CHAPTER 1

GENERAL INTRODUCTION
CHAPTER 1

SYSTEMIC SCLEROSIS

Systemic sclerosis (scleroderma, SSc) is an autoimmune connective tissue disease of unknown etiology characterized by cutaneous and visceral fibrosis, and widespread vascular pathology. Preliminary criteria for the classification of systemic sclerosis have been proposed by the American College of Rheumatology (formerly, the American Rheumatism Association) in 1980 (table 1). These proposed criteria had a 97% sensitivity and 98% specificity for definite systemic sclerosis in the presence of the major criterion, or two or more of the minor criteria. The main and major diagnostic criterion for SSc is fibrosis of the skin, whereas fibrosis is also a major part of the minor criteria.

Table 1. Preliminary clinical criteria for systemic sclerosis

<table>
<thead>
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<th>Major criterion</th>
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<td>Proximal scleroderma</td>
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<table>
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<th>Minor criteria</th>
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<td>Sclerodactyly</td>
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<td>Digital pitting scars or loss of substance of the distal finger pad</td>
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<td>Bibasilar pulmonary fibrosis on standard chest X-rays</td>
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A classification of SSc patients into 2 subsets was proposed by LeRoy et al. Limited cutaneous SSc (lcSSc) is defined by skin involvement limited to hands, face, feet, and forearms (acral). In diffuse cutaneous SSc (dcSSc) truncal and acral skin involvement is also present. Subsets differ also in the presence of autoantibodies and visceral disease (table 2).

Table 2. Subsets of systemic sclerosis

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<th>Diffuse cutaneous SSc (dcSSc)</th>
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<tr>
<td>Onset of Raynaud’s within 1 year of onset of skin changes (puffy or hidebound)</td>
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<tr>
<td>Truncal and acral skin involvement</td>
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<tr>
<td>Presence of tendon friction rubs</td>
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<td>Early and significant incidence of interstitial lung disease, oliguric renal failure, diffuse gastrointestinal disease, and myocardial involvement</td>
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<tr>
<td>Absence of anticientromere antibodies (ACA)</td>
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<td>Nailfold capillary dilation and capillary destruction</td>
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<td>Antitopoisomerase antibodies (30% of patients)</td>
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<table>
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<tr>
<th>Limited cutaneous SSc (lcSSc)</th>
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<tr>
<td>Raynaud’s for years (occasionally decades)</td>
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<tr>
<td>Skin involvement limited to hands, face, feet, and forarms (acral) or absent</td>
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<tr>
<td>A significant incidence of pulmonary hypertension, with or without interstitial lung disease, trigeminal neuralgia, skin calcifications, telangiectasia</td>
</tr>
<tr>
<td>A high incidence of ACA (70-80%)</td>
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<td>Dilated nailfold capillary loops, usually without capillary dropout</td>
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Skin thickening provides the definitive diagnostic criterion in SSc in over 90% of patients. However, the presence of vascular disease, such as Raynaud’s phenomenon, a major clinical manifestation in SSc patients, is not part of the classification criteria.
RAYNAUD’S PHENOMENON AND VASCULAR INVOLVEMENT

Raynaud’s phenomenon
Raynaud’s phenomenon (RP) is characterized by episodic digital vasospasm provoked by cold and/or emotional stress. Without evidence of an associated disorder RP is considered primary. With evidence of an associated disorder, like SSc, RP is considered secondary. A typical episode or attack is triphasic. First, vasospasm occurs with sharply demarcated white color changes of skin, then blue discoloration occurs (cyanosis), followed after rewarming by vasodilatation with erythema of reperfusion (red). In some patients only the white or cyanotic phase is seen, both always followed by the red phase. The signs of uncomplicated RP should be completely reversible after rewarming or reduction of stress. In severe secondary RP, pain or ulceration of the skin (typically the tips of the fingers and toes) may result from critical tissue ischemia.\(^3\),\(^4\) In patients diagnosed as primary RP, 7% to 13% eventually develop a secondary disorder, most frequently SSc, mixed connective tissue disease (MCTD), systemic lupus erythematosus (SLE) or Sjögren’s syndrome.\(^5\)-\(^7\) Otherwise, in patients with a connective tissue disease RP is a common manifestation. For example, RP is present in more than 95% of patients with SSc. Its presence together with other factors like the frequency and the severity of the attacks and the presence of digital ulcers affects the quality of life of these patients.\(^8\) The pathogenesis of RP is not yet fully understood, but is considered multifactorial. There is evidence that RP attacks are due to a local fault at the level of the digital microcirculation, in which digital arteries are hypersensitive to cold. Lewis observed in the first decades of the 20\(^{th}\) century that vasospastic attacks can still occur in RP patients after blockade of sympathetic nerves, such as after local anesthesia of the digital sympathetic nerves, or after sympathectomy, and suggested that a functional abnormality is responsible.\(^9\),\(^10\) Otherwise, evidence is present revealing an increased expression or activity of postjunctional \(\alpha_2\) adrenoreceptors.\(^11\)-\(^16\) Other, non-adrenergic mechanisms play an important role in the pathogenesis. Disturbances in the regulation of vascular tone with decreased or inadequate vasodilatory signals and increased or enhanced vasoconstrictive signals can result in RP,\(^16\),\(^17\) as in healthy state vascular tone is controlled by a delicate interplay between these factors.

Endothelial dysfunction
Campbell and LeRoy proposed their vascular hypothesis in 1975 considering the vascular abnormalities and the presence of RP prior to scleroderma skin manifestations.\(^18\) Hereby, vascular abnormalities and dysfunction are considered to be an important element in SSc. Vascular abnormalities are noted in the capillaries and small blood vessels. Using nailfold microscopy or digitised video capillaroscopy, characteristic nailfold capillary abnormalities are found, like decreased capillary density, enlarged and giant capillaries, haemorrhages, disorganization of the vascular array and ramified/bushy capillaries.\(^19\)-\(^23\) Besides structural abnormalities, vascular dysfunction plays an important role. The endothelial cell is responsible for vascular permeability, control of vascular tone, control of haemostasis and thrombosis, and can interact with leucocytes.\(^24\) Circulating markers of endothelial cell function, such as von Willebrand factor (vWF), tissue plasminogen activator, soluble E-selec tin (sE-selectin), soluble vascular cell adhesion molecule 1 (s-VCAM-1), soluble intercellular adhesion molecule 1 (s-ICAM-1), and endothelin, are raised in a significant
proportion of SSc patients.\textsuperscript{25-30} Also, the level of circulating endothelial cells (CECs), proposed to be a reliable marker of endothelial damage in different vascular diseases, is elevated in SSc, and related to disease activity and the presence of pulmonary hypertension.\textsuperscript{31} Increased capillary permeability of the nailfold capillaries has been found by using dynamic fluorescence videomicroscopy of the nailfold.\textsuperscript{32} Studying vascular tone, impaired endothelium-dependent vasodilation has been found in SSc using laser Doppler fluxmetry in combination with iontophoresis or other dynamic tests, like cooling and post-occlusive hyperemia.\textsuperscript{33-36} Laser Doppler fluxmetry is well established in the measurement of cutaneous microcirculatory flow. Other non-invasive methods for evaluating cutaneous microcirculatory flow have been described recently by Wright et al.\textsuperscript{37} To study endothelial function in conduit arteries, ultrasound has been used. Using brachial artery ultrasound-derived flow-mediated dilation (FMD), impaired endothelium-dependent vasodilation has also been found.\textsuperscript{38-40} Endothelial dysfunction is strongly implicated in the pathogenesis of atherosclerosis.\textsuperscript{41,42} An increased prevalence of atherosclerosis has been found in other auto-immune diseases, such as systemic lupus erythematosus, in which endothelial dysfunction is present.\textsuperscript{43-45} In SSc, vascular involvement has always been considered to be mainly microvascular,\textsuperscript{46} but in the last two decades macrovascular disease has also been described.\textsuperscript{47-49}

**Endothelin-1**

Endothelin is a potent vasoconstrictor peptide originally characterized from the culture supernatant of porcine aortic endothelial cells.\textsuperscript{50} The precursor of endothelin is a 212-residue prepropeptide cleaved in two steps into the active 21-amino acid endothelin. Three structurally and pharmacologically distinct isopeptides are present, produced by three separate genes.\textsuperscript{51} Endothelin-1 (ET-1) is the most significant isoform. Endothelin-2 (ET-2) is produced predominantly within the kidney and intestine, but has no specific physiological function. Endothelin-3 (ET-3) circulates in plasma, and has been found in the brain, gastrointestinal tract, lung and kidney. ET-3 is probably involved in the central nervous system. Endothelin-1 (ET-1) is produced in endothelial cells, but also in vascular smooth muscle cells. The plasma half-life is short and is approximately 4 to 7 minutes. As such, vascular tone can be rapidly adjusted depending on ET-1 concentrations. ET-1 binds to specific receptors on smooth muscle cells causing vasoconstriction.\textsuperscript{52} There are two endothelin receptors, ET\textsubscript{A} and ET\textsubscript{B}. The ET\textsubscript{B} receptor binds ET-1, ET-2 and ET-3 equally, while the ET\textsubscript{A} receptor has a strongly preferential binding affinity for ET-1 above ET-3.\textsuperscript{53} Increased plasma ET-1 levels have been found in patients with RP, primary and secondary, and in patients with SSc,\textsuperscript{54-60} suggesting that ET-1 plays a part in the pathogenesis of these conditions. However, others found no differences in ET-1 levels in plasma in resting conditions and after cold provocation in patients with primary RP and/or secondary RP compared with healthy controls.\textsuperscript{61-63} ET-1 may also be important in the pathogenesis of the fibrotic manifestations of SSc. ET-1 is profibrogenic by enhancing fibroblast proliferation and collagen synthesis,\textsuperscript{57} and ET-1 levels are found to correlate with several disease characteristics, amongst others skin fibrosis.\textsuperscript{64} ET-1 was shown to induce a fibrogenic phenotype in normal fibroblasts similar to that of lesional SSc fibroblasts.\textsuperscript{65} Since ET-1 plays an important role in vascular and fibrotic lesions in SSc, use of specific endothelin-receptor antagonists may be a promising therapy for both vascular and fibrotic lesions in
SSc. Bosentan is an orally active dual endothelin receptor antagonist, shown to be effective in the treatment of idiopathic pulmonary arterial hypertension and in SSc-related pulmonary hypertension. Bosentan was also found to be effective in the prevention of new digital ulcers in SSc patients, and case-series have suggested a positive effect of the drug on RP.

**OUTLINE OF THIS THESIS**

This thesis describes several aspects of vascular involvement in SSc, i.e. RP, microvascular involvement, endothelial cell dysfunction, and macrovascular involvement.

**Microvascular involvement and endothelial cell dysfunction**

In SSc treatment with vasodilating agents, including calcium-channel blockers, angiotensin converting enzyme inhibitors, serotonin receptor blockers and intravenous prostaglandin analogues, are used for RP. However, these agents showed only moderate reduction in severity and frequency of attacks. Side-effects frequently result in discontinuation of these agents.

Endothelin-1 (ET-1) has been suggested to play a role in the pathogenesis of RP. We hypothesized that bosentan, an ET receptor antagonist, could be a useful agent in the treatment of RP. Furthermore, we questioned whether these effects could be explained by the effects of bosentan on endothelial cell dysfunction in SSc patients with severe RP.

In **chapter 2** the results of treatment with bosentan on frequency, duration and severity of RP attacks are described in patients with RP secondary to SSc. These effect were assessed by diary and, more objectively, by photoelectric plethysmography during cooling and rewarming.

**Chapter 3** describes the effects of treatment with bosentan on endothelial cell dysfunction by assessment of vasodilatory microvascular responses using laser Doppler fluxmetry combined with iontophoresis, capillary permeability using fluorescence videomicroscopy, nailfold capillary microscopy, and serological markers of endothelial activation.

Vascular or endothelial cell dysfunction has been suggested to be a crucial element in the pathogenesis of SSc. In **chapter 4** we describe microvascular reactivity in SSc patients and healthy controls. We evaluated endothelial microvascular function, assessed by laser Doppler flowmetry combined with iontophoresis, and determined whether endothelium-dependent microvascular reactivity was related to the presence of SSc or other possible confounding variables, such as hypertension, diabetes and obesity.

In **chapter 5** the results are presented of a case-control study in which we investigated capillary permeability in SSc patients and healthy controls. The primary objective of this study was to investigate whether increased capillary permeability in nailfold capillaries found in patients with SSc, is a generalized phenomenon. We used sodium fluorescence videodensitometry of the ankle to test this hypothesis.
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Macrovascular involvement
Microvascular involvement is common in SSc and the frequency of vascular abnormalities was found to be inversely proportional to the size of blood vessels studied. However, less data are available on the prevalence of macrovascular involvement in SSc.

In chapter 6 the literature on macrovascular involvement in SSc is reviewed.

In chapter 7, we describe the prevalence of early signs of atherosclerosis by measuring intima media thickness (IMT) of the common carotid artery in patients with SSc compared to healthy controls. Outcome is related to the presence of disease-related and traditional cardiovascular risk factors.

Accumulation of advanced glycation endproducts (AGEs) is related to age, but occurs more rapid in conditions like diabetes mellitus, renal failure, atherosclerosis and inflammatory diseases, like rheumatoid arthritis. Tissue autofluorescence is related to the accumulation of AGEs. In chapter 8, we assessed skin autofluorescence by using the Autofluorescence Reader in patients with SSc in comparison with healthy controls. We related the extent of AGE accumulation to the presence of disease-related and traditional cardiovascular risk factors.

Finally, the results and conclusions of this thesis are summarized in chapter 9.
REFERENCES

10. Lewis T, Pickering GW. Observations upon maladies in which the blood supply to the digits ceases intermittently or permanently, and upon bilateral gangrene of digits; observations relevant to so-called Raynaud's disease. Clin.Sci. 1933;1:327.
CHAPTER 1


General introduction
