Biomarkers In Chronic Diseases: Diabetes, Obesity, Cardiovascular Diseases, And More…

State of the art, opinions, challenges from the 4th International Symposium on Integrated Biomarkers – Riyadh, Saudi Arabia, 4-6 May 2010. In the conference, scientists coming from Arabian Peninsula, India, USA, Europe, Egypt, Sudan, Eritrea, Tunisia, Somalia, and other Countries convened to a) discuss basic and clinical research devoted to support the methodological and technological approach to the discovery, validation, and qualification of Biomarkers; b) debate on cost/effectiveness evaluation, regulatory requirements, and health organization conditions, to facilitate the use of Integrated Biomarkers in chronic disease management.

Report by Andrea Peracino

Scenario

○ diabetes
The health-care burden of diabetes, especially type 2 diabetes mellitus, continues to increase. An estimated 285 million people worldwide have diabetes at present, and 439 million are expected to have diabetes by 2030 (International Diabetes federation. The diabetes atlas. brussels. International Diabetes Federation, 2009). Vascular complications are responsible for most of the associated morbidity, mortality, and excess costs (US Centers for Disease Control and Prevention. National diabetes fact sheet. http://www.cdc.gov/diabetes/pubs/pdf/ndfs/2007.pdf (accessed Jan 11, 2010)). Diabetes is the sixth-leading cause of death, with most deaths (nearly 70%) attributed to Cardiovascular Disease (CVD) and with ischemic heart disease being responsible for nearly 50% of these deaths (CMAJ 2003; 168: 1661-1667). Total mortality from Coronary Artery Disease (CAD) in subjects with type 2 diabetes mellitus, without a previous myocardial infarction, is as high as that of non-diabetic individuals with a previous infarction (Mol Cell Endocrinol 2009; 297 (1-2): 112-26). In a 1998 forecast (Diabetes Care 1998; 21: 1414-31) India, China, and USA, that were ranking in 1995 in the first worldwide positions for diabetes (19.9, 16.0, 13.9 million respectively), had been expected to keep such positions in 2025 (with 57.2, 37.6, 21.9 million respectively): nowadays there is no doubt that that forecast will hold true.

○ overweight, obesity
The alarming increase of overweight, obesity and diabetes in adults and in children in many Countries is considered even more a severe public health problem. Overweight and obesity are the result of a complex interaction between genetic and environmental factors (J Endocrinol Invest 2008; 31 (11): 979-84). The child's obesity may give rise to other important health problems: metabolic (such as type2 diabetes), cardiovascular, respiratory, orthopedic and psychological. The adverse health effects of obesity of children justify the need to look for efficient diagnosis, treatments, and prevention (Med Wieku Rozwoj 2006; 10 (1): 3-191 (N Engl J Med. 2007;357:2329-37). It is well known that the increasing prevalence of overweight and obesity is linked to an alarming epidemic increase of type 2 diabetes mellitus and its cardiovascular complications (Centers for Disease Control and Prevention. National Diabetes fact Sheet: general Information and National Estimates of Diabetes in the United States, 2007.
Atlanta, GA: US Department of Health and Human Services, Centers for Disease Control and Prevention; Circulation 2008; 118: 428-64 and Circulation 2009; 119: 3244-62). In the WHO European Region some 30-80% of the adults is overweight (BMI over 25). Almost 400 million adults in Europe are estimated to be overweight and about 130 million are obese. About 20% of children are overweight, and a third of these are obese. Obesity creates a major economic burden through loss of productivity and income, and consumes 2-8% of overall health care budgets. (http://euro.who.int/obesity/import/20060220_1). The INTERHEART study estimated that 63% of heart attacks in Western Europe and 28% of heart attacks in Central and Eastern Europe are due to abdominal obesity (Lancet 2004; 364: 937-52). INTERSTROKE study recently showed that the nine risk factors of INTERHEART (hypertension, smoking, abdominal obesity, diet, physical activity, diabetes mellitus, alcohol intake, psychosocial factors, and apolipoproteins levels) account for about 90% of the Population-Attributable Risks (PARs) for stroke, with differences between ischemic and hemorrhagic stroke. On the basis of an indirect comparison between the studies, these differences are most notable for hypertension, apolipoproteins levels, physical activity, and alcohol intake (Lancet 2010; 376: 112-23).

- **diabetes: a new approach?**

The rapid increase in the prevalence of obesity, type-2 diabetes and associated complications (diabesity) is a major global health problem. Approximately 33 million adults are estimated to have diabetes; obesity, which is a major recognised risk factor for type-2 diabetes, is rapidly increasing in prevalence resulting in a diabesity epidemic. The current cost of type-2 diabetes in the European Union is € 15 billion per year, and medical complications arising from diabetes account for up to 8% of total health costs in Europe (MedGenMed. 2007; 9: 39 – no link). More than 50% of the global number of diabetic patients inhabit in northern tropical area labeled as red zone: including North-African, Arabian, South-Asian countries. Besides ethnicity, family history, ageing, life style change within the frame of globalization, diet and sedentariness, are playing an increasing role. Urbanization is one of the constant factors, independently from the economic level of the subjects (Int J Diabetes Mellitus 2010;2:1-2 – no link).

**Integrated Biomarkers**

- **the project**

Clinical decision-making in the study of the individual patient or in risk stratification in populations, or in drug development in clinical trials demands increasingly more support from highly predictive diagnostic tools (for clinical trials see also http://www.fda.gov/oc/initiatives/criticalpath/). The highly predictive value is even more critical in the clinical approach to chronic diseases, such as diabetes, obesity, atherosclerosis and their development to CVD (www.healthyurope.org) where scholars and clinicians are facing an increasing number of qualified biochemical diseases markers, not always validated and context-specific, and the pressure of innovation in bio-technology. Multimarker analysis (Biomarkers Med 2007; 1: 1-13) and the integration of biochemical and bioimaging disease markers in CVDs support clinical decision-making more than the use of biomarkers alone (J Intern Medicine 2007; 261: 214-34). According to the FDA, pharmaco-genomics guidance for biomarkers (http://www.fda.gov/cder/guidance/6400fml.pdf,Oct25,2006), can be classified as exploratory, probably valid, and valid. There is a need for consensus criteria for validation and qualification of the integrated approach in the specific context of use of the tool (Circulation 2007; 115. 949-
Within the framework of the Project on Integrated Biochemical and Bioimaging Markers, the Lorenzini Foundation’s three previous meetings (Lugano 2005, Berlin 2007, and Seattle 2008) have highlighted the critical issues related to the use of biomarkers to support the clinical decision making in the cardiovascular area. The three meetings have confirmed the necessity and the advantages of an open discussion and peer-to-peer dialogue among clinicians, biochemists, mathematicians, and health organizations, bioimaging, bioinformatics, regulatory, health economics, and industry experts, in order to reach consensus and recommendations.

Additionally, it confirmed the need to analyze and to confront different clinical and research approaches and different best solutions in the use of biochemical and bioimaging markers.

Further on, different geographic and ethnic specific patterns are asking for devoted approach to local epidemiology realities to better design protocols for diagnosis, prevention and treatment of cardiovascular patients. Thus the Biomarker Research Centre of College of Science of the King Saud University in Riyadh (Riyadh) decided to ask the Lorenzini Medical Science Foundation (Milan, Houston) to co-organize the Conference that is under the auspices of the prestigious King Saud University, internationally known as Medical Master in science, medicine and health organization in favor not only of the Arab population. The 4th International Symposium on Integrated Biomarkers – was held on 4-6 May 2010 in Riyadh (Saudi Arabia), under the patronage of the Saudi’s Royal Family. The meeting, co-chaired by Prof. Omar Al-Attas (Riyadh, Saudi Arabia) and Prof. Rodolfo Paoletti (Milan, Italy), has been designed to provide a comprehensive and up-to-date overview of recent advances in the field of Integrated Biomarkers assessment and qualification in the context of diagnosis, treatment, and prevention of Chronic Disease such as Diabetes, Obesity, Metabolic Syndrome, Cardiovascular Disease and Cardiometabolic Disorders. In the conference, scientists coming from Arabian Peninsula, India, USA, Europe, Egypt, Sudan, Eritrea, Tunisia, Somalia, and other countries convened to a) discuss basic and clinical research devoted to support the methodological and technological approach to the discovery, validation and qualification of Biomarkers; b) debate on cost/effectiveness evaluation, regulatory requirements, and health organization conditions, to facilitate the use of Integrated Biomarkers in chronic disease management. Participants have been provided with insights into cutting-edge advances in using the Integrated Biomarkers for the evaluation and monitoring of current and new therapeutic strategies, with the goal of further improving patient outcomes.

The 4th International Symposium in a nutshell
(see also the slide library in http://www.lorenzinfoundation.org/biomarkers2010/slides/)
Clinical decision-making in the study of the individual patient, in risk stratification in populations, or in drug development, increasingly demands more support from highly predictive diagnostic tools. The highly predictive value is even more critical in the clinical approach to chronic disease, such as diabetes, obesity, atherosclerosis and their development to CVD where scholars and clinicians are facing an increasing number of qualified biochemical diseases markers, not always context-specific, and the pressure of bio-technology innovation.

Multimarkers panels and the integration of biochemical and bioimaging disease markers in CVDs support clinical decision-making more than the use of biomarkers alone (Integrated Biomarkers: Lugano-2005, Berlin-2007, Seattle-2008)
(for clinical trials see also http://www.fda.gov/oc/initiatives/criticalpath/)
The highly predictive value is even more critical in the clinical approach to the large pattern of metabolic diseases such as diabetes, obesity, metabolic syndrome, and their eventual evolution
towards cardiovascular events (Circulation 2006; 113: 2335-62). This highly predictive value demands the support of validated and context specific qualified markers of disease: the Biomarkers (J Intern Medicine 2007; 261: 214-34).

- pathological approach

The pathological approach (Andrea Peracino – Milan, Italy; Integrated Approach to Biomarkers Perspective http://www.lorenzinifoundation.org/biomarkers2010/slides/showPres.asp?IDSlide=233) to Acute Coronary Syndrome, Ischemic Stroke, and the combination of Obesity and Diabetes is a model for the evaluation of some common pathomechanisms, such as: glucose intolerance, insulin resistance, visceral adipose metabolism, adiponectin/leptin system, neuro-hormone, incretins, endothelial function, inflammation and acute reactant proteins, lipid disorders, atherosclerotic plaque development, thrombosis/coagulation, neurohormones activation, ischemia, myocardium conditioning, astrocytes activation, blood brain barrier damage. All these can be deeply explored through appropriate tools as context qualified biomarkers. Examples of biomarkers constellation related to specific pathophysiological moments are reported in the following three pictures:


Diabetes mellitus is strongly associated with oxidative stress (Syed Ibrahim Rizvi – Allahabad, India; Elevated erythrocyte plasma membrane redox system is an early marker of type 2 diabetes http://www.lorenzinifoundation.org/biomarkers2010/slides/showPres.asp?IDSlide=168). Human erythrocytes contain a plasma membrane redox system (PMRS) which transfers electrons from intracellular donors to extracellular acceptors. The PMRS, which incorporates an ascorbate free radical (AFR) reductase, provides a redox system that enables the cells to effectively counteract oxidative processes. The increase of erythrocyte PMRS and AFR reductase provides compensatory mechanisms to mitigate increased oxidative stress. Elevated erythrocyte PMRS and AFR reductase may be used as markers to predict the development of disease.

Vascular complications of diabetes (Faten Abdulhady Zakareia – Riyadh, Kingdom of Saudi Arabia; Correlation of Peripheral Blood Flow, Plasma Vascular Endothelial Growth Factor, Fibroblast Growth Factor, Fatty Acid Synthase, Intercellular Adhesion Molecules, and Adrenomedullin in Diabetic Peripheral Vascular Disease http://www.lorenzinifoundation.org/biomarkers2010/slides/showPres.asp?IDSlide=219) may be better understood with the use of biomarkers, as follows. The vascular protective role of plasma vascular endothelial growth factor (VEGF) in diabetic vasculopathy and its effects particularly in the development of proliferative retinopathy and its possible mitogenic effects in tumor, are opening a bridge between patho-mechanisms of atherosclerosis and some type of tumors. Soluble fatty acid synthase (sFas) could be a causative factor involved in the advancement of apoptotic changes in vasculopathy: the rise of sFAS in vasculopathy and its negative correlation with Ankle/Brachial Index (A/BI) indicate that apoptosis could be the cause of reduced vascular integrity and contribute to vascular complications in diabetes. The protective
role of *adrenomedullin* (ADM) in diabetic Peripheral Vascular Obstructive Disease (PVD), as shown by its high level in these cases, is still to be elucidated. The potential role of *intercellular adhesion molecules* (ICAM) as a predictor for early diagnosis of PVD, suggests its use as a marker for PVD, supporting the role of inflammation in the pathogenesis of diabetic PVD. In the evaluation of diabetic PVD, it may be appropriate to measure the levels of sICAM-1, as well as of marker of oxidative stress, in addition to routine laboratory assessments.

**Clinical approach**

The clinical approach ([Wolfgang Koenig – Ulm, Germany; The Role of Biomarkers in CVD: Where Do We Stand in 2010?](http://www.lorenzinifoundation.org/biomarkers2010/slides/showPres.asp?IDSlide=87) is still faced by an ongoing controversy regarding the clinical utility of various biomarkers. Current prediction of coronary heart disease (CHD) has certain limitations which relate mainly to the reduced number of risk factors evaluated by the various scores and, in particular, to the imperfect prediction based on LDL blood levels which still represents an important criteria for intervention and a target for therapy. Additional factors based on the INTERHEART study from one side may complement the view of potentially modifiable risk factors but, on the other, still leave an incomplete situation. This has formed the basis for a continued interest in a number of blood biomarkers, markers of subclinical disease, and genetic markers to further improving risk stratification for cardiovascular outcomes. Cardiovascular biomarkers can be used in the context of risk stratification, early detection of subclinical disease, monitoring of disease process, selection of therapy, and response to therapy (Circulation 2007; 115. 949-52). Biomarkers with greatest promise selected by the National Academy of Biochemistry (NACB LMPG = National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines Circulation) are Lp (a) = Lipoprotein(a), apo A= apolipoprotein A; apo B = apolipoprotein B; hsCRP = high sensitive C Reactive Protein; WBC = White Blood Cells. Among the markers of subclinical disease, interest focuses on the measurement of carotid intima-media thickness and coronary calcium scoring and Lipoprotein Associated Phospholipase A2 (Lp-LPA2). A prolonged elevated inflammatory response is associated with clinical progression of the disease. In fact, the JUPITER study’s design ([NEJM 2008; 359: 2280-2](http://www.lorenzinifoundation.org/biomarkers2010/slides/showPres.asp?IDSlide=87)) was based on the identification of a high risk group of patients with elevated CRP levels who otherwise had no indication for statin therapy. Please note that biomarkers like CRP can be used to monitor response to statin therapy ([NEJM 2005; 352: 20-8](http://www.lorenzinifoundation.org/biomarkers2010/slides/showPres.asp?IDSlide=87)).

**Proteomic approach**

The proteomic approach ([John J. Albers – Seattle, WA, USA; Phospholipid Transfer Protein and Selected Biomarkers in Cardiovascular and Alzheimer's Disease](http://www.lorenzinifoundation.org/biomarkers2010/slides/showPres.asp?IDSlide=10) addresses the questions whether *LDL-cholesterol* is the best initial biomarker to be targeted for the treatment or therapy of patients at high risk for CAD. Moreover, is low *HDL-cholesterol* the best biomarker to be targeted to reduce the residual risk remaining after LDL reduction therapy? Proteomic analysis of HDL reveals at least 48 HDL-associated proteins. One of the HDL proteins is *phospholipid transfer protein* (PLTP), an important modulator of lipoprotein metabolism. PLTP has been shown to be increased in CAD, obesity and type 2 diabetes. Studies in mice have suggested that PLTP is atherogenic, and efforts to develop drugs to inhibit PLTP are under consideration. However, increased plasma PLTP activity may not be a mediator of the
CAD. Therefore, targeting a biomarker associated with CAD may not necessarily be beneficial, as we observed that PLTP reduces pro-inflammatory cytokines in human macrophages and plasma PLTP complexes contain numerous proteins linked to immunity and inflammation. HDL promotes cholesterol efflux from peripheral cells and transports cholesterol back to the liver; reduces inflammation; acts as an anti-oxidant and reduces LDL oxidation. As a CAD biomarker, apolipoprotein B (apoB) that is a direct measure of the number of atherogenic particles in plasma, may be superior to LDL-C as a predictor of CAD risk and as a target for therapy. Measures of HDL functionality may have value beyond HDL-C or apolipoprotein A-I (apoA-I) in predicting CAD risk and as a target for therapy.

- **primary prevention approach**

  The role of inflammation biomarkers in primary prevention (Filippo Crea – Rome, Italy; Role of Inflammation Biomarkers in Primary Prevention [http://www.lorenzinifoundation.org/biomarkers2010/slides/showPres.asp?IDSlide=79](http://www.lorenzinifoundation.org/biomarkers2010/slides/showPres.asp?IDSlide=79)) is stressed by scientists and clinicians. For this purpose, CRP holds much promise. CRP reveals the presence of inflammation regardless of being one of the culprit players. This very ‘non-specificity’ may underlie much of CRP’s utility in risk prediction; CRP integrates an overall inflammatory status, capturing aspects otherwise difficult to be measured directly. For example, assessment of ‘lifestyle’ variables presents a practical challenge in the clinic. CRP may provide an overall readout that incorporates elements associated with risk otherwise difficult to quantitate. Indeed, CRP may reflect low-grade chronic infections in various sites such as bronchitis and periodontitis or genetically determined hyper-reactivity of inflammatory cells, which can exacerbate atherosclerosis. Furthermore, recent studies have implicated risk factors not included in the Framingham Heart risk score, like abdominal obesity, psychosocial factors, sedentary lifestyle, low fruit and vegetable intake, and lack of alcohol consumption in a large proportion of population attributable risk for myocardial infarction. CRP levels association with all these risk factors is not yet accounted for by most current risk algorithms. The INTERHEART study showed that risk factors not considered in current risk scores explain about 45% of the population attributable risk (PAR) of myocardial infarction. As CRP, a non specific marker of inflammation, is associated with these additional risk factors, it might identify subjects at risk not identified by traditional risk factors. The JUPITER trial confirmed that subjects with raised CRP levels and norm levels of LDL-C have a cardiovascular risk sufficiently high to be reduced by cholesterol-lowering treatment with a statin. Capturing these elusive risk components might contribute to the ability of hs-CRP to re-stratify many individuals deemed at intermediate risk of cardiovascular events by traditional risk calculators, into higher, or equally importantly, lower risk categories. This adjustment has major implications for preventive practice. Indeed, while high-risk subjects deserve a pharmacological treatment, the effectiveness of drug therapy remains uncertain in the intermediate risk category. Another potentially interesting biomarker is Lp-PLA2 which might have a pathogenetic role in atherosclerosis progression and, consequently, it might become a new therapeutic target.

- **diabetes in youth**

  Biomarkers of cardiovascular disease and inflammation in youth with type 1 and type 2 diabetes (Santica M. Marcovina - Seattle, WA, USA; Biomarkers of Cardiovascular Disease and Inflammation in Youth with Type 1 and Type 2 Diabetes
are even more entering in the evaluation of the so alarming explosion of diabetes and obesity in both young and adult populations. Until the early 1990s, diabetes in youth was almost exclusively type 1 diabetes (T1D). Type 2 diabetes (T2D) was almost exclusively diagnosed in older individuals and rarely observed in pediatric centers. However, concomitantly with the rising prevalence of obesity in youth, T2D is commonly diagnosed in the pediatric population. Early onset T2D (age of diagnosis 18-44 years) has been associated with more aggressive CVD than later onset T2D, suggesting that CVD complications in youth with T2D may be even more unfavorable. Lipid and lipoprotein levels, preponderance of small, dense LDL, apoB levels and other novel CVD risk factors such as IL-6, hsCRP, fibrinogen, adipocytokines and microalbuminuria are biomarkers evaluated in a cohort of young patients (age 10-22 years) with T1D or T2D and in a control group of non-diabetic youths. Youths with T1D and optimal glucose control had lipid levels comparable to those in the control group, while higher apoB levels and more small, dense LDL were observed regardless of glycemic control. Compared with controls, T1D youths had higher IL-6 and fibrinogen levels independent of glucose control while hsCRP levels were significantly higher only in T1D youths with poor glycemic control. Compared with controls, youths with T2D had a higher prevalence of all major CVD risk factors. Adjustment for body mass index and glucose control substantially lowered the differences in CVD risk factors between cases and controls except for fibrinogen and IL-6 which remained significantly higher. These results indicate that adiposity and blood glucose levels are important contributors to the observed differences in CVD risk profile among T2D and control youths while inflammatory and prothrombotic factors appear to play an independent role. Youths with T2D have a high prevalence of multiple CVD risk factors characteristic of adults with T2D. Adiposity and glycemic control are primary contributors to the unfavorable CVD risk profile in youths and additional mechanisms such as inflammation also play an independent role. Adiposity, particularly visceral abdominal obesity, is a well known risk factor for a cluster of metabolic abnormalities including insulin resistance, dyslipidemia and hypertension, in turn associated with CVD. The lifestyle intervention reduced the incidence by 58%. Metformin reduced the incidence by 31%. The methodology of biomarkers deserves better attention than it is now having: LDL-C screening programs are missing 12% of diabetic children with elevated apoB or small dense LDL. Public Health Strategies in youths should take into consideration: multi-level intervention; longer duration and greater intensity of physical activity; more comprehensive changes to school food environment; behavioral strategies including individual goal setting and achievement; integrated promotional and educational communications strategies; and more reliable screening methods for dyslipidemia.

○ insulin resistance and ageing

Studies in obesity have highlighted *adipocytokines* in the development of insulin resistance, which in turn may lead to accelerated aging. *Adiponectin* has protective effects against metabolic abnormalities that accelerate aging (insulin sensitizing and anti-inflammatory) possibly by improving insulin sensitivity and reducing systemic inflammation, ultimately mitigating endothelial dysfunction and atherosclerosis development.

In obese rats, *adiponectin* reversed endothelial dysfunction by increasing *nitric oxide* production by *endothelial nitric oxide synthase* (eNOS), and by decreasing *nitric oxide* inactivation by blocking superoxide production ([*Int J Obes* 2010; 24: 165-171 - no link]). *Body Mass Index* and *insulin resistance*, were associated with *telomere* loss, possibly explained as a result of cumulative psychological, metabolic, inflammatory and oxidative stress insults leading to accelerated physiological aging ([*Mech Ageing dev* 2008; 129: 745-51]).

**more on biomarkers in special populations**

*Heat shock protein 70* and *IgE* (Amal A. Baalash – Riyadh, Kingdom of Saudi Arabia; *Heat Shock Protein 70 and Ige are Early Predictors of Myocardial Ischemia and Recovery After Coronary Artery Disease Grafting* (cABG) [http://www.lorenzinifoundation.org/biomarkers2010/slides/showPres.asp?IDSlide=30](http://www.lorenzinifoundation.org/biomarkers2010/slides/showPres.asp?IDSlide=30) might be used as markers for detecting early minor myocardial damage and coronary insufficiency with less overt damage than myocardial infarction, as significant changes in their levels appear before occurrence of any changes in the levels of MMP-9, *CPK-MB* and LDH. Besides, heat shock protein 70, and *IgE* returning to the normal levels after *Coronary Artery Bypass Graft* (CABG) surgery, suggests that they could be helpful for evaluating the effect(s) of CABG surgery.

**Endothelial nitric oxide synthase** (eNOS) gene polymorphisms and their application as potential genetic biomarkers for coronary artery disease have been studied in Saudi population (*Khalid M. Alkharfy – Riyadh, Kingdom of Saudi Arabia; Endothelial Nitric Oxide Synthase (enos) Gene Polymorphisms and their Application as Potential Genetic Biomarkers for Coronary Artery Disease in Saudi Population* [http://www.lorenzinifoundation.org/biomarkers2010/slides/showPres.asp?IDSlide=20](http://www.lorenzinifoundation.org/biomarkers2010/slides/showPres.asp?IDSlide=20)). eNOS gene polymorphisms confer significant CAD risk in the Saudi population and the findings emphasize the data in a larger population to consider their potential clinical application.

Measuring *Urinary 8-hydroxydeoxyguanosine* (8-OHdG) (Shereen Hassan Atef – Cairo, Egypt; *Urinary 8-hydroxydeoxyguanosine as a Biomarker of Microangiopathic Complications in Type 2 Diabetic Patients* [http://www.lorenzinifoundation.org/biomarkers2010/slides/showPres.asp?IDSlide=1](http://www.lorenzinifoundation.org/biomarkers2010/slides/showPres.asp?IDSlide=1)) is a novel convenient method for evaluating oxidative DNA damage. Diabetic patients, especially those with advanced nephropathy and retinopathy, have significantly higher levels of oxidative modifications that may contribute to the development of microvascular complications of diabetes. This study, for the first time, suggests an independent association of 894G>T and -786T>C polymorphisms of endothelial nitric oxide synthase gene with coronary artery disease in Saudi population.

Association of *polymorphisms* related to *methyltetrahydrofolate reductase* (MTHFR) and *angiotensin converting enzyme* (ACE) with hypertension genes has been studied in Saudi cases from Qassim region (*Ahmad A. Settin – Buraydah, Saudi Arabia; Polymorphisms in MTHF and ACE Genes and the Association with Hypertension Among Saudi Population from Qassim*
Saudi population from Qassim Region are carriers of a relatively high amount of genetic alleles predisposing them to hypertension and cardiac diseases. Adoption of competent programs for prevention and/or early detection of cardiovascular disorders is required. In this respect we suggest to screen for serum homocysteine levels.

**Atrial natriuretic peptide (ANP)** is involved in blood pressure regulation due to its vasodilator and natriuretic effects *(Hayet Soualmia – Tunis, Tunisia; ScaI Atrial Natriuretic Peptide Gene Polymorphism and Hypertension in Tunisian Population [http://www.lorenzinifoundation.org/biomarkers2010/slides/showPres.asp?IDSlide=202]*) . Abnormalities in the ANP system could play a key role in the genesis of hypertension. Several variants of ANP gene have been identified in humans. The ScaI is a restriction site loss in the ANP precursor gene as the substitution of a T for C at position 2238 led to translation of ANP with two additional arginines. In this study, the authors evaluated the impact of the ScaI ANP gene polymorphism on hypertension in a sample of the Tunisian population.

- **damages to the proteome**

  **Damage to the proteome by glycation, oxidation and nitration** is a continuous process in human tissues and body fluids *(Paul J. Thornalley – Coventry, UK; Protein Damage-based Biomarkers for Metabolic Control, Risk of Vascular Complications and Therapeutic Monitoring in Diabetes [http://www.lorenzinifoundation.org/biomarkers2010/slides/showPres.asp?IDSlide=211]*) . Glycation by both glucose and reactive dicarbonyl metabolites such as methylglyoxal increases in diabetes. Oxidative and nitration damages also increase as a consequence of oxidative stress in diabetes. Damaged proteins undergo cellular proteolysis releasing in plasma and urine markers of protein damage such as glycated, oxidised and nitrated amino acids. Protein damage is characterised and quantified by stable isotopic dilution analysis tandem mass spectrometry (LC-MS/MS), considered to be the gold standard for specificity and high sensitivity evaluation. Urinary fluxes of modified amino acids relate to whole body protein damage. Specific damaged proteins and sites within them are identified by mass spectrometric proteomics. The results of the studies show that damage to the proteome is increased in diabetes linked to metabolic control and development of vascular complications; some protein damage markers are sensitive to both fasting and postprandial hyperglycaemia and offer a route to improved indicators of glycaemic control; urinary flux of some markers of protein damage relates to risk prediction of renal function decline; angiotensin II receptor blockers (ARBs) decrease angiotensin-converting enzyme (ACE) and nitration exposure – probably by indirectly affecting oxidative and dicarbonyl stress; metformin decreases the modification of apolipoprotein B100 of LDL by dicarbonyl-derived AGEs oxidation; markers of protein damage may provide useful biomarkers in chronic disease as they often relate directly to metabolic dysfunction and mechanism of morbidity

- **from athero-vascular diseases to central nervous system**

Even more attention is given to some possible links between atherosclerosis and Alzheimer Disease *(AD)* *(John J. Albers – Seattle, WA, USA; Phospholipid Transfer Protein and Selected Biomarkers in Cardiovascular and Alzheimer's Disease [http://www.lorenzinifoundation.org/biomarkers2010/slides/showPres.asp?IDSlide=10]*) .
A combination of cerebrospinal fluid (CSF) biomarkers, such as phosphorylated tau and beta-amyloid 1-42 (Aβ42), magnetic resonance imaging, and positron-emission tomography, show promise in the early detection and diagnosis of Alzheimer’s disease. Low CSF phospholipid transfer protein (PLTP), an important modulator of lipoprotein metabolism, activity is associated with AD and inflammatory neurological diseases. Furthermore, PLTP reduces the level of phosphorylated tau in neuronal cells. These findings suggest that inclusion of other markers, such as PLTP, may increase the ability to detect pathological processes associated with neurodegeneration, as well as provide an opportunity to monitor treatment responses. Other genetic biomarkers for late-onset AD have yet to withstand the test of time such as presence of the apolipoprotein E (APOE) isoform ε4. In CSF, biomarkers under study are: beta-amyloid 1-42 (Aβ42), total tau (Tau), phosphorylated tau (pTau), Aβ42/pTau and Aβ42/Tau ratios. Other CSF biomarkers are isoprostanes, markers of inflammation, and markers of neuro-degeneration. In plasma no single plasma biomarker fits the criteria for a successful AD biomarker. A study by Ray and colleagues suggested that combination of multiple markers in plasma may be of prognostic and/or diagnostic value (Nature Medicine 2007; 13: 1359-62). Imaging may open a new way to integration between biochemical and bioimaging markers. Brain atrophy measured by magnetic resonance imaging (MRI) is a valid marker of AD and its progression. The degree of atrophy of medial temporal structures is a diagnostic marker of AD at the mild cognitive impairment (MCI) stage and is a sensitive marker of progression of neuro-degeneration. The integration of structural markers and functional imaging with CSF biomarkers will provide optimal diagnosis and monitoring of the disease process and therapeutic success.

- **transcriptomic approach**

A transcriptomic approach is useful for evaluating gene expression changes that occur during progression from one cell state to another, and identify individual gene products meriting further investigation as therapeutic targets, biomarkers, or both. (Lee E. Eiden - Bethesda, MD, USA; Microarray-based Gene Discovery and Its Application to Chronic Disease [http://www.lorenzinifoundation.org/biomarkers2010/slides/showPres.asp?IDSlide=265](http://www.lorenzinifoundation.org/biomarkers2010/slides/showPres.asp?IDSlide=265)).

The focus on cell culture systems may facilitate the understanding of a transition condition associated with disease (neurodegeneration, transformation, insulin resistance, etc). Signaling pathways for enhancement, or repression, of gene transcription identified in cell culture models provide potential targets based on pharmacological abrogation of expression of specific target genes. Linking gene expression to cellular outputs can identify genes whose silencing confirms functional significance, a first step in target validation for therapeutics development versus biomarker development. An emergency response peptide pituitary adenylate cyclase (PACAP) discovered in 1989 (Handbook of Neurochemistry and Molecular Neurobiology: Neuroactive Peptides and Proteins (Lim R ed) pp 1-36, Springer, Heidelberg) is presented as a model of signaling. PACAP signaling is required for peripheral and central responses to multiple forms of stress related to disease; microarray identifies genes activated by PACAP; these are mapped to function with pharmacology and siRNA; PACAP-responsive genes mediating survival in cells are examined as PACAP-dependent genes in vivo. Microarray analysis allows the opportunity to discover novel drug targets, mechanisms of disease, and biomarkers for disease progression. Pharmacological dissection of signaling pathways involved in chronic disease modulation may allow manipulation of individual features, e.g. stress transduction versus neuroprotection.
stress, inflammation, and metabolic disorders
Some biomarkers are recognized common denominators of sleep disorders, metabolic, inflammatory and stress states (George P. Chrousos - Athens, Greece; Biological Markers of Sleep Disorders, Metabolic, Inflammatory and Stress States: What are the Common Denominators? http://www.lorenzinifoundation.org/biomarkers2010/slides/showPres.asp?IDSlide=39).

Sleep and wakefulness are linked to the circadian clock and its zeitgebers and function diurnally in succession and mutual opposition of each other. Sleep and wakefulness are both located in the central nervous system (CNS), particularly the hypothalamus and the brainstem. Wakefulness is regulated by the Arousal System, located in the Locus Ceruleus (LC) and the reticular formation, which together with the autonomic system and the hypothalamic-pituitary-adrenal (HPA) axis, are also components of the Stress System, that is activated when any stressor exceeds a certain threshold. The homeostasis between sleep and wakefulness is modulated by a large number of psycho-physical actions where environmental and biochemical factors may drive the subject to allostatic of several levels. Adipokines and cytokines, such as TNF-a and Interleukin-6 (IL-6), are both somnogenic and fatiguegenic, while Stress System mediators, including the key neurotransmitter of the Locus Ceruleus and sympathetic system nor-epinephrine, as well as corticotropin-releasing hormone (CRH) and cortisol, are stimulating wakefulness and arousal.

States associated with hypercytokinemia, such as infections, inflammatory disorders and central obesity, are frequently associated with excessive daytime sleepiness (EDS) and fatigue, while states associated with Stress System activation, such as situational apprehension, anxiety disorders and melancholic depression, are frequently associated with sleep disturbances, including insomnia, early morning awakening, frequent awakenings, etc. Lack of sleep in normal individuals is associated with elevated circulating somnogenic cytokines, such as IL-6, while stress is associated with elevated CRH, catecholamines and cortisol, all of which promote wakefulness and disturb sleep. Patients with central obesity and insulin resistance, or even lean patients with polycystic ovaries and insulin resistance, suffer from sleep apnea, have increased circulating cytokines/adipokines, and suffer from EDS and fatigue. Thus, sleep apnea and EDS and fatigue appear to be components of the Dysmetabolic Syndrome. The conclusion by George Chrousos is that sleep disorders share biological markers with metabolic, inflammatory and stress states.

Drug development
Drug development (Rodolfo Paoletti – Milan, Italy; Biomarkers in Clinical Practice and Pharmacodiagnostics http://www.lorenzinifoundation.org/biomarkers2010/slides/showPres.asp?IDSlide=126) can benefit from an appropriate use of biomarkers. Critical areas in drug development could be represented as it follows: the development of a new molecule and the launch in the market of a new drug requires higher and higher investments in resources and time than before; the breakeven between income and investment is even more postponed, and the need for a return on investment is affecting the price of marketed drugs; the entry of a new drug in the medical protocols requires strong support by large clinical trials and by the even more demanding criteria of evidence-based medicine; the approval by regulatory authorities of a new drug is based even more on stringent and documented advantages in the risk/benefit ratio for patients, and in the cost/benefit ratio for health organizations; the maintenance of a consolidated drug in the medical
protocols is linked to even more stringent pharmaco-economic comparison with alternative approaches.

Three models are presented to support biomarkers choice. Imaging is represented by carotid Intima Media Thickness (cIMT). The progression of carotid IMT together with the assessment of new vascular events will allow the validation of IMT progression as a biomarker of atherosclerosis. Carotid IMT may be proposed as surrogate end point in drug development studies (J Am Coll Cardiol 2007; 49: 925-32, Vasc Med 2004; 9 (1): 46-54, Current Cardiology Reports 2008; 10: 521-525, Postgrad Med 2010; 122 (1): 10-8, Cerebrovasc Dis 2007; 23 (1): 75-80, Int J Clin Pract 2007; 61 (6): 951-62). The second model refers to HDL: it is a class of heterogeneous, high-density lipoproteins, with outer shell consisting of apolipoproteins, phospholipids, and free cholesterol, and inner hydrophobic core consisting largely of triglycerides (TGs) and cholesterol esters (CEs). A better understanding of HDL response to specific drugs evaluation, can help in avoiding failure of new drug assessment (NEJM 2007; 356: 1620-30).

The third model is following the pathways by which vascular and extravascular sources of inflammation result in circulating levels of serum markers that provide a reflection of the underlying inflammatory response. CRP is expected to achieve even more a prognostic value of pharmacological modulation of inflammatory patho-mechanisms in atherosclerosis (Curr Opin Lipidol 2005; 17: 495-501, NEJM 2008; 359: 2280-2).

- unmet needs in metabolic disorders


The improvement of the management of hyperglycaemia in type 2 diabetes and of obesity requires earlier diagnosis, better risk profiling and the development of personalised medicine. Novel approaches to the development of biomarkers, including the use of imaging and the use of gases as biomarkers are required. To reach a personalised medicine may still be a dream for metabolic diseases, but the intermediate step of “stratified” medicine may be possible in the near future. The unmet needs are e.g.: measurement of beta cell function and mass; measure of insulin resistance; markers of prediabetes/dysglycaemia; better early markers of renal disease; predictors of weight gain; markers that add or improve upon current risk profiling ethnic specific in CVD; nonalcoholic steatohepatitis (NASH). Critical points among others are paucity of integrated development programmes for biomarkers; true personalised medicines still unlikely for most; stratified medicine or nichebusters are most likely the next step; biomarkers may include unconventional methods such as molecular imaging for beta cells, PET MR, eNose, etc. There is the need to train clinicians differently to ensure the delivery of personalised healthcare.

- the choice

The choice between consolidated or novel biomarkers (Andrea Peracino – Milan, Italy; Consolidated or Novel Biomarkers: How to choose? http://www.lorenzinifoundation.org/biomarkers2010/slides/showPres.asp?IDSlide=150 ) is asking accepted criteria to better qualify biomarkers in specific contexts. The challenge of evaluating and developing consensus criteria for the application of new imaging and biochemical technologies to choose biomarkers, is demanding a common understanding among the research,
industry, and regulatory communities. Specifically, biomarkers can aid in patient stratification (risk assessment), treatment response identification (surrogate markers), or differential diagnosis - identifying individuals who are likely to respond to specific drugs (J Biopharm Stat 2009; 19 (3): 530-42). The pathway between discovery to clinical practice has been largely consolidated in its several steps, and the necessity to integrate among them the several competences and methodologies is mandatory. Using athero-thrombosis as a model of underlying patho-mecanism of several interlinked diseases (e.g: cardiocerebrovascular disease, diabetes, obesity, some tumours), the integration process is demanding high context-specific qualified biomarkers, and a consensus in the use of their predictive value under the differentiated requirements of users. Many questions still open on the qualification of biomarkers can impact with the expected reliability of the outcomes of both the experimental and clinical processes. Critical areas and barriers to be better understood are e.g: Qualification versus validation of methods; the level of evidence and the technical performance required for qualification are dependent on the context of the proposed use; barriers in transferring integrated solutions to the benefit of the patients; barriers in transferring integrated solutions to the benefit of the society; role of regulatory authorities in defining rules; role of stakeholders in reaching consensus; role of scientific societies in the development of guidelines for method qualification. Validating is done preferably by different researchers in different centers, using a different case mix, and using slightly different definitions and measurements of both predictors and outcome. In case of reduced predictive accuracy, the model can be adjusted based on the validation study data, without having to develop an entirely new model (Clinical Chemistry 2010; 56: 537-541). Surrogate end points are biomarkers intended to be substitute for a clinically meaningful endpoint: biological plausibility, epidemiological data, qualify-quantitative relationship are requirements to support the definition of surrogate end points, in their ability to measure the drug treatment effect. Further understanding is required in evaluation of imaging. Critical points are now under consideration in the Health Technology Assessment (HTA) methodology: modality to evaluate accuracy of the imaging evaluation; studies on characteristics of the population (age, gender, concomitant diseases); goals of bioimaging application (diagnosis/prognosis); prevalence of disease/event in the study populations; pathophysiological background for bioimaging marker (adequacy, and integration); round the clock reporting; technolgical assessment by external experts; guidelines by Scientific Societies.

**Take home Messages**

**Integration of Biomarkers in chronic diseases**

A Coronary Artery Disease biomarker, such as apolipoprotein B, that is a direct measure of the number of atherogenic particles in plasma, may be superior to LDL-C as a predictor of Coronary Artery-CAD risk and as a target for therapy. Measures of HDL functionality may have value beyond HDL-C or apolipoprotein A-I in predicting CAD risk and as a target for therapy. (John J. Albers – Seattle, WA, USA)

Brain atrophy measured by MRI I is a valid marker of Alzheimer Disease and its progression. The degree of atrophy of medial temporal structures is a diagnostic marker of Alzheimer Disease at the MCI stage and is a sensitive marker of progression of neuro-degeneration. The integration
of structural markers and functional imaging with CSF biomarkers will provide optimal
diagnosis and monitoring of the disease process and therapeutic success. (John J. Albers –
Seattle, WA, USA)
Overall data support the concept that carotid-IMT is a biomarker of atherosclerotic disease:
the progression of carotid IMT together with the assessment of new vascular events will allow to
validate IMT-progression as biomarker of atherosclerosis. (Rodolfo Paoletti – Milan, Italy)

**Biomarkers in population studies and health policy**

Two decades ago, type 2 diabetes and obesity were very rarely observed in youth; presently
obesity in youth has reached epidemic proportions. Obesity together with insulin resistance
and relative insulin deficiency has resulted in a dramatic increase of Type 2 diabetes in youth: it has
been estimated that 20 – 30% of children now being diagnosed with diabetes have diabetes Type
2. (Santica M. Marcovina – Seattle, WA, USA)
A substantial proportion of young diabetic participants in the SEARCH study (see also J Clin
Endocrinol Metab 2010; 95: 2868-76) have abnormal lipids and lipoproteins: the frequency of
dyslipidemia is significantly higher in youth with Type 2 diabetes than in those with Type 1;
elevated apo B and dense LDL, both strong predictors of CVD, are the most frequent
abnormalities in youth with Type 2 diabetes. (Santica M. Marcovina – Seattle, WA, USA)

Saudi populations from Qassim Region are carriers of a relatively high amount of genetic alleles
predisposing them to hypertension and cardiac diseases. Adoption of competent programs for
prevention and/or early detection of cardiovascular disorders is required: e.g. screening for
serum homocystein levels to support supplementation either from dietary sources or from
medications especially to women during pregnancy. (Ahmad A. Settin – Buraydah, Saudi Arabia)

No association between ScaI ANP gene polymorphism and hypertension was found:
Sca I ANP gene variant is not a marker in Tunisian hypertensive subjects. (Hayet Soualmia –
Tunis, Tunisia)

**Biomarkers and primary prevention**

CRP is a marker but not a cause of CVD, nevertheless it can be useful in the identification of
patients at high risk of CV events due to “non traditional” risk factors.
Other markers of inflammation like Lp-PLA2 might play a pathogenetic role and become a
therapeutic target. (Filippo Crea – Rome, Italy)
Consolidated and Novel biomarkers

The increase of erythrocyte plasma membrane redox system (PMRS) and ascorbate free radical (AFR) reductase represent compensatory mechanisms to mitigate the increased oxidative stress. Elevated erythrocyte PMRS and AFR reductase may be used as markers to predict the development of diabetes. (Syed Ibrahim Rizvi – Allahabad, India)
Measuring Urinary 8-hydroxydeoxyguanosine (8-OHdG) is a novel convenient method for evaluating oxidative DNA damage. Diabetic patients, especially those with advanced nephropathy and retinopathy, have significantly higher levels of oxidative modifications that may contribute to the development of microvascular complications of diabetes. (Shereen Hassan Atef – Cairo, Egypt)

Heat shock protein 70 and IgE could be used for detection of early minor myocardial damage, as significant changes in their levels appear before any changes in the levels of MMP-9, CPK-MB and LDH. Besides, heat shock protein 70, and IgE returning to the normal levels after CABG surgery, thus suggesting that they could be helpful for evaluating the effect of CABG surgery. (Amal A. Baalash – Riyadh, Kingdom of Saudi Arabia)

Adiposity and insulin resistance collectively lead to accelerated aging that is associated with development and progression of chronic non-communicable diseases. Obesity and insulin resistance collectively influence chromosomal telomere length among adult Arabs with and without DMT2. The positive association of adiponectin to Telomeres has clinical implications due to the possible protective effects of this hormone against accelerated aging. (Shaun Louie B. Sabico)

Diabetic vasculopathy is the result of multiple factors, and the better understanding of the effects of sFas or intercellular adhesion molecules (ICAM) in halting the vasculopathy, targeting multiple mechanisms simultaneously by administering combination treatments with vascular endothelial growth factor (VEGF), anti-apoptotic drugs together with/or adrenomedullin-ADM may be prospective. (Faten Abdulhady Zakareia – Riyadh, Kingdom of Saudi Arabia)

Unmet needs in metabolic disorders

Among others are e.g.: measurement of beta cell function and mass; measure of Insulin resistance; markers of prediabetes/dysglycaemia; better early markers of renal disease; predictors of weight gain; ethnic specific markers of current risk profiling in CVD. (Sudhesh Kumar – Coventry, UK)
Critical points among others are paucity of integrated development programmes for biomarkers; true personalised medicines still unlikely for most; stratified medicine or nichebusters are most likely the next step. Biomarkers may include unconventional methods such as molecular imaging for beta cells, PET MR, eNose etc. There is the need to train clinicians differently to ensure delivery of personalised healthcare. (Sudhesh Kumar – Coventry, UK)

Methodlogical critical points

In the Type 2 group, the frequency of elevated apo B and dense LDL is significantly higher than the frequency of elevated LDL-cholesterol (36%, 36% and 23% respectively). These results indicate that the use of LDL-C to identify youth who will benefit from intervention, will miss a
significant proportion of those with high apo B and/or dense LDL thus resulting in an inadequate management of young Type 2 diabetics with dyslipidemia. It is well established that the impact of dyslipidemia on the development and progression of CVD is determined by the degree of dyslipidemia as well as by the duration of exposure. (Santica M. Marcovina – Seattle, WA, USA)

**Advanced technology**
The use of advanced technology in proteomics may help to better understand some pathomechanism. *Proteomic analysis* of HDL reveals at least 48 HDL-associated proteins. One of the HDL proteins is PLTP, an important modulator of lipoprotein metabolism. (John J. Albers – Seattle, WA, USA)

Damage to the proteome is increased in diabetes linked to metabolic control and development of vascular complications: some protein damage markers are sensitive to both fasting and postprandial hyperglycaemia and offer a route to improved indicators of glycaemic control. Urinary flux of some protein damage markers relates to risk prediction of decline in renal function. Protein damage markers may provide useful biomarkers in chronic disease as they often relate directly to metabolic dysfunction and mechanism of morbidity. (Paul J. Thornalley – Coventry, UK)

*Microarray analysis* allows the opportunity to discover novel drug targets, mechanisms of disease, and biomarkers for disease progression. Pharmacological dissection of signaling pathways involved in chronic disease modulation may allow the manipulation of individual features, e.g. stress transduction versus neuroprotection. (Lee E. Eiden - Bethesda, MD, USA)

**Clinical trials**
The development of a new molecule and the launch on the market of a new drug requires higher investments in resources and time than before; the breakeven between income and investment is even more postponed, and the need for a return on investment is affecting the price of marketed drugs. The entry of a new drug in the medical protocols requires strong support by large clinical trials and by the even more demanding criteria of evidence-based medicine. The approval by regulatory authorities of a new drug is based even more on stringent and documented advantages in the risk/benefit ratio for patients, and in the cost/benefit ratio for health organizations. The maintenance of a consolidated drug in the medical protocols is linked to even more stringent pharmaco-economic comparison with alternative approaches. (Rodolfo Paoletti – Milan, Italy)

**Key points in the use of biomarkers**

According to the US Food and Drug Administration, *pharmaco-genomics guidance for biomarkers* (http://www.fda.gov/cder/guidance/6400fnl.pdf; Oct25, 2006), biomarkers can be classified as exploratory, probably valid, and valid. There is a need for consensus criteria for validation and qualification of the integrated approach in the specific context of use of the tool (Circulation 2007; 115. 949-52).

Criteria are proposed for a standard reporting of biomarker studies and also describing the necessary statistical performance measures for risk estimation besides determination of relative risk (Circulation 2007; 116: 3-5. Clin Chem 2009; 55: 378-384). Such measures include e.g.
discrimination based on c-statistics, calibration looking into the closeness of predicted probability to the observed one, and possibly, most importantly for the clinical situation, reclassification of events based on additional testing. (Wolfgang Koenig – Ulm, Germany)

**ACC/AHA Classification of Recommendations**

There is an increasing concern in using ACC/AHA Recommendations in the evaluation of biomarkers. There is a need to revise the criteria underlined by the mentioned classification mainly in the evaluation of an increasing combination of biochemical and bioimaging markers. Decision making in front of the patients, in the studies of population, in drugs development (see also surrogate end-points) etc. are demanding an integration of competence and expertise of several experts and are urging new approaches to qualify diagnostic tools as biomarkers.

(Andrea Peracino – Milan, Italy)

*June 26, 2010*