S4.1

BIOCHEMICAL MARKERS OF SUCCESSFUL AGING. THE MODEL OF CENTENARIANS

Pes G.M.

Chair of Clinical Biochemistry, University of Sassari – Italy

The elderly population is rapidly increasing all over the world. However, it is well known that the pattern of aging has a marked inter-individual heterogeneity. There are subjects who become frail and rapidly dependent during aging, while others are able to maintain health and autonomy up to the later life. This pattern of 'successful aging' (1) is particularly remarkable in healthy centenarians, exceptional individuals who are characterised by a delayed aging process and particularly resistant against common age-related disease. In the last decade a lot of research has been carried out on centenarians, which now are recognized as a useful model to study the determinants of human longevity and to identify biochemical and genetic markers of successful aging. Blood chemistry analyses reveal that in centenarians most of the parameters are within the reference range for adult in general, and are related with the health status. However, centenarians may show paradoxically the presence of 'risk factors' for disease such as atherosclerosis, impaired hemostasis and cancer.

Nutritional status. Plasma levels of albumin, electrolytes, liver enzymes, B12 vitamin and folate as well as amino acids and vitamins are useful for the assessment of nutrition. The nutritional status in centenarians is quite variable and depends on the cognitive level and autonomy of the subject. According to the oxidative stress hypothesis successful aging is due to the ability of the organism to self-protect from damage caused by free radicals. However, the measurement of antioxidant compounds (e.g. vitamin A and E) in plasma of centenarians produced controversial results and the antioxidant enzymes (superoxide dismutase, glutathione peroxidase, catalase) are often in the same range as in young adults.

Markers of cardiovascular risk. Centenarians seem to be protected from vascular disease even though a considerable percentage of them carry a lipid profile considered at risk for atherosclerosis (high levels of total and LDL cholesterol, high levels of TG and normal/low levels of HDL). The serum level of Lp(a) is high in 25% of centenarians although not associated with sign of vascular disease. On the other hand, centenarians may show a low frequency of genetic factors causing hypercholesterolemia and neurodegeneration (e.g. ApoE å4).

Hyperfibrinogenemia and markers of impaired hemostasis are often present. These findings support the hypothesis that in subjects who age 'successfully' a complex remodelling of physiological functions and metabolism occurs.

Inflammation. Several markers of inflammation, such as high plasma concentrations of CRP and IL-6, are found in centenarians and correlate with the health status.

Bone metabolism. Aging is associated with a decrease in bone density. Accordingly, changes in markers of bone metabolism are frequently observed (increased BS-ALP activity, undetectable vitamin D).

Hormones. During the aging process an overall decline in hormone concentration occurs. Selective changes of plasma GH, IGF-1, and sexual hormones may be responsible for impaired functional status of arteries and brain.

In conclusion, centenarians are highly selected individuals who may allow us to identify biologic predictive markers related to increased survival and good quality of life. This model may prove crucial also to understand gender-specific differences in health and functional status at the extreme end of life. To achieve these goals we probably need more specific and appropriate laboratory strategies.

[1] Rowe and Kahn. Science 1987;237:143-149.

S4.2

IMMUNOLOGY AND GENETICS OF HUMAN LONGEVITY

Franceschi C.[1], Valensin S.[2], Monti D.[3], Sansoni P.[4], Bonafè M.[2], Prolisso G.[5], Caruso C.[6], Deiana L.[7], Pes G.[7], Carru C.[7], De Benedictis G.[8], Baggio G.[7,9]

[1] Italian National Research Centre on Aging, Ancona, Italy
[2] Dept. of Experimental Pathology, University of Bologna, Italy
[3] Dept. of Oncology and Experimental Pathology, University of Florence, Italy
[4] Dept. of Internal medicine, University of Parma, Italy
[5] Dept. of Gerontology and Geriatrics, II University of Naples, Italy
[6] Dept. of Biopathology, University of Palermo, Italy
[7] Inst. Of Clinical Biochemistry, University of Sassari, Italy
[8] Dept. of Biology, University of Calabria, Cosenza, Italy
[9] Dept. of Geriatrics, University Hospital Padua, Italy

In the last ten years a large group of Italian scientists became interested in the biological determinants of human aging , with particular attention to longevity and successful aging. To this aim several hundreds of people older than 100 years of age (centenarians) were traced and recruited in continental Italy, Sardinia and Sicily, interviewed, characterized for their health status and thoroughly studied for a variety of biological parameters, and compared with people of younger age from 20 to a 100 years. The most important finding can be summarized as follows: 1. the immune system undergo complex and profound changes (immunosenescence) we proposed to indicate with the term "remodelling" to stress that it is not a random deteriorative phenomenon, but rather a reshaping which apparently derives from the evolutionary architecture of the immune system and appears to inversely recapitulate its evolutionary pattern. Indeed, as age progress innate immunity, the most ancestral type of immunity centered on macrophage, is activated while clonotypical, adaptive immunity, the most evolutionary recent and sophisticated type of immunity centered on lymphocytes, deteriorates. Both these characteristics of immunosenescence are likely the consequence of the lifelong antigenic stress, i.e. the continuous, unavoidable exposure to bacteria, viruses, but also food and self molecules, among others. The activation of innate immunity is related to another major characteristics of aging, i.e. the presence of a chronic inflammatory status we suggested to refer to as "inflamm-aging", a biological situation which likely favours the onset of major age-related pathologies such as atherosclerosis and neurodegeneration, and diseases such as diabetes and osteoporosis, all sharing a consistent inflammatory pathogenesis. The results of the study of the genetics of aging and longevity are in agreement with this general scenario. In recent years association studies on more than 40 candidate genes have been performed in our laboratories on centenarians and younger control subjects, and the results indicate that indeed most of the positive results we obtained regard inflammation and stress response, as well as glucose utilization and energy production, all phenomena related to the response to damaging and threatening agents, and which require a readjustment of metabolic pathways and energy redirecting.
Last but not least, during these studies on centenarians we realized that, in order to correctly interpret the immunological and particularly the genetic data, it was absolutely necessary to take into account the demographic characteristics of the population we were interested in. Thus, in collaboration with scientists of the Max Plank Institute for Demographic Research in Rostock, Germany, new analytic tools and models were generated which greatly help the comprehension of such difficult and complex phenomena such as human aging and longevity.

S4.3

I MECCANISMI PATOGENETICI DELLA PATOLOGIA AUTOIMMUNITARIA

Indiveri F.

Dip. di Medicina Interna, Div. di Medicina Interna ad orientamento Immunologico, Universita di Genova

Both genetic and environmental factors are involved in the pathogenesis of autoimmune diseases. These factors act by altering the homeostasis of the immune system increasing the stimulating activity of autoreactive B and T lymphocytes and/or impairing the function of regulatory lymphocytes. The study of genetic of autoimmune diseases has allowed the identification of specific gene loci in animal and human chromosomes that are strictly associated with the predisposition to the autoimmune disease. This is the case for systemic lupus erythematosus (SLE) in which 3 loci (named sle1, sle2, sle3) must be present in mice to make the animal prone to SLE.

Furthermore, alterations of specific genes (i.e., the IL2 and the CTLA-4 genes) and genetic alteration of specific functions (i.e., phagocytosis of apoptotic bodies) have been identified in individuals affected by autoimmune diseases. In particular, the impairment of phagocytosis of apoptotic bodies is relevant in diseases, such as SLE, in which the autoantigens are endocellular (nuclear). In fact, it abolishes the regulatory mechanisms consequent to phagocytosis of apoptotic cells, allows the conversion of apoptotic bodies into pro-inflammatory necrotic debris and exposes the nuclear antigens, thus providing stimulation of autoreactive, pathogenic lymphocytes.

Environmental factors act by activating the innate immunity and/or by unbalancing the equilibrium between autoreactive clones and regulatory lymphocytes. It is now clear that immune responses are consequent to the existence of dangerous signals (constituted, i.e., by toxins, necrotic cells, bacterial DNA) which interact with the TOLL receptors on the surface of dendritic cells, macrophages, B lymphocytes, natural killer cells, neutrophils, activating inflammatory functions. Some viruses or bacteria may induce a strong stimulation of innate immunity favouring polyspecific effector immune responses and causing the onset of autoimmune phenomena.

The stimulation of autoreactive clones can also be due to exogenous antigens that mimick self antigens. It is now clear that it is enough that two peptides may have few or single aminoacids in common to can induce antigenic mimicking. Hence, this phenomenon is more frequent than before thought.

Impairment of regulatory cell function have been demonstrated in subjects with autoimmune diseases. The causes of these alterations are presently unknown. However, such findings have provided new insights for the comprehension of pathogenesis of autoimmune diseases.

S4.4

THE AUTOIMMUNE DIAGNOSIS IN ENDOCRINE DISEASES

Betterle C., Pedini B., Zanchetta R.

Chair of Clinical Immunology and Allergy, Department of Medical and Surgical Sciences. University of Padova, Padova Italy

Introduction

In 1957 Witebsky proposed the criteria for defining a disease as "autoimmune". All the organs may be virtually affected, although certains seem particularly susceptible (e.g., endocrine tissues). In this case, thyroid, stomach, pancreatic islet-cells, adrenals, parathyroid, gonads, adeno- and neurohypophysis can be subject to autoimmune attack. The autoimmune endocrine diseases (AED) are characterized by lymphocytic infiltrations into target tissues and by circulating autoantibodies. Identification and characterization of autoantigens are integral parts of research into the understanding of diagnosis, pathogenesis and preventive therapy of these diseases. The prevalence of AED is very different varying from <0.5-10%, but surely it is underestimated because many diseases can be present in subclinical forms.

Thyroid autoimmune diseases (TAD)

The spectrum of TAD includes two main clinical entities: chronic autoimmune thyroiditis and Graves' disease. Autoantibodies to thyroid peroxydase (TPO-Ab) and thyroglobulin are present, at different combinations, titers and frequency in chronic thyroiditis and Graves' disease. These antibodies are also present in patients without clinical TAD and correlate with lymphocytic infiltration and/or thyroid subclinical dysfunction. TSH-receptor blocking antibodies are additionally present in 25-40% of chronic thyroiditis and correlate with severe clinical hypothyroidism. TSH-receptor stimulating antibodies are also present in about 95% of patients with Graves' disease at onset inducing hyperthyroidism. The techniques for the detection of these antibodies are: RIA, ELISA or bioassay with recombinant autoantigens.

Stomach autoimmune diseases

The autoimmune diseases of stomach are atrophic body gastritis and pernicious anemia. Parietal cell (PCA) and intrisc factor autoantibodies (IFA) are the markers of these diseases. The frequency of PCA varies from about 65% in isolated body gastritis until 100% in pernicious anemia, but in this later disease IFA are additionally present in most of the patients. PCA and IFA may be present also in patients without any clinical sign, but they are at high risk of developing atrophic gastritis or pernicious anemia. The main autoantigens of PCA and IFA are $ATPaseH^+/K^+$ and intrinsic factor, respectively. The techniques for the detection of these antibodies are IFI on normal tissue and ELISA or RIA with recombinant or native antigens.

Pancreas autoimmune diseases

Type 1 diabetes mellitus is a disease characterized by an immuno-mediated destruction of the beta-cells. Islet-cells antibodies (ICA), anti-GAD, anti-IA2, and anti-insulin autoantibodies are the best serological markers of this disease. One or more of these are present in 80-95% of patients at onset. These antibodies may be present in some susceptible subjects without diabetes mellitus, and they confer a risk of diabetes directly correlated with the number of the markers and indirectly with the age of patients. The main techniques for their detection are IFI on human pancreas and RIA using recombinat autoantigens.

Adrenal autoimmune disease

Autoimmune Addison's (AD) disease is the most frequent cause of primary adrenocortical insufficiency. Adrenal cortex autoantibodies (ACA) are present in about 100% of patients at onset of disease, while they are absent in patients with non-autoimmune AD. ACA may be present also in patients without hypoadrenalism, but these patients must be followed up because they are at high risk to develop AD (mainly if children). 21-hydroxylase has been identified as the main autoantigen of ACA and now 21-OHAbs can be determined by IPA using recombinant antigens. ACTH-R blocking antibodies may be additionally present in patients with AD and stimulating ACTH-R antibodies with a rare form of autoimmune Cushing's disease, but the role of these autoantibodies needs to be confirmed. Antibodies against adrenal medulla or sympathetic ganglia were recently described by IFI but their existence and clinical value have to be established.

Autoimmune diseases of gonads

Premature ovarian failure (POF) is difined as a hypergonadotropic hypogonadism in an amenorrhoeal women before 40 yrs. Autoimmunity may contribute to develop two distinct forms of POF: a) lymphocytic oophoritis and b) Savage syndrome. Steroid-producing cells autoantibodies (StCA) are positive in almost all the patients with POF associated to AD, while they are present in only 10% of POF isolated or associated to other autoimmune diseases. The autoantigens of StCA are steroidogenic enzymes (17α-hydroxylase or P450 side chain cleavage). Gonadotropin receptors blocking autoantibodies were demonstrated in many of the patients affected by Savage syndrome, but these autoantibodies need to be confirmed by bioassay using recombinant autoantigens. In males, the autoimmune insufficiency of the testis is very rare and, if associated to AD, is positive for StCA.

Parathyroid autoimmune disease

Chronic hypoparathyroidism is an autoimmune disease where autoantibodies reacting with cytoplasm or surface of parathyroid tissue or cytotoxic to human parathyroid cells have been reported but they are not specific being absorbed by non-organ specific autoantigens. About half of patients with chronic hypoparathyroidism were reported to have autoantibodies reacting with calcium-sensing receptor, but also these data need to be confirmed.

Autoimmune disease of adeno- and neuro-hypophysis

Lymphocytic hypophysitis is a disease showing clinical symptoms of pituitary mass associated with deficient secretion of singular or multiple tropins. The detection of pituitary autoantibodies is difficult to perform, and the diagnosis of lymphocytic hypophysitis remains elusive without a hystological demonstration. Lymphocytic neuro-hypophysitis can manifest as diabetes insipidus. 37% of patients with idiopathic ID has vasopressin-producing cells autoantibodies by IFI. These antibodies may be also found in 1.2% of patients with EAD without any sign of ID. Some of these patients showed presence of partial ID, demonstrating the importance of these antibodies as markers of subclinical or potential disease.

Autoimmune polyendocrine syndromes (APS)

APS are the association between endocrine and non-endocrine autoimmune diseases observed in some patients. The APS do not appear randomly but in particular combinations and on the basis of these combinations they are defined as Type 1, Type 2 and Type 4. The autoantibody screening performed in patients at onset of one autoimmune disease is usefull to identify subclinical or potential APS. In this way many patients with potential or subclinical autoimmune APS can be precociously treated.

Reference

1. C. Betterle, Gli Autoanticorpi, Ed. Piccin, Padova, 1997
2. C. Betterle, Le Malattie Autoimmuni, Ed. Piccin, Padova, 2001

S4.5

STUDY OF GLUTATHIONE TRANSFERASE P1-1 POLYMORPHISM IN A CENTENARIAN POPULATION FROM SARDINIA

[1]Bellincampi L., [4]Carru C., [1,2]Bernardini S., [4]Pes G., [1]Dessì M.R., [1]Cortese C., [1,2]Federici G., [4]Baggio G., [3]Franceschi C., [4]Deiana L.

[1]Dept. of Internal Medicine, Univ. of Rome Tor Vergata, Rome, [2]Bambino-Gesù Children's Hospital-IRCCS Rome, [3]Italian National Research Centers on Aging, INRCA, Ancona, [4]University of Sassari, Italy.

Drug metabolizing enzyme genes such as cytochrome P450-1A1 or Glutathione S-transferases (GSTs,) have been recently implicated as candidate longevity genes. GSTs belong to a large family of functionally different enzymes which catalyzes the S-conjugation of glutathione (GSH) with a wide variety of electrophilic compounds including carcinogens, anticancer drugs, reactive oxygen species, and products of cellular metabolism. The GSTP1-1 class is polymorphic and four alleles have been described at the GSTP1 locus located on chromosome 11q13 at the electrophilic "H" site level: GSTP1**A*, GSTP1**B*, GSTP1**C*, and GSTP1**D*. Two sites in the cDNA sequence are variable and are characterized by an A → G transition at nucleotide 313 (point mutation in exon 5) and a C → T transition at nucleotide 341 (point mutation in exon 6). The resulting codon variants result in the amino acids Ile105 or Val105 and Ala114 or Val114. Consequently four allelic variants can be generated which have been conventionally defined as *A*,B*,C* or D**.

The aim of our investigation was to determine the relative allelic and genotype frequencies of this polymorphism in a population of 115 centenarians from Sardinia and two control populations of a younger age coming from Sardinia and continental Italy respectively

The *GSTP1-1* polymorphism was determined using a real time polymerase chain reaction and fluorescence resonance energy transfer with a Light-Cycler Instrument monitoring the PCR with specific hybridization probes.

Preliminary results:

	Centenarian (n=115)	CTRL from Sardinia (n=65)	CTRL from Rome (n=250)
A*	*64 %*	*70 %*	*71 %*
B*	*33 %*	*30 %*	*24 %*
C*	*3 %*	*0 %*	*5 %*

All the observed genotypes were in Hardy-Weimberg equilibrium. We did not find significant difference between centenarians and control subjects from Sardinia, although we found a trend in the Roman area toward a decrease in the frequency of the *B** variant. In the populations studied we did not find the *D** allele.

S4.6

GLYOXAL PLASMA LEVEL EVALUATION IN DIABETES AND NEPHROPATHY DISEASES BY A NEW GC/MS METHOD

Lapolla A.[1], Fedele D.[1], Senesi A.[1], Reitano R.[1], Aricò N.C.[1], Marotta E.[2], Pace G.[2], Deraglia R.[2], Traldi P.[2]

[1]Dipartimento di Scienze Mediche e Chirurgiche, Università di Padova, Via Giustiniani 2, 35100 Padova (Italy)
[2]CNR, Istituto di Scienze e Tecnologie Molecolari, Corso Stati Uniti 4, 35100 Padova (Italy)

A prevailing theme in diabetes research is that diabetic complications may not be a direct consequence of insulin deficiency or resistance, but an indirect result of chemical or metabolic sequelae of hyperglycemia. Twenty years ago, glycation of proteins was considered a reasonable source of diabetic complication, but this view gradually evolved into the advanced glycation end product (AGE) hypothesis.[1,2] AGEs production increases with high glucose level and long-lived proteins (e.g. collagen and crystallins) were recognized as important target proteins which accumulate AGEs in affected tissues. The Amadori patterns show the production of highly reactive AGE, as glyoxal and methylglyoxal are. The levels of these products show significant increase in renal failure irrespectively from the concurrence of diabetes disease.

For such reason the development of analytical methods able to quantify these molecules in plasma is surely of interest. The main requirements of these methods must be their reliability, confidence and simplicity and for such scope GC/MS seems to be particularly appealing. In this case a suitable derivatization procedure is required, and we have studied the reaction of glyoxal with O-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (PFBOA), leading to the corresponding PFBOA-oximes.[3]

The reaction, pH dependent, results quantitative in presence of PFBOA in excess. The detection limit in plasma of glyoxal was found in the order of ppb. Preliminary results on plasma samples for healthy, diabetic and nephropathic subjects show that the developed method is highly effective, allowing to put in evidence higher glyoxal levels in the last class of subjects. These results can be of interest considering that inhibition of AGE formation, rather than glycation, was recognized as an important goal for pharmaceutical management of diabetes.

1. H. Vlassara, R. Bucala and L. Striker Lab Invest. 1994, 70, 138-151.
2. M. Brownlee, H. Vlassara, A. Kooney, P. Ulrich and A. Cerami Science 1986, 232, 1629-1632.
3. S. Devon, A. Cancilla and S. Shane J. Chromatogr. 1992, 627, 1-16.

S4.7

PREVALENCE OF ORGAN AND NON ORGAN-SPECIFIC AUTOANTIBODIES IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

Tampoia M., Delia M.*, Capalbo S.*, Pansini N., Liso V.*

Clinical Pathology I, Policlinic of Bari
*Hematology, University of Bari

The association between autoimmunity and hematological malignancies has already been reported including the detection of autoantibodies in linphoproliferative disorders. CLL-cells from these patients seem to make autoantibodies reacting with antigens present on red blood cells and platelets causing autoimmune hemolitic anemia or immune thrombocytopenia (5%).
The aim of our study was to investigate non hematological autoimmunity in patients affected by Chronic Lymphocytic Leukemia.
We studied 115 sera from CLL-patients (44 women and 71 men of median age of 68 years; 86 in stage A; 15 in stage B and 14 in stage C) and 75 age/sex matched healt controls. We studied positivity for non organ-specific autoantibodies:antinuclear (ANA), anticardiolipin (ACA), anti reumatoid factor (RA-test) and for organ – specific autoantibodies: anti-thytroid (antiTPO), anti-thyroglobulin (antiTG), antiliver kidney microsomal (antiLKM), AMA, ASMA, APCA, ARA.
Twentytwo sera (19%) by CLL-patients and 6 sera (8%) by controls displayed ANA with a title > 80. This difference between the two groups was found to be significant ($p<0.05$) with a higher prevalence of positivity in men (16 patients) than in women (6 patients). We did not find any significant difference between the two groups about organ-specific autoimmunity. Four patients (3%) presented monoclonal protein in blood but none of these patients displayed ANA positivity and 7 patients (6%) had autoimmune disease as autoimmune hemolitic anemia or immune thrombocytopenia, but only one of these patients showed ANA positivity (with a title of 80).
In conclusion we found ANA-positivity in a percentage of CLL-patients significantly higher than in health controls. These autoantibodies do not contribute directly to the types of autoimmune disease that frequently are observed in patients with CLL.

S4.8

EVALUATION OF DIAGNOSTIC PERFORMANCE AbTg AND AbTPO AUTOANTIBODIES ASSAY USING THE AIA1200 ANALYZER

Grasso L., Mammoli C., De Marco G, Del Grosso A., Nencetti C., Albertini S., Chiovato L., Pinchera A.

Department of Endocrinology,University of Pisa-Pisa.

Accurate and sensitive methods are required to identify patients with and or risk for autoimmune thyroid disease. Thyroid peroxidase AbTPO and thyroglobulin antibodies AbTg assay are developed for the AIA 1200 Analyzer .Briefly human antibodies are captured on Tg or TPO human antigen coated microparticles and washed before the addition of human IgG conjugate with alcaline phosphatase; after a second incubation and wash step the fluorogenic 4-Metilumbelliferon is added. The titers are measured quantitavely by WHO derived standard curve. The precision, limit of detection, dynamic range suitability and frequency of antibody titers have been evaluated in a group from of 80 normal controls and in 210 patients with various thyroid diseases: toxic diffuse goiter (n 80), Hashimoto thyroiditis, (n .80) and differentiated thyroid carcinoma (n.50). Abtg and AbTPO are quantitated within a range from 1-2000 IU/ml for AbTg and 1-1000 IU/ml for AbTPO. The interassay coefficients of variation for AbTg at the concentrations of 1, 70, 270, IU/ml were 14, 10, 18 %, respectively. The interassay coefficients of variation for AbTPO at the concentrations of 10, 100, 500, IU/ml were 9.4,13.0,13.3 %, respectively. Calculated analytical sensitivity for AbTg is 0.8 IU/ml and for AbTPO is 1 IU/ml. In normal controls the range was from <1 to 10 IU/ml for AbTPO and <1 to 29 IU/ml for AbTg .
In the group of patients with toxic diffuse goiter serum AbTg was detected in55% and 78% for AbTPO respectively; in the group of patients with Hashimoto thiroiditis serum AbTg was detected in 60% and 84% for AbTPO respectively. In the group of patients with differentiated thyroid carcinoma serum AbTg was detected in 8% and 8% for AbTPO respectively.The results of the present study indicate that the AIA 1200 Analyzer is a precise and sensitive method for serum AbTg and AbTPO determinations and it is useful for the diagnosis of autoimmune thyroid diseases.

LP3

THROMBOTIC RISK INDEXES

Davì G.

Dipartimento di Medicina e Scienze dell'Invecchiamento, Università degli Studi "G. D'Annunzio", Chieti

There isn't a widely accepted definition of thrombophilia. In the past years, this term has been used to identify those disorders of hemostasis that predispose to thrombosis. Recently, it has been defined as a tendency to develop thrombosis as a consequence of both congenital or acquired predisposing factors. Thrombophilia may be caused by several conditions which can be investigated by laboratory testing. Thus, in the last few years, it has been responsible for an increased pressure on clinical laboratories, although demands for testing are not always justified.

There are many established congenital conditions associated with an increased risk for venous thromboembolism, such as antithrombin (AT), protein C (PC), and protein S (PS) deficiencies, and factor V (FV) Leiden mutation, leading to the activated PC (APC) resistance phenomenon. The relative risks associated with these abnormalities range from AT deficiency (the most severe), to PC/PS deficiencies (intermediate severity), to APC resistance (the least severe). These abnormalities are generally investigated by plasma-based assays, mainly because they are attributable to different mutations. Moreover, another condition associated with an increased thromboembolic risk is congenital dysfibrinogenemia, a rare risk factor for both venous and arterial thrombosis, whether abnormalities of the fibrinolytic system have not been confirmed as risk factor for thrombosis. By contrast, the mutation G20210A in the prothrombin gene, which may lead to hyperprothrombinemia, is firmly associated with an increased tendency to develop thromboembolism. Hyperhomocysteinemia may be caused by a congenital deficiency of the enzymes involved in the metabolic pathways of the essential aminoacid methionine, such as cysthationine β-synthase (CβS), methionine synthase (MS) or methilenetetrahydrofolate reductase (MTHFR). Interestingly, it is a graded risk factor, as the risk increases by 40% for every 5 μmol/L increase in homocysteine levels. Homocysteine can be measured by high performance liquid chromatography (HPLC) or by immunoassays that are as reliable as the HPLC method.

Among the acquired conditions associated with an increased risk for venous and arterial thrombosis, the anti-phospholipid antibody syndrome and hyperhomocysteinemia are the most important. The first syndrome is characterized by repeated positive test for lupus anticoagulant (LA) and/or solid-phase anti-phospholipid antibodies and, from a clinical standpoint, by thrombocytopenia and fetal loss. Acquired hyperhomocysteinemia is mainly caused by a poor dietary intake of folic acid and vitamin B12, acting as cofactors in the metabolism of methionine. The Leiden Thrombophilia Study revealed that high concentrations of procoagulant factors such as XI, VIII, IX, and fibrinogen were associated with an increased risk for venous thrombosis. Similarly, elevated plasma levels of thrombin activatable fibrinolysis inhibitor (TAFI) represent an independent risk factor for venous thrombosis. All the above, except factor VIII, seem to be weak risk factors and their value in the investigation of thrombophilic patients has not been established yet. In fact, the role of factor VIII as a thrombotic risk factor has been confirmed, and assays for both the antigen and the activity are suitable for screening thrombophilic patients. There are several coagulative markers, such as prothrombin fragment 1+2, fibrinopeptide A, thrombin-antithrombin complexes, and factor VIIa, which reflect coagulative activation, contributing to a pro-thrombotic state in many clinical settings. In addition, an impairment of anticoagulant mechanisms, reflected by a decrease in circulating levels of soluble thrombomodulin, protein C, protein C activation peptide, or activated protein C, may also predispose to the development of atherothrombosis. Moreover, a state of platelet activation, leading to an increased thrombotic risk, can be revealed by measuring urinary excretion of 11-dehydro-thromboxane (TX)B_2, a major tromboxane A_2 enzymatic metabolite, or plasma levels of P-selectin, that may give a comprehensive indication on platelet and endothelial cell functional status.

The oxidative modification of low density lipoproteins (LDL) plays a central role in atherosclerosis. Among the variety of biologically active lipids that result from LDL oxidation, a new family of prostaglandin isomers (isoprostanes), resulting from oxidative modification of arachidonic acid through a free radical–catalyzed mechanism, is emerging. They might operate as transduction mechanisms linking oxidant stress to specialized forms of cellular activation, such as platelet activation, as reflected by urinary 11-dehydro-TXB_2 excretion, and smooth muscle cell proliferation in human vascular disease. One of these compounds is 8-iso-$PGF_{2\alpha}$, which has attracted considerable attention because of its biological activity. Both stable-isotope dilution assays using gas chromatography/mass spectrometry (GC/MS) and immunoassays for 8-iso-$PGF_{2\alpha}$ have been developed. Measurable concentrations of the unmetabolized compound are present in peripheral venous blood, and a relatively reproducible fraction is excreted unchanged in uman urine. Given that mass spectrometers are confined to relatively specialized laboratories, the application of this methodology to clinical investigation is likely to remain restricted. An alternative approach is to use an immunoassay. Using an antibody previously described, we have published a correlation between levels of 8-iso-$PGF_{2\alpha}$ determined by both immunoassay and GC/MS.

Several studies have been completed addressing the formation of F_2 isoprostanes in clinical settings associated with oxidative damage, either as a result of acute ischemia/reperfusion or long-standing metabolic abnormalities. Free radicals are thought to mediate the reperfusion injury that characterizes thrombolytic therapy after myocardial infarction.

Enhanced formation of F2 isoprostanes has been reported in association with several cardiovascular risk factors, including cigarette smoking, diabetes mellitus, hypercholesterolemia, and hyperhomocysteinemia, conditions characterized by increased lipid peroxidation in response to cigarette smoke or complex metabolic abnormalities. Accelerated LDL oxidation, as well as platelet activation, are common features of these conditions. We have recently demonstrated that increased oxidant stress in obesity would induce enhanced generation of 8-iso-$PGF_2\alpha$, which in turn contribute to platelet activation in this setting.

However, the role of antioxidant therapy in the prevention of vascular disease is not well established; in fact, results from placebo-controlled primary prevention studies, such as CHAOS or CARET, failed to show any benefit from the use of antioxidant vitamins. On the other hand, some secondary prevention studies have suggested potential beneficial effects of antioxidants.

It has been demonstrated by our group that two-week dosing with vitamin E (100 to 600 mg daily) reduces urinary 8-iso-$PGF_{2\alpha}$ excretion rate in a dose-dependent fashion in hypercholesterolemic subjects, with all measurements falling within the range of healthy subjects at 600 mg daily. Similar changes in urinary 8-iso-$PGF_{2\alpha}$ have been reported with high-dose vitamin E supplementation in patients with diabetes mellitus.