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Introduction

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1 Focus and rationale

Systemic sclerosis, or “scleroderma”, derives from the Greek scleros (hard) and derma (skin), pointing towards the most visible physical characteristic of the disease. However, multiple organ systems are implicated in systemic sclerosis. Involvement of systemic sclerosis in the pulmonary vascular bed can result in pulmonary arterial hypertension. Pulmonary arterial hypertension is a severe condition characterised by vascular remodelling, leading to a rise in pulmonary vascular resistance, elevated pulmonary artery pressures, right heart failure and ultimately death.

Well-documented systemic sclerosis-associated pulmonary arterial hypertension (SScPAH) has been scarcely studied. In histopathologic and clinical classifications, in diagnostic and therapeutic algorithms and in clinical trials, SScPAH is grouped together with other forms of PAH (see Table 1). However, SScPAH has a different disease course compared with other forms of PAH, as demonstrated by lower transfer factor for carbon monoxide (TLCO), poorer response to therapy and premature death. Furthermore, there are indications of a worse adaptation of the right ventricle (RV) to the increased pressure load in SScPAH. Considering these findings, it could be suggested that SScPAH should be set apart from other PAH forms, with implications for, for example, classification, research and therapy.

SScPAH is the focus of this thesis. In well-documented SScPAH, it studies yet unexplored characteristics of both the pulmonary vasculopathy, from which the PAH syndrome arises, and the RV, a close interactor with the pulmonary circulation and the major determinant in the prognosis of PAH. Findings are extended as they are compared with IPAH, in order to explore the hypothesis that SScPAH displays distinct histopathological and pathophysiological features. Moreover, a possible screening tool is evaluated for its usefulness in detecting PAH in SSc.

2. Systemic sclerosis

2.1 Epidemiology of systemic sclerosis

Systemic sclerosis (SSc) is a rare, progressive and life-threatening disease, with a marked geographic variation with prevalences in Europe of 80 cases per million, compared with 240 cases per million in the United States of America[1,2]. Incidences are reported to be 4 per million in the UK and 19 per million in the United States[1,3]. The disease presents generally in the 4th-5th decade and female gender is a risk factor[4].

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2.2 Pathogenesis of systemic sclerosis

A widely accepted hypothesis is that in SSc a not yet elucidated complex sequence of pathogenic events occurs, on a genetic basis and following the impact of unknown environmental stimuli[5]. Initially, changes in the microvasculature take place, manifesting as altered vascular tone, endothelial dysfunction and oxidative stress. Vasculopathy is followed by immunological activation and perivascular inflammation, initially from the innate immune system and later from activated T-cells; complex networks and cascades of intercellular interactions, involving growth factors, cytokines, chemokines, drive the pathologic events that result in uncontrolled fibrosis[6]. Excessive fibrosis replaces normal tissue architecture and often leads to organ compromise (ischemia, limitation of motility) and failure[6,7]. Important mediators of vascular injury and fibrogenesis in SSc include transforming growth factor (TGF)- β , endothelin-1 (ET-1), connective tissue growth factor, matrix metalloproteinases, and platelet-derived growth factor (PDGF)[8,9].

2.3 Clinical features of systemic sclerosis

The clinical picture of SSc is heterogeneous. The amount of skin thickening is used for the determination of patients in different categories. At present, a widely accepted subclassification is based on cutaneous involvement of SSc: limited SSc, limited cutaneous SSc (LcSSc) and diffuse cutaneous SSc (DcSSc)[10,11]. Specific autoantibodies have been shown to correlate closely with various clinical and laboratory manifestations of the disease, and, therefore, are also used as part of the diagnostic panel.

Both DcSSc and LcSSc patients often exhibit early signs of vasculopathy with more than 85% of patients experiencing the Raynaud's phenomenon[12]. In DcSSc, there is often widespread inflammation in the skin and musculoskeletal system[13]. Pulmonary interstitial fibrosis is common early in the disease [14], but pulmonary vascular involvement is also seen. Visceral organ involvement compromises skin, renal, cardiac and gastrointestinal tract, generally occurring in the first 3 years in the disease and affecting mortality with a 9-year-cumulative survival rate of 38%, vs. 72% without visceral involvement[15]. LcSSc generally has better outcome numbers with 5-year survival rates of 86%[16]. In LcSSc, morbidity and mortality occur from digital vascular disease, gastrointestinal involvement, and pulmonary involvement, related both to pulmonary fibrosis and to pulmonary vascular disease. Raynaud's phenomenon often pre-exists for several years. Organ complications generally occur in later disease phases as compared with DcSSc[17].

Pulmonary involvement in the SSc-group as a whole, comprising interstitial lung disease and pulmonary arterial hypertension (PAH), is the leading cause of morbidity and mortality[18]. Interstitial lung disease in SSc may account for 16%

The third type of PH is associated with hypoxaemia and lung disease. Hypoxaemia due to interstitial lung disease is common in patients with SSc. As such, it is important to evaluate the presence of interstitial lung disease for either classification in group 1 or 3, although overlap of interstitial lung disease and PAH can occur[38].

Table 1. Revised clinical classification of pulmonary hypertension (Dana point classification 2008)[37]

Group 1: Pulmonary arterial hypertension (PAH)	
1.	Pulmonary arterial hypertension (PAH)
1.1.	Idiopathic PAH
1.2.	Heritable
1.2.1.	BMPR2
1.2.2.	ALK1, endoglin (with or without hereditary hemorrhagic telangiectasia)
1.2.3.	Unknown
1.3.	Drug- and toxin-induced
1.4.	Associated with
1.4.1.	Connective tissue diseases
1.4.2.	HIV infection
1.4.3.	Portal hypertension
1.4.4.	Congenital heart diseases
1.4.5.	Schistosomiasis
1.4.6.	Chronic haemolytic anaemia
1.5.	Persistent pulmonary hypertension of the newborn
1'. 1.	Pulmonary veno-occlusive disease (PVOD) and/or pulmonary capillary hemangiomatosis (PCH)
Group 2: Pulmonary hypertension owing to left heart disease	
2.1.	Systolic dysfunction
2.2.	Diastolic dysfunction
2.3.	Valvular disease
Group 3: Pulmonary hypertension owing to lung diseases and/or hypoxia	
3.1.	Chronic obstructive pulmonary disease
3.2.	Interstitial lung disease
3.3.	Other pulmonary diseases with mixed restrictive and obstructive pattern
3.4.	Sleep-disordered breathing
3.5.	Alveolar hypoventilation disorders
3.6.	Chronic exposure to high altitude
3.7.	Developmental abnormalities
Group 4: Chronic thromboembolic pulmonary hypertension (CTEPH)	
Group 5: Pulmonary hypertension with unclear multifactorial mechanisms	
5.1.	Hematologic disorders: myeloproliferative disorders, splenectomy
5.2.	Systemic disorders: sarcoidosis, pulmonary Langerhans cell histiocytosis: lymphangioleiomyomatosis, neurofibromatosis, vasculitis
5.3.	Metabolic disorders: glycogen storage disease, Gaucher disease, thyroid disorders
5.4.	Others: tumoral obstruction, fibrosing mediastinitis, chronic renal failure on dialysis

3.2 Epidemiology of systemic sclerosis-associated pulmonary arterial hypertension

Registries from referral centers estimate prevalence rates of PAH between 15 and 25 and incidence rates between 2.4 and 7.6 annual cases per million adult inhabitants[39,40]. As for SScPAH, prevalence rates are estimated between 3 and 10 and incidence rates between 0.4 and 2.8 annual cases per million adult inhabitants [39,40]. More recent data demonstrated the incidence of PAH to be 0.61 per 100 patient-years[41]. For the Netherlands Vonk *et al.* estimated the combined prevalence of PAH and PH associated with fibrosis in patients with SSc to be 9.9%[42].

Risk factors are the limited cutaneous form of SSc, with long-standing Raynaud’s phenomenon (> 10 years), and the presence of anticentromere antibodies[43]. An older age at onset of SSc has been associated with an increased risk of developing PAH as well[44]. TLCO values of less than 50% of predicted have been detected in patients with LcSSc without interstitial fibrosis 4.5 years before PAH was diagnosed[43].

Before the newer therapies, 1-year survival of SScPAH was 50-55%.[28,45]. Newer cohorts show better survival, but prognosis is still poor with a 3-year survival rate of 47-56% despite therapy. Notably, this is worse than other forms of PAH such as IPAH[25-28].

3.3 Pathogenesis of systemic sclerosis-associated pulmonary arterial hypertension

3.3.1 PAH pathogenesis

The pathogenesis of PAH is only partly elucidated. Genetic involvement is demonstrated by mutations in the bone morphogenetic protein receptor-2 (BMPR2) in the majority of patients with familial PAH, but also in some IPAH cases[46-48]. Irrespective of its underlying cause, PAH demonstrates disturbances in the normal balance between endogenous vasoconstrictors and vasodilators in response to endothelial dysfunction or injury[49,50]: there is increased production of vasoconstrictors such as thromboxane A2 and ET-1, and reduced production of vasodilators such as nitric oxide (NO) and prostacyclin. These abnormalities elevate vascular tone and promote remodeling of the vascular wall, which leads to the persistent increase in PVR and its adverse clinical sequelae.

Vascular remodeling in PAH involves a broad array of cell types residing in all layers in the vessel wall, including endothelial cells, smooth muscle cells, fibroblasts, platelets and inflammatory cells. The biological pathways leading to vascular remodeling are poorly understood. Inflammatory mechanisms are prominent in various types of PAH and are recognised as important components in the pathogenesis[51-56]. Increasing evidence demonstrates that cytokines, chemokines, and growth factors such as vascular endothelial growth factor, platelet derived

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growth factor (PDGFR) and epidermal growth factor (EGFR) play a direct role in the development of vascular remodeling[57,58]. Some PH models suggest that adventitial fibroblasts, thought to be derived from bone marrow-derived progenitor cells, react to the pulmonary hypertensive stimulus by proliferation, extra-cellular matrix production and migration to the media and intima of the vessel[59,60].

3.3.2 SScPAH pathogenesis

There are limited data on specific pathogenesis of SScPAH. Little is known about genetic involvement in SScPAH. Two small cohorts did not identify BMPR2 mutations as found in familiar PAH and IPAH[47,61]. An association has been found between SScPAH and endoglin gene (ENG) polymorphism. ENG is present on human endothelium and part of the TGF- β complex, but its function is unknown[62].

Increasing evidence indicates that autoantibodies are implicated in the pathogenesis of SScPAH. For example, antifibrillar antibodies (anti-U3-RNP) are found in SScPAH[63], and antibodies to fibrin-bound tissue plasminogen activator are demonstrated in LcSSc patients as well as in IPAH patients[64,65]. Anti-endothelial cell antibodies (AECAs) correlate with digital infarcts in SSc and are suggested to play a role in PAH pathogenesis[66,67]. *In vitro* it has been shown that autoantibodies from patients with connective tissue diseases can upregulate adhesion molecules and histocompatibility complex class II molecules on human pulmonary artery endothelial cells[68]. Fibroblasts are present in the remodeled pulmonary vessel wall. Auto-antibodies against fibroblasts, which have been found in SScPAH and IPAH, could be of pathogenic importance by activating fibroblasts to induce aberrant extra-collagen matrix deposition[69,70].

3.5 Therapy in systemic sclerosis-associated pulmonary arterial hypertension

The vasodilator/vasoconstrictor imbalance is the target of current PAH therapies. Present treatment targets in PAH are relative prostacyclin- and NO deficiencies as well as increased endothelin (ET)-1 levels, and provide symptomatic relieve and some improvement in prognosis in PAH patients[22,71-74]. None of these therapies, however, cures PAH.

Prostacyclin compound therapy has shown roughly equal functional improvement in SSc-PAH subsets compared with IPAH groups[75]. However, whereas continuous intravenous epoprostenol reduces mortality in IPAH, the survival benefit in SScPAH appears to be lower[76,77]. Survival rates of 58% and 41% have been reported of 1- and 2-year survival rates, respectively[78]. The only non-invasive prostacycline-compound evaluated in the CTD-PAH group is treprostinil[79]. Six-minute-walking distance (6MWD), a common outcome

parameter in PAH trials, and some haemodynamic parameters showed a significant decrease. Only half of the CTD-group, however, consisted of patients with SSc.

The ET-1 receptor antagonist bosentan showed improvement of haemodynamics and New York Heart Association (NYHA) functional class, and increased time to deterioration in PAH[74,80,81]. Most SScPAH-patients, however, did not improve in functional class and tended to have a higher mortality compared to IPAH patients[74,82]. Study of the selective ET_A blocker sitaxsentan in the subgroup with CTD-PAH demonstrated both improvement in 6MWD and haemodynamics. However, no conclusions on SScPAH can be made as the placebo group only comprised 2 SScPAH patients, and placebo-treated patients were in worse condition[83].

Inhibition of phosphodiesterase type-5 with sildenafil, thereby increasing NO-mediated pulmonary vasodilation, showed roughly equal improvement of 6MWD and haemodynamics in a mixed group of patients with CTD[84]. Posthoc-analysis of the CTD group found improvements in exercise capacity, functional class, and hemodynamics. Again, generalizability to the SScPAH group is limited as less than half of the CTD patients had SSc. The results of tadalafil, another phosphodiesterase type 5 inhibitor, have been recently reported[85]. The treatment effect upon 6MWD, time to clinical worsening, and quality of life was significant in PAH. Statistically significant improvements in 6MWD occurred in the CTD-group, though the proportion of patients with SSc was not reported, thus, again, these results cannot easily be extrapolated to the SScPAH group. As for the evaluation of combination of therapies in SScPAH, there is limited data. Mathai *et al.* demonstrated that addition of bosentan to sildenafil in SScPAH resulted in less improved exercise capacity as compared to IPAH[86]. Recently, a report of long term follow-up of SScPAH patients treated with bosentan with or without addition of sildenafil demonstrated stabilisation of NYHA functional class and exercise capacity[87]. Survival estimates were still poor with 1-, 2- and 3-year-survival rates of 80%, 56% and 51%, respectively.

Taken together, currently approved therapeutic modalities of PAH have not been studied extensively in SScPAH as a separate entity. As such, treatment effectiveness in this group is insufficiently known yet. However, there are clear indications that SScPAH patients respond poorer to treatment than IPAH patients.

3.5 The right ventricle in systemic sclerosis-associated pulmonary arterial hypertension

Despite the importance of the interaction between the pulmonary vasculature and the right ventricle (RV) in PAH[88], demonstrated by the influence of the RV on PAH morbidity and mortality[89], the RV in SScPAH has scarcely been studied. In SSc patients without established PAH, several studies demonstrated altered

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diastolic[90-92] and systolic function[93]. This might be due to involvement of SSc-specific disease in the myocardium. Autopsy studies demonstrated fibrosis in hearts of SSc patients [94-99], and structural and functional abnormalities of the small coronary arteries are known features of SSc[100-104]. These studies did not include SSc patients with PAH, but it can be speculated that SSc-specific disease is involved in RV function of SScPAH.

It is of importance to note that there are indications that the RV in SScPAH is more impaired than in IPAH. Fisher *et al.* showed that SScPAH patients have lower mPpa than IPAH patients, despite similar levels of cardiac index (CI)[26]. Such differences

3.6 Screening for pulmonary arterial hypertension in systemic sclerosis

Diagnosing PAH in SSc is difficult as initial symptoms such as fatigue, dyspnoea and exercise intolerance are non-specific. Moreover, there are no optimal screening tools yet, whereas there are indications that early initiation of treatment might modify the course of disease in SScPAH[105].

Screening programs comprise an integrated evaluation of risk factors, echocardiography, pulmonary function tests and the biomarker brain natriuretic peptide (NT-proBNP), in selected cases leading to right heart catheterization which confirms or rejects the diagnosis.

Transthoracic Doppler echocardiography (TTE) estimates RV systolic pressures as an approximation of systolic artery pressures (sPpa). In patients with advanced lung disease sPpa estimations are known to have a poor positive predictive value [106]. Once sPpa is > 45 mmHg, there is a > 90% correlation with right heart catheterisation values, particularly if there are other RV changes[107]. The use of echocardiography alone for the diagnosis of PAH may result in a misleading diagnosis in almost 10% of the cases, and screening programs with echocardiography also have demonstrated that left-heart disease is common in SSc[22]. This emphasizes the importance of proper evaluation of haemodynamics by right heart catheterisation, as classification of SSc patients in group 1 (PAH) or group 3 (PH associated with left heart diseases) has major therapeutic implications. However, in screening algorithms for PAH in SSc, TTE has an important role[22,108].

Pulmonary function testing in SScPAH is hallmarked by an isolated decreased TLCO [109,110]. However, TLCO has not been established yet as a marker for SScPAH as, for example, it is not clear how interstitial lung disease influences TLCO in these patients, as patients with severe interstitial lung disease; moreover, only a weak correlation between mean pulmonary arterial pressures and TLCO has been demonstrated in SSc patients[111].

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filling function of the RV are examined and compared with IPAH. To increase the understanding of pathologic mechanisms involved in altered RV function, in **Chapter 6**, hallmarks of SSc pathology, including fibrosis, inflammation and vasculopathy, are evaluated in the myocardium of SScPAH, and compared with IPAH.

In an effort to contribute to the search for adequate screening parameters of PAH in SSc, in **Chapter 7** the partitioned TLCO is tested for its utility as a tool in the diagnostic work-up of SScPAH.

Finally, **Chapter 8** is a summary of this thesis and includes suggestions for future research.

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