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Autoimmune and angiogenic biomarkers in autoimmune atherosclerosis

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ABSTRACT

Several inflammatory, proteolytic, angiogenic and bone-associated factors play a role in the development of autoimmune, accelerated atherosclerosis in rheumatic diseases. Some of these may serve as biomarkers of vascular pathology and may be useful in the follow-up of vascular damage and outcome. Multi-biomarker profiles rather than a single markers would likely be optimal in this respect.

1. Introduction

Accelerated "autoimmune" atherosclerosis leading to increased cardiovascular (CV) morbidity and mortality has been associated with rheumatoid arthritis (RA), as well as other autoimmune-inflammatory rheumatic diseases [1–4]. Perpetuation of angiogenesis supported by the abundant production of angiogenic factors has also been recognized as a key event in the pathogenesis of RA [5–9]. Numerous immune cells, pro-inflammatory cytokines, chemokines, growth factors, proteases, soluble and cell-expressed adhesion molecules and other mediators have been implicated in the development of inflammatory atherosclerosis and angiogenesis [2,3,5,6,10,11]. Some of these cells and molecules may serve as biomarkers of these vascular events [3,10,12]. In this review, we briefly summarize the current view on the value of these biomarkers. We will focus on inflammatory and angiogenic molecules and will not discuss metabolic factors (lipids, adipokines, etc).

2. The brief pathogenesis of autoimmune atherosclerosis

In general, arthritis-associated atherosclerosis and CVD involves both traditional and inflammatory risk factors. In RA, systemic inflammation may be the key driver of the development of vascular pathology [1,2,11,13]. With respect to biomarkers, the inflammatory pathways are highly relevant, so we will not further discuss the role of traditional risk factors.

Arthritis and inflammatory atherosclerosis may share numerous pathogenic mechanisms. Endothelial activation promotes inflammatory cell infiltration of the synovium, as well as the vessel wall. Inflammatory macrophages and T-cells are involved in synovial inflammation and initiation, progression and rupture of atherosclerotic plaques. Neovascularization mainly based on tissue hypoxia and the production of vascular endothelial growth factor (VEGF) facilitates synovial inflammation. In addition, angiogenic factors and newly formed vessels have been associated with increased rupture risk. Finally, tissue degradation mediated by matrix metalloproteinases (MMP) is a key event in joint destruction and the erosion of vascular fibrous caps leading to rupture and thrombosis [2,3,8,10,11,14].

Both synovial inflammation and atherosclerosis involve genetic, environmental and lifestyle-related factors, as well as autoimmunity. The initiation of synovial and vascular pathology is highly based on this "Bermuda triangle" (Fig. 1). These three initiating factors trigger systemic inflammation, which further leads to endothelial dysfunction, arterial stiffness and atherosclerosis [2,3].

3. Genetic factors

Both RA and atherosclerosis or CVD have strong genetic background. In RA, genome-wide association studies (GWAS) revealed numerous susceptibility alleles, among others, *HLADRB1*, *PTPN22*, *TRAF1/C5*, *STAT4*, *PADI4*, *IRF5*, *FCGR*, *IL2RA*, *IL2RB*, *CD40*, *CCR6*,

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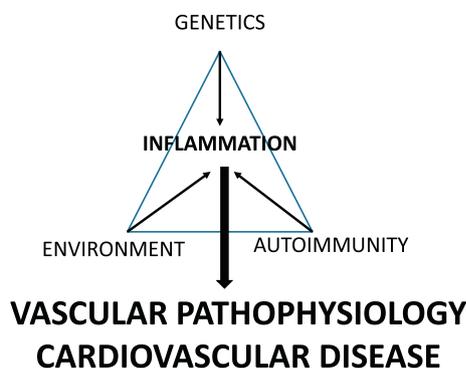*E-mail address:* szekanecz.zoltan@med.unideb.hu (Z. Szekanecz).

Fig. 1. The "Bermuda triangle" of autoimmune-inflammatory atherosclerosis.

CCL21. More than 40 SNPs have been associated with RA [15,16]. In atherosclerosis, a GWAS study carried out on > 100,000 Europeans revealed association of > 30 genes with coronary atherosclerosis. These included matrix molecule (e.g. *ADAMTS7*, *ANKS1A*, *COL4A1*), lipid (e.g. *LPA*, *LDLR*), chemokine (e.g. *CXCL12*) and other genetic loci [17,18]. The role other "lipid-related genes" including *ABCA1*, *APOA5*, *LCAT*, *CETP*, *SORT1*, as well as > 50 "non-lipid" genes have also been identified in atherosclerosis by GWAS [19]. *HLADRB1* has been associated with both RA [15,20] and CVD [19].

It is also possible, that RA and the associated CVD may have common genetic denominators. Mostly single-allele studies have yet been performed. For example, in comparison to RA patients carrying no or one SE allele, those with HLA-DRB1*01/*04 exert a 3-times higher risk for CV death [21]. Studies on single non-HLA alleles such as *MTHFR*, *SMAD3*, *CD40*, *IRF5*, *OPG* and others were primarily performed by the group of Gonzalez-Gay (reviewed in [22,23]). As the association of RA and atherosclerosis with complex genetic signatures has not yet been elucidated, we have recently performed the analysis of genetic signatures that may determine RA-associated atherosclerosis [24].

Theoretically, single genetic alleles or complex signatures may reflect autoimmune atherosclerosis, however, the assessment of genes as biomarkers may have several limitations.

4. Environmental factors

Numerous environmental risk factors including smoking, alcohol, diet, infections, silica and others have been implicated in the pathogenesis of RA and atherosclerosis [2,3,25,26]. With respect to biomarkers, only infectious agents may have some relevance. In recent years, the microbiome associated with RA [27–29], as well as atherosclerosis [27,29] have been discussed and, at least in part, characterized. Periodontitis and bacteria, such as Porphyromonas gingivalis may link RA and atherosclerosis [29,30]. As one example, bacterial species were identified in the aortic adventitia of patients with CVD only and in RA patients with associated CVD. 16S rRNA gene sequencing was used. Numerous species were only detected in CVD without RA. In RA patients with CVD, some species (e.g. Pedobacter, Methylobacterium, Veilonella, etc) showed moderate expression (detectability in < 10% of clones). There was one species, Methylobacterium oryzae (*M. oryzae*), which was detectable in 45% of clones in RA patients with CVD, but not in CVD patients. Moreover, human primary macrophages showed a pro-inflammatory response when infected by this bacterium. Upon *M. oryzae* infection, macrophages produced significant amounts of IL-1α, IL-1β, IL-6 and IL-8 and CCR7 expression was also induced on these cells. Thus, *M. oryzae* may be a good microbial marker of RA-associated atherosclerosis compared to CVD in the general population [31].

5. Autoantibodies

Like in most autoimmune diseases, genetic and environmental factors may be associated with the production of autoantibodies. Certainly in RA, rheumatoid factor (RF), as well as anti-citrullinated (ACPA) and anti-carbamylated protein (anti-CarP) autoantibodies have been in focus. Citrullinated proteins have been detected in the atherosclerotic plaque [32], which may drive ACPA-dependent atherogenesis in RA. We and others have found association of RF and ACPA with vascular pathology in RA [33–38]. Higher percentage of patients with myocardial infarction (MI) were ACPA positive compared to healthy controls [33]. Also, high ACPA levels were associated with carotid plaque formation [39], CVD, fatal CVD and stroke [37], as well as increased CV mortality [38]. Recently, anti-CarP antibodies have been correlated with subclinical atherosclerosis [40] and CV mortality in RA [38]. RF positivity has also been associated with accelerated atherosclerosis [41] and CV mortality in RA [38].

Among other autoantibodies, antibodies to oxidized LDL (anti-oxLDL), the phospholipid cofactor β2GPI (anti-β2GPI), anti-phosphorylcholine and anti-heat shock protein 60/65 (anti-hsp60/65) have also been implicated in the pathogenesis of autoimmune atherosclerosis [3,42–48]. These antibodies may be highly relevant in systemic lupus erythematosus (SLE) and antiphospholipid syndrome (APS) [42,46,47].

We have found increased production of antiphospholipid [48] and anti-oxLDL antibodies [47] in acute coronary syndrome. These antibodies may serve as good biomarkers of endothelial dysfunction and acute endothelial damage. Oxidized LDL and β2GPI may form complexes. Increased amounts of antibodies to oxLDL/β2GPI (AtherOx) were detected in the sera of autoimmune, as well as vascular patients [49].

Anti-hsp60/65 antibody levels have been associated with atherosclerosis and CVD [50]. Previously, we detected elevated anti-hsp65 levels in the sera of AS patients [51]. Recently, we have found significant correlation between anti-hsp60/65 levels and arterial stiffness indicated by PWV in RA and AS patients. Anti-hsp60/65 also correlated with triglyceride levels [52].

6. Inflammatory and vascular mediators in the development of vascular pathology

The central event of synovitis and autoimmune atherosclerosis is the accumulation of inflammatory cells and mediators in the synovial tissue and vessel wall, respectively [2,3]. A great amount of inflammatory cells and factors have been described in this respect [3]. Most pro-inflammatory mediators also stimulate angiogenesis [5,6].

We and others have assessed the role of multiple inflammatory mediators in the development of autoimmune atherosclerosis and CVD. Possibly the most important marker is CRP, which is an independent predictor of CV risk [53]. In earlier studies, we described the production of TNF-α, IL-6, IL-8 and interferon γ (IFN-γ), as well as the abundant expression of the ICAM-1 adhesion molecule in vascular tissues taken from patients with inflammatory aortic aneurysms [54–56]. In RA, endothelial activation was associated with the increased production of von Willebrand factor antigen (vWF:Ag) [3,57]. Recently, increased expression of TNF-α and IL-18 was found in the aortic adventitia of RA patients with CVD compared to non-RA CVD patients [58].

Endothelial dysfunction, arterial stiffness and carotid atherosclerosis can be detected by ultrasound evaluation of brachial artery flow-mediated vasodilation (FMD), arterial pulse-wave velocity (PWV) and common carotid intima-media thickness (cIMT)/plaque analysis, respectively [34,59,60]. Impaired FMD was associated with increased serum TNF-α, IL-6 and CRP levels. Moreover, increased, abnormal cIMT correlated with RA disease duration, higher TNF-α and CRP, as well as lower IL-4 and IL-10 levels [34].

With respect to angiogenesis, increased neovascularization in the arterial wall is associated with atherosclerosis. Pro-angiogenic growth

factors, primarily VEGF, but also PDGF, FGF and HGF stimulate inflammatory angiogenesis. In addition, numerous pro-inflammatory cytokines, chemokines, adhesion molecules, matrix-degrading proteases and other factors have been implicated in neovascularization associated with arthritis and atherosclerosis [3,6,61]. In a recent study, circulating angiopoietin 2 (Ang2) and PDGF correlated with RA disease duration. Moreover, Ang1 and Ang2 levels also correlated with CRP. Finally, PDGF-BB concentrations correlated with cIMT [62].

Cell adhesion molecules, such as VCAM-1, ICAM-1 and *E*-selectin have been associated with endothelial dysfunction in RA [63]. All these adhesion receptors are involved in inflammatory and angiogenic processes underlying RA and atherosclerosis [6,8,55,64].

Proteases, such as the urokinase plasminogen activator (uPA) system is important in fibrinolysis, inflammation, matrix degradation and angiogenesis [6,65]. uPAR (CD87) is a cell-bound, GPI-anchored protein, which may be cleaved from the cell membrane (soluble uPAR, suPAR) [65,66]. We have previously detected uPAR in the RA synovial tissue, mostly on leukocytes and endothelial cells [65]. High levels of suPAR have been associated with various autoimmune diseases [67]. In a recent study, we found significant positive correlation between suPAR and RF or anti-CCP levels in RA patients [68].

Regarding other mediators and biomarkers, NT-proBNP is a marker of myocardial function. Higher NT-proBNP levels were associated with increased all-cause and CV mortality in early arthritis patients [69].

Osteoprotegerin (OPG) is a well-known bone marker, a decoy receptor for RANKL, which plays a role in inflammatory joint destruction in RA [70,71]. Interestingly, OPG has also been associated with atherosclerosis and CVD [70,72,73]. High OPG levels correlated with CVD and CV mortality [72]. OPG levels were higher in RA patients with CVD compared to those without CVD [73]. On the other hand, the OPG ligand TRAIL may rather be atheroprotective [74]. Sclerostin (SOST) and Dickkopf-1 (DKK-1) are TNF-α-mediated inhibitors of Wnt- and β-catenin-dependent bone formation. They play an important role in joint destruction [75]. Both SOST [76] and DKK-1 [77] have been associated with the promotion of vascular calcification.

7. Use of cardiovascular and angiogenic biomarkers in monitoring therapeutic efficacy

EULAR has published recommendations on arthritis-related CVD [1]. These recommendations, similarly to the general population, include primary prevention. However, as systemic inflammation is the major driver of vascular pathology, treatment of the underlying rheumatic disease is essential [1,11].

Indeed, several studies carried out in large cohorts suggest that both traditional (e.g. methotrexate) and biologic DMARDs may suppress the development of secondary CVD. After studies using surrogate markers, some hard endpoint clinical trials have also been successfully conducted [78–80].

With respect to biomarkers, targeted therapies may decrease the production of numerous markers described above. In our early RA study, adalimumab significantly decreased soluble vWF:Ag levels [57]. Recently, we have treated RA and ankylosing spondylitis (AS) patients with TNF inhibitors. Etanercept or certolizumab pegol significantly decreased VEGF levels in RA and AS, as well as PDGF-BB levels in RA patients [62].

Anti-oxLDL/β2GPI antibodies (AtherOx) were described above. Anti-TNF therapy significantly decreased serum levels of these antibodies in RA and AS patients [52].

With regards to the above mentioned NT-proBNP, in a Dutch cohort, adalimumab decreased NT-proBNP levels [81]. In our study, etanercept or certolizumab pegol treatment significantly suppressed NT-proBNP production in RA and AS patients. Furthermore, NT-proBNP levels were higher in RF and ACPA seropositive vs seronegative patients [52].

We described the importance of the uPA system in inflammation and vascular pathology. In our cohort, anti-TNF therapy was able to decrease suPAR levels in RA. Furthermore, the most striking effect was observed in patients with very high baseline suPAR levels [68]. Among hemostasis markers, tocilizumab significantly decreased fibrinogen and D-dimer levels in RA [82].

Serum amyloid A (SAA) and pro-inflammatory HDL (piHDL) containing SAA are very important pathogenic factors in inflammation, atherogenesis and angiogenesis [3,6,83]. IL-6 receptor blockade significantly decreased piHDL/SAA levels in RA patients [82].

Finally, as mentioned above, several bone markers including OPG, SOST and DKK-1 play a role in inflammatory joint destruction. These molecules have been implicated in vascular calcification as well. Targeted therapies may differentially regulate the expression of these biomarkers in RA and AS [71,84].

8. Single biomarker or multi-biomarker approach?

Several potential biomarkers of autoimmune atherosclerosis were described above. It is a question, whether to apply a single biomarker or use a set of biomarkers. Until now, no "gold standard" biomarker has been identified. Therefore it is likely that a multi-biomarker approach could be preferable.

As one example, Paramo et al. [12] assessed soluble biomarkers and imaging technologies in the identification of vulnerable atherosclerotic patients. Biomarkers of inflammation (e.g. TNF-α, IL-1, IL-6, soluble ICAM-1 and VCAM-1), proteolysis (various MMPs) and thrombosis (soluble CD40L, P-selectin, PAI, etc) were grouped as possible members of a multi-biomarker platform. In association studies, some biomarkers exerted good association with vascular MRI (e.g. ICAM-1, VCAM-1, P-selectin, MMP-2, MMP-9), intravascular ultrasound (e.g. IL-6, VCAM-1, ICAM-1, *E*-selectin, CRP) or cIMT (e.g. IL-6, IL-10, oxLDL, MMP-10, CRP). Thus, laboratory biomarker profile assessment may reflect vascular changes otherwise identified by imaging [12].

Acknowledgements

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