

DIAGNOSIS AND MECHANISMS OF
CARDIAC INVOLVEMENT IN PATIENTS
WITH SYSTEMIC SCLEROSIS

Ph. D. thesis

by

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Abbreviations

A	late diastolic velocity of the transmitral Doppler flow
ACA	Anti-Centromere Antibody
ACEI	Angiotensine Converting Enzyme Inhibitor
ANA	Antinuclear Antibody on human Hep-2 cells
BMI	Body Mass Index
CAD	Coronary Artery Disease
CFR	Coronary Flow Reserve
CFR _{echo}	Coronary Flow Reserve measurement with Doppler signal augmented echocardiography
CO ₂	Carbon dioxide
CT	Computer Tomography
CTD	Connective Tissue Disease
CTEPH	Chronic Thrombembolic Pulmonary Hypertension
dcSSc	diffuse cutaneous Systemic Sclerosis
DICOM	Digital Imaging and Communication in Medicine
DLCO	Diffusion Capacity of the Lung for Carbon monoxide
DLCO/VA	Carbone monoxide diffusing capacity adjusted for alveolar volume
E	Early diastolic velocity of the transmitral Doppler flow
ET-1	Endothelin 1
FFR	Fractioned Flow Reserve
FFR _{cor}	coronary Fractional Flow Reserve
FFR _{myo}	myocardial Fractional Flow Reserve
FVC	Forced Vital Capacity
HAQ-DI	Health Assessment Questionnaire Disability Index
HRCT	High Resolution Computer Tomography

hsCRP	high-sensitivity C-Reactive Protein
IMR	Index of Myocardial Resistance
IMR _{bas}	basal Index of Myocardium Resistance
IMR _{hyp}	hyperaemic Index of Myocardium Resistance
iPAH	idiopathic Pulmonary Arterial Hypertension
lcSSc	limited cutaneous Systemic Sclerosis
LV	Left Ventricular
LVEF	Left Ventricular Ejection Fraction
MCTD	Mixed Connective Tissue Disease
mPAP	mean Pulmonary Arterial Pressure
MRI	Magnetic Resonance Imaging
MVD	Microvascular Dysfunction
NO	Nitric oxide
P _a	Aortic pressure
PAH	Pulmonary Arterial Hypertension
PAH-CTD	Connective tissue disease associated Pulmonary Arterial Hypertension
PAP	Pulmonary Arterial Pressure
P _d	distal Coronary Pressure
PH	Pulmonary Hypertension
PM	Polymyositis
PVR	Pulmonary Vascular Resistance
P _w	wedge Pressure
QCA	Quantitative Coronary Angiography
RA	Rheumatoid Arthritis
RHC	Right Heart Catheterization
RP	Raynaud's Phenomenon

RV	Right Ventricle
SD	Standard Deviation
SHD	Scleroderma Heart Disease
SLE	Systemic Lupus Erythematosus
SPECT	Single Photon Emission Tomography
SPSS	Statistical Package for the Social Sciences
SSc	Systemic Sclerosis
SYNTAX	Synergy between Percutaneous Coronary Intervention with TAXUS stent and Cardiac Surgery trial
TDE	Tissue Doppler Echocardiography
TFC	TIMI Frame Count
TIMI	Thrombolysis in Myocardial Infarction trial
Tmn	mean transit Time
TxA ₂	Thromboxane A ₂

1 Introduction

1.1 Systemic sclerosis

Systemic sclerosis (SSc) is a connective tissue disease (CTD) characterized by vascular abnormalities and excessive fibrosis. Manifestations of SSc may occur in numerous tissues and organs resulting in significant morbidity and mortality¹. In an individual case variable combination of organ damage or, less frequently, a severe, single organ involvement is responsible for SSc morbidity and mortality. The functional impairment of different organs shows variable, frequently subclinical progression and overlap that makes the long-term outcome of SSc extremely unpredictable. The life expectancy of SSc patients was considerably improved with the introduction of angiotensin-inhibitory treatment that reduced the emergence of the scleroderma renal crisis. More recently with the advent of novel effective therapies and with regular screening of SSc patients for pulmonary hypertension, its impact on mortality has been decreased. Recent cohorts report that the primary cause of death in scleroderma is related to pulmonary and cardiac causes. While the overall long-term prognosis of patients with SSc seems to have improved in recent years, the proportion of deaths due to heart disease has not changed significantly².

Cardiac manifestations are common in SSc, with an estimated clinical prevalence of 15–35%³.⁴. In the majority of SSc patients, however, cardiac manifestations may remain subclinical^{5, 6}. Individuals who develop clinically apparent myocardial manifestations are recognized to be at greater risk of clinical deterioration⁷, and monitoring of myocardial involvement represents an important aspect of their disease management⁸.

Cardiac manifestations may affect patients with either lcSSc or dcSSc and, when clinically evident, are often associated with increased mortality irrespective of disease subtype^{3, 4, 9, 10}.

Vlachoyiannopoulos et al.¹¹ retrospectively analysed the clinical files of 254 patients over 4

years. They estimated that the mortality rate is 2% per year, and the incidence of cardiac disease is between 7% in lcSSc and 21% in dcSSc patients¹¹. Similarly, a review of 1095 SSc patients between 1959 and 1988 estimated the overall mortality of SSc to be 33%, with deaths of 42 patients (4.5%) resulting from cardiac manifestations¹². Another review of 405 SSc patients followed for 5 years determined that 21 out of 145 (14%) patient deaths were due to cardiac manifestations, at a rate of 1% per year¹³.

1.1.1 Scleroderma Heart Disease

Cardiac involvement in systemic sclerosis may affect the endocardium, myocardium and pericardium, separately or concomitantly. As a consequence, it may result in pericardial effusion, arrhythmias, conduction system defects, valvular impairment (in rare cases), myocardial ischemia, myocardial hypertrophy and heart failure⁸. Renal and pulmonary involvement can also adversely affect cardiac status.

These primary myocardial manifestations—those without systemic or pulmonary hypertension and without significant pulmonary or renal disease—predominantly result from the underlying vascular pathology of SSc, i.e. the characteristic vascular lesions and fibrosis that impair microcirculation and myocardial function, respectively⁸. The signs of early myocardial manifestations of SSc are often non-specific and subclinical. Consequently cases with cardiac manifestations may remain undiagnosed, potentially enabling the disease to progress silently. This particular progression manifests only as a slowly reducing physical capacity, and limiting effort related dyspnoea. Conversely, progressive deterioration of cardiac dysfunction with clinically, overt cardiac decompensation may occur in some SSc patients. Early diagnosis is therefore very important. Currently several recommendations support the regular echocardiographic screening of SSc cases and for patients with SSc undergoing autologous haematopoietic stem cell transplantation, a full cardiological

assessment before and during the transplant is recommended, as patients with cardiac abnormalities are known to be at increased risk of mortality¹⁴. The presence of ischemia in scleroderma heart disease (SHD) is supported by several in vivo studies, using SPECT, echocardiography and MRI and post-mortem histologic findings^{5, 6, 8, 15, 16}. These results suggest the existence of reversible functional and vasospastic abnormalities (demonstrated by SPECT, echocardiography and MRI) as well as fixed abnormalities due to fibrosis or organic abnormalities of small coronary vessels (demonstrated by post-mortem studies and the methods used in vivo)^{5, 6, 8, 16}. In a study of 52 consecutive patients with SSc (mean disease duration since first non-RP 6.6±6.1years) cardiac abnormalities were observed in 75%. Abnormal findings were as common in limited as in diffuse disease. Thinning of the left ventricular myocardium was found in 29% of the patients and pericardial effusion and systolic and diastolic ventricular dysfunction were also common.¹⁷ A multicenter study of SSc showed a 5.4% prevalence of LV dysfunction and identified age, male gender, myositis, digital ulcers, lung involvement and absence of previous treatment with calcium channel blockers as predictors of reduced left ventricular ejection fraction (LVEF)¹⁸. Right ventricular dilation was noted in seven patients in whom pulmonary hypertension was excluded by catheterization. Myocardial fibrosis in a non-coronary distribution was found in 21% of the patients¹⁶.

1.1.1.1 Abnormalities in cardiac microcirculation

Numerous abnormalities in the cardiac microcirculation may affect patients with SSc. Alterations of the microvasculature and microvascular dysfunction (MVD) may play a crucial role in the development of SHD¹⁹. Observations suggest that coronary flow reserve (CFR) is reduced in SSc. Kahan et al. measured first invasively the coronary sinus blood flow, and described that some patients had markedly reduced coronary vasodilatory response despite the

lack of atherosclerotic coronary artery disease (CAD)^{20, 21}. This observation has been confirmed with several alternative methodologies.

Vasospasm of the small coronary arteries and arterioles is a major, important and early cardiac manifestation that can be observed at rest or induced in both symptomatic and asymptomatic SSc patients following cold pressor testing²². Interestingly, this type of microvascular ischemia has been investigated in other rheumatic diseases associated with Raynaud phenomenon (RP), but has not been detected in those cases²³.

One abnormality of the myocardial microcirculation that is typically exhibited late in patients with SSc is reduced coronary flow reserve, which is believed to result from fixed, structural abnormalities of the small coronary arteries and arterioles. This has been investigated in patients with dcSSc with established myocardial involvement and normal coronary arteriograms²¹. These patients were not different to controls at baseline, but exhibited strikingly reduced vasodilator reserve following maximal coronary vasodilation with intravenous dipyridamole²¹. Thallium-201 SPECT and MRI enable abnormalities in cardiac perfusion to be investigated in greater detail²⁴. Studies using these techniques have demonstrated that SSc patients may benefit from the administration of the calcium channel blockers nifedipine^{6, 25-27}, the angiotensin-converting enzyme (ACE) inhibitor captopril, as well as intravenous dipyridamole used as a pharmacodynamic test inducing maximal coronary artery vasodilatation²⁵. Treatment of SSc patients with these agents has been observed to improve myocardial perfusion; however, not all abnormalities in myocardial perfusion are reversible. It is possible that ischemic lesions amenable to vasodilator therapy may co-exist with irreversible lesions, such as those with organic abnormalities or associated with fibrosis⁸.

Several studies have attempted to characterize the coronary vasoreactivity in SSc patients using non-invasive methods²⁸⁻³⁰. Measurement of the diastolic coronary flow obtained by transthoracic echocardiography initially has been reported to exhibit suboptimal feasibility, which can be improved by using contrast augmentation of the Doppler signal and second harmonic technology (CFR_{echo})³¹⁻³³. Sulli et al. found significantly lower CFR_{echo} values among patients with diffuse SSc than in subjects suffering from the limited form of the disease. Furthermore, CFR_{echo} values in SSc differed significantly from healthy controls³⁰. Montisci et al recently reported beneficial effect of single intravenous administration of L-propionylcarnitine to the CFR_{echo} . The authors noted that the peak diastolic velocity was significantly decreased in the left anterior descending coronary artery in basal conditions and the CFR_{echo} measured during adenosine infusion increased significantly after the L-propionylcarnitine application. They explained that the decreased resting coronary flow velocity may be the result of the “anti-endothelin” effect of L-propionylcarnitine for microvascular tone and this agent could cause improved sensitivity for adenosine infusion³¹. D’Andrea et al analyzed the supposed correlation among left ventricular (LV) function, CFR examined by echocardiography and endothelial function investigated by flow mediated dilatation method. The authors conclude that endothelial dysfunction in SSc patients was not confined to coronary microvessels, but also extends to peripheral arteries²⁸. Similar results were found earlier by Andersen et al³⁴.

1.1.1.2 Abnormalities in myocardial tissue

Myocardial fibrosis is a hallmark cardiac manifestation of SSc that can remain subclinical for a considerable period of time⁸. The fibrotic lesions are patchy, distributed in both ventricles and are inconsistent with regards to coronary artery distribution. Myocardial fibrosis occurs later in SSc and can lead to systolic and diastolic dysfunction, including exercise-induced

dysfunction, segmental dysfunction, decreased peak filling rate and reduced global left and right ventricular ejection fractions¹². Myocardial fibrosis in SSc can affect both ventricles, leading to increased ventricular mass, decreased movement of the ventricular walls and impaired relaxation of myocardial tissue during diastole^{9, 35}. The combined effects of myocardial fibrosis and reduced perfusion can be investigated using tissue Doppler echocardiography (TDE), which enables sensitive and non-invasive measurement of myocardial contractility. Using this technique, myocardial contractile velocities and strain rates in SSc patients have been found to be reduced, although the exact pathological mechanism underlying such reductions remains unclear⁸. Vignaux et al.²⁷ investigated the effects of nifedipine in 18 SSc patients with reduced systolic and diastolic contractile strain rates. In these patients, nifedipine significantly improved MRI perfusion index, and the systolic and diastolic TDE strain rates²⁷. Further investigations to evaluate the effects of nifedipine in a greater number of patients with cardiac manifestations of SSc are, however, necessary. Short-term treatment with the dual endothelin receptor antagonist bosentan has been evaluated in 18 SSc patients without clinical heart failure and with normal pulmonary arterial pressure³⁶. Short-term treatment with bosentan simultaneously improved myocardial perfusion and function. It remains to be seen if long-term treatment might result in additional remodelling effects.

1.2 The coronary circulation

1.2.1 The arterial system

The arterial tree consists of large arteries ranging from several mm to 400 μ m and branching into small arteries and arterioles (with a diameter <400 μ m). The arterioles are surrounded by smooth muscle cells. The distinction between large and smaller arteries is not sharp; it corresponds to physiological and clinical differences.

The epicardial vessels can be the sites of atherosclerotic narrowings which are the main cause of the myocardial ischemia. Normal epicardial arteries do not create any significant resistance to blood flow. Even at high flow rates induced by intravenous adenosine, only a negligible pressure difference exist between central aorta and the most distal part of epicardial coronaries, therefore these are called conductance vessels³⁷. The arterioles called as resistance vessels are able to dilate under physiological and pharmacological stress. This modulation of resistance is paramount for matching myocardial blood flow to variable energy requirements. The ability to dilate may be impaired before the development of visible atherosclerosis.³⁸⁻⁴⁰

This two-compartment model of the coronary circulation does not account for the heterogeneity in the distribution of coronary resistance with respect to regulation of coronary blood flow⁴¹. Based on sequential differences in the response to shear stress and adenosine, resistance vessels have been divided into two groups:

The proximal compartment consists of vessels with a diameter ranging from 100-400 μ m. Their tone is controlled by coronary flow, distending pressure and myogenic tone, and modulated by the autonomic nervous system and endothelial function.

The distal compartment consists of vessels with a diameter of less than 100 μm and influenced primarily by the perfusion pressure and by myocardial metabolism.

1.2.2 Capillaries

The diameter of the capillaries is approximately 5 μm ; their density is 3500-4000/ mm^2 in human heart. The myocytes are connected to the capillary wall by collagen struts. Each myocyte is lined by at least one capillary in close anatomic structure. Capillaries are not uniformly patent because precapillary sphincters regulate flow according to oxygen demand.

1.2.3 Coronary flow characteristic, regulation of myocardial blood flow

The coronary arterial blood flow occurs predominantly during diastole. The myocardial perfusion represents approximately 5% of cardiac output under baseline conditions. In case of normal epicardial coronary arteries, coronary flow equals myocardial flow. The myocardial oxygen demand is high (8-10 ml/min/100 g of tissue) as compared to skeletal muscles (0.5 ml/min/100g)⁴². The high density of coronary capillaries is optimally suited to meet high oxygen consumption. The extraction of oxygen by the myocardium is close to maximum.

Hence, since oxygen extraction cannot increase much further, the coronary circulation can only meet increasing oxygen demand by increasing blood flow, so there is a close linear relation between the metabolic demand and myocardial blood flow⁴³. The flow is regulated by a changing balance of interacting mechanisms, including intrinsic autoregulation, external compressive forces, neural regulation, metabolic demand and endothelial factors.

1.2.3.1 Autoregulation

Myocardial perfusion is maintained relatively constant when perfusion pressure varies from 45 to 130 mmHg approximately in humans⁴⁴. When coronary perfusion pressure falls below

this limit, small additional reductions in pressure are associated with marked reduction in myocardial flow and wall thickening. Despite mild or moderate epicardial stenoses, coronary blood flow is maintained by compensatory vasodilatory regulation of the microcirculation (autoregulation).

The main supposed mechanism is the local metabolic regulation. Several metabolic mediators have been studied, e.g. oxygen, CO₂, hyperosmolarity, H⁺, K⁺, and Ca⁺⁺ concentration, adenosine and nitric oxide (NO).

1.2.4 Coronary reserve

Coronary vasodilator reserve is the ability of the coronary vascular bed to increase flow above the basal level in response to a mechanical or pharmacological stimulus (e.g. reactive hyperemia, exercise, pharmacologic agents), to a maximal hyperemic level.

1.2.4.1 Fractional flow reserve (FFR)

Fractional flow reserve is an index specific for the degree of epicardial coronary artery stenosis. FFR is calculated as a ratio of distal and aortic pressure during hyperemia. FFR measurements demonstrated no significant variation during various hemodynamic conditions in a recent study⁴⁵.

1.2.4.2 Coronary flow reserve (CFR)

Coronary flow reserve is expressed as the ratio of maximal hyperemic flow to resting coronary flow – a ratio that averages from 2 to 5 in humans^{46, 47}.

The feasibility of CFR to reflect the functional state of the microcirculation independently is limited because CFR is influenced by the flow status of both the epicardial artery and the microcirculation hence does not allow discrimination between these two components⁴⁸.

Furthermore, CFR is limited by its dependence on heart rate and blood pressure, therefore its reproducibility seems to be questionable.

Currently used devices, such as the pressure-temperature sensor-tipped coronary wires allow to measure pressure and to estimate coronary artery flow simultaneously⁴⁹. Based on thermodilution principles, the mean transit time (Tmn) of room-temperature saline injected down a coronary artery can be determined and has been shown to correlate inversely with absolute flow⁴⁹. Use of CFR to evaluate the microcirculation is limited by the fact that CFR interrogates the entire coronary system, including the epicardial artery and the microcirculation⁵⁰. For this reason, a patient with epicardial disease but with normal microcirculatory function can have an abnormal CFR, which potentially limits the applicability of CFR when assessing microvascular disease. Furthermore, because CFR represents a ratio between peak hyperemic and resting coronary flow, factors that affect resting hemodynamics, such as heart rate and contractility, may affect the reproducibility of CFR⁵¹. Previous studies have shown that Doppler flow velocity– derived CFR is significantly reduced by tachycardia⁵¹⁻⁵³ and by increased contractility but is not significantly affected by changes in blood pressure due to compensatory changes in coronary blood flow⁵¹⁻⁵³. Consistent with these previous studies, study of Ng et al. documented the hemodynamic dependence of thermodilution-derived CFR. In this study, hypotension induced by nitroprusside infusion had no effect on thermodilution-derived CFR. In contrast, right ventricular pacing–induced tachycardia and dobutamine infusion were both associated with significant reductions in thermodilution-derived CFR values, largely due to an increase in resting coronary blood flow (and hence a reduction in resting Tmn)⁴⁵.

1.2.5 Index of myocardial resistance

In normal coronary vessels there is a big difference in resistance between baseline and hyperemia in the arterioles. These vessels have an ability to adjust, relax and contract their tone in response to wide range of stimuli. Contrary, there is no significant difference regarding the resistance between rest and hyperemia in the large epicardial vessels and in the small capillaries. At hyperemia the capillaries offer the highest resistance. Conditions associated with lesser capillaries, such as myocardial infarction, hypertension and diabetes, are associated with reduced blood flow despite the absence of coronary stenosis.

Myocardial resistance equals the pressure drop across the myocardium, divided by the myocardial blood flow during hyperemia. Assuming venous pressure close to zero, the pressure drop equals distal coronary pressure (Pd). Using the thermo-dilution principle, blood flow equals $1/T_{mn}$ then $IMR = Pd \times T_{mn}$, at maximal hyperemia.

Mean transit time (T_{mn}) can be measured by injecting saline into the coronary artery and it is inversely proportional to coronary blood flow. This simplified formula assumes that the coronary flow is identical to myocardial flow. In the presence of a severe stenosis this assumption is not correct, since collateral flow will increase, and IMR will be overestimated using this formula. To compensate for the collateral flow, wedge pressure (Pw), must be incorporated into the formula⁵⁴:

$$IMR = Pd \times T_{mn} \times FFR_{cor} / FFR_{myo}$$

$$IMR = T_{mn} \times Pa \times (Pd - Pw) / (Pa - Pw)$$

Pa = Aortic Pressure, Pw = Wedge Pressure (distal pressure under total occlusion), Pd = Distal Pressure

If collateral flow (wedge pressure) is not accounted for, IMR increases with increasing stenosis severity. By measuring wedge pressure and incorporating this in the calculation, IMR remains fairly constant even with increasing stenosis severity. IMR has significantly lower variation as compared to CFR after changes in pressure, heart rate and contractility.

The state of the coronary microcirculation is an important determinant of patient outcomes in a number of clinical settings, including acute coronary syndromes, percutaneous coronary interventions, and other microcirculatory perturbations. The index of myocardium resistance (IMR) has been shown in animals to correlate with true microvascular resistance and, unlike coronary flow reserve (CFR), to be independent of the epicardial artery⁵⁵. In animal model, IMR, defined as the distal coronary pressure divided by the inverse of the hyperemic mean transit time, correlated well with an accepted experimental method for measuring microvascular resistance⁵⁵.

Unlike CFR, IMR is derived at peak hyperemia, thereby eliminating the variability of resting vascular tone and hemodynamics. Ng et al. evaluated the intrinsic variability of CFR and IMR under baseline conditions and after a series of hemodynamic interventions that included right ventricular pacing at 110 bpm, nitroprusside infusion, and dobutamine infusion. In the resting state, heart rate, mean coronary transit times, and systemic and distal coronary pressures were similar between the two baseline measurements. They compared the IMR with thermodilution-derived CFR in terms of reproducibility and dependence on hemodynamic changes and also concurrently measured FFR under different hemodynamic conditions. The salient findings of the present study were as follows: (1) IMR demonstrates less intrinsic variability and better reproducibility at baseline than CFR, and (2) whereas CFR is very sensitive to hemodynamic changes, IMR is largely independent of variations in hemodynamic state. Furthermore, FFR (when simultaneously measured with IMR and CFR) is highly

reproducible and also largely independent of hemodynamic state. These findings suggest that IMR could be reliably applied in the catheterization laboratory for assessment of microcirculatory resistance. Furthermore, simultaneous measurement of FFR and IMR with a single pressure-temperature sensor-tipped coronary wire may provide a simple means for comprehensive and specific assessment of coronary physiology at both epicardial and microvascular levels, respectively⁴⁵.

In a recent study using a porcine animal model, Ng et al. found that IMR distinguished between normal and abnormal microcirculatory function and correlated well with true microcirculatory resistance as measured by an external flow probe and pressure wire⁵⁵. Furthermore, IMR, in its simplest form, was not significantly affected by the presence of a moderate to severe epicardial stenosis and is therefore a specific measure of the state of the microcirculation, unlike CFR. In more severe stenoses, in which collateral flow may be contributing to myocardial perfusion, a more complex form of IMR, which incorporates the coronary wedge pressure, is necessary to accurately determine microvascular resistance^{50, 54}. Variations in hemodynamic status, including changes in heart rate, blood pressure, and contractility, do not significantly affect IMR measurements. During all hemodynamic interventions in the present study (rapid right ventricular pacing, nitroprusside infusion, and dobutamine infusion), IMR exhibited greater hemodynamic stability than CFR⁴⁵.

1.3 Pulmonary hypertension

Pulmonary arterial hypertension (PAH), a disease of the pulmonary circulation, is defined as a mean pulmonary arterial pressure (mPAP) of > 25mmHg at rest or > 30mmHg during exercise together with a pulmonary capillary wedge pressure of <15mmHg⁵⁶. Sustained increases in PAP result in an increase in pulmonary vascular resistance (PVR), which in turn leads to right ventricular overload, and ultimately right ventricular failure and death⁵⁷. Irrespective of its

underlying cause, the elevations in PAP that characterize PAH are believed to occur at least in part from disturbances in the normal balance between endogenous vasoconstrictors and vasodilators in response to endothelial dysfunction or injury⁵⁸. Simultaneously in the vasculature, there is increased production of potent vasoconstrictors such as thromboxane A2 and ET-1 and reduced production of vasodilators such as nitric oxide (NO) and prostacyclin synthase, which is required to convert arachidonic acid to prostacyclin. These abnormalities elevate vascular tone and promote remodelling of the vascular wall, which leads to the persistent increase in PVR and its adverse clinical sequelae⁵⁹. PAH is a devastating and often fatal complication of CTDs, especially within the scleroderma (SSc) patient population, which has the highest mortality of all the rheumatic disorders⁶⁰.

1.3.1 Classification of pulmonary hypertension

An increased understanding of the pathophysiology of PAH has enabled important advances in the classification of pulmonary hypertension (PH). This classification has progressed from its initial separation into primary and secondary PH, via the Evian classification of 1998⁶¹, to today's Venice classification. The Venice classification, which arose from the 2003 Third World Symposium on PAH⁵⁷, recognizes five subclasses of chronic PH. Recent modification of this is the Dana Point Classification that was published in 2009, maintained the main pathogenesis based structure of the earlier versions and included the latest research results. The first of these main groups is the pulmonary arterial hypertension (PAH) that is frequently encountered in patients with SSc. The second category is pulmonary venous hypertension, in which increased PAP arises from elevated pressures on the left side of the heart. Anything that elevates left heart filling pressures, left ventricular end-diastolic pressure or wedge pressure is transmitted back to the pulmonary vasculature and can cause PH. Potential contributors to this type of PH include systolic dysfunction, diastolic dysfunction, which is

not uncommon in SSc patients, and valvular heart disease such as aortic valve disease or mitral valve disease. Because the treatment of PH associated with left heart disease is very different to that for PAH, it is important to differentiate this form of PH from PAH. The third type of PH is associated with hypoxemia and lung disease. Anything that causes hypoxemia can lead to modest increases in mPAP to 25–35 mmHg, whereas any type of lung disease, such as interstitial lung disease and pulmonary fibrosis, which are common in patients with SSc, and sleep apnoea can also cause this type of PH. Chronic thromboembolic PH (CTEPH), which is thought to develop from single or recurrent thromboembolism at sites of venous thrombosis that fail to be reabsorbed, represents the fourth category of PH. Again, it is very important to differentiate CTEPH from PAH as CTEPH patients with proximal, surgically accessible disease may be cured with pulmonary endarterectomy. Finally, the Venice classification recognizes a fifth subclass of chronic PH that includes sarcoidosis and some unusual diseases that affect the pulmonary vasculature directly⁶².

1.3.2 PAH associated with CTDs

The vascular remodelling that occurs in PAH arises from the extension of muscle into peripheral non-muscular arteries following the differentiation of pericytes and intermediate cells into mature smooth muscle cells⁵⁹. In normally muscular arteries, medial hypertrophy develops as a result of cellular hypertrophy/hyperplasia accompanied by extracellular matrix deposition. Loss or impaired growth of arterioles may also occur and, as the disease progresses, plexiform lesions, fibrosis and in situ thrombosis may develop. Thus, there are three main events in the pathophysiology of PAH: vasoconstriction, thrombosis and most importantly remodelling. PAH is a significant and often fatal complication of CTDs, including not only SSc but also SLE, MCTD and, to a lesser extent primary SS, PM and RA¹⁴. Data from the French National Registry of 674 patients with PAH show that 95% of all

the cases of PAH-CTD are associated with SSc (76%), SLE (15%) or MCTD⁶³. Thus, PAH is a rare complication in patients with RA, SS and PM. Within the SSc patient population, prevalence of confirmed PAH is ~10–16%¹⁴. This is a finding borne out by Wigley's recent surveys⁶⁴. Data from the French National Registry show that about two-thirds of the cases of PAH-SSc are patients with limited SSc and a third of cases are patients with diffuse SSc⁶³. PAH is a leading cause of disease-related death in SSc patients⁶⁵, in whom outcome has historically been much worse than in patients with iPAH⁶⁶. With median survival of just 1 year, the survival rate for patients with PAH-SSc on conventional therapy is poor⁶⁷. Diagnosis of PAH in this patient group is difficult as clinical symptoms and findings considerably overlap with several other internal organ involvements.

1.3.3 Diagnostic approach to patients with suspected PAH

Assessment of PAH is based on a logical sequence of determining whether there is a risk of PAH being present, whether PAH is likely to be present based on initial, noninvasive evaluation, clarifying the underlying etiology of PAH in an individual patient, and delineating the specific hemodynamic profile, including the acute response to vasodilator testing. Risk groups warranted to screen for PH include the following: patients with known genetic mutations predisposing to PH, first-degree relatives in FPAH family, patients with scleroderma spectrum of disease, patients with portal hypertension prior to liver transplantation, and patients with congenital heart disease with systemic-to-pulmonary shunts. Patients with PAH generally present with a spectrum of symptoms attributable to impaired oxygen transport and reduced cardiac output. Although PAH may be asymptomatic, particularly in its early stages, exertional dyspnoea is the most frequent presenting symptom. Dyspnoea is eventually present in virtually all patients as the disease progresses. Fatigue, weakness, or complaints of general exertion intolerance are also common complaints. As

PAH progresses, dyspnoea may be present at rest. Chest pain and syncope are each reported by approximately 40% of patients during the course of the disease. Since the symptoms of PH are nonspecific, the initial evaluation of patients with these symptoms is often appropriately directed at diagnosing or excluding more common conditions.

Signs of PH on physical examination are subtle and often overlooked. Although no rigorous analysis of the sensitivity and specificity of findings on physical examination has been performed, experience suggests that the likelihood of PAH is increased when certain findings are present. PAH results in right ventricular hypertrophy and right-heart dilation. Since these processes produce ECG abnormalities, the ECG may provide a signal of hemodynamically significant PH.

Echocardiography with Doppler ultrasound is the most portable and widely available technology among the noninvasive imaging methods. Echocardiography provides both estimates of pulmonary artery pressure and an assessment of cardiac structure and function. These features justify its application as the most commonly used screening tool in patients with suspected PAH.

Although Doppler echocardiographic methodology may provide a proxy measurement of pulmonary hemodynamics by estimating right ventricular pressure, a more accurate value for pulmonary artery pressure obtained by right-heart catheterization is strongly advised. Right-heart catheterization also provides direct and accurate measurements of RAP, pulmonary venous pressure (pulmonary capillary wedge pressure), pulmonary blood flow, mixed venous oxygen saturation, and allows for the calculation of pulmonary vascular resistance. Right-heart catheterization not only provides important indices of disease severity, but it also enhances the diagnostic process by excluding other etiologies such as intracardiac or

extracardiac shunts and left-heart disease, and provides an assessment of the degree of right-heart dysfunction through measurement of RAP and cardiac output. It should be acknowledged that right-heart catheterization also has limitations: measurements are generally obtained only under resting conditions in the supine position, which may not be representative of hemodynamic responses to upright posture, activity, or sleep. Even under these static circumstances, measurements may vary^{62, 68}.

2 Aims

The cardiac and pulmonary manifestations represent an important diagnostic challenge and a huge prognostic burden in patients with systemic sclerosis. Our goal was to design and execute a prospective screening program for SSc patients that included classical, non invasive screening investigations as well as invasive methods for characterization of cardiopulmonary circulatory parameters among high risk cases. We aimed

- to estimate the prevalence of pulmonary hypertension, atherosclerotic coronary artery disease and microvascular dysfunction among SSc patients
- to characterise the proportion and distribution of these alterations behind the overlapping symptoms
- to investigate the mechanisms of the microvascular impairment by means of analyzing the myocardial resistance of SSc patients compared to patients with angina pectoris having mild coronary artery disease

3 Cross-sectional study of SSc patient for elucidation of the prevalence of cardiopulmonary involvement

3.1 Patients and methods

A total of 120 consecutive SSc cases were enrolled in the study. SSc was diagnosed by standard criteria¹, and the patients were asked to provide informed consent before the entry. Those with an ejection fraction <30% on echocardiography or with known severe valvular disease were excluded. Patients with severe lung fibrosis (forced vital capacity <50% on a pulmonary function test) were also excluded from further studies (pulmonary fibrosis group). Each patient underwent a baseline physical examination. In addition, electrocardiogram, echocardiography and a 6-min walk test were also performed. Lung involvement was investigated by using chest X- ray, pulmonary function tests and high-resolution CT in cases where interstitial lung disease was suspected. Cardiac catheterisation was initiated in the presence of abnormalities suggestive of PAH (“suspected PAH” group) or suggestive of CAD (“suspected CAD” group). Criteria for the “suspected PAH” included signs of right ventricular involvement on echocardiography; tricuspidal insufficiency diagnosed by flow velocity over 3 m/s, or consistent with 2.5–3 m/s in the presence of unexplained dyspnoea; signs of right ventricular hypertrophy/dilatation, or right ventricular D sign; and effort related dyspnoea with disproportional decrease of CO diffusion capacity (DLCO) compared to the forced vital capacity (FVC/DLCO >1.8). Patients were included in the “suspected CAD” group if they reported recent deterioration in physical activity, evolving effort dyspnoea or evolving chest pain, if they fulfilled the criteria of the New York Heart Association functional class III–IV, or if their 6-min walking distance was <380 m, but did not fulfilled the criteria for PAH¹⁴. Right heart catheterisation (RHC) and coronary angiography, supplemented with

thermodilution coronary flow reserve (CFR) assessment, were performed at the same time. The trial protocol was approved by the Medical Research Council Scientific and Ethical Committee.

3.1.1 Echocardiography

Echocardiography was performed on every patient using Aloka ProSound 5500 ultrasound equipment. Ejection fraction was measured by biplane Simpson's method. Peak of the early (E) and late (A) velocities of the transmitral Doppler flow were measured from the apical 4-chamber view. Systolic pulmonary artery pressure was estimated by using the simplified Bernoulli equation, and calculated from the peak tricuspidal regurgitation velocity ($4v^2$ plus estimated right atrial pressure according to the diameter and collapse index of the inferior vena cava).

3.1.2 Right heart catheterization

A Swan-Ganz catheter (B. Braun, Melsungen, Germany) was introduced into a main pulmonary artery branch. If the resting mean pulmonary pressure (mPAP) was lower than 30 mmHg, a 3-minute bench-fly physical stress test was performed with 1kg dumbbells. Mean PAP was measured at rest, and at peak exercise. mPAP values of >25 mmHg at rest or >30 mmHg upon exertion were considered abnormal.

3.1.3 Coronary angiography

Angiograms were recorded digitally (Philips Integris, Amsterdam, The Netherlands). Coronary lesions were assessed by visual estimation as well as with quantitative angiography (QCA). The SYNTAX score, a validated comprehensive angiographic scoring system, was used to describe the extent of the CAD⁶⁹. The target artery was instrumented with an

intracoronary pressure wire (RADI Medical, Uppsala, Sweden). After baseline measurements of aortic pressure (Pa), distal coronary pressure (Pd) and mean transit time of a room temperature 3 ml bolus of saline, measurements were performed in triplicate in the presence of maximal hyperaemia induced by intracoronary papaverine (12 mg bolus). Fractional flow reserve (FFR) was calculated as Pd/Pa ratio during hyperaemia. CFR was calculated from the ratio of the mean transit times in hyperaemia and at rest. MVD was defined as reduced flow reserve (CFR<2) in the absence of significant stenosis (FFR>0.75). Coronary flow velocity was assessed by the ‘‘Thrombolysis in Myocardial Infarction (TIMI) frame count’’ (i.e., counting the cine frames required for contrast to leave the catheter tip and to reach standardised distal landmarks in the coronaries). TFC was measured off-line using a frame counter on a digital DICOM viewer (Inturis Suite ViewerLite v1.0, Philips, Holland) by a single observer who was blinded to clinical diagnosis.

3.1.4 Statistical analysis

For the in-group comparisons, unpaired t tests, χ^2 tests, or Fisher exact tests were used. Correlation analysis was performed with the Pearson rho test.

3.2 Results

Of the 120 cases, 2 patients were excluded due to severe pulmonary fibrosis. Characteristics of the patients are depicted in table 1a, 1b.

	Systemic sclerosis (n=120)	Patients with suspected PAH (n=20)	Patients with suspected CAD (n=10)	Non catheterized patients (n=90)
Antibodies:				
Anti-centromere antibody	24 (20%)	6 (30.0%)	2 (20.0%)	16 (17.8%)
Anti Scl-70 antibody	43 (35.8%)	5 (25.0%)	3 (30.0%)	35 (38.9%)
Biochemical data:				
Blood sedimentation (mm/h)	24.6±19.5	28.5±20.0	20.8±19.5	24.2±19.5
hsCRP (mg/l) (norm.: <5mg/l)	10.4±15.4	9.6±10.6	11.9±15.2	10.4±16.4
hemoglobin (g/l) (norm.:120-170g/l)	126.9±12.5	127.9±11.7	130.8±13.7	126.2±12.6
lactate-dehydrogenase (U/l) (norm.:<450 U/l)	339.3±81.2	341.8±108.3	339.8±68.6	338.7±76.8
creatinine (µmol/l) (norm.: 44-80 µmol/l)	83.8±63.1	81.8±26.5	73.6±12.0	85.4±71.7

Table 1a: Patient characteristics of the 120 systemic sclerosis patients included in the study.
Abbreviations: PAH: pulmonary arterial hypertension, CAD: coronary artery disease, hsCRP: high-sensitivity C-reactive protein.

	Systemic sclerosis (n=120)	Patients with suspected PAH (n=20)	Patients with suspected CAD (n=10)	Non catheterized patients (n=90)
Treatment history:				
Cyclophosphamide	24 (21.7%)	3 (15.0%)	3 (30.0%)	18 (20.0%)
Low dose corticosteroid	23 (19.2%)	4 (20.0%)	2 (20.0%)	17 (18.9%)
Pentoxifylline	116 (96.7%)	19 (95.0%)	9 (90.0%)	88 (97.8%)
Ca-channel blocker	78 (65.0%)	15 (75.0%)	9 (90.0%)	54 (60.0%)*
ACE inhibitor	48 (40.0%)	7 (35.0%)	5 (50.0%)	36 (40.0%)
Spirolactone	42 (35.0%)	10 (50.0%)	8 (80.0%)*	24 (26.7%)*
H ₂ blocker and/or proton-pump inhibitor	101 (84.2%)	16 (80.0%)	8 (80.0%)	77 (85.6%)
Pulmonary status:				
Pleuritis (in case history)	5 (4.2%)	0 (0.0%)	1 (10.0%)	4 (4.4%)
Pulmonary fibrosis (X Ray. diffuse)	8 (6.7%)	2 (11.1%)	1 (10.0%)	6 (6.7%)
Pulmonary fibrosis (HRCT)	75 (62.5%)	11 (55.0%)	8 (80.0%)	56 (62.2%)
Pulmonary fibrosis (HRCT diffuse)	18 (15.0%)	2 (10.0%)	3 (30.0%)	13 (14.4%)
Forced vital capacity (FVC)	96.2±19.3	94.7±15.4	92.5±20.5	97.0±20.0

CO diffusion capacity (DLCO)	63.5±17.8	55.1±18.8	60.5±16.8	65.7±17.2*
DLCO calculated to alveolar volume (DLCO/VA)	78.0±17.8	71.6±27.1*	78.9±10.4	79.4±15.6
FVC/DLCO	1.6±0.6	2.1±1.2*	1.6±0.2	1.5±0.4*
Echocardiography:				
Ejection fraction	61.2±6.4	62.0±6.8	60.8±3.8	61.3±6.9
Diastolic function abnormality	95 (79.2%)	20 (100.0%)*	9 (90.0%)	66 (73.3%)*
Calculated RV systolic pressure (mmHg)	30.8±6.2	38.8±7.6**	32.1±6.8	29.9±5.1*

Table 1b: Patient characteristics, lung function and echocardiographic findings of the 120 systemic sclerosis patients included in the study. Abbreviations: PAH: pulmonary arterial hypertension, CAD: coronary artery disease, ACE: angiotensin converting enzyme, HRCT: high resolution computer tomography, RV: right ventricle, *: p<0.05 **p<0.001 unpaired t test or Fisher's exact test as appropriate. Cyclophosphamide intake was encoded when cyclophosphamide treatment for at least 12 months, with an average dose of 1000 mg/month, was recorded in the patient's medical history. Steroid intake was marked when low or medium dose corticosteroid treatment was recorded in the patient's medical history for at least 12 months.

Concerning the cardiovascular risk profile, hypertension and diabetes were more frequent in the “suspected PAH” group, whereas the distribution of hypercholesterolemia, male gender and obesity were similar among groups. No significant difference was detected in any other laboratory results. Further details are included in the table 2a, b.

	All patients (n=30)	Pulmonary hypertension (PAH) (n=14)	Coronary stenosis (CAD) (n=15)	Microvascular disease (MVD) (n=10)
Age (years)	59.5±11.2	62.4±12.1	62.6±10.7*	61.2±13.6
Echocardiography:				
Ejection fraction	60.8±3.4	60.1±3.6	60.6±3.4	60.4±3.5
Left ventricular mass (LVM)	191.6±36.5	199.8±25.8	197.9±32.9	184.7±43.8
E/A	1.0±0.3	0.9±0.3	0.9±0.3	0.9±0.2
Lung function:				
FVC	93.9±17.0	101.4±12.7*	97.4±15.5	99.4±12.3
DLCO	56.9±18.0	48.9±13.2*	55.9±17.4	53.6±22.3
DLCO/VA	74.0±23.0	64.5±19.7*	70.1±19.4	63.5±22.7
HRCT (performed in 22 out of the 30 cases)				
Basal fibrosis	18 (60.0%)	8 (57.1%)	8 (53.3%)	6 (60.0%)
Diffuse fibrosis	5 (16.7%)	1 (7.1%)	3 (20.0%)	2 (20.0%)
Honeycombing	3 (10.0%)	1 (7.1%)	1 (6.7%)	0 (0%)

Table 2a: Findings of non-invasive investigations in 30 patients. Abbreviations: E/A: ratio of the early (E) and late (A) velocities of the transmitral flow, FVC: forced vital capacity, DLCO: diffusing capacity for carbon monoxide, DLCO/VA: carbon monoxide diffusing capacity adjusted for alveolar volume, *: p<0.05 compared to the rest of the catheterized cases using Fisher's exact or unpaired t-test as appropriate.

	All patients (n=30)	Pulmonary hypertension (PAH) (n=14)	Coronary stenosis (CAD) (n=15)	Microvascular disease (MVD) (n=10)
Basic hemodynamics:				
Systolic pressure (mmHg)	144.7±24.7	154.3±23.3*	145.9±27.7	146.6±28.6
Diastolic pressure (mmHg)	76.4±13.9	77.1±15.5	75.0±15.3	72.2±19.5
Frequency (beats/min)	81.2±16.2	81.6±15.4	82.7±16.9	83.2±12.6
Pulmonary capillary wedge pressure (mmHg)	12.5±4.2	13.0±4.3	13.3±4.3	11.8±4.6
Cardiac output (l/min)	5.1±1.1	5.0±0.9	5.1±1.3	5.0±0.9
Pulmonary vascular resistance (dynes*sec/cm ⁵)	152.0±103.0	214.6±110.4**	151.2±114.5	207.5±112.5*
Systemic vascular resistance (dynes*sec/cm ⁵)	1516.4±459.8	1478.6±535.5	1528.6±386.8	1538.6±560.4
Angiographic findings :				
Calcification	8 (26.7%)	3 (21.4%)	8 (53.3%)**	2 (20.0%)
Plaques	18 (60.0%)	11 (76.9%)	15 (100%)*	7 (70.0%)
Stenosis	15 (50.0%)	9 (64.3%)	15 (100%)*	6 (60.0%)
Occlusion	1 (3.3%)	0 (0%)	1 (6.7%)	0 (0%)
Diffuse lesions	7 (23.3%)	3 (21.4%)	7 (46.7%)*	3 (30.0%)
Aneurysm	1 (3.3%)	0 (0%)	1 (6.7%)	1 (10.0%)

Slow coronary flow	6 (20.0%)	4 (28.6%)	3 (20.0%)	1 (10.0%)
Bridge	2 (6.7%)	0 (0%)	1 (6.7%)	2 (20.0%)
Hinge	19 (63.3%)	8 (57.2%)	9 (60.0%)	8 (80.0%)
SYNTAX score (A)	6.8±11.3	6.0±5.5	13.7±12.9***	6.0±6.4
Coronary flow velocity (B):				
Left anterior descending artery	29.1±14.8	25.7±13.8	25.6±11.8	19.5±9.7**
Circumflex artery	21.4±10.6	22.5±12.7	19.7±11.3	16.3±5.0*
Right coronary artery	17.8±14.4	18.7±10.7	17.1±11.3	13.4±9.2
Average frame count	23.2±10.9	22.3±10.5	22.0±9.0	16.4±4.7*
Coronary flow reserve:	2.7±1.1	2.6±1.3	2.6±1.2	1.5±0.3***

Table 2b: Findings of combined left and right heart catheterization in 30 patients. Abbreviations: (A) SYNTAX score, a validated comprehensive angiographic scoring system to describe the extent of CAD. (B) Coronary flow velocity, assessed by the TIMI (thrombolysis in myocardial infarction) frame count method, which counts the number of angiographic frames required for dye to reach standardized distal landmarks: for details see methods, *: p<0.05, **: p<0.01, *: p<0.0001 compared to the rest of the catheterized cases using Fisher's exact or unpaired t-test as appropriate.**

Cardiac catheterisation was performed in 30 cases. In all, 20 patients were included in the “suspected PAH”, and 10 cases in the “suspected CAD” group. Among the 120 patients with SSc, the prevalence of PAH was 11.6% (14/120), while the prevalence of verified CAD and of severe CFR reduction were 12.5% (15/120) and 8.3% (10/120), respectively. Normal coronary vessels and pulmonary pressure, as well as preserved CFR, were found in eight cases. There was a considerable overlap among these groups. In all, 12 patients in the “suspected PAH” group and 2 in the “suspected CAD” group had PAH. Coronary

angiography was positive in 9 cases in the “suspected PAH” group. Severely reduced CFR was found in seven cases in the “suspected PAH” and in three patients in the “suspected PAH” group (fig 1).

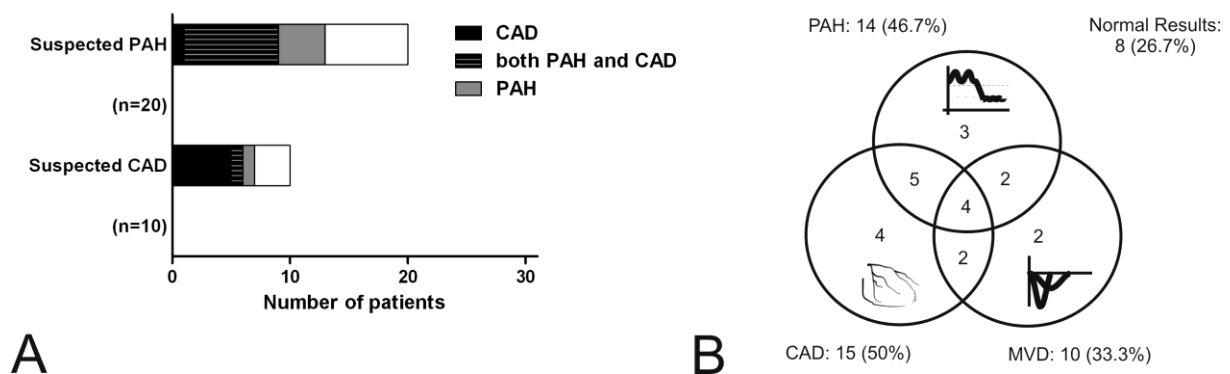


Figure 1: Catheterization findings in 30 systemic sclerosis patients. Panel A shows the frequency of coronary artery disease (CAD) and pulmonary hypertension as indications for catheterization. Panel B illustrates the considerable overlap found between abnormalities of the pulmonary circulation and the coronary micro- and macrovasculature. Abbreviations: PAH: pulmonary arterial hypertension, CAD: coronary artery disease, MVD: microvascular disease

Significant correlation was found between the coronary flow velocity and flow reserve values, indicating better CFR in patients with slower resting flow ($p < 0.01$, $r^2 = 0.24$). Of the 15 patients with coronary lesions detected by morphological and functional assessment, 8 underwent revascularisation (1 bypass grafting, 7 percutaneous coronary stent implantations). These procedures resulted in improvement in the patients’ physical activity (Figure 2).

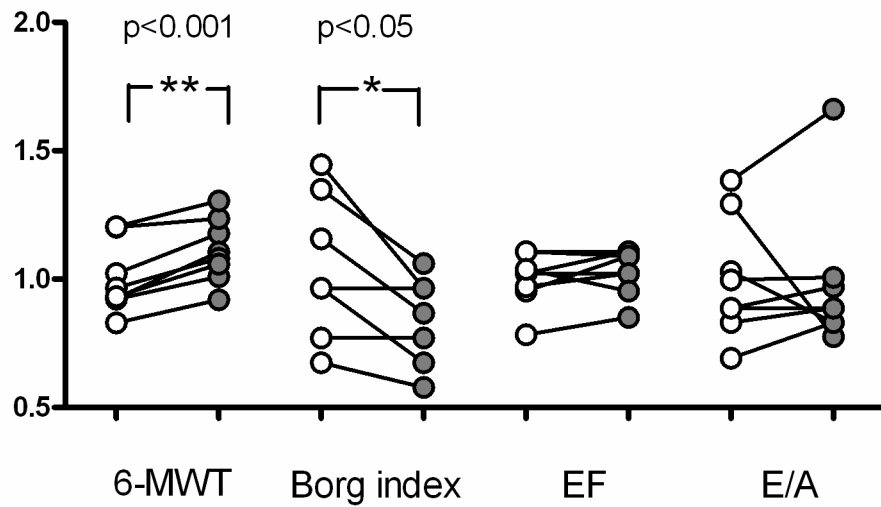


Figure 2: Effect of coronary revascularization on physical competence and echocardiographic parameters. Dots represent percentage relative to initial mean values. Open circles show baseline state, while grey circles refer to 30-day evaluation after coronary intervention. Revascularization improved walking distance in 6-minute walk test (6-MWT, $p < 0.001$) and decreased Borg dyspnoea index in 8 SSc patients ($p < 0.05$). Ejection fraction (EF) and E/A values representing systolic and diastolic left ventricular function did not show significant difference between time points.

4 Investigation of coronary microcirculatory parameters and mechanisms of coronary microvascular dysfunction in SSc

4.1 Patients and methods

Diffuse and limited subset of SSc cases were diagnosed by the commonly used criteria¹. The trial protocol was approved by the Medical Research Council Scientific and Ethical Committee (330/PI/2007) and patients provided informed consent before the study. Each patient underwent a baseline physical examination. Lung involvement was investigated by using chest X-ray, pulmonary function tests, and high resolution computed tomography if interstitial lung disease was suspected. Duration of Raynaud's phenomenon (RP) at the time of the study entry was evaluated by clinical interview, while duration of SSc was determined from the time of the onset of the first SSc-related non-Raynaud symptom. Patients with decreased systolic function (ejection fraction <30% on echocardiography), with known severe valvular disease or with severe lung fibrosis (forced vital capacity <50%) were excluded from further investigations. Cardiac catheterization was initiated in the presence of abnormalities, which were suggestive of pulmonary arterial hypertension (PAH) or of coronary artery disease. Detailed criteria for catheterization are published elsewhere⁷⁰.

Briefly, criteria for the 'suspected PAH' included signs of right ventricular involvement on echocardiography (tricuspid insufficiency diagnosed by flow velocity over 3 m/s, or consistent with 2.5-3m/s in the presence of unexplained dyspnoea, signs of right ventricular hypertrophy/dilatation, or right ventricular D sign) and effort related dyspnoea with disproportional decrease of CO diffusion capacity (DLCO) compared to the forced vital capacity (FVC/DLCO >1.8). Patients were included in the 'suspected CAD' group if they

reported recent deterioration in physical activity, evolving effort dyspnoea, or chest pain, or fulfilled the criteria of the New York Heart Association functional classes III-IV, or if their 6-minute walking distance was <380m, but it did not fulfilled the criteria for PAH^{14, 70}. Coronary angiographic findings and coronary flow reserve data of the first ten consecutive patients were included in our previous publication⁷⁰. Subsequent seven SSc patients were enrolled according to the same protocol for investigation of the myocardial resistance levels. The Ca-antagonist therapy was stopped for more than twenty four hours before cardiac catheterization. Right heart catheterization (RHC) and coronary angiography, supplemented with thermodilution CFR assessment were performed at the same time. Intracoronary pressure-wire measurements of 17 patients with intermediate coronary lesions were used as control.

4.1.1 Echocardiography and Right Heart Catheterization

The methods of Echocardiography and Right Heart Catheterization applied during the study have been explained in details in chapter 3.1.1. and 3.1.2.

4.1.2 Coronary angiography

The coronary arteriography was performed as described in chapter 3.1.3.

IMR was calculated as a product of mean transit time and Pd, in hyperemia and at rest. (Figure 3)

$$(A.) \quad FFR = \frac{Pd_{hyp}}{Pa_{hyp}} \quad (B.) \quad CFR = \frac{Tm_{bas}}{Tm_{hyp}}$$

$$(C.) \quad IMR_{bas} = Tm_{bas} \times Pd_{bas}$$

$$IMR_{hyp} = Tm_{hyp} \times Pd_{hyp}$$

Figure 3: Calculation of hemodynamic parameters. During the pressure-wire measurements aortic pressure at the coronary orifice (Pa), distal coronary pressure at the tip of the wire (Pd) and mean transit time of 3 ml room temperature saline (Tm) were measured at basal conditions and during hyperemia (subscript ‘bas’ and ‘hyp’, respectively) A: Fractional flow reserve (FFR) was calculated as a Pd/Pa ratio during hyperemia. B: coronary flow reserve (CFR) was calculated from the ratio of the mean transit times in hyperemia and at rest. C: Index of myocardial resistance (IMR) was calculated as a product of mean transit time and Pd, in hyperemia and at rest.

4.1.3 Statistical analysis

Continuous variables are presented as mean \pm SD. All analyses were performed with SPSS Software (version 16.0, SPSS Inc., Chicago, IL, USA). In group comparison unpaired t-test, chi-square test or Fisher’s exact test were used as appropriate. Pearson r test was used to examine correlations.

4.2 Results

Demographics, treatment and echocardiographic characteristics of the included patients are depicted in Table 3, while patient characteristics and pulmonary findings of the systemic sclerosis patients are summarized in Table 4a, b.

	SSc patients (n=17)	Controls (n=17)
Age (years)	59.9±12.0	58.3±7.5
Gender (male:female)	5:12	9:8
Hypertension	9(52.9%)	13 (65.0%)
Diabetes mellitus	0 (0%)	5 (27.8%)*
Hypercholesterolemia	3 (17.6%)	7(41.1%)
BMI (kg/m ²)	25.2±5.3	27.6±3.8
Smoker	0 (0%)	1 (5.9%)
Treatment:		
Ca-channel blockers	13 (76.5%)	4 (23.5%)**
Nitrates	3 (17.6%)	6 (35.5%)
β-blockers	5 (29.4%)	9 (52.9%)
ACE inhibitors	4 (23.5%)	7 (41.2%)
Statins	2 (11.8%)	9 (52.9%)*
Pentoxifylline	17 (100.0%)	1 (5.9%)**
Cyclophosphamide†	6 (35.5%)	0 (0.0%)**
Low dose corticosteroid†	9 (52.9%)	0 (0.0%)**
Echocardiography:		
Ejection fraction	58.88±6.32	55.55±2.82
Calculated RV systolic pressure	34.43±7.10	35.33±6.00
E/A	1.01±0.39	0.75±0.23

Table 3: Data are presented as mean ± SD or number of patients (percent). Abbreviations: BMI: body mass index, ACE: angiotensin converting enzyme, HRCT: high resolution computer tomography, RV: right ventricle, E/A: ratio of the early (E) and late (A) velocities of the transmitral flow, *: p<0.05 **p<0.001 unpaired t test or Fisher's exact test as appropriate. † Cyclophosphamide intake was encoded when cyclophosphamide treatment for at least 12 months, with an average dose of 1000 mg/month, was recorded in the patient's medical history. Steroid intake was marked when low or medium dose corticosteroid treatment was recorded in the patient's medical history for at least 12 months.

	SSc patients (n=17)
LcSSc:DcSSc	8:9
Disease duration (years)	9.6±8.3
Follow-up (months)	48.0±52.0
SSc Disease Activity Index	3.3±2.1
Medsger Severity Scale	8.2±3.2
HAQ-DI	0.8±0.6
ANA screen	16 (94.1%)
ACA	3 (17.6%)
Scl-70	6 (35.3%)
Clinical manifestations, case history:	
Raynaud's phenomenon	17 (100.0%)
Subcutaneous calcinosis	2 (11.8%)
Myositis	3 (17.6%)
Modified Rodnan skin score	8.5±10.0
Abnormal Shirmer's test	5 (29.4%)
Pericarditis	0 (0%)
Renal involvement	1 (5.9%)
Esophageal involvement	11 (64.7%)

Table 4a: Data are presented as mean ± SD or number of patients (percent). Abbreviations: lcSSc: limited cutaneous systemic sclerosis, dcSSc: diffuse cutaneous systemic sclerosis, SSc: systemic sclerosis, HAQ-DI: Health assessment questionnaire disability index, ANA: antinuclear antibody on human HEp-2 cells, ACA anti-centromer antibody.

	SSc patients (n=17)
Lung function test:	
FVC	98.6±16.6
DLCO	56.3±16.4
DLCO/VA	68.6±16.6
HRCT:	
Basal fibrosis	10 (58.8%)
Diffuse fibrosis	5 (29.4%)
Honeycombing	2 (11.8%)
Right heart catheterization:	
Pulmonary artery mean pressure (mmHg)	23.6±10.6
Pulmonary capillary wedge pressure (mmHg)	12.3±6.0
Pulmonary arterial resistance (dynes*sec/cm ⁵)	256.9±272.6
Systemic vascular resistance (dynes*sec/cm ⁵)	1600.7±431.9
Cardiac output (l/min)	5.1±1.4
Lung function test:	
FVC	98.6±16.6
DLCO	56.3±16.4
DLCO/VA	68.6±16.6
HRCT:	
Basal fibrosis	10 (58.8%)
Diffuse fibrosis	5 (29.4%)
Honeycombing	2 (11.8%)

Right heart catheterization:	
Pulmonary artery mean pressure (mmHg)	23.6±10.6
Pulmonary capillary wedge pressure (mmHg)	12.3±6.0
Pulmonary arterial resistance (dynes*sec/cm ⁵)	256.9±272.6
Systemic vascular resistance (dynes*sec/cm ⁵)	1600.7±431.9
Cardiac output (l/min)	5.1±1.4

Table 4b: Data are presented as mean ± SD or number of patients (percent). Abbreviations: SSc: systemic sclerosis, FVC: forced vital capacity, DLCO: diffusing capacity for carbon monoxide, DLCO/VA: carbon monoxide diffusing capacity adjusted for alveolar volume

The control group was matched for gender and age. Cardiovascular risk profile was similar except for the more frequent diabetes in control group. All SSc patients but none of the controls reported the presence of Raynaud’s phenomenon (RP). Regarding therapy, the patients in the control group used significantly less calcium channel blockers, pentoxifylline, cyclophosphamide and corticosteroids. Baseline hemodynamic data were similar between the two groups. The severity of coronary atherosclerosis as expressed by the QCA and SYNTAX scores did not differ significantly (P=0.830, and P=0.821, respectively). The SSc patient group and the control group were similar with regard to TFC, FFR and CFR (P=0.604, P=0.651 and P=0.117, respectively, Table 5).

	SSc patients (n=17)	Controls (n=17)
Basic hemodynamic data:		
Systolic pressure (mmHg)	137.1±25.9	148.3±32.6
Diastolic pressure (mmHg)	77.9±15.6	84.3±16.2
Frequency (beats/min)	75.9±13.1	81.9±19.7
Angiographic findings:		
Calcification	6 (35.6%)	4(22.2%)
Plaques	12 (70.6%)	14 (77.8%)
Occlusion	1 (5.9%)	0 (0%)
Diffuse sclerosis	4 (23.5%)	5 (27.8%)
Aneurysm	1 (5.9%)	0 (0%)
Bridge	2 (11.8%)	1 (5.6%)
SYNTAX score	10.7±10.7	9.9±8.4
Coronary flow velocity:		
Left anterior descending artery	28.4±16.8	24.6±9.5
Circumflex artery	23.9±14.2	23.0±9.0

Right coronary artery	18.8±16.4	19.7±9.9
Average frame count	23.7±13.2	21.7±7.3
Intracoronary pressure measurement:		
Coronary flow reserve (CFR)	3.1±1.7	2.3±1.0
Fractional flow reserve (FFR)	0.9±0.1	0.9±0.1
Quantitative Coronary Angiography stenosis (QCA) (%)	48.4±15.1	49.3±8.2
Index of myocardial resistance:		
At rest (IMR _{bas})	67.7±40.4	51.1±33.0
At hyperemia (IMR _{hyp})	19.0±7.5	18.1±8.4

Table 5: Data are presented as mean ± SD or number of patients (percent). Abbreviations: SYNTAX score: a validated comprehensive angiographic scoring system to describe the extent of coronary artery disease. (IMR_{bas}): Basal myocardial resistance. (IMR_{hyp}): Myocardial resistance at maximal vasodilatation

The IMR_{bas} was not significantly higher in the SSc group (P=0.207), and in response to maximal vasodilatation there was no significant difference between SSc patients and controls (P=0.731) (Fig.4).

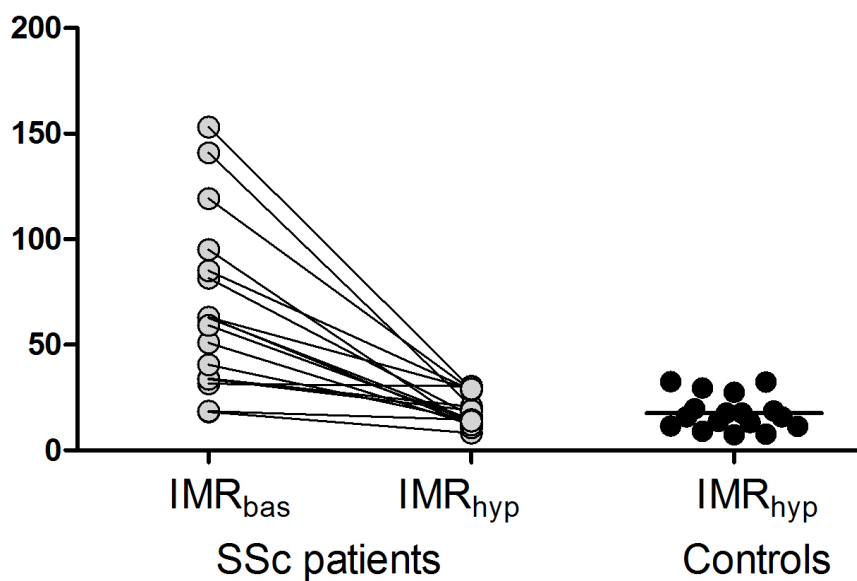


Figure 4: In systemic sclerosis (SSc) patients the myocardial resistance at maximal vasodilatation (IMR_{hyp}) was not significantly different from controls ($p=0.99$). The difference of the magnitude of the response to vasodilatation (i.e. the coronary flow reserve) is dominated by the differences in the resting resistance (IMR_{bas}) i.e. in the vascular tone.

CFR values lower than 2 were detected in six SSc patients. These patients had higher basal coronary flow velocities than SSc patients with normal CFR (10.63 ± 5.1 vs. 27.53 ± 14.8 $p < 0.05$) (Figure 5).

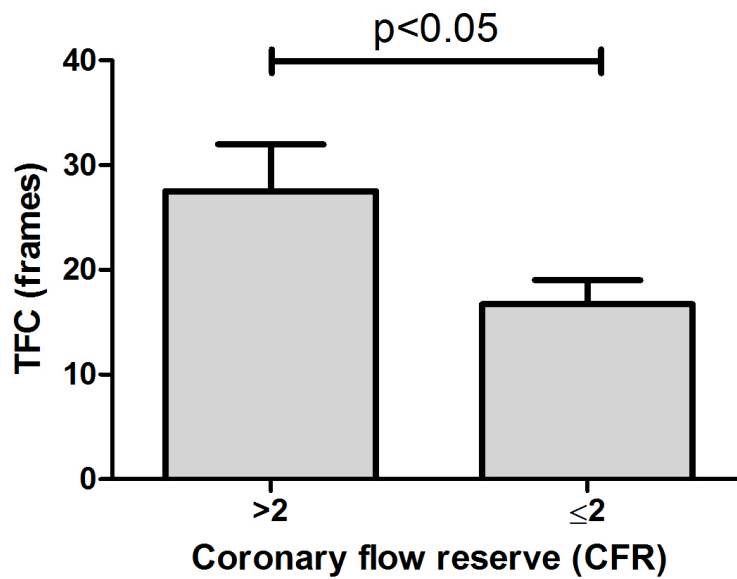


Figure 5: TIMI frame count (TFC) of SSc patients with normal CFR were significantly higher, than in SSc patients with decreased CFR, which indicate accelerated coronary flow among patients with restricted coronary reserve.

We found a trend for lower IMR_{bas} values in these patients (43.8 ± 23.6 vs. 80.7 ± 42.5 , $P=0.07$).

IMR_{hyp} differed significantly neither from the SSc patients with normal CFR nor from the control group (21.68 ± 6.61 , 17.23 ± 7.83 and 17.80 ± 8.17 , respectively, $P=0.292$ and $P=0.308$).

The IMR_{bas} correlated to the coronary flow velocity ($R=0.56$, $p<0.05$) (Figure 6).

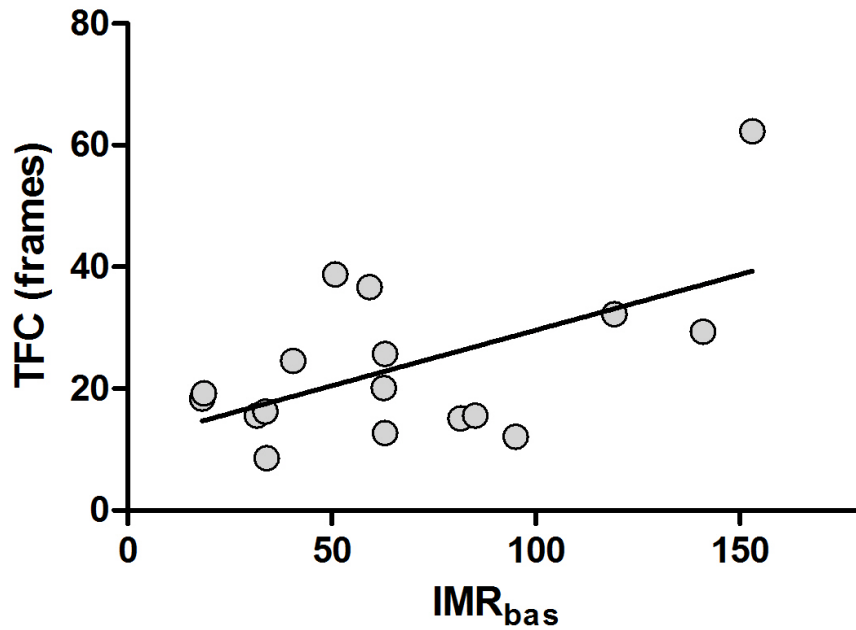


Figure 6: The baseline myocardial resistance shows significant linear correlation with the coronary flow velocity – assessed by the TFC. ($R_{\text{Pearson}}=0.56$, $p<0.02$)

There was no significant difference between limited cutan (lcSSc) and diffuse form (dSSc) of SSc as regards CFR, IMR_{bas} and IMR_{hyp} results. Clinical complaints showed neither relation to these parameters (data not shown). Mean transit time values during the baseline conditions in SSc patients were 0.75 ± 0.39 , 0.72 ± 0.50 , and 0.67 ± 0.41 sec, in the controls 0.56 ± 0.37 , 0.55 ± 0.36 , 0.51 ± 0.41 sec in baseline and 0.27 ± 0.11 , 0.27 ± 0.14 , 0.22 ± 0.10 versus 0.25 ± 0.14 , 0.22 ± 0.12 , and 0.23 ± 0.12 sec in hyperemia. No trend for deceleration was found (Figure 7).

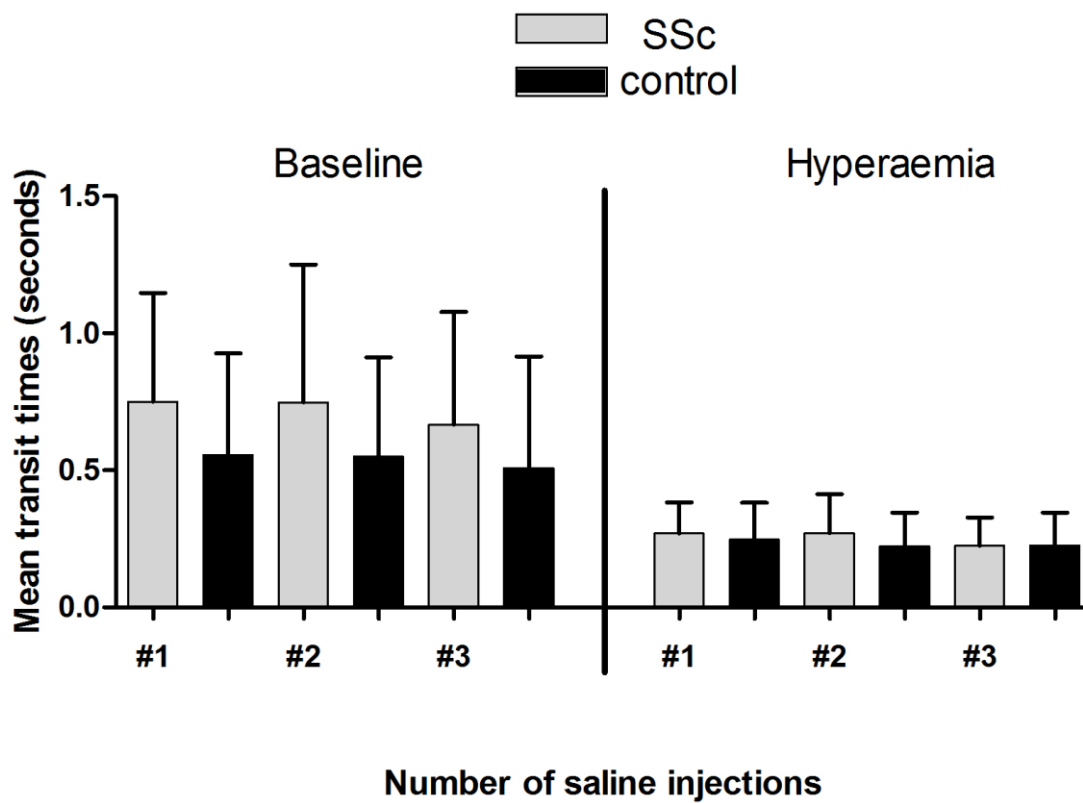


Figure 7: Mean transit time values in baseline and in hyperemia. After injection of room-temperature saline we observed longer transit times among SSc patients, however, this difference was not significant. In response to the repeated injections, lack of deceleration of the coronary artery flow suggests the absence of coronary Raynaud's phenomenon.

5 Novel findings of the thesis

Our studies demonstrated that coronary artery disease may mimic, and can appear in combination with PAH in patients with SSc. Patients with SSc with reduced physical capacity and exertional dyspnoea showed a considerable overlap between PAH and CAD. We found that among patients who showed signs of PAH by non-invasive investigations, the prevalence of CAD and PAH was comparable.

We found that, coronary revascularisation successfully eases symptoms and improves physical capacity. Therefore, screening for the presence of coronary artery disease by invasive methods seems to be indicated in symptomatic cases of SSc.

In our cohort coronary flow velocity showed inverse relation to the CFR, indicating that coronary flow deceleration is reversible in patients with SSc.

The index of myocardium resistance in hyperemia (IMR_{hyp}) is not increased in SSc patients compared to controls having no significant epicardial coronary artery stenosis suggesting that there is no irreversible damage at the level of the coronary arterioles in SSc.

In SSc patients with reduced CFR we demonstrated the decrease of the basal IMR, which suggests together with accelerated flow velocity the presence of a compensatory mechanism to maintain myocardial perfusion at rest already.

6 Discussion

This study demonstrated that coronary artery disease may mimic, and can appear in combination with PAH in patients with SSc. Patients with SSc with reduced physical capacity and exertional dyspnoea showed a considerable overlap between PAH and CAD. The incidence of PAH in our study was similar to that observed previously^{14, 71}. Intriguingly, in patients who showed signs of PAH by non-invasive investigations, the prevalence of CAD and PAH was comparable. Patients with suspected CAD also showed similar overlap, although this was less prominent. “Pure” PAH without coronary disease was a rather rare finding in our cohort, only affecting 10% of the cases. These findings suggest that the current screening methods are not ideal for distinguishing CAD and PAH in patients with SSc.

Thus a more invasive approach, such as conventional or CT coronary angiography, may be necessary to properly characterise heart involvement in SSc. Moderate pulmonary fibrosis was equally distributed among the groups, supporting recent studies indicating that pulmonary vascular and interstitial involvement progresses independently⁷². Incorporating the examination of the coronaries into the catheterisation protocol resulted in a reduction of the number of negative examinations. Still, a reasonable explanation for findings suggestive of cardiopulmonary involvement was lacking in a remarkably high number of patients (8/30) with normal RHC values, normal coronaries and without severe pulmonary fibrosis. These data indicate that a more complex approach should be developed for the evaluation of cardiopulmonary involvement in SSc. Angina in scleroderma is a weak predictor of CAD, as patients with SSc with chest pain are half as likely to have CAD compared to the general population. In a retrospective analysis of 172 SSc cases, 47% and 50% standard prevalence ratios were found for CAD in the typical and atypical angina group, while in the non-anginal or breathlessness group the ratio was 93%⁷³. Compared to our findings, the overall prevalence

of CAD was lower in this study. The main difference between the two studies was the selection of patients for coronary angiography, as our selection criteria covered a wider patient group. Indications for angiography may influence the incidence of CAD, and the more extensively coronary angiography is performed, the larger number of affected patients may be found⁷⁴. In this study the coronary flow velocity was quantified by using the TIMI frame count (TFC). Slow coronary flow has been hypothesised to be the result of microvascular loss and characterised by an increased TFC, reduced CFR and microvascular angina. Restriction of coronary adaptation in patients with SSc is attributed to involvement of microvasculature and considered to be a characteristic of primary myocardial disease^{21, 29, 30}. We found that this feature does not apply uniformly to all patients. In our cohort, none of the patients had abnormally high TFC, but interestingly, velocity showed inverse relation to the CFR, indicating that coronary flow deceleration is reversible in patients with SSc. Alternatively, the use of vasodilator agents may improve the myocardial perfusion in those patients in whom coronary flow reserve is maintained. We conclude that CAD can be found in a considerable proportion of patients with SSc and overlaps substantially with PAH. In selected patients, coronary revascularisation successfully eases symptoms and improves physical capacity. Therefore, screening for the presence of coronary artery disease by invasive methods seems to be indicated in symptomatic cases of SSc.

To our knowledge our work was the first study which assessed the myocardial resistance in SSc patients by intracoronary pressure-wire measurement. The main finding of our investigation is that the index of myocardium resistance in hyperemia (IMR_{hyp}) is not increased in SSc patients compared to controls having no significant epicardial coronary artery stenosis suggesting that there is no irreversible damage at the level of the coronary arterioles in SSc. Coronary arterioles are responsible for the distribution of the myocardial

blood flow. Their function is subject to delicate regulation by different signals of local ischemia. The cornerstone of myocardial vascular resistance is the active tone of the arterioles. It has been hypothesized on the basis of recent studies that in SSc patients the loss of the arterioles and consequent reduction of the arteriolar bed's cross-section result in an increased hyperemic resistance^{20, 75}. This concept however, was not confirmed by our results. The next step of the regulation is the level of microcirculation. Capillary field is exceptionally dense in the heart providing one capillary to one myocyte⁷⁶. Capillaries are not uniformly patent because the precapillary sphincters regulate flow according to demand.

In some conditions, i.e. left ventricular hypertrophy, ischaemic heart disease and diabetes mellitus, the impaired microcirculation with increased resistance and elevated resting blood flow is known. This process inspired to insure myocardial oxygen demand, resulting in reduced CFR.

In our study, in SSc patients with reduced CFR we demonstrated the decrease of the IMR_{bas} , which suggests together with accelerated flow velocity the presence of a compensatory mechanism to maintain myocardial perfusion at rest already. This process may cause exhaustion of the adaptive capacity and results in ischemia at a lower threshold. Consistently with other studies that consequently demonstrated myocardial perfusion defects in SSc we hypothesize that these ischemic signals arise from the microcirculatory bed distally from the level of resistance arterioles.

Thus loss of capillaries would not influence the hyperemic resistance, yet may lead to ischemia of the contractile elements. We found that SSc patients with reduced CFR exhibited decreased basal resistance. At the presence of coronary stenosis the reduction of the basal tone at rest is a compensatory mechanism that intends to maintain the myocardial perfusion despite

the decreased distal coronary perfusion pressure^{50, 54}. In our SSc cohort FFR values were normal; supporting the absence of hemodynamically significant stenosis, consequently compensation for the diminished distal coronary pressure can be excluded. However, this fact mentioned above together with the signs of accelerated flow in rest in patients with low CFR suggests that decrease of resting resistance may be a consequence of compensatory mechanisms alerted by ischemic signals from the myocardium.

Previous studies could only estimate the coronary flow parameters in SSc patients. Although Nitenberg et al. have investigated the coronary blood flow and myocardial resistance levels invasively²⁰, the coronary perfusion pressure was calculated and the coronary blood flow was evaluated using thermodilution measurements in the coronary sinus. Our data are based on direct invasive measurements of intracoronary pressures, which provide more accurate pressure values enhancing the strength of our findings. Without direct measurement of Pd, a diffuse but hemodynamically significant stenosis cannot be excluded.

Several studies have attempted to characterize non-invasively the coronary vasoreactivity in SSc patients²⁸⁻³⁰. Measurement of the diastolic coronary flow obtained by transthoracic echocardiography initially has been reported to exhibit suboptimal feasibility, which can be improved by using contrast augmentation of the Doppler signal and second harmonic technology (CFR_{echo})³¹⁻³³.

Sulli et al. found significantly lower CFR_{echo} values among patients with diffuse SSc than in subjects suffering from the limited form of the disease. In our cohort such a difference has not been found. Furthermore, CFR_{echo} values in SSc differed significantly from healthy controls while we found similar CFR values when compared to the patients referred to cardiac catheterization in order to exclude CAD³⁰.

Montisci et al found that the peak diastolic velocity was significantly decreased in the left anterior descending coronary artery in basal conditions and the CFR_{echo} measured during adenosine infusion increased significantly after single intravenous L-propionylcarnitine application. It may be interpreted as the “antiendothelin” effect of L-propionylcarnitine for microvascular tone and this agent could cause improved sensitivity for adenosine infusion³¹.

D’Andrea et al conclude that endothelial dysfunction in SSc patients was not confined to coronary microvessels, but also extends to peripheral arteries²⁸. This concept is in line with recent observations that found impairment of endothelium dependent vasodilatation in SSc, while endothelium independent vasodilatation is preserved.⁷⁷ Similar results were found earlier by Andersen et al³⁴. Limitation of these studies is the lack of strict pressure measurement allowing only indirect determination of the coronary flow. Further limitation is the lack of information about the coronary anatomy. Coronary heart disease in forms of coronary artery sclerosis and microvascular disease can be found frequently in combination in symptomatic SSc patients⁷⁰. Furthermore, clinical presentation of atherosclerosis is atypical in this patient group and coronary abnormalities may be found among symptom free patients⁷³,

74 .

Studies investigating the myocardial perfusion in SSc have found irreversible as well as reversible perfusion defects. These have no coronary artery anatomy related distribution and calcium channel blockers, dipyridamole or captopril treatment resulted in their partial reversibility^{25, 29, 30, 78}. These findings altogether support the idea that abnormal vasoreactivity causing episodic focal ischemia is associated with the myocardial fibrosis related structural myocardial disease⁷⁵.

Cardiac magnetic resonance studies demonstrated that the presence of areas with late-enhancement corresponding to the fibrotic areas earlier found by autopsy studies are common finding^{15, 16, 79, 80}. Distribution of the fibrosis did not show a direct relationship to the coronary anatomy, however, coronary artery disease is a frequent finding in SSc patients presenting with cardiac symptoms⁷⁰. In a recent study fibrosis was found in 66% of SSc cases. These lesions were found more frequently in cases with long history of RP. There was no different incidence found between diffuse and limited cutan forms of SSc⁸⁰. Allanore et al. demonstrated that the myocardial perfusion index can improve after administration of endothelin receptor antagonist bosentan³⁶. This finding confirms our hypothesis that the improvement of endothelial function which is mainly represented by capillary system can repair the myocardial perfusion.

In our study we did not find any correlation with history of the RP the basal resistance (IMR_{bas}) of SSc patients did not differ significantly from the controls. The concept that Raynaud-like mechanisms may be involved in the evolution of the scleroderma specific internal organ damage is a tempting hypothesis. Mukerjee et al. investigated the effect of central and peripheral cold challenges in 21 SSc patients with PAH. They did not verify significant changes in hemodynamic parameters and in the level of vasoactive peptides after a two- minute long hand immersion into cold water or after administration of 4C° saline into the right atrium, so they excluded that cold provocation could alter the pulmonary circulation⁸¹. Theoretically, the hemodynamic changes caused by low temperature in the heart are even less probable. Our results strengthen this statement since the injection of room temperature saline into the coronaries did not provoke angina or spasm and we did not find any increase of the transit times. The IMR_{bas} was not elevated in SSc patients despite the stress situation, and the cool temperature of the cathlab.

Our study has certain limitations. The gender distribution was different in the two groups although this difference was not statistically significant probably due to the small sample size. Considering the cardiovascular risk factors, the incidence of the hypercholesterolemia was significantly higher in the control group; both of them may influence the vasoreactivity of the coronary arteries. We did not find significant difference in the IMR_{bas} between the $CFR \leq 2$ and $CFR > 2$ SSc groups. One possible explanation can be the high standard deviation of those values. Furthermore in our current study we assessed neither the myocardial metabolisms nor the myocardial perfusion.

In conclusion, reduced CFR can be considered as a sign of SScMI. Our observations support that in SSc the reduction of the CFR is mainly attributable to a decrease of the basal myocardial resistance i.e. with lower arteriolar tone and with an accelerated coronary flow velocity. These findings suggest a sort of compensatory state. Vasodilatory treatment may cause further decrease in the tone of the arterioles, which may be primarily effective among patients with retained CFR. Further studies are needed to characterize the mode of action of these vasodilatory drugs in SSc.

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8 List of publications

Articles

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