some results obtained with the proteome technologies on clinical analysis. These researches are in progress in our laboratory and consist in:

1- cancer-associated spots in breast carcinoma;
2- study of the human antibody responses to infectious diseases;
3- new markers for pathology in biological fluids;
4- tyrosine-phosphorylated proteins in hypoxic versus normoxic synaptosomes.

References:

1- Bini L. *et al.* (1992), *Electrophoresis* 13, 743-746.
2- Bini L. *et al.* (1996), *Electrophoresis* 17, 612-616.
3- Bini L. *et al.* (1996), *Electrophoresis* 17, 185-190.
4- Bini L. *et al.* (1997), *Electrophoresis* 18, 2832-2841.
5- Bjellqvist B. *et al.* (1993), *Electrophoresis* 14, 1357- 1365.
6- Magi B. *et al.* (1999), *Methods Mol. Biol.* 112, 431-443.

S8.3

Gasparro C., Aguzzi F., Zorzoli I., Moratti R., Merlini G.

AGAROSE GEL ELECTROPHORESIS VERSUS CAPILLARY ZONE ELECTROPHORESIS IN LIGHT CHAIN RELATED AMYLOIDOSIS

Biotechnology Research Lab., Servizio Analisi Chimico Cliniche, IRCCS Policlinico San Matteo, Pavia, Dip. Biochimica, Università di Pavia

The aim of this work was to compare the sensitivity of agarose gel electrophoresis (AgEP) and capillary zone electrophoresis (CZE) in detecting monoclonal components (MC) in light chain related amyloidosis (AL), a severe disease caused by low-concentration MC.
Forty-eight sera from consecutive, unselected, patients with AL amyloidosis were collected at the Center for Systemic Amyloidoses, IRCCS Policlinico San Matteo, Pavia. The fresh sera were analyzed in parallel by AgEP (performed on home-made gels, according to Jeppsson et al[1], with electrophoretic run of at least 8 cm, and evaluated by visual inspection) and by CZE (performed on Paragon CZE 2000, Beckman, according to the manufacturer's instructions). All sera were also analyzed by immunofixation (IF) which was considered the gold standard.
Immunofixation detected a monoclonal component in 46 patients, of these patients AgEP showed a MC in 44 (96%) and CZE in 41 patients (89%) (p = ns). The MC concentration was below the threshold of densitometric evaluation in 25 patients, and the MC median concentration in the remaining 19 patients was 10 g/L (range 4.9-18.9). All these discrete MC were detected by AgEP and CZE. As expected, there was a large preponderance of lambda over kappa light chain (38/12), with 4 patients having both kappa and lambda MC. In 15 patients (33%) the MC was composed by light chains only.
CZE showed a good sensitivity in detecting small, but clinically very relevant, MC. It gave results similar to those of AgEP carried out in optimal conditions.

1. Jeppsson JO, Laurell CB, Franzen B. Agarose gel electrophoresis. Clin Chem 1979;25:629-38

S8.4

BrunoFranco M., Montani A., Vimercati M.E., Vanelli S.

IDENTIFICATION AND TYPIFICATION OF LITTLE MONOCLONAL COMPONENTS BY CAPILLARY ELECTROPHORESIS

Dip. Pat. Clin., ASL Provincia di Lodi, Presidio di Casalpusterlengo (LO)

In this study the use of immunosubtraction capillary electrophoresis for the typification of little monoclonal components has been evaluated, comparing this method with the , well consolidated , immunofixation (Paragon gel IFE). In our experience we have already proved the good quality of the immunosubtraction in the typification of big monoclonal components, and we are carrying out a continous study to estabilish the utility of application also for little monoclonal components. We have used an electrophoresis immunosubtraction kit Paragon CZE 2000, applied to dedicated capillary electrophoresis system. Sample serum reacts with a specific antibody (against light and heavy chains) fixed on sepharose granules. The instrument performs automatically reference capillary electrophoresis and subsequent series of electrophoresis after serum reaction with different antibodies. The changes in the electrophoretic profile allow the typification of the monoclonal component, according to antibody specificity causing the disappearance of the monoclonal peak. The cases we have investigated concern little monoclonal components found in routine electrophoresis. For each sample we have performed both the immunosubtraction and the immunofixation.
RESULTS: For the estimation of this method 12 healthy subjects (blood donors AVIS) and 16 pathological subjects with an evident,even if little monoclonal component, have been submitted to immunofixation and immunosubtraction. The negativity of 12 blood donors, for monoclonal components, obtained with capillary electrophoresis/immunosubtraction has been totally confirmed by agarose gel electrophoresis/ immunofixation. The specificity of capillary electrophoresis/ immunosubtraction compared with agarose gel electrophoresis/immunofixation is 100%. A peculiarity of the immunosubtraction of healthy subjects is that the reference electrophoretic profile is pratically identical to those of different immunosubtractions, in spite of a reduction of the gamma band, particulary evident for use of antibodies against IgG and K chains. This behaviour confirms that the most antibodies of an healthy subject is represented by an heterogeneous population of IgG-K. The concordance of immunosubtraction versus immunofixation have been evaluated by typifying the monoclonal components of 16 pathological subjects with two different methods. We have found a complete concordance. In the present study we have identified: 6 components IgG-K, 6 IgG-λ, 1 IgG-K/λ, 1 IgM-K, 2 IgA-λ. From these results we can assert that immunosubtraction certainly represents a valid technique both in the typification of big and little monoclonal components. In our laboratory experience we are trying to introduce the immunosubtraction as first level method for monoclonal components typification and to use immunofixation only for uncertain cases.

S9.1

Conti A.A., Gensini G.F.

EVIDENCE BASED MEDICINE: OVERVIEW AND APPLICATIONS IN CLINICAL BIOCHEMISTRY

Department of Internal Medicine and Cardiology, University of Florence, and Italian Centre for Evidence Based Medicine, Florence, Italy.

Evidence Based Medicine (EBM) is a scientific-cultural movement that is nowadays implemented in an international setting. In the critical interpretation of the Italian Centre for Evidence Based Medicine it represents the methodological search of the best possible solution for a clinical problem, using the most robust evidence available in the biomedical literature, collected and critically appraised in the light of physician's experience and expertise, in order to improve individual patient care. This approach takes place in the frame of the risk/benefit and cost/effectiveness ratios.

The EBM method largely disseminated in the last few years at the same time as other phenomena that have characterised the medical world. We refer to the powerful development of the information technologies in biomedicine, to the rapid and often uncontrolled proliferation of medical scientific information, to the growing proofs showing that practical health care does not mirror research acquisitions.

To provide a solution to the problems deriving from these phenomena, i.e. the incorrect and inappropriate use of electronic databases, the poor quality control of research evidence and the provision of health care not evidence based, EBM has elaborated a methodology useful for the multi-dimensional solution of clinical issues.

In this scenario individual preparation, culture and sensibility are not ruled out; on the contrary, they gain value, as they are placed in the perspective of modern clinical epidemiology. Actually, Evidence Based Medicine has the purpose of building a therapeutic physician-patient alliance, alliance based on the largest share of robust and up-dated evidence, to provide an integrated management of the individual patient and not of the sole disease management.

With special reference to clinical biochemistry, we believe that every of its traditionally recognised phases (the pre-analytical one, the analysis laboratory and the post-analytical phase) can take advantage of the correct implementation of the method of Evidence Based Medicine.

Papers recently published in leading medical journals point to the fact that the studies evaluating the characteristics of laboratory assays do often not satisfy methodological quality standards dealing with the independent evaluation of the tested assay, the judgement on the precision and the reproducibility of the method studied, the analysis of the correctness in the description and application of laboratory procedures. The ever-growing implementation of EBM method in the biochemistry world appears particularly appropriate also from the clinician standpoint, in order to prompt the communication among biochemists and clinicians using a common scientific language.

Biomedical literature shows that many clinical biochemistry areas urgently need quantitative systematic reviews according to EBM criteria, criteria that should be elucidated in educational packages precious for the increase of the professional qualification of the clinical biochemist.

Clinical biochemistry is a really important and promising field for the promotion of the EBM diagnostic features, and this is even more important given that EBM has been implemented up to now almost only in the therapeutic realm.

The shared critical appraisal of sensitivity, specificity and predictive values of biochemical diagnostic tests in the light of EBM appears as the common benchmark for the more and more requested integration among laboratory medicine and clinical practice.

References:

- Gensini GF, Galanti G, Conti AA. La medicina basata sulle evidenze: prospettive, applicazioni e confini. Il Policlinico 1998; 105: 592-601.
- Gensini GF, Conti AA. EBM e pratica clinica: quanto è simile il paziente dello studio clinico al paziente della realtà quotidiana? Evidence-Based Medicine (edizione italiana) 1999; 3(2): 3-4.
- Gensini GF, Conti AA. Il ragionamento bayesiano alla luce della medicina basata sulle evidenze: implicazioni in clinica. Evidence-Based Medicine (edizione italiana) 2000; 4: 35-6.
- Moore RA. Evidence-based clinical biochemistry. Ann Clin Biochem 1997; 34: 3-7.
- Moore RA. On the need for evidence-based clinical biochemistry. Evidence-Based Medicine (English edition) 1998; 3: 7-8.
- Reid MC, Lachs MS, Feinstein AR. Use of methodological standards in diagnostic test research. Getting better but still not good. JAMA 1995; 274: 645-51.

S9.2

Freedman D.B.

EVIDENCE BASED LABORATORY MEDICINE – AN OVERVIEW

Luton & Dunstable Hospital NHS Trust, Luton, Bedfordshire, UK

Evidence based laboratory medicine (EBLM) aims to advance clinical diagnosis and management of disease through researching and disseminating generalisable new knowledge which meets the standards of critical review on clinically effective practice of laboratory investigations. EBLM combines methods from epidemiology, statistics and social sciences with traditional molecular 'bench' science to evaluate the role of investigations in clinical decision-making and outcomes for patients.

The way forward:

In general terms, the approach of evidence based medicine (EBM) identifies real clinical problems which are translated into questions answerable by literature search and critical review, or by appropriate research. The results are then properly interpreted to yield a solution which is applied and evaluated in practice. Any reasons for requesting a laboratory investigation on a patient should be to confirm or exclude a diagnosis, to monitor disease progress or treatment, or to detect changes that are not clinically obvious. To achieve this, the appropriate test should be selected and, if a test cannot provide the information desired, it is unnecessary, wasteful and potentially misleading.

This sounds a very simple and logical concept, but, for decades, Clinical Biochemists have been concerned about the steady increase in requests for laboratory tests, the increasing costs and, more importantly, feel that many of the requests are inappropriate or unnecessary and not based on sound evidence.

If an approach is beneficial, cost effective and affordable, it should be available – if it is not, it should be abandoned. *(UK Department of Health, 1992) 1*

The systematic evaluation of diagnostic tests could be expected to provide benefits in several areas:

1. Elimination of poor or useless tests before they become widely available
2. Improve the quality of diagnostic test information
3. Reduce health care costs, and
4. Most importantly, improve patient care

A topical example is that of increasing the workload of many laboratories in the measurement of Prostatic Specific Antigen (PSA) as a screening test for prostate cancer. This is despite the fact that the recent 'Effectiveness Matters' bulletin from the UK NHS Centre for Reviews and Dissemination has recommended 'routine testing of asymptomatic men for prostate cancer should be discouraged, irrespective of family history'. Purchasers should not fund screening services for prostate cancer and, most importantly, patients enquiring about PSA should be clearly informed of the current state of evidence about the benefits and harms of screening and treating asymptomatic men.

EBLM should be defining when laboratory tests are appropriate and those tests that are not appropriate, and automatically generate evidence on the sensitivity and specificity for each investigation.

We in the laboratory need to review and change our current thinking. Analytical and post-analytical phases are often detached from the pre-analytical phase, i.e. 'why should you do the investigation in the first place?' It must also be understood that many investigations (tests) do not give a 'yes/no' answer.

What do clinicians want from a laboratory?

1. To be able to discuss a patient's illness with a colleague
2. To abandon the reporting of normal ranges
3. To have evidence available to support the validity, importance and clinical usefulness of a test.

In the UK, the Association of Clinical Biochemists, through the Scientific Committee, had led very many task forces where evidence has been sought to justify the use of a biochemistry test. However, it is important that the profession becomes more involved and this will include undertaking systematic reviews of the evidence in important areas of clinical biochemistry, understanding how to critically appraise the literature, seek ways in which the reports of diagnostic tests published in biochemistry journals can reflect the acceptable standards of reporting, using standards cited by *Read et al* and, finally, examine ways in which the clinical and cost effectiveness information of a laboratory can be best disseminated and subsequently implemented.

The Oxford/East Anglia Evidence Based Clinical Biochemistry Initiative has attempted to address this issue. The objective of the group was to show that evidence based clinical biochemistry (EBCB) can be achieved, formulate rules for promulgation of EBCB and undertake a systematic review of a commonly requested laboratory test. The Group discovered some very interesting findings in monitoring of hypothyroid patients on thyroxine replacement. This initiative has resulted in some understanding of the rules of applying EBM to laboratory investigations and it should be recommended that modules on EBM, systematic reviews and critical appraisal of the literature are included in educational programmes for those preparing for professional qualifications in clinical biochemistry.

It has to be appreciated that diagnostic testing will need to be approached differently; there is a need to provide more and better information about the effectiveness of tests and how that can be built into a diagnostic process will be a big challenge.

Diagnostic tests are, or should be, an integral part of EBM.

It is about incorporating good science into everyday clinical practice and must be explored for pathology and laboratory medicine in general.

References

1. The Advisory Group on Health Technology Assessment, Department of Health: 'Assessing the effects of health technologies'. 1992: 8
2. The University of York and NHS Centre for Reviews and Dissemination.
3. Moore, R.A. 'On the need for Evidence Based Clinical Biochemistry' *Evidence Based Medicine*. 1998; 1:7-8
4. Read, M.C., Lachs, M.S., Feinstein, A.R. 'Use of methodological standards in diagnostic test research: Getting better but still not good'. JAMA, 1995; 174: 645-51

S9.3

Trenti T., Baraldi E., Campioli D., Casolari C., Marchegiano P., Roncaglia R.

APPROPRIATENESS BASED CRITERIA IN THE SELECTION AND INTRODUCTION OF NEW DIAGNOSTIC LABORATORY TESTS

Dipartimento di Medicina di Laboratorio, Azienda Ospedaliera di Modena, via del Pozzo 71, Modena

In Laboratory Medicine an increasing number of diagnostic tests are proposed to be introduced when the old tests are seldom removed, this together with the limited founds underlines the challenge to provide strong evidence for the utility of new and/or different tests. In this light care maps, clinical practice guidelines and outcomes measure are tools used to provide the best care with the best use of resources, however often there has been a minimal involvement of the laboratory professionals in managing this process. In this light professionals working in the Department of Laboratory Medicine developed a team to improve the culture and the discipline of an evidence based approach to laboratory medicine working on patient focused outcomes. As first action the group focused the importance to define criteria in evaluating the implementation of new diagnostic tests by design a way to measure test impact on patients outcomes. As the first step was introduced a new application form that have to be filled by the actor of the new test demands (physicians outside laboratory or laboratory professionals). In the first part of this form it was asked to illustrate the proposed new test not only in terms of analytical procedures as appropriate reference intervals, acceptable CV and analytic considerations but to demonstrate the expected outcomes for patients, the prevalence of the disease, the level of demand, the cost effectiveness. The second part focused on the presence of systematic review, the validity of the question, the adequacy of the proposed use including cost. The group decide to introduce or not the test in the second step where, nevertheless the importance to evaluate parameters as sensitivity and specificity and predictive value, an increasing emphasis is placed in the use of likelihood ratio and acceptable evidence of both clinical and cost effectiveness coupled with systematic assessment of outcomes using source of information as Cochrane Database, Evidence Based Medicine Publications and Clinical Guidelines etc. In the case of new test introduction in laboratory, the third step is to place the test into diagnostic and management protocols or clinical guidelines working cooperatively with clinicians. The final stage should be the identification of outcomes or the relative cost effectiveness of alternative means. This to promote and disseminate the recognition of the question that is being asked when a test is requested and the availability or not of evidence to answer the question that is the core of this culture.

S9.4

Pagani F., Panteghini M.

EVALUATION OF SENSITIVITY OF CARDIAC PROTEINS IN THE DETECTION OF ACUTE MYOCARDIAL INFARCTION THROUGH AN EVIDENCE-BASED APPROACH

Laboratorio Analisi Chimico Cliniche 1, Azienda Ospedaliera 'Spedali Civili', Brescia, Italy

Systematic reviews are scientific investigations with planned methods that use original studies as subjects and synthesize the results of multiple studies using strategies to limit bias and random error. The aim of this study was to determine whether, using this evidence-based approach, the results of the papers found in the literature are valid and scientifically rigorous to be used to definitely address the problem of the cardiac marker sensitivity in detection of acute myocardial infarction (AMI). In particular, we have reviewed the biomedical literature published over the last 10 years in order to try to establish the diagnostic sensitivities of myoglobin, creatine kinase (CK)-MB isoenzyme, determined as mass concentration, CK-MB isoforms, and of the two cardiac troponins, troponin I and troponin T. We used the methodology recommended by the IFCC Committee on Systematic Reviewing in Laboratory Medicine, which can be summarized as follows: 1. retrieve the relevant literature; 2. validate the studies found using *a priori* formulated inclusion/exclusion criteria for judging their eligibility to be included in the analysis; 3. extract information from the eligible studies and analyze the data; 4. identify areas for further research.
A clear final message derived from this systematic analysis is the unacceptable poor diagnostic sensitivity of all evaluated markers at patient admission with substantial failure to rule out AMI at this time. Decisions for triage just based on an admission sample have the potential to result in significant diagnostic errors. Only serial marker measurements are useful for accurate triaging of chest pain patients. Myoglobin is at present the most sensitive of the markers studied for excluding *early* AMI with an optimum timing of sampling at patient presentation and approximately 4 hours later. However, this marker cannot be used by oneself as the portion of patients admitted to the hospital with a late infarction could be missed. The early rise of CK-MB mass and troponin T is similar. Maximum sensitivity of these two parameters is achieved by the analysis of a second sample 6 to 12 hours after admission. Additional larger comparative studies are needed to definitively address the question of which troponin shows earlier release after myocardial damage, and to clarify the role of CK-MB isoforms as possible early marker of myocardial infarction.

S9.5

Canini S.[1], Venturini P.[2], Famularo L.[1], De Biasio P.[2]

A COST/EFFECTIVENESS COMPARISON OF PRENATAL SCREENING FOR DOWN'S SYNDROME IN THE FIRST AND SECOND TRIMESTER OF PREGNANCY

[1] Clinical Chemistry Laboratory; [2] Obstetrics and Gynaecology Unit "G.Gaslini" Institute – Genova

The purpose of our study is to compare the cost/effectiveness ratio of two kinds of prenatal screening for the estimation of Down's syndrome risk, in order to decide on the opportunity of replacing the classical second-trimester Tri-test with the new "combined test" performed at the first trimester of pregnancy. Second trimester screening performance was evaluated on 17168 women, who underwent Tri-test in 1991-1998 and whose gestation outcome is known. Alfa-fetoprotein, unconjugated oestriol and total hCG were assayed by RIA methods (Johnson & Johnson Ortho). The gestational age was determined by ultrasound measurement of BPD and ranged from 15 to 19 weeks. Combined test at first trimester of pregnancy performance was evaluated on a 1467 women retrospective study. Nuchal translucency and CRL for gestational age evaluation (10-13 weeks) were measured by trans-abdominally ultrasound scan. Pregnancy associated plasma protein A and free beta hCG subunit were determined by IRMA methods (Johnson & Johnson Ortho). The median maternal age of the two populations was similar (31 y.). Down's syndrome risk evaluation was performed by software (LMS Ltd. London) and the chosen risk cut-off level was 1 in 350 at term. There were 27 Down's syndrome affected pregnancies in the second trimester screened population and 20 resulted at risk. There were 7 Down's syndrome affected fetus in first trimester retrospective study and 6 resulted at risk by the combined test. The affected pregnancy unit cost was calculated by dividing the sum spent for all the performed tests and for the consequent prenatal diagnosis by the number of affected cases, who resulted at risk on the basis of the screenings. The Tri-test sensitivity resulted 74 % and its efficiency was 94 %. Combined test sensitivity was 86% and its efficiency was 97 %. The cost of one combined test appeared greater than that of one Tri-test, because of specialized ultrasound scan and kits larger cost. But the identification of one affected fetus costed about 50 millions Italian lire by Tri-test and only 19 millions Italian lire by first trimester combined test. In fact this new screening shows a higher detection rate and a lower false positive rate. Even if these result are based on two populations a little different, it seems to be advisable to replace Tri-test by "combined test", that is performed in early pregnancy, with advantages on woman psychological and physical well-being.
Essential reference: Prenat Diagn 1999; 19:360-63.

WORKSHOP: I MARCATORI DI LESIONE MIOCARDICA
Sala C
Venerdì 15 settembre, ore 9.30-10.30

WS.1

Panteghini M.
(for the ANMCO-SIBioC-SIMeL Working Group on Markers of Myocardial Damage)

RECOMMENDATIONS FOR THE USE OF CARDIAC MARKERS

Laboratorio Analisi Chimico Cliniche 1, Azienda Ospedaliera 'Spedali Civili', Brescia, Italy

This presentation is intended to give evidences and suggestions from the ANMCO – SIBioC – SIMeL Working Group on Markers of Myocardial Damage on the use of biochemical markers for the evaluation of patients with acute coronary syndrome (ACS). There is general agreement that both 'early' and 'definitive' markers of myocardial damage are necessary for the optimal triage of patients presenting in the emergency room with suspected myocardial infarction (MI). Currently, myoglobin is the marker that most effectively fits the role as an 'early' marker (i.e. increased in blood within 6 h after onset of symptoms), whereas 'definitive' markers (i.e. increased later but abnormal for several days) are cardiac-specific troponins. This two-marker approach results especially useful in those hospitals attempting to triage patients between emergency department holding areas and inpatient beds. Since the sensitivity of the initial electrocardiogram (ECG) is only 50% for detecting MI, the use of biochemical markers may significantly contribute to the early diagnosis and become relevant just when ECG is not diagnostic. Conversely, there is no need for the use of any biochemical marker when the clinical diagnosis of MI is unequivocal, other than for diagnosing reinfarction, estimating the infarct size, and monitoring thrombolytic therapy. These applications are however optional and not definitively supported by scientific evidence.
New more sensitive biochemical markers, particularly the cardiac troponins, are presently also the best criterion to detect the presence of small myocardial cell damage. With regard to this point, two decision limits are probably needed at present for the optimum use of troponins: a low abnormal value suggesting the presence of myocardial damage and a higher value suggesting the diagnosis of MI according to traditionally WHO-criteria. Chest pain patients with results for troponin between these two limits should be clearly labeled as having 'myocardial damage' and appropriately managed on a prospective basis in virtue of their worse prognosis. If in the next future the biochemical WHO-criterion for MI diagnosis will be changed using increased troponin values in the setting of acute ischemia instead of the classic CK-MB findings, there would be only a single cutoff limit for troponin corresponding to the upper limit of the reference distribution, reflecting the incremental risk associated with increasing concentrations of the marker, consistent with the continuous injury concept of ACS.

WS.2

Galvani M.

BIOCHEMICAL CRITERIA IN THE DIAGNOSIS OF MYOCARDIAL INFARCTION: SHOULD THEY BE REVISITED AND HOW?

Coronary Care Unit, Ospedale G.B. Morgagni, Cardiovascular Research Unit, Fondazione Sacco, Forlì, Italy

There has been nearly three decades of progress since the original WHO criteria for acute myocardial infarction (AMI) were proposed. These criteria, originally elaborated for epidemiological purposes, have widely entered clinical practice. They consist of different clinical, electrocardiographic, and biochemical findings which are classified as unequivocal or equivocal; in general, an AMI is diagnosed when two out of three criteria are present. The biochemical criterion consists of the presence of characteristic serial changes of the so-called "cardiac enzymes", particularly the MB isoenzyme of creatine kinase (CK-MB). In the past years the WHO criteria have been progressively refined in the direction of a more precise quantification of the degree of CK-MB elevation required to satisfy the AMI diagnosis. It is now accepted that an AMI is present when a two-fold elevation of CK-MB is observed. The significant amount of elevation required for the diagnosis reflects the sub-optimal sensitivity and specificity of CK-MB for the detection of myocardial damage. However, the discovery of new biomarkers of myocardial damage, i.e. cardiac troponin I (cTnI) and T (cTnT), with enhanced sensitivity and specificity, has raised the question whether they should become the new "golden standard" for the AMI diagnosis. The improved tissue specificity of the troponins, compared to CK-MB and other conventional markers, is well established. This specificity is coupled with an improved sensitivity for the detection of cardiac injury. Along with the prolonged time window during which cardiac troponins are elevated, this allows the detection of a larger number of patients at risk for subsequent adverse cardiac events.
The first issue to take into consideration when cardiac troponins are proposed as the new diagnostic paradigm is their specificity for the detection of irreversible cardiac injury. The troponin subunits are present in the cytosol (3% for cTnI, 5% for cTnT) and they are smaller than CK-MB. Therefore, they could theoretically be released into the circulation even in the presence of a reversible damage to the cell membrane. In a recent experimental study, increases in plasma CK were invariably associated with histologic evidence of cardiac injury detected by electron microscopy in the experimentally-induced ischemic bed. Given the difficulty of detecting injured cardiac myocytes even in this model, it is likely that if one used markers and/or criteria with even greater sensitivity, it would have been impossible

to find evidence of cardiac injury morphologically. Similar studies have not been accomplished for the troponins. However, the troponins, and particularly cTnI, are released as complexes which are similar in weight to CK-MB. Thus, they are likely to be similar to CK-MB in terms of release from the injured myocytes. Finally, the available clinical and experimental information suggests that troponin release represents irreversible injury: 1 - there is a relationship between the amount of troponin released and the amount depleted from myocardium ; 2 - there is no increase of troponin in response to ischemia; and 3 - in experimental models of vital exhaustion where minor troponin increases have been observed, they are invariably associated with histologic evidence of injury. Unfortunately, only one case report has been published which shows pathologically confirmed coagulation necrosis with a subtle increase of cTnT in the absence of CK-MB elevation. From the clinical perspective, since prognosis is related to detection and the extent of myocardial injury, an operational definition of AMI based on the detection of troponin elevations seems to be much more effective than the traditional one. The second issue is the amount of elevation of cardiac troponin required for the diagnosis of AMI. This issue has become very controversial and topical in regard to cTnI measurements. Many assays exist for cTnI which use different antibodies recognizing different epitopes, some of which are more and some less stable, have different analytical imprecision, and demonstrate differences with calibration materials. False-positive increases are related: 1 - to the use of serum, since fibrin can confound several of the cTnI assay formats; 2 - the presence in the serum of heterophilic antibodies, which appear in the plasma of patients who make autoantibodies and in those who receive therapy with extrinsic antibodies; 3 - the presence of rheumatoid factor. Among the methods available for cTnI measurement, there is differential antibody recognition of degradation products due to degradation of the epitopes on the C-terminal of cTnI assays. Thus, central region epitope-based assays are preferred. In addition, conservative thresholds should be utilized for the diagnosis of cardiac injury, which need to take into consideration the variability of the single assay around the cutoff limit. The third issue is related to the clinical, social, and economic implications related to the shift of the diagnosis of AMI to a troponin standard. It is clear that the adoption of cardiac troponin will lead to an increase of the absolute number of patients discharged with the diagnosis of AMI; in fact near 30% of patients with unstable angina show troponin elevations suggestive of ongoing myocardial damage. This fact needs to be further addressed in the next few years.
Generally speaking, an AMI is thought to occur when there is evidence of cardiac damage, as detected by marker proteins, in the setting of acute ischemia. Troponin increase is therefore not synonymous of AMI since it does not define the mechanism of injury. If the clinical circumstance suggests that an ischemic mechanism is unlikely, other etiologies for cardiac injury should be pursued. This occurs, for example, in patients with congestive heart failure, pulmonary embolism, myocarditis, direct trauma to the heart, or as a result of myocardial toxins, such as adriamycin, or related to endogenous substances released in critically ill patients, e.g. patients with septic shock. Furthermore, mechanical injury, such as ablations, cardioversions, etc., may also induce cardiac injury.
In conclusion, the WHO criteria are going to be revised; in the meanwhile, there is need to keep the AMI diagnosis uniform despite the use of different biomarkers which identify the presence of myocardial damage in variable proportions of patients with acute myocardial ischemia. This can be done by the prospective "ad hoc" identification of cutoff limits for the different new biomarkers which correspond to WHO definition. This work has been done in part but needs to be completed.

WS.3

Zaninotto M.
(for the ANMCO-SIBioC-SIMeL Working Group on Markers of Myocardial Damage)

EXTERNAL QUALITY ASSESSMENT SCHEME FOR BIOCHEMICAL MARKERS OF CARDIAC DAMAGE: A NATIONAL EXPERIENCE

Department of Laboratory Medicine, University-Hospital of Padova, Padova, Italy

Several immunoassay systems for quantification of cardiac proteins have recently been introduced. However, the lack of standardization has become a critical point in the test validation and in comparison of method's results. In 1999, the Working Group on Markers of Myocardial Damage proposed a National External Quality Assessment Scheme (NEQAS) in order to verify and monitor the performance of immunoassay methods used for the measurement of creatine kinase (CK)-MB mass, myoglobin, cardiac troponin I (cTnI) and troponin T (cTnT), widely recognized as the most useful biochemical markers for diagnosis of myocardial damage as well as for treatment monitoring. The NEQAS provides several useful information, such as the analytical performance of the assays used, the bias from the consensus value, and the intra- and interlaboratory imprecision, but also permits to compare the reference limits and the decisional cutoffs adopted in different laboratories. Laboratories participating in the NEQAS (n=60) used seven different platforms for cTnI and CK-MB assays, two for cTnT, and ten for myoglobin. The within-method imprecision (CV) ranged, in the same cycle, from 6-17% (Beckman Access) to 16-46% (Dade Opus) for cTnI, from 5-10% (Abbott AxSYM) to 25-35% (Opus) for CK-MB, and from 4-7% (Access) to 12-25% (Opus) for myoglobin. A very high variation in the recovery for cTnI between instruments was showed: AxSYM assay produced the highest concentrations, approximately 25-fold higher than those obtained by Dade Dimension. Intermediate values were observed with Access, Opus, and Stratus systems. Between-assay differences were lower (about two times) for CK-MB and myoglobin. The reports demonstrated a wide dispersion of the reference limits adopted by various laboratories, also for the same analytical system. A significant number of laboratories uses reference limits that are different from those suggested by the manufacturers (possibly showing a critical approach to this problem), one-half of participants declaring to also adopt for troponins a decisional cutoff for 'minimal' myocardial damage. These findings show the impellent need to promote a wide diffusion of NEQAS for cardiac markers, being unsatisfactory not only the quality of the assay results and the standardization of the methods but also the performances of the laboratories. The obtained data seem to suggest a very different approach between laboratories to the calibration of systems as well as to the internal quality control procedures.