REVIEW

Novel biomarkers of atherosclerosis and cardiovascular risk in autoimmune diseases: Genomics and proteomics approaches

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Atherosclerosis (AT) and cardiovascular disease (CVD) are enhanced in autoimmune diseases such as antiphospholipid syndrome (APS), systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA). The reason for this accelerated process is still debatable and, although traditional risk factors are more prevalent in those patients than in general population, they do not fully explain that enhanced risk. Inflammatory components of the immune response, mainly interleukins, TNF-α, and IFN-γ, as well as some autoantibodies, including anti-oxidized low density lipoproteins (anti-oxLDL), anti-beta-2-Glycoprotein 1 (anti- β2GPI), anti-Heat shock proteins 60/65 (anti-HSP60/65), and anti-oxLDL/β2GPI have been shown to play a leading role in the pathogenesis of both, AT and CVD. However, the role of the autoantibodies in accelerated AT in autoimmune disease patients is still controversial. Recently, DNA microarray and proteomic-based approaches have made substantial breakthrough into the study of various rheumatic diseases, thus allowing for the discovery of previously unknown proteins involved in CVD including some that may be suitable to be used as biomarkers. Herein, we review recent genomics and proteomic approaches that have been applied to the study of autoimmune diseases with atherosclerotic and CV risk. The pharmacogenomics and pharmacoproteomics studies given over to the analysis of ancient and new drugs used to relieve the physiopathology associated to these complex diseases are also discussed.

Keywords:
Atherosclerosis / Autoimmune diseases / Cardiovascular disease / Genomics / Proteomics

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Abbreviations: AGPAT-1, 1-acylglycerol-3-phosphate *O*-acyltransferase 1; **anti-oxLDL,** anti-oxidized low density lipoproteins; **anti-β2GPI,** anti-β-2-Glycoprotein 1; **anti-HSP60/65,** anti-heat shock proteins 60/65; **aPL,** antiphospholipid antibodies; **APS,** antiphospholipid syndrome; **AT,** Atherosclerosis; **CV,** cardiovascular; **CVD,** cardiovascular disease; **DMARDs,** disease-modifying antirheumatic drugs; **HDL,** high density lipoproteins; **HMG,** 3-hydroxy-3-methylglutaryl; **Hp,** haptoglobin; **IFN,** interferon; **IL,** interleukin; **MAPK,** mitogen activated protein kinase; **miRNA,** microRNA; **MTX,** methotrexate; **NFκB,** nuclear factor kappa B; **PBMCs,** peripheral blood mononuclear cells; **PDI,** protein disulfide isomerase; **RA,** rheumatoid arthritis; **RAGE,** receptor for advanced glycation end products; **SAA,** serum amyloid A; **SLE,** systemic lupus erythematosus; **SNPs,** single nucleotide polymorphisms; **TF,** tissue factor; **TNF-α,** tumor necrosis factor alpha; **VEGF,** vascular endothelial growth factor

1 Autoimmune diseases: Relationship with atherosclerosis and cardiovascular risk

Atherosclerosis (AT) is a chronic inflammatory disease of the arteries associated with various risk factors that promote lipid abnormalities (*i.e.*, dyslipidemia), development and progression of atherosclerotic lesions, plaque rupture, and vascular thrombosis [1]. AT is enhanced in autoimmune diseases; noninvasive investigations show increases in intima-media thickness, carotid plaque, and coronary artery calcifications in patients with antiphospholipid syndrome (APS), systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA) compared to controls [2]. The reason for this accelerated process is still debatable and, although traditional risk factors (such as hyperlipidemia, smoking, obesity, hypertension, diabetes mellitus, postmenopausal status, and sedentary lifestyle) are more prevalent in those patients than in general population, they do not seem to fully explain that enhanced risk [3].

Experimental studies and human observations suggest that innate and adaptive immune responses participate in the pathogenesis of both AT and autoimmune diseases. Actually, some autoantibodies, including anti-oxidized low density lipoproteins (anti-oxLDL), anti-β-2-Glycoprotein 1 (anti-β2GPI), anti-Heat shock proteins 60/65 (anti-HSP60/65), and anti-oxLDL/β2GPI, have been shown to be associated to the pathogenesis of AT [4, 5]. However, their role in accelerated AT in APS, SLE, and RA patients is still controversial. In fact, some of them seem to be proatherogenic and other protective; moreover, it has been demonstrated that induced oral tolerance has a protective role against AT [6, 7]. Thus, many other studies are required to explain the role of autoantibodies in the pathogenesis of AT in these patients, because the characteristics of these autoimmune diseases seem to mask their effects for atherogenesis.

AT and heart failure are characterized by a long period of silent disease progression, allowing early diagnosis and the potential of early therapeutic intervention. Recently, DNA microarray and proteomic-based approaches have made steady inroads into the study of various rheumatic diseases [8]. The transcriptome analysis has two main objectives in rheumatology: (i) identifying a gene expression profile that is a hallmark of a pathology and using it for a diagnostic or prognostic purpose and (ii) gathering genes with similar changes of expression, which allows one to specify the identity of novel proteins involved in a well-known intracellular cascade of regulation or even to identify new cascades [9]. Protemic approaches that have been applied to the study of rheumatic diseases include 2-DE/MS, multidimensional high-pressure LC-MS/MS, CE-MS/MS, SELDI–TOF-MS/MS, and a variety of targeted antibody-based protein arrays [8]. Although each of these different methods has its own advantages and disadvantages, the application of these proteomic tools to rheumatology has given birth to a continuously increasing panel of molecules that may have the potential to serve as early biomarkers in various rheumatic diseases.

2 Genomic and proteomic studies developed in systemic autoimmune diseases

2.1 APS, AT and cardiovascular disease (CVD)

APS was defined as a clinical disorder characterized by thrombosis and pregnancy morbidity associated with the persistent presence of antiphospholipid (aPL) antibodies including anti-β2GPI, and/or lupus anticoagulant (LA) [10]. Thrombosis is the major manifestation in patients with aPL, but the spectrum of symptoms and signs associated with aPL has considerably broadened, and other manifestations such as thrombocytopenia, nonthrombotic neurological syndromes, psychiatric manifestations, livedo reticularis, skin ulcers, haemolytic anemia, pulmonary hypertension, cardiac valve abnormality, and AT have also been related to the presence of those antibodies [11].

Numerous mechanisms have been proposed to explain the thrombotic tendency of patients with APS, but the pathogenesis seems to be multifactorial. Procoagulant cell activation, accompanied with tissue factor (TF) expression, and TF pathway upregulation is one of the key events considered explaining the pathophysiology of thrombosis in patients with APS. Previous studies showed elevated plasma levels of soluble TF in APS patients, and thereafter we reported that monocytes prepared from APS patients had high TF expression [12–14]. At molecular level, the signal transduction mechanisms induced by aPL have been recently explored. In a recent study, we showed that aPL induced TF in monocytes from APS patients by activating, simultaneously and independently, the phosphorylation of mitogen-activated protein kinase/extracellular regulated kinase (MEK-1/ERK) protein, and the p38 mitogen-activated protein kinase (MAPK)-dependent nuclear translocation and activation of nuclear factor κ B (NFκB)/Rel proteins [15]. Similar results have been reported in platelets, monocyte cell lines, and on *in vivo* models of aPL-induced thrombogenicity [16–18]. Parallel studies performed in endothelial cells (ECs) further concluded that: (i) NFκB plays an essential role in aPL activation by aPL [19]; (ii) p38 MAPK phosphorylation and NFκB activation are involved in the aPL-induced increase of TF transcription, function and expression, IL6 and IL8 upregulation, and inducible nitric oxide syntase (iNOS) expression [20].

Previous reports indicate a close relationship between TF and vascular endothelial growth factor (VEGF), a family of proteins involved in normal vascular development, and in relevant pathologies including cancer, wound healing, and inflammation [21]. Precedent studies had reported increased plasma levels of VEGF in APS patients [22]. In a recent study, we analyzed the VEGF and fms-related tyrosine kinase 1 (Flt-1) expression levels in monocytes of APS patients, the molecular mechanisms involved in their aPL-induced expression, and their association with the elevated TF expression found in these patients [23]. Our data primarily showed that monocytes from APS patients expressed increased levels of both VEGF and Flt-1 in comparison with monocytes from healthy donors. Furthermore, *in vitro* results indicated that this cytokine was produced by monocytes when treated with aPL and that the p38 MAPK signaling pathway played an important role. Thus, VEGF might act as a regulatory factor in aPL-mediated monocyte activation and TF expression, thereby contributing to the proinflammatory–prothrombotic phenotype of the APS patients.

Experimental studies and human observations suggest that APS is associated with AT. In fact, innate and adaptive immune responses participate in the pathogenesis of both diseases. Anti-oxLDL, anti-aPL, anti-β2GPI, anti-HSP antibodies, among others, has been found in patients with APS and AT [24]. Endothelial dysfunctions, oxidative stress,

increase in cell adhesion molecules, and active platelets are common findings in both diseases. In addition, macrophages, dendritic cells, T-cell activation, CD40–CD40 ligand interaction are considered as pathogenic mechanism of AT and APS [25, 26]. Furthermore, premenopausal female patients with APS have a higher prevalence of cerebrovascular disease in comparison with male patients. Accelerated AT and hormones could be the explanation of these findings. High levels of aPLs significantly predict the risk of future ischemic stroke in women but not in men [27]. Thus, AT is one of the main features of systemic APS and offer opportunities for new treatment strategies.

2.1.1 Genomics and proteomics biomarkers for APS

A recent genomic study has been developed with peripheral blood mononuclear cells (PBMCs) in order to search for patterns of gene expression that can predict the risk for venous thrombosis in APS patients [28] (Table 1). Authors were able to describe gene-expression patterns from patient peripheral blood that can predict an individual's predisposition to developing thrombosis. Interestingly, theses results were confirmed in independent cohorts of patients. Some of the genes identified in this study revealed processes that we would infer from our current understanding of thrombosis including APOE, factor X, and thromboxane. In addition, other genes were identified that have thus far not been directly linked to venous thrombosis, including those encoding for hypoxia inducible factor (HIF-1α), zinc finger proteins, matrix metalloproteinase19 (MMP19), interleukin 22 (IL22) receptor, and hematopoietic progenitor cell antigen (CD34) precursor, among others.

We have also addressed the question of predicting thrombotic risk in APS patients by following a proteomic approach on pure monocytes [29]. The proteins more significantly altered among monocytes from APS patients with thrombosis were functionally related to the induction of a procoagulant state. Two annexins were upregulated in monocytic APS samples: annexin 1 and annexin 2. Annexins are a family of phospholipids and calcium-binding proteins which modulate inflammation, immune response, and blood coagulation. Annexin 1 may play a role in the regulation of macrophage activity and its levels are raised in some autoimmune diseases [30, 31]. Annexin 2 is a receptor for fibrinolytic activation localized on the cell surface of ECs, monocytes, and syncytiotrophoblasts [32]. By functioning as a receptor for β2GPI, annexin 2 is a target not only for anti-annexin 2 antibodies but also for anti-β2GPI antibodies, which are direct inductors of TF overexpression and thus significantly associated with thrombosis in the setting of APS [33]. Protein disulfide isomerase (PDI), a multifunctional protein catalyzing the oxidation, reduction, and isomerization of disulfide bridges, was significantly decreased in monocytes isolated from APS with thrombosis patients. The surface-accessible, extracellular Cys186–Cys209 disulfide bond of TF is critical for coagulation, and PDI disables coagulation by targeting this disulfide [34]. Thus, reduced expression of PDI in monocytes from APS patients with thrombosis might contribute to their prothrombotic state. Ubiquitin-like protein nedd8 was significantly increased in monocytic APS cells. This protein is involved in the proteolytic destruction of IκB (inhibitor of NFκB) [35]. Rho A was also significantly increased in the APS with thrombosis group. Rho A proteins are modulators of gene expression, adhesion, and migration of activated macrophages, which also play critical roles in inflammatory signal pathways such as those required for activation of NFκB [36].

Proteins reported to be connected to recurrent pregnancy loss (*i.e.*, fibrinogen and haemoglobin), were also found significantly deregulated in APS patients without thrombosis. Interestingly, *in vitro* treatment with IgG fractions purified from thrombotic APS patient plasmas changed the pattern of protein expression of normal monocytes in the same way that was observed *in vivo* for monocytes from APS patients with thrombosis, thus demonstrating a causal relationship between aPL and alterations in gene/protein expression.

2.2 SLE, AT and CVD

SLE is a complex multisystem inflammatory disease caused by autoimmune dysregulation [37]. Patients with SLE have an increased risk of AT that persists even after accounting for traditional cardiac risk factors. Recent studies strongly suggest, that the mechanism is due in part to a combination of inflammatory and immune mechanisms. Contributory factors include increased levels of oxidized lipids (such as oxidized low density lipoproteins (LDL) and proinflammatory high density lipoproteins (HDL), upregulation of adhesion molecules, and upregulation of cytokines such as monocyte chemotactic protein-1 (MCP-1), tumor necrosis factor α (TNF-α), IL-1, IL-12, and interferon γ (IFN-γ) [38, 39]. In that way, recent reports have demonstrated that elevated levels of type I interferons (IFN-I, cytokines with potent antiproliferative and anti-angiogenic effects, and associated with active SLE disease, and positivity for some autoantibodies) could lead to endothelial dysfunction through the promotion of a reduction in the number of endothelial progenitor cells (EPCs, responsible for the neovascularization in sites of endothelial injury), thus contributing to the increased CV risk observed in SLE [40].

Additional mechanism for the predisposition of these patients to premature CVD and the development of cell-mediated and humoral-immune response included the existence in SLE subjects of high levels of circulating apoptotic ECs, which strongly correlated with elevated levels of TF [41]. Those mechanisms constitute a potential link between endothelial apoptosis, thrombosis, and abnormal endothelial tone, with its implications for atherothrombotic events in SLE.

Table 1. Genomic and proteomic markers of CVD risk in autoimmune diseases

Disease	Genes/proteins associated with CVD			Technique utilized	Change	References
	Gene	Protein	Accession			
APS	**Pattern of 50 genes expressed in PBMCs that predict thrombotic risk in APS patients**			DNA microarrays		[26]
	PTGIR	Prostacyclin receptor	D38127		Down	
	MMP19	Matrix metalloproteinase-19	U89651		Down	
	F10	Coagulation factor X	L00390		Down	
	HSPCA	Heat shock protein-90	M27024		Down	
	APOE	Apolipoprotein E	M10065		Down	
	PDIA6	PDI a6	Q15084		Down	
	REQ	Zinc-finger protein UBI-D4	U43920		Down	
	HIF1A	Hypoxia-inducible factor 1 α	AF050127		Down	
	IL22RA1	Interleukin 22 receptor	Q8N6P7		Down	
	Proteins differentially expressed in blood monocytes from APS with thrombosis patients			2-DE MALDI TOF		[28]
		Annexin 1	P04083		Up	
		Annexin 2	P07355		Up	
		Ubiquitin-like protein nedd8	Q15843		Up	
		Transforming protein RhoA	P61586		Up	
		PDI	P07237		Down	
SLE	**Genes differentially expressed in PBMCs from SLE patients**			DNA microarrays		[43–50]
	IFN target genes					
	IFIT1	Interferon-induced protein with tetratricopeptide repeats-1	X06559		Up	
	G1P3	Interferon, α-inducible protein (IFI-6-16)	U22970		Up	
	IFIT4	Interferon-induced protein with tetratricopeptide repeats 4	O14879		Up	
	PLSCR1	Phospholipid scramblase 1	O15162		Up	
	G1P2	Interferon, α-inducible protein (IFI-15K)	M21786		Up	
	IRF7	Interferon regulatory factor 7	Q92985		Up	
	THBS1	Thrombospondin 1	J04835		Up	
	FCGR1A	Fc fragment of IgG, high-affinity Ia receptor	M63830		Up	
	USP20	Ubiquitin-specific protease 20	Y17457		Down	
	MATK	Megakaryocyte-specific tyrosine kinase	S75164		Down	
	TNF and TNF receptor families					
	TNFSF10	TNF-related apoptosis inducing ligand (TRAIL)	U37518		Up	
	TNFRSF10C	TRAIL receptor 3	O14798		Up	
	FAS	Fas (TNF receptor superfamily, member 6)	D31968		Up	
	Chemokines and chemokine receptors					
	CCR7	Chemokine (C–C motif) receptor 7	L31584		Up	
	IL8RB	Interleukin 8 receptor, β	M99412		Up	
	Cell surface activation antigens					
	CD69	CD69 molecule	Z30426		Up	
	Fc receptors					
	FCGR1A	Fc-γ receptor I A1	M63830		Up	
	FCGR2A	Fc fragment of IgG, low affinity IIa, receptor (CD32)	A21604		Up	
	Metalloproteinases					
	MMP3	Matrix metallopeptidase 3	J04732		Up	
	MMP9	Matrix metallopeptidase 9	D10051		Up	
	Defensins					
	DEFA3	Defensin 3, neutrophil-specific	L12691		Up	

Table 1. Continued

Disease	Genes/proteins associated with CVD			Technique utilized	Change	References
	Gene	Protein	Accession			
	Proteomic analysis of plasma from SLE patients			2-DE MALDI-TOF MS nano-(n) ESI-IT MS/MS		[54]
		Hpα2 isoform	P00738		Up	
RA	**Genes differentially expressed in PBMCs from RA patients**			DNA microarrays		[43, 65–68]
	Calcium binding					
	S100A12	S100 calcium-binding protein A12	D83657		Up	
	Cytokine/chemokine					
	CCL5	Chemokine (C–C motif) ligand 5	M21121		Up	
	TGFB1	Transforming growth factor, β1	M38449		Up	
	PF4	Platelet factor 4 (chemokine (C–X–C motif) ligand 4)	M25897		Up	
	DNA binding					
	HIST2H2AA	Histone 2, H2aa	L1977		Up	
	Enzyme					
	SAT	Spermidine/spermine N1-acetyltransferase	U40369		Up	
	GPI	Glucose phosphate isomerase	K03515		Up	
	PIB5PA	Phosphatidylinositol (4,5) bisphosphate 5-phosphatase, A	U45975		Up	
	PTGS1	Prostaglandin-endoperoxide synthase 1	M59979		Up	
	HUMNOSB	iNOS	D29675		Up	
	GLA	Galactosidase, α	X14448		Up	
	Integral plasma membrane					
	SELPLG	Selectin P ligand	U25956		Up	
	CD63	CD63 antigen	X62654		Up	
	CSF3R	Colony stimulating factor 3 receptor	M59820		Up	
	HLA-DQA1	MHC, class II, DQ α 1	M34996		Up	
	CD7	CD7 antigen	D00749		Up	
	SELP	Selectin P	M25322		Up	
	HDLBP	HDL-binding protein (vigilin)	M64098		Up	
	ITGAX	Integrin, αX	M81695		Up	
	ITGB2	Integrin, β 2 (antigen CD18 (p95)	M15395		Up	
	Mitochondrial					
	UCP2	Uncoupling protein 2	U94592		Up	
	Signal transduction					
	CDC25B	Cell division cycle 25B	S78187		Up	
	PLCB2	Phospholipase C, β 2	M95678		Up	
	IKBKE	Inhibitor of κ light polypeptide gene enhancer in B-cells, kinase epsilon	D63485		Up	
	TRAF1	TNF receptor-associated factor 1	U19261		Up	
	STAT5A	Signal transducer and activator of transcription 5A	U43185		Up	
	RHOG	Ras homolog gene family, member G (rho G)	X61587		Up	
	Structural					
	MYL9	Myosin, light polypeptide 9, regulatory	J02854		Up	
	GFAP	Glial fibrillary acidic protein	S40719		Up	
	MYH9	Myosin, heavy polypeptide 9, non-muscle	M31013		Up	
	Transcription					
	FOS	V-fos FBJ murine osteosarcoma viral oncogene homolog	V01512		Up	
	JUND	Jun D proto-oncogene	X56681		Up	
	PML	Promyelocytic leukemia	M79462		Up	
	YY1	YY1 transcription factor	M77698		Up	

Table 1. Continued

Disease	Genes/proteins associated with CVD			Technique utilized	Change	References
	Gene	Protein	Accession			
	Ubiquitin					
	UBC	Ubiquitin	M26880		Up	
	Proteomic analysis of plasma/serum from RA patients					
		Transthyretin	P02766	SELDI-TOF MS	Up	[74]
		SAA	P02735		Up	
	Proteomic analysis of PBMCs from RA patients					
		Fibrinogen γ-chain	P02679	2-DE-MALDI TOF	Up	[80, 81]
		Hsp60	P10809		Down	

Some examples of genes from each category are given. See cited references for a complete list of genes identified in the genomic studies.

Autoantibodies to oxidized lipids and immune complexes, including aPL and anti-β2GPI, anti-oxLDL, anti-oxidized palmitoyl arachidonoyl phosphocholine (anti-oxPAPC), anti-HSP antibodies, lipoprotein(a) [Lp(a)], and HDL, may also play a role in the development of AT in SLE [42]. Thus, the increased risk of AT seen in SLE is likely due to the complex interplay of many of these inflammatory and immune mediators.

2.2.1 Genomics and proteomics biomarkers for SLE

Several genomic studies have been developed on SLE PBMCs [43–49] (Table 1). Genomic studies have consistently given strong support to the involvement of a dysregulation of IFN-dependent pathways in the pathogenesis of SLE [50, 51]. Analysis of SLE upregulated genes showed a predominance of genes known to be upregulated in response to IFN. In some cases, expression results obtained using DNA microarrays were also confirmed by independent methods such as quantitative real-time reverse transcription PCR [49, 52]. Additional changes that may be of great importance in determining the high thrombotic risk of SLE patients include the upregulation of other gene families such as TNF and TNF receptor, chemokines and chemokine receptors, cell surface activation antigens, FC receptors, metalloproteinases, and defensins [50]. Interestingly, many of the expression changes observed in PBMCs isolated from SLE patients were reproduced in healthy PBMCs cultured with IFN-α [46]. The lack of detection of significant IFN-α transcripts in the SLE patient's PBMCs supported that this cytokine may be mainly produced by plasmacytoid dendritic cells located in the patient's tissues [46].

A recent study using a microRNA (miRNA) chip has been carried out in PBMCs isolated from SLE patients [53]. The analysis identified 16 miRNAs differentially expressed in SLE, which can be potential diagnosis biomarkers and probable factors involved in the pathogenesis of SLE. Nevertheless, the possible relationship between miRNAs expression pattern and thrombotic risk of SLE patients remains to be established.

The proteomic analysis of plasma samples from SLE patients has allowed an important observation in order to understand the higher susceptibility of SLE patients to suffer CV disorders. Pavón *et al.* [54] have studied by 2-DE plasma samples from SLE patients and healthy controls of initially unknown haptoglobin (Hp) phenotype, and tryptic digests of the excised Hpα polypeptide chain spots were analyzed by MALDI-TOF/MS. Selected tryptic peptides were then sequenced by nano- (n)ESI-IT MS/MS. There were remarkable interindividual differences in the Hp patterns of SLE patients compared with those of healthy controls. Thus, Hpα1F protein was only present in one of the patients studied, whereas the Hpα2 isoform was detected in all but one SLE patients studied, resulting in an Hp^2 allele frequency significantly higher than that in healthy controls. Hp functions as an antioxidant and an essential endothelial protector by binding to free haemoglobin, avoiding oxidative stress [55]. Both the hemoglobin-binding and the antioxidant capacity of Hpα1 is higher compared with that of Hpα2 [56] and Hp genotype plays a critical role in the oxidative and inflammatory response to intraplaque hemorrhage [57]. Moreover, Hp genotype modulates the balance of inflammatory (Th1) and anti-inflammatory (Th2) cytokines produced by macrophages exposed to free haemoglobin, which may have implications in understanding interindividual differences in the inflammatory response to hemorrhage [58]. Moreover, large-artery elasticity index and small-artery elasticity index were significantly lower and systemic vascular resistance was higher in homozygotes for the 2 allele (Hp 2-2) compared with patients with Hp 2-1 or Hp 1-1 phenotypes [59].

The knowledge of the Hp phenotypes and their PMF by 2-DE and MS in SLE patients can help predicting or preventing CV disorders and determining a more precise prognosis and better treatment [54].

2.3 RA, AT and CVD

RA is a chronic systemic inflammatory disease affecting approximately 1% of the adult general population. One of the

most important causes of death in patients with RA is CVD, so that RA is associated with increased risk of stable angina, myocardial infarction (MI), heart failure and stroke. Although persistent inflammation and immune dysregulation of RA may contribute to favor other well-known CV risk factors, such as dyslipidemia, it is now clear that the disease itself represents an independent risk factor for CV disease by the action of RA chronic inflammatory process as well as humoral- and cell-mediated immune mechanisms [60].

Pathogenic mechanisms include pro-oxidative dyslipidemia, insulin resistance, prothrombotic state, hyperhomocysteinemia, and immune mechanisms such as T-cell activation that subsequently leads to endothelial dysfunction, a decrease in EPCs, and arterial stiffness, which are the congeners of accelerated AT observed in RA patients [61, 62].

Among immunological and metabolic laboratory markers, anticyclic citrullinated peptide (anti-CCP) antibodies, IgM rheumatoid factor, circulating immune complexes, proinflammatory cytokines including TNF-α and interleukin-6 (IL-6), Th0/Th1 cells, decreased folate and vitamin B12 production, and impaired paraoxonase activity may all be involved in the development of vascular disease in RA [38, 63, 64].

There are only scarce data in the literature regarding the benefit of CV-risk reduction therapies in this group. Thus, further studies are required for the refinement of the CV-risk stratification algorithms and for the improvement of the CV-risk management in RA.

2.3.1 Genomics and proteomics biomarkers for RA

Several genomic studies have been developed to compare transcript levels in PBMCs obtained from RA patients with active disease and normal individuals [43, 65–68] (Table 1). Among the different genes that were differentially regulated in PBMCs from RA patients compared with healthy controls, a number of transcripts encoded for proteins which are relevant for AT. Several genes related with inflammation at different levels were significantly increased in RA PBMCs. These include the transcripts for cytokine receptors (TNF-α receptor), important members of signalling pathways (NFκB), and proteins related with cell adhesion (*SELP*, selectin P; and SELPLG, selectin P-ligand). SELP contributes to many inflammatory diseases and has been shown to mediate leukocyte interaction with EC wall [69].

A significant increase in S100A12, the ligand of the receptor for advanced glycation end products (RAGE), has been observed in RA PBMCs in several genomic studies, and this has highlighted the RAGE pathway as potentially important in RA [65, 68]. This change may have important consequences for atherothrombotic risk because the expression of RAGE and the RAGE-binding protein may further compromise cell survival and promote plaque destabilization [70].

Several genes not previously known as being differently regulated in RA were also identified by genomic analysis. INPP5E is a member of the inositol polyphosphate 5-phosphatase family, which regulates PI-3 kinase signal transduction [71]. 1-Acylglycerol-3-phosphate *O*-acyltransferase 1 (AGPAT-1) catalyzes the conversion of lysophosphatidic acid (LPA) to phosphatidic acid (PA), two phospholipids involved in signal transduction and phospholipid synthesis [72]. Overexpression of AGPAT-1 in cell lines leads to the expression of both TNF-α and IL-6 in cells stimulated with IL-1β, suggesting that AGPAT-1 overexpression may amplify cellular signalling responses from cytokines [73].

The fact that many of the genes identified by genomic analysis have been previously associated with RA validates this kind of analysis. However, it is important to note that not all genes are primarily regulated by changes in mRNA levels, with many being subject to post-transcriptional regulation. For instance TNF-α, the best-validated molecular therapeutic target in RA, did not emerge from these genomic studies [68].

The proteomic approach has been also utilized to investigate putative changes at the protein levels in blood samples (plasma or PBMCs) from RA patients. A recent proteomic study has been performed on plasma from RA patients and healthy donors [74]. Through differential profiling of plasma proteins, two prospective candidate biomarkers were selected. Transthyretin distinguished patients with RA from healthy controls; and serum amyloid A (SAA) distinguished inactive patients with RA from patients with active RA. Interestingly, apoA-I cleavage by transthyretin may affect HDL biology and the development of AT by reducing cholesterol efflux and increasing the apoA-I amyloidogenic potential [75]. Furthermore, the acute-phase protein SAA interacts physically with cystatin C, which may results in cystatin C deficiency. Decreased systemic cystatin C levels predispose to accelerated AT and development of amyloidosis in patients with RA [76]. Another proteomic study developed in serum has identified myeloid-related protein 8 as a biomarker of RA [77]. This finding may be of great importance to understand higher susceptibility of RA patients to CVD. Myeloid-related protein 8/14 complex is released by monocytes and granulocytes at the site of coronary occlusion and induces a thrombogenic, inflammatory response in human microvascular ECs by increasing the transcription of proinflammatory chemokines and adhesion molecules and by decreasing the expression of cell junction proteins and molecules involved in monolayer integrity [78]. This complex has been recently proposed as a novel, early, and sensitive marker of acute coronary syndromes [79].

Two recent studies have been focused on the comparison of proteomic patterns of PBMCs from RA patients and healthy donors [80, 81]. These studies allowed the identification of protein expression patterns that were characteristic of RA patients. Interestingly, fibrinogen γ-chain levels were upregulated in RA patients. Fibrinogen is strongly, consistently, and independently related to CV risk [82]. The heat shock protein 60 (Hsp60) was downregulated in PBMCs from APS patients, which agrees with a similar decrease we have observed in monocytes from APS patients [29].

3 Pharmacogenomic and pharmacoproteomic studies developed in systemic autoinmune diseases with cardiovascular risk

Autoimmune conditions often require multiple lifelong drugs and it can be challenging to identify drugs to which and individual patient will respond to. The field of pharmacogenomics seeks to identify genetic factors responsible for individual differences in drug efficacy and adverse drug reactions [83]. Pharmacogenomics can improve the utility of drugs for autoimmune diseases by determining in whom drugs will be most effective by identifying patients that are more likely to respond to a specific drug. Pharmacogenomics can also make medications safer by applying genetic diagnostic tests that predict which individuals are at a high risk for a severe adverse drug reaction and adjust an individual's dosage according to their likely response to treatment [84].

For autoimmune diseases, pharmacogenomics has led to several DNA-based tests to improve drug selection, optimize dosing, and minimize the risk of toxicity. An example of that includes the surveys performed to assess the treatment with warfarin, the most commonly prescribed oral anticoagulant drug in the USA for APS patients. Too high dose may lead to serious risks of excessive bleeding and intracranial hemorrage, while a subtherapeutic low dose may lead to the dangerous formation of blood clots [85]. Variations in warfarin dose are partially explained by environmental factors such as the intake of vitamin K, illness, age, gender, concurrent medication and body surface area, and also by genetic factors [86]. Cytochrome P450 (CYP) 2C9 normally inactivates warfarin, and upto 20% of the population carry variants in the CYP2C9 gene that confer low enzyme activity (called *2 or *3 variants). Carriers of these variants require significantly lower doses of warfarin and are at increased risk of serious and life-threatening bleeding when administered a standard dose [87]. Overall, the genetic variations in CYP2C9 account for 6–10% of warfarin dose variability [88]. In sum, CYP2C9 pharmacogenetic testing could protect individuals from unnecessarily high and potentially dangerous doses of warfarin, and thus genotype-based dose prediction prior to starting warfarin therapy will permit personalized dosage modifications.

A number of recent publications have demonstrated the pleiotropic effects of the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors or statins, on cultured ECs, platelets, monocytes/macrophages and *in vivo* models, and human studies of several autoimmune and CVD [89].

Statins act by affecting cholesterol synthesis, through the competitive inhibition of HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis. In addition, the therapeutic potential of statins goes further hypolipemic properties, as shown by pleiotropic effects exerted by this drug class. Indeed, by altering isoprenylation, which in turn induces the inhibition of small GTP-binding proteins Rho, Ras, and Rac, statins are able to improve the endothelial function, to enhance the stability of atherosclerotic plaques, to decrease oxidative stress and inflammation, inhibit the thrombogenic response, and to have beneficial effects on immune system [90].

Various studies have used genomics and proteomics to analyze modifications in the protein map of plasma and blood cells after statins treatment of hypercholesterolemic patients, patients with autoimmune diseases associated to CVD, and patients with AT. Data obtained suggested that these drugs respond differently in human depending on multiple polymorphisms. In addition to genes that are classically described when talking about statins (for example cholesteryl ester transfer protein (CETP) and APOE genes), recent genes have been recently reported to exhibit polymorphisms that influence the response to this class of drugs. Recently, two common and tightly linked single nucleotide polymorphisms (SNPs) in the 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) gene (a A>T substitution at position 74726928 and a T>G substitution at position 74739571) were related to response to pravastatin treatment [91]. Individuals with a single copy of the minor allele of these SNPs had their overall efficacy for modifying total cholesterol concentration reduced to 22%.

On the other hand, pleiotropic genes whose variations have been studied with statins are the angiotensin-converting enzyme (ACE) gene, the β-fibrinogen (FGB) gene, the glycoprotein IIIa (GPIIIa) gene, the stromelysin-1 (MMP3) gene, the CD36 gene, and the estrogen receptor α (ESR1) gene [92].

Pharmacoproteomics approaches are clearly very useful during the development of new drugs to control some toxicity including drug interactions and in different pathological status. The modulation of the levels of proteins secreted by cultured atherosclerotic plaques and in the blood of patients with AT after atovastatin treatment has been recently evaluated [93]. This study showed 24 proteins that were increased and 20 that were decreased after statin treatment. Some of the increased proteins, like Cathepsin D (which could play a significant role in plaque stability), reverted to control values after atorvastatin administration, thus becoming a potential therapeutic target for statin treatment. Furthermore, recent studies by Grobbee and Bots [94] have examined the evidence for imaging studies showing the efficacy of statins in slowing AT progression and promoting regression of disease.

A very recent study developed by our group [95], with the aim to analyze changes in protein expression of monocytes of APS patients after statin treatment, showed that the *in vivo* treatment for 1 month with fluvastatin reversed the changes produced in the expression levels of proteins altered in APS patients with thrombosis *versus* healthy donors, such as annexin II, Rho A proteins, or PDI, thus pointing to that proteins as new potential targets for rational pathogenesis-based therapies.

Finally, the group of Anderson and Anderson [96], by proteomic studies, has described key modification after sta-

tin treatment in carbohydrate metabolism, stress protein, calcium homeostasis, and protease activity. It has been also suggested to follow other enzymes from the mevalonate pathway, an important information which could have avoid the problem with different statin derivatives or some of their side effects.

Disease-modifying antirheumatic drugs (DMARDs) are the mainstay of treatment in RA. DMARDs not only relieve the clinical signs and symptoms of RA but also inhibit the radiographic progression of disease. Recently, a new class of disease-modifying medications, the biologic agents, has been added to the existing spectrum of DMARDs in RA. However, patient's response to these agents it not uniform, with considerable variability in both efficacy and toxicity [97]. TNF-blocking strategies are widely used in the treatment of RA. Three anti-TNF agents are registered for use in RA: etanercept, infliximab, and adalimumab. Although anti-TNF therapy is very effective in controlling disease activity and slowing down radiological damage, prolonged response is only seen in approximately 70% of the patients [98]. The causes for nonresponse in the remaining patients have not yet been elucidated. Pharmacogenetic studies focusing on genes involved in RA aetiology (and/or progression) and in the pharmacokinetics of TNF-blocking agents have shown that so far, no clinical predictors of response to TNF inhibitors have been identified, but genetic variation in the MHC, class II, DR β 1 (HLA-DRB1), and the lymphotoxin α (LTA)-TNF regions was shown to influence the response [99].

Methotrexate (MTX) has been also widely used for the treatment of RA. Many studies have focused on the adenosine-mediated anti-inflammatory effects of MTX. In that way, a recent study has developed a clinical model to predict the efficacy of MTX in RA. In this study [100], (including 205 patients with newly diagnosed RA), the model for MTX efficacy consisted of sex, rheumatoid factor, and smoking status, the disease activity score (DAS), and four polymorphisms in the adenosine monophosphate deaminase 1 (AMPD1), 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase (ATIC), inosine triphosphatase (ITPA), and methylenetetrahydrofolate dehydrogenase 1 (MTHFD1) genes.

4 Conclusions

AT is a chronic inflammatory disease of the arteries associated with various risk factors that promote lipid abnormalities, development and progression of atherosclerotic lesions, plaque rupture, and vascular thrombosis. Several autoimmune diseases, such as APS, SLE, and RA, exhibit increased overt CVD prevalence as well as findings of advanced subclinical AT. This phenomenon cannot only be attributed to traditional risk factors for AT and use of specific drugs, such as corticosteroids, but also might be the result of other autoimmune and inflammatory mechanisms that are aggravated in autoimmune diseases.

Pathophysiological mechanisms that might connect AT and CVD with APS, SLE, and RA, respectively, have been greatly broadened with the application of genomics and proteomics technologies, which have allowed explaining how these pathologies might be linked in each autoimmune disease. For example, new genes and proteins differentially expressed in blood monocytes from APS patients with thrombosis, such as annexin II or PDI (which are also related to the effect of specific autoantibodies on that disease), have been found. In SLE patients, various genomic studies have allowed the identification of new genes enconding for proteins directly related to inflammation as well as to the development of AT and CVD such as IFN target genes, TNF and TNF receptors, chemokines, metalloproteases, *etc.* Finally, in RA patients, where the number of proteomic and genomic studies developed is significantly greater, a relevant number of genes differentially expressed in the PBMCs (and also related to the development of AT and CVD) have been described, including those associated to cell signalling, DNA binding, transcription, and calcium binding, among others.

Animal studies indicate interesting connections between AT and autoimmune diseases. For example, it has been demonstrated that the interaction of CD40 with CD40L plays an important role in humoral- and cell-mediated immune responses, and ligation of this interaction ameliorates AT and autoimmune disease in animal models [101, 102]. Furthermore, intravenous Ig, often used for treatment of severe autoimmune manifestations, protects against AT in an animal disease model [103]. However, further studies are still needed to determine whether atherosclerotic plaques in autoimmune disease have special features, or to analyze which autoantigens, autoantibodies, and autorreactive cells are most important *in vivo* for promotion of AT.

Genomics and proteomics biomarkers are biological measurements that serve as indices for disease progression, pharmacology, or safety. Thus, as a result of pharmacogenomics and pharmacoproteomics studies, genomic and proteomic markers for antithrombotic drugs should be used to distinguish individuals at high risk for the disease and at risk for the drug side effects, or with a probability of nonresponse. All that new data, after the appropriate preclinical and clinical assays, should improve the ability of the physicians to lower the death rate from AT and CVD in patients with autoimmune diseases.

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