Premature atherosclerosis in systemic autoimmune diseases
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CHAPTER 1

ACCELERATED ATHEROSCLEROSIS IN PATIENTS WITH SYSTEMIC AUTOIMMUNE DISEASES

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ABSTRACT
Systemic autoimmune diseases such as systemic lupus erythematosus and Wegener’s granulomatosis are associated with a significantly increased prevalence of cardiovascular disease (CVD) compared to age- and sex-matched controls. Many risk factors are involved in the pathogenesis of atherosclerosis, the major underlying cause of CVD. In patients with systemic autoimmune diseases, it has been shown that traditional risk factors for CVD cannot completely explain the prevalence of atherosclerosis. Therefore, in addition to these traditional factors, non-traditional risk factors are suggested to contribute to atherogenesis. All risk factors, traditional and non-traditional, contribute to endothelial activation that, followed by endothelial dysfunction, is seen as one of the first steps in this process. This review updates information on the factors that contribute to accelerated atherosclerosis in patients with systemic autoimmune diseases, such as disease-related factors, inflammatory mediators and advanced glycation endproducts.
INTRODUCTION
Atherosclerosis can be considered as a progressive process that starts as early as in adolescence, becoming manifest clinically at a much later age. Accumulating evidence suggests that inflammation plays a fundamental role in each stage of atherogenesis, from the initial phases, starting with endothelial cell (EC) activation, to eventual rupture of the vulnerable atherosclerotic plaque. EC activation results in upregulation of adhesion molecules such as P-selectin, E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) on the EC surface, leading to attachment and, finally, migration of circulating leukocytes. Differentiation of migrated monocytes into macrophages, and the subsequent uptake of lipids by these cells, results in foam cell generation and fatty streak formation. Further recruitment of inflammatory cells and proliferation of smooth muscle cells lead to the development of an atherosclerotic plaque.¹²

Despite growing knowledge of the pathogenesis of atherosclerosis, the exact cause is not known yet. Many traditional risk factors, such as hypercholesterolemia, hypertension, and smoking, can injure and activate the endothelium. Infectious agents, such as bacteria and viruses, have also been indicated as potential triggering pathogens that start the cascade resulting in plaque formation.³

In some situations, the development of atherosclerosis is accelerated, for example in systemic autoimmune diseases. Increased prevalence of cardiovascular morbidity and mortality due to atherosclerosis, which cannot fully be explained by traditional risk factors, has been observed in patients with systemic autoimmune disease, indicating that additional “non-traditional” factors are also involved.⁴¹¹ A simplified overview of factors contributing to atherosclerosis in systemic autoimmune diseases is presented in table 1. In this review, atherosclerosis in systemic autoimmune diseases is discussed. In addition, a selection of more or less established non-traditional risk factors and, last, the role of other hypothetical contributing factors in the pathogenesis of atherosclerosis will be discussed.

Systemic autoimmune diseases
Systemic autoimmune diseases such as systemic lupus erythematosus (SLE) and Wegener’s granulomatosis (WG) are characterized by inflammation of many organ systems. The course of these diseases is very variable among patients. The disease can be quiescent for years after an active period, but also can have a relapsing/remitting
Table 1. Traditional and non-traditional risk factors in patients with systemic autoimmune diseases

<table>
<thead>
<tr>
<th>Traditional risk factors</th>
<th>Non-traditional risk factors in systemic autoimmune diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Auto-antibodies against phospholipids, endothelial cells,</td>
</tr>
<tr>
<td>Gender</td>
<td>ds-DNA or ANCA</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>Systemic inflammation</td>
</tr>
<tr>
<td>Smoking</td>
<td>Enhanced lipid oxidation</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Altered vascular remodeling?</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Levels of HSPs and antibodies against HSPs?</td>
</tr>
<tr>
<td>Obesity</td>
<td>Renal impairment?</td>
</tr>
<tr>
<td>Positive family history for CVD</td>
<td>Elevated levels of homocysteine?</td>
</tr>
<tr>
<td></td>
<td>Medication, especially steroids?</td>
</tr>
<tr>
<td></td>
<td>Exhaustion of endothelial progenitor cells?</td>
</tr>
<tr>
<td></td>
<td>Advanced glycation endproducts?</td>
</tr>
</tbody>
</table>

course. The long-term outcome of these diseases is receiving increasing focus now that the ability to control recurrences has improved remarkably. In 1976 Urowitz et al reported that mortality in SLE follows a bimodal pattern. Due to the better treatment modalities now available, more patients die late instead of early in the course of their disease. Early death is associated with active disease, large doses of steroids and a marked incidence of infection, while in the late stage death is associated with inactive lupus, long duration of steroid therapy, and a striking incidence of myocardial infarction due to atherosclerotic heart disease.

In SLE as well as in WG this increased prevalence of cardiovascular disease (CVD) cannot be explained by the presence of traditional risk factors such as hypertension, dyslipidemia, diabetes mellitus, and smoking. To determine other factors that participate in the development of atherosclerosis, the initial steps of the process should be investigated. EC activation and EC dysfunction precede atherosclerosis. As in systemic autoimmune diseases vessels are often involved in the inflammatory process, increased EC activation may be expected. Indeed, EC activation has been demonstrated indirectly by the elevation of circulating soluble adhesion molecules and thrombomodulin. Also, in skin biopsy specimens of SLE patients, endothelial expression of E-selectin, VCAM-1 and ICAM-1 is increased, most markedly in active disease. The next step in the process is EC dysfunction, which is considered crucial in the early stages of atherogenesis. Indeed, EC dysfunction is described in CVD, as well as in systemic autoimmune diseases. EC dysfunction can be measured by different methods. The most commonly used is flow-mediated dilation, but
increasingly, laser Doppler fluxmetry in combination with iontophoresis is used. To determine endothelial function, both methods compare the ability for endothelium-dependent vasodilatation with the ability for endothelium-independent vasodilatation. Thus, considering the presence of diffuse EC dysfunction in autoimmune diseases, it can be assumed that these patients have a higher risk of developing CVD. This hypothesis is supported by several studies that demonstrated increased prevalence of clinical and subclinical atherosclerosis in patients with SLE and WG. The contribution in this process of non-traditional risk factors is discussed in the remainder of this review.

**Autoantibodies**

A hallmark of systemic autoimmune diseases is the presence of autoantibodies. In SLE, many autoantibodies can be found, such as autoantibodies directed to phospholipids, endothelial cells, or double-stranded DNA. Anti-neutrophil cytoplasmic antibodies (ANCA), particularly those directed to proteinase 3 (PR3-ANCA), are characteristic autoantibodies for WG. Binding to and subsequent activation of ECs by these antibodies has been shown in several studies giving a direct explanation for the presence of accelerated atherosclerosis.

Furthermore, systemic low-grade inflammation, which is reflected by increased levels of C-reactive protein (CRP), even when disease activity is low, is present in systemic autoimmune diseases. CRP has been found to be an independent prognostic marker for CVD. Several studies have suggested that CRP may contribute directly to the development of atherosclerosis, as it induces expression of adhesion molecules on the endothelial surface and promotes the adherence of leukocytes. Note that EC dysfunction in systemic vasculitis is related to inflammation, as reflected by CRP levels and Birmingham Vasculitis Activity Score.

Furthermore, heat shock proteins (HSPs) have been suggested to contribute to the cascade that leads to atherosclerosis. It has been demonstrated that traditional risk factors, such as hypertension, smoking, diabetes, and chemically altered lipoproteins, act as stress factors for ECs, which express HSPs upon activation. Owing to activation, ECs may die, resulting in release of HSPs, then called soluble HSPs (sHSPs), into intercellular spaces. These sHSPs seems to be directly proatherogenic, as they stimulate macrophages to produce proinflammatory cytokines and ECs to express adhesion molecules. Simultaneously, macrophages present antigens to B cells, which
may induce the production of autoantibodies against HSPs, which, in turn, can react with HSPs expressed on activated ECs. Increases in both HSPs and autoantibodies against HSPs have been associated with atherosclerosis.\(^{33,36,37}\) Also, in systemic autoimmune diseases a defective HSPs response could accelerate the development of atherosclerosis. However, data on this latter effect are controversial, for example, Cederholm et al could not find any differences in levels of HSP60, HSP70 or antibodies against HSP60, HSP65 and HSP70 between controls and SLE patients with or without a history of CVD.\(^{38}\)

Another disease-related factor, especially in SLE, is suggested to be the enhanced oxidation of lipids.\(^ {39,40}\) Oxidized LDL (oxLDL) plays an important role in atherogenesis and may contribute to the immune activation and inflammation present in atherosclerotic lesions, because it is chemotactic, activates T cells, and is taken up by macrophages in the atherosclerotic plaque.\(^ {41}\) OxLDL could, therefore, be a common pathogenic factor in SLE and atherosclerosis. Furthermore, autoantibodies to oxLDL have been found to be related to atherosclerosis.\(^ {42-45}\) Elevated antibodies against oxLDL are found in atherosclerotic lesions and in plasma of patients with atherosclerosis.\(^ {46,47}\) SLE patients also have elevated anti-oxLDL antibodies.\(^ {40,48,49}\) However, several studies have suggested that antibodies against oxLDL prevent plaque formation. The precise mechanisms remain unclear. Some authors have suggested that autoantibodies to oxLDL may act as scavengers and clear oxLDL from the circulation.\(^ {50}\) So far, it has not been well established whether the immune response to oxLDL is predominantly proatherogenic or antiatherogenic.\(^ {44,45}\)

**Matrix metalloproteinases**

Vascular remodeling, in which matrix metalloproteinases (MMPs) play a major role, is important in atherosclerosis.\(^ {51}\) Excessive tissue remodeling and increased MMP activity have been demonstrated during atherosclerotic lesion progression, including plaque disruption. Increased MMP-1, MMP-3, MMP-9, and one of their inhibitors, TIMP-1, were found in human atherosclerotic plaques.\(^ {52}\) Also, in acute coronary syndromes, increased serum MMPs have been detected.\(^ {53-55}\) Various cells present in plaques produce MMPs, including smooth muscle cells and basophils, but the major source is the activated macrophage. Production is stimulated by cytokines, such as inflammatory mediators. Therefore, some studies suggest that MMPs can also be used as markers for disease activity, for example, in Takayasu’s arteritis\(^ {56}\) and giant cell
arteritis.\textsuperscript{57} Also, in WG and SLE patients, increases in MMPs and their inhibitors are found.\textsuperscript{8,58,59} Whether this is a reflection of atherosclerotic changes of the vessel wall or of smouldering disease activity in these patients, which results in endothelial activation and, eventually, in atherosclerosis, is unknown yet.

**Medication**

Treatment, especially the use of steroids, also influences the process of atherosclerosis. Steroid treatment is often believed to be atherogenic, because of effects on plasma lipoproteins. Because inflammation is implicated in atherosclerosis, steroid treatment could actually prevent atherosclerosis as well. Indeed, there are conflicting studies. Several studies did not find an association between long-term treatment with glucocorticoids and atherosclerosis.\textsuperscript{60,61} Wei \textit{et al} demonstrated in a large group of patients that those who received high-dose glucocorticoids, defined as more then 7.5 mg daily for 1 year or more, were more than twice as likely to have had a cardiovascular event than patients who used no glucocorticoids.\textsuperscript{62} In contrast, Roman \textit{et al} found a negative association between carotid plaque and a high mean daily dose of prednisolone in SLE patients.\textsuperscript{10} Clearly, the role of prednisolone treatment in the development of CVD in SLE deserves further study.

**Others**

Last, two factors that may play a role in induction of EC activation and EC dysfunction in systemic autoimmune diseases are discussed briefly, namely, renal impairment and homocysteine. It has been demonstrated that EC dysfunction is related to renal impairment.\textsuperscript{63} However, this is debatable, as in SLE no relationship could be found between renal function and presence of plaque, and in WG patients no relation could be detected between renal function and EC dysfunction.\textsuperscript{10,18} Furthermore, elevated levels of homocysteine have been associated with CVD in the general population\textsuperscript{64,65} as well as in SLE patients, who have even higher levels of homocysteine compared to controls.\textsuperscript{40,60,66,67}

**Hypothetical non-traditional risk factors; advanced glycation endproducts**

Recently, the accumulation of advanced glycation endproducts (AGEs) has been recognized as a contributing factor in the progression of atherosclerosis.\textsuperscript{58} AGEs are a class of compounds resulting from glycation and oxidation of proteins, lipids or nucleic
acids. Glycation is the non-enzymatic addition or insertion of saccharide derivatives to these molecules. This leads to the formation of intermediary Schiff bases and Amadori products, and, finally, to irreversible AGEs. The formation of AGEs occurs ubiquitously and irreversibly in patients with diabetes mellitus, and the presence of those compounds contribute to the pathogenesis of accelerated atherosclerosis found in diabetes.\textsuperscript{69-71} Apart from AGE formation in the extracellular matrix, resulting in decreased elasticity and increased thickness and rigidity of the vascular wall, the interaction of AGEs with their receptors (RAGEs) has been implicated in endothelial dysfunction.\textsuperscript{72} The interaction between AGEs and RAGEs on macrophages induces oxidative stress and activation of intracellular signaling, causing secretion of cytokines and mediators of inflammation, vasoconstriction because of reduced production of nitric oxide, and coagulation.\textsuperscript{73} Furthermore, in a model of accelerated and advanced atherosclerosis in diabetic mice, treatment with soluble RAGE completely suppressed diabetic atherosclerosis.\textsuperscript{74}

Although the primary causal factor leading to the formation of AGEs and their pathophysiological effects in diabetes is chronic exposure to high blood levels of glucose, in recent years it has been suggested that AGEs can be formed in multiple milieus, such as in inflammation and renal failure.\textsuperscript{75,76} In systemic autoimmune diseases, such as SLE and WG, where inflammation of vessels is common, increased AGE formation at these sites can be expected, which could contribute to accelerated atherosclerosis. Currently, this hypothesis is under investigation in our laboratory.

\textbf{Circulating progenitor cells}

A healthy endothelium is important to prevent atherogenesis. Therefore, disturbance in the equilibrium between vascular injury and vascular repair will be followed by atherosclerosis. Recent insights suggest that injured endothelium can be regenerated by circulating bone marrow-derived endothelial progenitor cells (CPCs). Exhaustion of these cells in number, function, or both, due to chronic exposure to cardiovascular risk factors, can result in atherosclerosis.\textsuperscript{77,78} In apolipoprotein E \textsuperscript{-/-} mice after transplantation with wild-type bone marrow from younger animals, reduction in atherosclerotic lesions was found, because of regeneration by the transferred cells.\textsuperscript{79} Furthermore, possible anti-inflammatory effects of these cells have been suggested. After transfer, interleukin-6 levels were decreased. Considering that atherosclerosis is an inflammatory disease,
these anti-inflammatory effects of CPCs, next to vascular repair, could prevent
development or progression of atherosclerosis.

In animal studies, as in humans, it has been demonstrated that the number of CPCs
correlates negatively with established risk factors for atherosclerosis and endothelial
function. However, not only number, but also functionality is important. It has been
shown that in diabetes, which is characterized by accelerated atherosclerosis, the
capacity of CPCs to adhere is compromised.80

How might CPCs be a link between accelerated atherosclerosis and systemic
autoimmune disease? It is possible that in SLE and WG, both characterized by chronic
vascular and systemic inflammation that cause continuous vascular injury, the pool of
CPCs is exhausted. Furthermore, because of this continuous demand of CPCs, quality
and functionality of these cells could be compromised, resulting in decreased capacity
to repair vascular damage, thereby disturbing the equilibrium between vascular injury
and repair.

CONCLUSION

In summary, in systemic autoimmune diseases, which are associated with increased EC
activation, EC dysfunction and accelerated development of atherosclerosis, not only
traditional risk factors, but also non-traditional factors play an important role in
atherosclerosis. Clearly, at present, questions remain as to whether all non-traditional
risk factors have already been elucidated and which factors play the most important
role in the accelerated development of atherosclerosis in these patients. Further insights
into the pathophysiological role of these factors may result in new strategies for
interference in these pathological processes.

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Introduction, aim and outline of the thesis


AIM AND OUTLINE OF THE THESIS

In *chapter 1* an overview is given of the possibly involved risk factors in the development of accelerated atherosclerosis in patients with systemic lupus erythematosus (SLE) and Wegener’s granulomatosis (WG).

We hypothesized that patients with SLE and WG are prone to develop cardiovascular disease due to traditional and non-traditional risk factors by inducing endothelial activation and endothelial dysfunction, which are one of the earliest steps in the development of atherosclerosis.

However, until now, it is not clear which risk factors are most contributing to the process of cardiovascular disease in systemic autoimmune disease. Furthermore, it would be worthwhile to have diagnostic or screening tools to determine vascular function and, possibly, cardiovascular risk, in order to identify patients who might benefit from preventive measures such as improving lifestyle and risk-factor modification.

In this thesis, the prevalence of atherosclerosis and contributing factors are investigated, as well as methods to assess endothelial function. The thesis is divided in two parts. Part one involves SLE patients and part two WG patients.

**Systemic lupus erythematous**

*Chapter 2* and *3* describe the prevalence of premature atherosclerosis in SLE as measured by intima-media thickness (IMT) of the common carotid artery. In *chapter 2* we evaluate whether atherosclerosis is, indeed, more prevalent among our SLE patients. In addition, we investigated whether its prevalence can be explained by the presence of traditional and non-traditional risk factors for cardiovascular disease, including markers of endothelial activation and vascular remodeling. *Chapter 3* is a longitudinal study, in which IMT measurement is repeated after three years of follow-up to determine predictors of progression of IMT.

*Chapter 4* describes the assessment of endothelial function using laser Doppler fluxmetry in combination with iontophoresis of acetylcholine and sodium nitroprusside, an endothelium-dependent and endothelium-independent vasodilator, respectively.

The accumulation of advanced glycation endproducts (AGEs), compounds which have been implicated to be involved in the development of atherosclerosis, can be measured non-invasively with the AGE-reader. In *chapter 5* we aim to investigate whether AGEs...
are increased in SLE patients, and whether AGEs are related to atherosclerosis, and its traditional and non-traditional, disease-related risk factors. Chapter 6 concerns the number and functionality of circulating endothelial progenitor cells in SLE, which might be involved in repair of damaged endothelium and thereby, when altered, contribute to accelerated development of atherosclerosis.

**Wegener’s granulomatosis**

In chapters 7 and 8 IMT is measured in WG patients to assess the prevalence of increased premature atherosclerosis. Chapter 7 is a cross-sectional study, as chapter 8 is a follow-up study, in which IMT is determined after a follow-up of six years. To elucidate whether endothelial function of the microcirculation is impaired in WG, endothelial function is measured using laser Doppler fluxmetry in combination with iontophoresis, as described in chapter 9.

Finally, the results of this thesis are summarized and discussed in chapter 10.