Echocardiographic assessment of cardiac and pulmonary manifestations in patients with systemic sclerosis

Ph.D. Thesis

by

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Table of contents

Publications related to the subject of the Thesis: 3

Other publications 3

Abbreviations: 6

1. Background 8

1.1 Immunological and pathophysiological background of SSc 8

1.2 Clinical manifestations and classification of SSc 12

1.3 Cardiac manifestations of the SSc 13

1.4 Pulmonary manifestations of the SSc 14

2. Primary goals of the thesis 18

3. Methods 19

3.1. Patient selection 19

3.2. The role of echocardiography in the assessment of cardiac and pulmonary manifestations of systemic sclerosis 21

3.2.1. New techniques to assess myocardial involvement 21

3.2.2. Detailed methodological description of the LA study 22

3.2.3. Methods of the exercise echo study 25

4. Statistical analysis 27

5. Results 28

5.1. Results of the LA study 28

5.2. Results of the exercise echo study 31

5.3. Results of the follow up study 35

6. Discussion 37

6.1. Discussion of the LA study 37

6.2. Discussion of the EDE study 40

6.3. Discussion of the follow-up study 43

7. New observations 45

8. References 46

9. Acknowledgements 61

Publications related to the subject of the Thesis:

1. **Agoston G**, Gargani L, Miglioranza MH, Caputo M, Badano LP, Moreo A, Muraru D, Mondillo S, Moggi Pignone A, Matucci Cerinic M, Sicari R, Picano E, Varga A. Left atrial dysfunction detected by speckle tracking in patients with systemic sclerosis. Cardiovasc Ultrasound. 2014 ;12:30**. IF:1.32**

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1. Gargani L, Doveri M, Bazzichi M, **Agoston G**, Costantino M.F, Nigro A, Bombardieri S, Sicari R, Varga A, Picano E. Ultrasound lung comets as a long-term prognostic determinants in systemic sclerosis. European Heart Journal, 2009 September, Supplement 1.

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Abbreviations:

SSc: systemic sclerosis, scleroderma

PAH: pulmonary arterial hypertension

PAP: pulmonary artery pressure

ILD: interstitial lung disease

LV: left ventricle

LA- left atrium

ECM: extracellular matrix

TGF-β Transforming growth factor beta

ET- 1 endothelin one

CTGF- connective tissue growth factor

PDGF - Platelet-derived growth factor

RHC- right heart catheterization

mPAP - mean pulmonary arterial pressure

ACR- American College of Rheumatology

EULAR- European League Against Rheumatism

PCWP - pulmonary capillary wedge pressure

PVR- pulmonary vascular resistance

NYHA- New York Heart Association

TDI: Tissue Doppler Imaging

RV- right ventricle

RVOT- Right ventricular outflow tract

TAPSE- Tricuspid Annular Plane Systolic Excursion

PASP- estimated pulmonary systolic pressure

TRV- tricuspid Doppler Tracing

EDE - Exercise Doppler Echocardiography

CO- cardiac output

CI- cardiac index

WU - Wood Units

DLCO- diffusing capacity of the lungs for carbon monoxide

STE- two-dimensional speckle tracking strain echocardiography

BSA - body surface area

$\varepsilon_{\text{pos peak-}}$ positive peak left atrial longitudinal strain

sec $\varepsilon_{\text{pos peak-}}$ second positive peak left atrial longitudinal strain

$\varepsilon_{\text{neg peak}}$ – negative peak left atrial longitudinal strain

1. Background

Systemic sclerosis (SSc; scleroderma) is a heterogeneous, systemic, autoimmune disease. The pathogenesis of SSc is characterized by 3 hallmarks: small vessel vasculopathy, production of auto-antibodies and fibroblast dysfunction leading to increased deposition of extracellular matrix (ECM)[1]. The clinical manifestations and the prognosis of SSc vary with the majority of patients having skin thickening and variable involvement of internal organs. Organ manifestations of SSc can be particularly problematic when present in the lungs, kidneys or heart.

The majority of patients with SSc are believed to have subclinical cardiac involvement [2 3 4]. Overt cardiac manifestations of SSc are associated with poor prognosis [5, 6, 7, 8], and they can be difficult to manage [9].

Pulmonary arterial hypertension (PAH) is also a frequent complication of SSc and is one of the leading causes of morbidity and mortality in patients with this disease[10 11 12]. SSc–related PAH is the result of an isolated pulmonary arteriopathy, but in these patients, elevated pulmonary artery pressure (PAP) may occur also as a consequence of interstitial lung disease (ILD) or left ventricular (LV) systolic and/or diastolic dysfunction.

The thesis is focused on the early detection of cardiac and pulmonary manifestations of SSc using different echocardiographic modalities.

1.1 Immunological and pathophysiological background of SSc

The precise etiology of SSc is an expanding area of research, since the accurate nature of the mechanisms underlying this disease remains unclear [13]. The disease may be initiated in the vascular bed of the organs, the evidence suggesting that some pathomorphological changes may be apparent before the onset of the disease [13]. The pathological events in SSc may include impaired communication between endothelial cells, epithelial cells and fibroblasts; lymphocyte activation; autoantibody production; inflammation; and connective tissue fibrosis [13]. These events result in an accumulation of constituents of the ECM, which replaces the normal tissue architecture, and it can turn into the culmination of organ failure.

It has been widely recognized that the tissue fibrosis seen in scleroderma patients is the end result of a complex biologic process involving immune activation and

vascular injury [14]. Clinical and biological evidence suggests that the primary target for both initiating and propagating the disease is the epithelium of blood vessels. Following tissue injury, the epithelium plays an essential role in repairing wounds and re-surfacing tissue. In patients with SSc, there is evidence that this regeneration may be dysregulated. Epithelium-derived factors influence the behavior of fibroblasts [15] [16] (Table 1). Epithelial to mesenchymal transdifferentiation occurs in lung fibrosis, and this process is known to be influenced or mediated by transforming growth factor beta (TGF-β), and potentially endothelin-1 (ET-1) [17] [18] [19].

Mediator	Role in fibrogenesis
TGF-β	ECM production, fibroblast proliferation and differentiation
CTGF	Regulation of fibroblast proliferation and migration and TGF-β- dependent ECM synthesis
ET-1	Regulation of ECM synthesis and contraction
Fibroblast growth factor	Regulation of fibroblast growth
IL-1	Inflammatory mediator
IL-4	Regulation of collagen synthesis
IL-6	Regulation of α-SMA expression in myofibroblasts
IL-12	Regulation of collagen synthesis
IL-13	Induction of TGF-β
IL-17	Fibroblast proliferation
MCP-1	Inflammatory mediator and regulation of collagen synthesis
MCP-3	Regulation of collagen synthesis
PDGF	Regulation of TGF receptor expression, and fibroblast and progenitor cell recruitment

Table 1 Key cytokines in the induction of fibrosis of SSc (from Abraham DJ et al Overview of pathogenesis of systemic sclerosis Rheumatology 2009 [20])

Fibroblasts maintain the structural integrity of connective tissue, secreting fibrillar procollagens, fibronectin and regulating the turnover and composition of the ECM via highly specific proteases such as collagenase. In SSc patients, activated fibroblasts are responsible for the development of fibrosis and accumulation of ECM molecules. These fibroblasts are characterized by an overproduction of collagen and the induction of collagen-modifying enzymes. Fibroblasts can be activated by different mechanisms, including direct cell–cell contact, stimulation by soluble mediators following induction of the appropriate receptor expression including TGF-

β, connective tissue growth factor (CTGF), platelet-derived growth factor (PDGF) or ET-1 [21] [22], or by modulation of cell–matrix interactions (Figure 1).

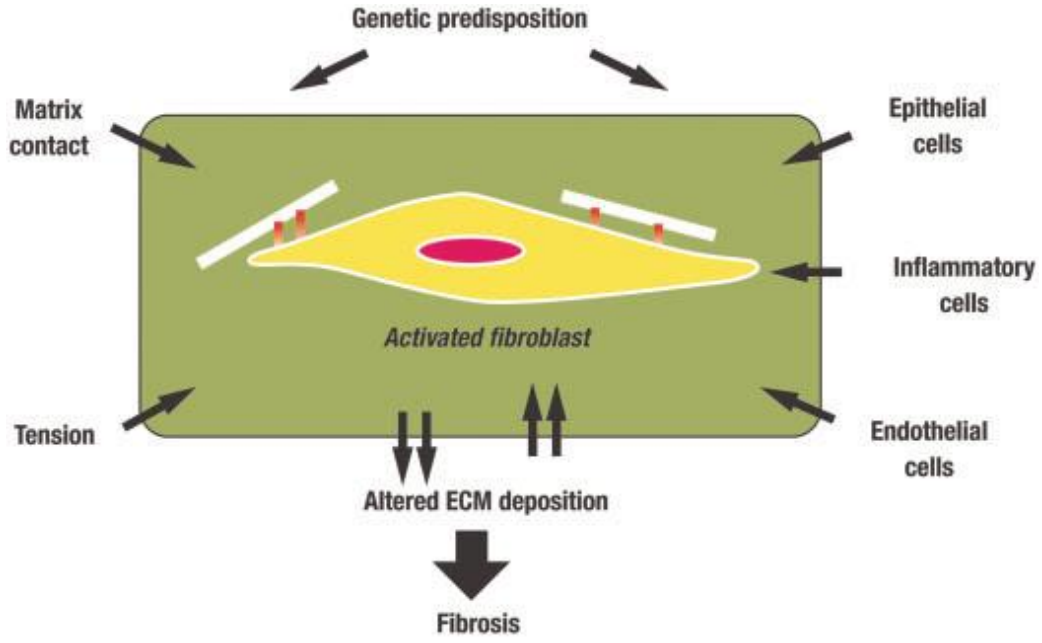

Figure 1 The role of fibroblasts in the pathogenesis of SSc (D. J. Abraham et al Overview of pathogenesis of systemic sclerosis Rheumatology 2009 [20])

The immune system also plays a role in the pathology of SSc. Activated lymphocytes, auto-antibodies, chemokines, interleukins are detectable in both the circulation and the affected organs of SSc patients. The majority of patients with SSc reveal circulating levels of highly specific auto-antibodies: one auto-antibody group is directed against nuclear antigens (topoisomerase, RNA polymerase), others may have a supposed pathogenic role. This latter group includes anti-endothelial cell antibodies, which are estimated to occur in 44–84% of SSc patients and may induce apoptosis [23] [24] [25] [26]. In patients with SSc, vascular remodeling is dysregulated. Vasculopathy may result from a disrupted or inappropriate repair process following endothelial cell insult or injury. These patients may exhibit up-regulation of vasoconstrictive, thrombogenic, mitogenic and pro-inflammatory factors, and down-regulation of vasodilatatory, anti-thrombogenic and anti-mitogenic factors. These results in vasculopathy are characterized by vasoconstriction, adventitial and intimal proliferation, inflammation and thrombosis. Vasculopathy involves all layers of the vessel wall and is characterized by fibrotic intimal hyperplasia [27]. As a result, vessels

lose their elasticity and become narrower. In time, the arterial intima may thicken and occlusion of the small arteries can facilitate the formation of in situ thrombosis. Fibrosis typically begins in the media of medium-sized arteries extending into the intima and adventitia and disrupting elasticity. The typical plexiform lesions of pulmonary arterial hypertension comprising endothelial cells and myofibroblasts can occur at all stages of development and healing [20].

Vascular remodeling appears to be preceded by endothelial dysfunction. Consistent evidence suggests that microvascular endothelial cell activation and damage is ubiquitous and occurs early in SSc [20].

The possible triggers of endothelial cell activation are:

- cross-reactivity between cytomegalovirus epitopes
- presence of anti-endothelial cell antibodies
- oxidative stress and the presence of elevated levels of reactive oxygen species.

The primary cause of the initial activation results in endothelial cell damage. Apoptosis is an early event, and if endothelial cells are not replaced by new cells, capillary breakdown and the typical clinical manifestations of vasculopathy can develop. A physiological reaction pattern to capillary breakdown and resulting tissue hypoxia is angiogenesis, a finely balanced process involving both angiogenic and angiostatic factors. In patients with SSc, angiogenesis becomes dysregulated [20].

ET-1 is a potent and important mediator of vasculopathy. It is a highly potent vasoconstrictor that is produced by endothelial cells and is a key mediator of vasculopathy, which also promotes cell growth, arterial wall thickening and endothelial cell dysfunction resulting in decreased levels of nitric oxide. Moreover, it can stimulate the proliferation of pulmonary artery smooth muscle cells and fibroblast collagen production [28] [29]. Increased ET-1 production can trigger an inflammatory cascade elevating the plasma levels of pro-inflammatory cytokines in patients with pulmonary arterial hypertension [30]. Thus, ET-1 promotes vasoconstriction and contributes to cardiac and vascular hypertrophy, inflammation and fibrosis [31]. It is over-expressed in both early- and late-stage SSc [13]. A role in the pathogenesis of SSc is also suggested by the finding that circulating levels of ET-1 correlate with skin fibrosis and the duration of disease [32].

1.2 Clinical manifestations and classification of SSc

The diagnosis of SSc was established according to the classification of American Rheumatism Association [33]. However, due to the insufficient sensitivity of the 1980 criteria and advances in knowledge about SSc, the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) established a committee to provide new classification criteria for SSc [34].

The keystones of recent criteria are:

- including broader spectrum of SSc, these are patients whose disease is in an early stage as well as those in late stages
- including vascular, immunologic, and fibrotic manifestations
- adapted to daily clinical practice usage

The score system classification criteria based on symptoms and clinical findings are shown in Table 2.

Item	Sub-item(s)	Weight/score
Skin thickening of the fingers on both hands extending proximal to the metacarpo-phalangeal joints (sufficient criterion)	-	9
Skin thickening of the fingers (only count the higher score)	Puffy fingers	2
	Sclerodactylity of the fingers (distal to the metacarpo-phalangeal joints but proximal to the proximal interphalangeal joints)	4
Fingertip lesions (only count the higher score)	Digital tip ulcers	2
	Fingertip pitting scars	3
Telangiectasia	-	2
Abnormal nailfold capillaries	-	2
Pulmonary arterial hypertension and/or	Pulmonary arterial	2
		2

interstitial lung disease (maximum score is 2)	hypertension Interstitial lung disease	
Raynaud's phenomenon	-	3
SSc-related autoantibodies (anticentromereanti-topoisomerase I (anti-Scl-70), anti-RNA polymerase III) (maximum score is 3)	Anticentromere Anti-topoisomerase I Anti-RNA polymerase III	3

Table 2 Score system of the classification in SSc - from van den Hoogen F at al Arthritis Rheum. 2013 [34].

The total score is determined by adding the maximum weight (score) in each category. Patients with a total score of >9 are classified as having definite SSc.
In our population, we also consider the major subsets of SSc:

- Limited cutaneous SSc: defined by skin thickening in areas solely distal to the elbows and knees, with or without facial effects, such as telangiectases.
- Diffuse cutaneous SSc: patients have skin thickening over both proximal and distal limbs, as well as the face and the trunk. Reduction in the oral aperture is common.

1.3 Cardiac manifestations of the SSc

Cardiac manifestation of SSc is particularly problematic in the clinical practice. In the majority of patients, cardiac manifestations are subclinical. Systolic and/or diastolic dysfunction can develop very early in the course of the disease, even years before becoming clinically evident. Cardiac involvement is common in SSc, with an estimated clinical prevalence of 15–35% while at post-mortem examination the heart was affected in up to 80% of the patients [5 6]. The development of overt myocardial manifestations is recognized as powerful adverse prognostic factors and may affect patients with both limited cutaneous SSc and diffuse cutaneous SSc. When clinically evident, these features are often associated with increased mortality [5 6 7 8]. All cardiac structures: the endocardium, myocardium, pericardium, valves, coronary arteries, electrical and autonomic nervous system, may be involved, potentially leading to

heart failure. Primary myocardial involvement, without systemic or PAH and without significant renal or pulmonary involvement, implicates different pathophysiological mechanisms, including the characteristic vascular lesions and fibrosis deposition, which may impair coronary microcirculation and myocardial function.

Myocardial fibrosis is a hallmark of cardiac manifestation of SSc. In SSc the myocardium is often characterized by patchy fibrosis, secondary to both repeated ischemia and/or immuno-inflammatory damage, inexorably leading to myocardial dysfunction. In SSc, the cause of ischemia is usually not the consequence of the epicardial coronary artery stenosis, but rather to microvascular dysfunction [35]. Characteristic vascular lesions in SSc result in the impairment of the microcirculation [9]. In addition to these fixed abnormalities, vasospasm of the small coronary arteries or arterioles may play a significant role in the development of early myocardial alterations [36]. Furthermore, diastolic dysfunction is frequent in SSc [37] [38] [39], and is correlated with disease duration [40], whereas systolic dysfunction is present in only a minority of patients [41]. 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 [7] [37].

1.4 Pulmonary manifestations of the SSc

Pulmonary involvement is a prominent feature of the SSc and occurs more frequently in SSc than in any other connective tissue disease [42]. The two most frequent types of lung involvement are ILD and pulmonary arterial hypertension (PAH) [43]. SSc-related PAH is the result of an isolated pulmonary arteriopathy, but in these patients, elevated PAP may occur also as a consequence of ILD or LV systolic and/or diastolic dysfunction, a condition that should be more generically addressed as PH, not as PAH. The diagnosis of PAH is established during right heart catheterization (RHC), when mean pulmonary arterial pressure (mPAP) exceeds 25 mmHg with a pulmonary capillary wedge pressure (PCWP) below 15 mmHg [44]. Additional diagnostic criteria may include normal or reduced cardiac output (CO) or a pulmonary vascular resistance (PVR) over 3 Wood units[45].

PAH is a severe vascular complication of the SSc and it has a dramatic impact on the clinical course and overall survival of the patients. It is the single most common cause of death in patients affected by this syndrome. The prevalence of

hemodynamically proven PAH in large cohorts of patients with SSc was about 5% to 10% [46] [47]. The three-year survival for SSc patients with PAH has been estimated to be 56% compared with 94% in those without PAH [48]. Observational studies have demonstrated that mortality remained high in SSc patients with PAH even when new therapeutic options (new drugs, new methods) were applied [49] [50] [51] [52] (Figure 2).

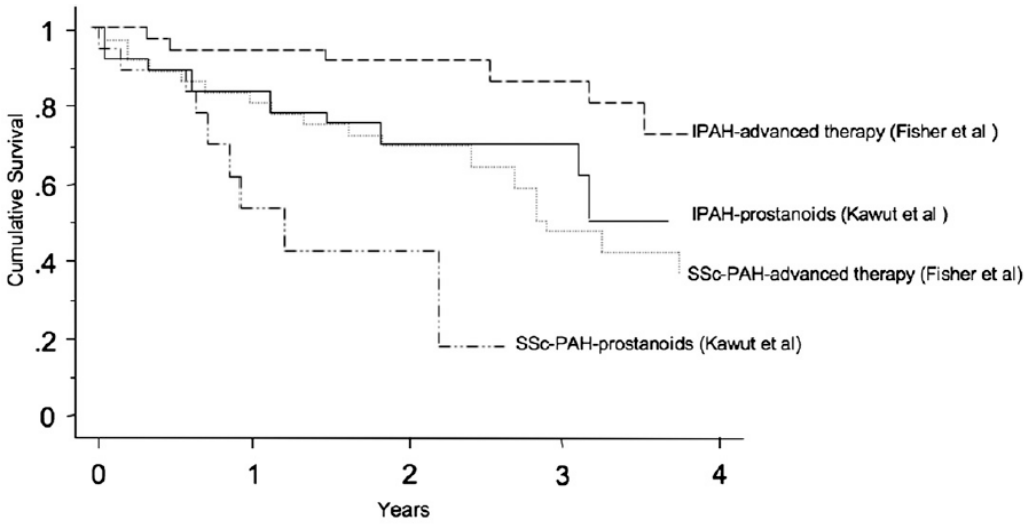

Figure 2 Kaplan–Meier analysis showing the survival in patients with SSc-related pulmonary arterial hypertension (SSc-PAH) and idiopathic pulmonary arterial hypertension treated with prostanoids or advanced therapy. Fisher MR at al Arthritis Rheum 2006 [51], Kawut SM at all, Chest 2003 [52]

Markers of worse prognosis include male gender, late age at diagnosis, pericardial effusion, functional severity based on the New York Heart Association (NYHA) functional class, right heart dysfunction, and hyponatremia [49] [52]. Poor outcome of PAH in SSc may be partially explained by disease-related co-morbidities but also by a delay in diagnosis. Signs and symptoms of SSc-PAH are generally aspecific, and thus often underestimated. The establishment of the diagnosis is frequently delayed to the advanced phases of the pathological process, characterized by structural and not reversible damage of the pulmonary vasculature. RHC is the criterion standard for PAP measurement. However, RHC is not applicable to large populations because of its invasive nature and costs. This is another reason why PAH is usually recognized at advanced stages, when the treatment does not significantly change the clinical course of the disease [52] [53]. Therefore, efforts to promote an early recognition of PAH have been expended in the last few years [46]. One recent study observed

better prognosis in subjects identified in an active screening program compared with those identified in the course of routine practice [54] suggesting a potential benefit of the intervention earlier in the course of disease (Figure 3).

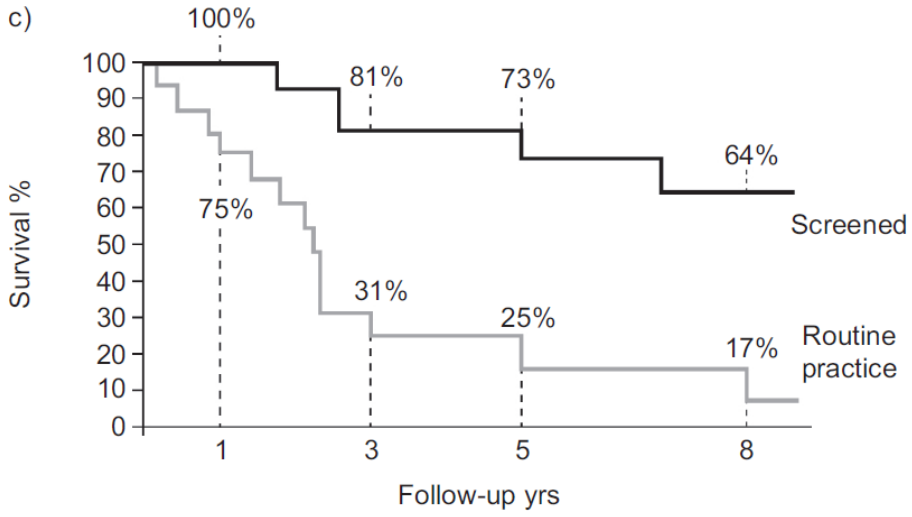

Figure 3 Prognosis for PAH-SSc patients detected in routine practice or via a screening program Humbert M at al. Eur Respir Rev. 2012 [54]

The subsequent important issue is the presence of an associated ILD in patients with SSc. ILD is a common and serious form of pulmonary involvement is SSc and characterized by various patterns of inflammation and fibrosis on high-resolution CT scan and in lung biopsy specimen (Figure 4). ILD was an important prognostic factor in patients with SSc and PAH [49 55 56]. SSc patients with ILD-associated PAH have a worse prognosis. However, ILD either limited or extensive, even without PAH, also has an impact on the overall prognosis, and ILD is also one of the leading causes of death in SSc [11 57]. Patients with limited SSc disease will typically develop isolated PAH 10 to 15 years after the onset of their disease [58]. In contrast, patients with diffuse SSc are at greater risk for ILD, usually within the first 5 years after the establishment of the diagnosis when the most rapid rate of decline in forced vital capacity is observed, but PAH may develop at any stage in the course of their disease. Although PAH is generally modest (mPAP 25–35 mmHg) in patients with ILD, PAP elevations can be more substantial.

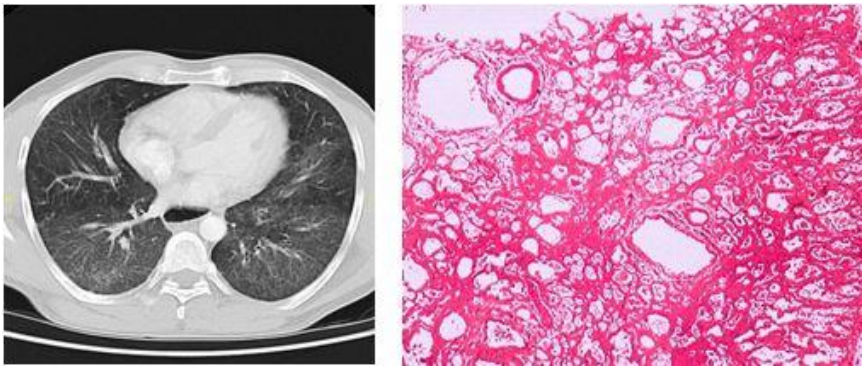

Figure 4 shows pulmonary fibrosis in high resolution computed tomography scan and in a lung specimen.

Courtesy; Robert C Mellors, M.D. PhD. Immunopathology, 1995

2. Primary goals of the thesis

Considering the fact that cardiac and pulmonary manifestations are very frequent in SSc leading to rapid progression of the disease and to fatal outcome in a substantial number of the patients, it is essential to recognize the early signs of these manifestations. Therefore, our goals of the present research were:

1. To assess whether two dimensional speckle tracking echocardiography (STE) parameters may detect early alterations in left atrial (LA) function in SSc patients.

2. To evaluate the clinical and echocardiographic determinants of exercise-induced increase in pulmonary artery systolic pressure (PASP) in a large population of patients with SSc.

3. To evaluate whether the exercise-induced PASP increase may predict resting PASP increase in a population of SSc patients.

3. Methods

3.1. Patient selection

For the study of the early involvement of the LA function, from September 2009 to January 2010, 42 consecutive patients affected by SSc (Group 1, age 50 ± 14 years, 95% females), admitted to the Department of Rheumatology in Florence, and 42 age and gender-matched control subjects (Group 2, age 49 ± 13 years, and 95% females) were enrolled. Patients in Group 1 underwent a thorough clinical assessment, pulmonary function test [59], assessment of pulmonary fibrosis by standard chest X-ray, lung ultrasound [60], when clinically indicated, by thoracic high-resolution computed tomography scan [61]. Inclusion criteria were: 1) age > 18 and < 85 years; 2) a previous diagnosis of SSc according to the European Scleroderma Trial and Research (EUSTAR) recommendations [62]. Exclusion criteria were: 1) inability to provide informed consent; 2) known history of coronary artery disease, electrocardiographic signs of myocardial ischemia, LV ejection fraction <55%, regional wall motion abnormalities, LV hypertrophy, more than mild valvular heart disease, pericardial effusion, and evidence or clear history of atrial fibrillation, or inadequate LA tracking for strain analysis. Anticentromere antibodies (ACA by indirect immunofluorescence on Hep-2 cells and by ELISA for CENP antigen) and antitopoisomerase I antibodies (anti-Scl70 by immunoblot analysis) were determined. All operators were unaware of the results of the other tests. The local Ethical Committee of Pisa, Italy, protocol number 2849, approved the study, and all patients gave informed consent. For the exercise echo study, from May 2007 to June 2009, 220 patients with SSc admitted to the Department of Rheumatology in Florence (n = 53), Milan (n = 76), Pisa (n = 47), Potenza (n = 5), Szeged (n = 9), and Udine (n = 30) underwent a thorough clinical and instrumental assessment, according to the European Scleroderma Trial and Research (EUSTAR) recommendations [62]. Inclusion criteria were as follows: 1) age ≤18 and ≥85 years and 2) a previous diagnosis of SSc according to the American Rheumatism Association classification criteria. Exclusion criteria were as follows: 1) inability to provide informed consent and 2) known history of coronary artery disease, more than mild valvular heart disease, and evidence or clear history of atrial fibrillation. All operators were

unaware of the results of the other tests. The Ethical Committee of Pisa approved the protocol (no. 2849), and all patients gave informed consent. The selection process is depicted in Figure 5.

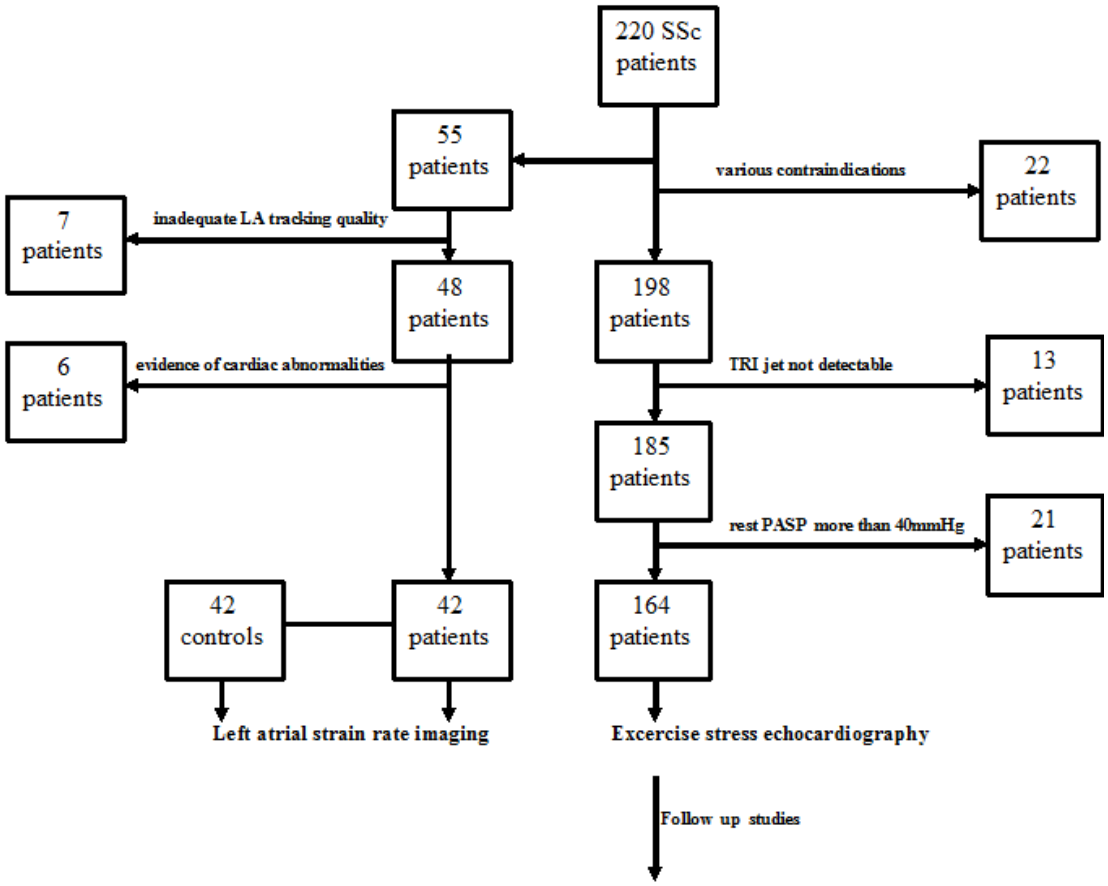

Figure 5 Flowchart showing patient selection process.

37 SSc patients (age = 58±13 years, 82% females, 74% limited cutaneous form) with normal resting PASP (<40 mmHg), who had developed exercise-induced PASP increase at a previously graded bicycle semi-supine exercise Doppler echocardiography (EDE). They underwent the same examination after at least six months. Patients having had a resting PASP > 40 mmHg did not perform the exercise. The average follow-up period was 21 ± 12 months.

3.2. The role of echocardiography in the assessment of cardiac and pulmonary manifestations of systemic sclerosis

Echocardiography is crucial in the evaluation of patients affected by SSc because it makes it possible to detect not only cardiac abnormalities responsible for the symptoms but subclinical dysfunctions as well. Echocardiography is also frequently used in vasculitis to assess valvular abnormalities, hypertension-related damage, sequelae of vasculitis related ischemia, and of course, systolic and diastolic functions. A main limitation of echocardiography is that ejection fraction is often the only parameter provided to evaluate cardiac function, whereas more subtle myocardial dysfunction cannot be assessed. Several studies have recently proposed novel echocardiographic techniques such as Tissue Doppler Imaging (TDI) and STE as effective tools for early detection of right and LV systolic and diastolic dysfunction [63].

Echocardiography is routinely employed in SSc for the screening of PAH, although right heart catheterization is necessary for proper diagnostic confirmation. Exercise Doppler echocardiography could be useful for the screening and the understanding of the pathophysiological mechanisms leading to PAH in patients with SSc. In the present research, we employed new echocardiographic techniques and methods – detailed below – for the evaluation of early cardiac manifestation of SSc.

3.2.1. New techniques to assess myocardial involvement

Strain describes myocardial deformation and appears in the fractional change in the length of the myocardial segment. Strain is usually expressed as a percentage. Strain rate imaging is a relatively new, largely angle-independent technique used for the detailed evaluation of myocardial function. The speckles seen in grayscale B-mode images are the result of constructive and destructive interference of ultrasound backscattered from structures. With this technology, random noise is filtered out, while keeping small temporally stable and unique myocardial features referred to as speckles. Blocks or kernels of speckles can be tracked from frame to frame (simultaneously in multiple regions within an image plane) using block matching, and provide local displacement information from which parameters of myocardial function such as velocity, strain, and strain rate can be assessed [64]. Assessment of 2D

strain by STE can be applied to both ventricles and atria and it provides sophisticated, detailed information about myocardial function in SSc**.**

3.2.2. Detailed methodological description of the LA study

All patients underwent comprehensive two-dimensional transthoracic echocardiography examinations at rest, using conventional methods with a commercially available ultrasound machine (Vivid 7, GE Medical Systems, Horten, Norway) equipped with a 2.5–3.5 MHz phased array sector scan probe, second harmonic technology, and coupled with tissue TDI. LV end-diastolic and end-systolic diameters were measured from the internal dimensions obtained from parasternal long axis view. LA diameters were measured from the apical four-chamber view. LA areas and volumes were measured using the biplane method of disks (modified Simpson's rule), in the apical 4- and 2-chamber view at end-systole (maximum LA size), and a mean value of area and volume was obtained [65]. LA volumes were subsequently indexed to body surface area (BSA). LV mass was calculated by the Devereux formula and then indexed to body surface area [65]. Mitral regurgitation was assessed semi-quantitatively (0 = absent or trivial, 1 = mild, 2 = moderate, 3 = severe), including evaluation of vena contracta, regurgitant volume and effective regurgitant orifice area, when indicated [66]. TDI was evaluated, as previously described, in the pulsed-wave Doppler mode, to assess longitudinal myocardial regional LV function. A volume was sampled centrally to the basal segment of infero-septal and antero-lateral wall for the LV, and then the mean value of the velocity profiles was recorded. Gain and filters were adjusted as needed, to eliminate background noise and to obtain a clear tissue signal. TDI signals were recorded at a sweep of 100 mm/s. Each parameter was measured as the average of at least three consecutive beats. LV diastolic function was determined from the pattern of mitral flow velocity by pulsed Doppler echocardiography, complemented by mitral annular velocity by TDI and LA volumes [67]. PASP was estimated from peak tricuspid regurgitation jet velocities, adding right atrial pressure estimated from inferior vena cava diameter and respiratory changes [68]. All measurements were performed according to the recommendations of the European Association of Echocardiography/ American Society of Echocardiography [65] [66] [67] [68].

Assessment of the left atrial function

Particular attention was paid to obtaining an adequate grayscale image, allowing reliable delineation of myocardial tissue and extracardiac structures. During breath hold, 3 consecutive heart cycles were recorded and averaged. The frame rate was set between 60 and 80 frames per second. These settings are recommended to combine temporal resolution with adequate spatial definition, and to enhance the feasibility of the frame-to-frame tracking technique [69].

Recordings were processed by using an acoustic-tracking software (EchoPac PC version 108.1.4, GE Healthcare, Horten, Norway), allowing off-line semi-automated analysis of STE strain. In the end-diastolic/systolic frame, the atrial endocardial border was traced by a point-and-click method. After automatic creation of a region of interest, the LA wall was divided into six subregions, and segmental tracking quality was analyzed. We analyzed LA from apical two- and four-chamber views, so we used a 12-segment model. The dashed curve represents the average strain (Figure 5). The tracking settings allow distinguishing three LA strain values. If the reference point is set at the onset of the QRS, we can measure positive peak atrial longitudinal strain (ε pos peak), which corresponds to LA reservoir function (Figure 5). If the reference point is set at the onset of the P wave, we can measure both negative atrial longitudinal strain (ε neg peak), which mirrors LA pump function and second positive peak atrial strain (sec ε pos peak), which corresponds to LA conduit function (Figure 6). Inter- and intra-observer variability of strain parameters has been previously assessed [70].

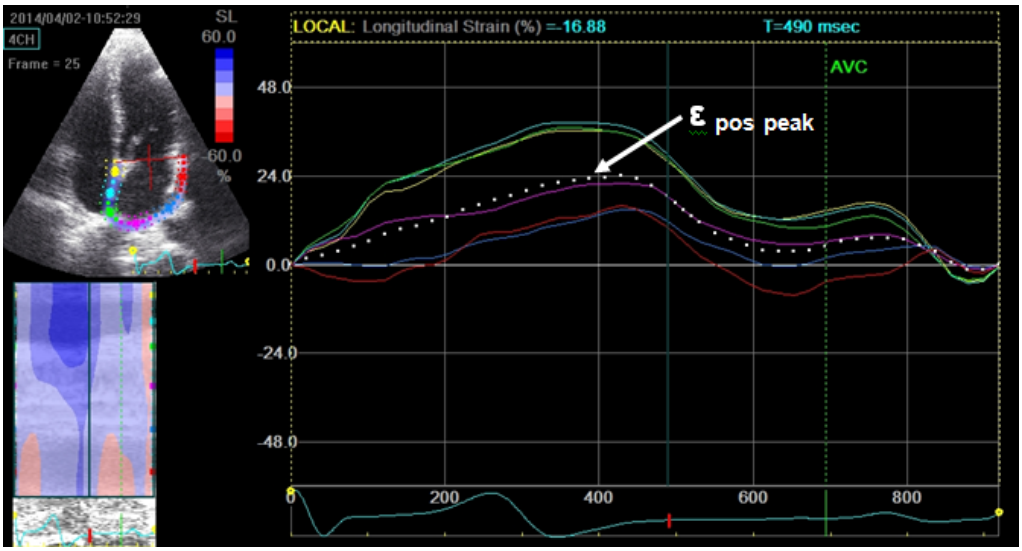

Figure 5 2D speckle tracking derived LA peak, positive longitudinal strain ($\varepsilon_{\text{pos peak}}$) measurement form apical 4 chamber view (the reference point is the beginning of the QRS complex).

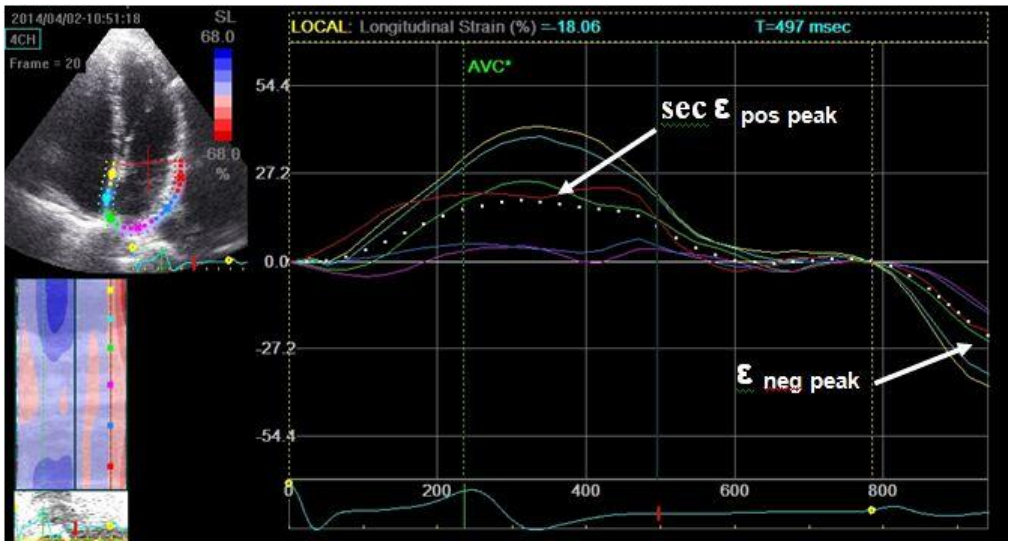

Figure 6 Measurement of second positive peak longitudinal LA strain (sec $\varepsilon_{\text{pos peak}}$) and negative peak longitudinal strain ($\varepsilon_{\text{neg peak}}$) from apical four chamber view (the reference point is the onset of the p wave).

3.2.3. Methods of the exercise echo study

All patients underwent a comprehensive transthoracic echocardiogram using conventional methods with commercially available ultrasound devices (Sonos 7500 and IE33 [Philips Medical Systems, Andover, MA], Sequoia C256 Acuson [Siemens, Mountain View, CA], Famiglia Mylab25 [Esaote, Genoa, Italy], Vivid System 7 [GE/Vingmed, Milwaukee, WI]) equipped with 2.5- to 3.5-MHz phased array transducers, second harmonic technology, and coupled with TDI. Pulmonary artery systolic pressure was derived from the maximal velocity of tricuspid Doppler tracing (TRV) according to the Bernoulli equation and adding the value of right atrial pressure, estimated on the basis of collapsibility index of the inferior vena cava [68]. Stroke volume, CO and cardiac index (CI) assessments were performed by the LV outflow track Doppler method. PVR was estimated by the following formula: TRV/RVOT (right ventricular outflow tract) time velocity integral × 10 + 0.16 [71]. PCWP was noninvasively derived by the following formula: PCWP = (1.24 × [E/e′]) + 1.9, where E/e′ represents peak early diastolic velocity to early diastolic velocity at basal mitral annulus ratio [72], and mPAP was calculated as 0.6 × PASP + 2 [73]. We measured the ratio PASP/CI to normalize pulmonary pressure increases for increases in CO [74 75].

All measurements were performed according to the recommendations of the European Association of Echocardiography (EAE)/American Society of Echocardiography [65].

Exercise stress echo was conducted using a graded semisupine bicycle ergometer with 25-W incremental loading every 2 minutes [76]. A 12-lead electrocardiogram and a blood pressure measurement were performed at baseline and every minute thereafter. Transthoracic 2-dimensional echocardiographic monitoring was performed throughout and up to 5 minutes after the end of stress. Tricuspid Doppler tracing and RVOT time-velocity integral were estimated at peak exercise, while the patient was still pedaling. Immediately after that, while the patient was stopping pedaling, LV outflow track time-velocity integral and measurements of left and RV function were performed because PASP and CO return rapidly back to normal after exercise, although it is less relevant for CO [77]. A cutoff value of PASP ≥50 mmHg at peak exercise was considered a significant exercise-induced increase in PASP. A cutoff value of peak PVR ≥3.0 Wood Units (WU) was considered a significant PVR

increase [68]. The stress test was symptom limited. Criteria for test positivity and terminating the test followed EAE recommendations [76].

Quality control

Almost all echocardiographic examinations (90%) were performed by the same operator (G.A.). The remaining examinations were performed by operators with long experience in echocardiography, who passed the quality control in stress echocardiography, as previously described [78]. This resulted in homogeneous data and image acquisition.

4. Statistical analysis

Data are presented as mean ± standard deviation (SD) unless otherwise stated. Comparisons between SSc patients and controls in the LA study were performed by using Student's t-tests or by non-parametric Mann-Whitney U-test, as appropriate. Comparisons between categorical variables were made with χ^2 test. All tests were two-sided, and p-values < .05 were considered statistically significant. Correlations were tested by Pearson or Spearman's correlation tests, as appropriate. Univariate comparisons between patients with and without exercise induced increase in PASP were made with $\chi 2$, 2-sample t test or similarly Mann-Whitney U test, as appropriate. The association of selected variables with the development of exercise-induced increase in PASP and PVR was assessed by logistic regression analysis using univariate and stepwise multivariate procedures. The following variables were included into the analysis: age, gender, body mass index, diffuse form, Scl-70 antibody positivity, Raynaud's phenomenon, Rodnan skin score, ILD, pulmonary function test data, resting echocardiographic parameters (ejection fraction, wall motion score index, indexed LV and LA volumes, Tricuspid Annular Plane Systolic Excursion (TAPSE), TDI systolic LV and RV waves, E/e′, RV tricuspid annulus early to late diastolic velocity ratio [E′/A′], PASP, acceleration time, CI, PCWP), stress echocardiographic parameters (peak wall motion score index, peak CI, peak MAPSE, peak TAPSE, peak TDI systolic LV and RV wave), peak workload, and peak oxygen saturation. Variables were selected according to their clinical relevance and potential impact on PASP and/or PVR, as shown by earlier studies. Categorical variables are presented as counts and percentages. All analyses were performed by using SPSS 20.0.0.0 (IBM Inc, Chicago, IL, USA) and GraphPad Prism version 6 (GraphPad Software Inc., San Diego, CA, USA).

5. Results

5.1. Results of the LA study

The clinical and echocardiographic characteristics of Groups 1 and 2 are summarized in Tables 3 and 4. From the initial population of 55 patients, 7 were excluded for inadequate LA tracking quality, and 6 were excluded for the evidence of cardiac abnormalities (2 patients for EF<55%, 3 patients for pericardial effusion, 1 patient for LV hypertrophy) (Figure 5).

The two groups did not differ in systolic function of the LV (Group 1 EF = 63.1±4%, vs. Group 2 = 66.1±4%, p=ns). SSc patients did not show significantly different LA indexed volumes (Group 1 = 24.9 ± 5.3 ml/m^2 vs. Group 2 = 24.7 ±4.4 ml/m^2, p = .8) (Figure 7), but showed significantly different LV diastolic and LA longitudinal strain parameters. E/e' ratio was higher in SSc patients (Group 1 = 7.6±2.4 vs. Group 2 = 6.5±1.7, p<.05, Figure 8) and $\varepsilon_{\text{pos peak}}$ and sec $\varepsilon_{\text{pos peak}}$ were significantly decreased (Group 1 = 31.3 ± 4.2% vs. Group 2 = 35.0 ± 7.6%, p < .01 and Group 1 = 18.4 ± 4 % vs. Group 2 = 21.4 ± 7.6%, p < .05) (Figure 9). $\varepsilon_{\text{neg peak}}$ did not show significant differences (Group 1 = 12.9 ± 2% vs. Group 2 = 13.6 ± 3%, p=ns). Interestingly, we found significant correlation between $\varepsilon_{\text{pos peak}}$ and age, but only in the control group (R = -.59, p < .001) and not in SSc patients (R = -.09, p = .57). Among echocardiographic parameters, no correlations were found between LA $\varepsilon_{\text{pos peak}}$ and LV EF, nor E/e', or LA indexed volume or PASP. No significant difference in LA $\varepsilon_{\text{pos peak}}$ values were found between patients with and without Scl-70 antibodies (31.1 ± 4.2 vs. 30.6 ± 4.1, p =.75). No echocardiographic, STE or clinical parameter was different between patients in NYHA class I and NYHA classes II–III, including diffusing capacity of carbon monoxide (DLCO).

Table 3 Clinical characteristics

Clinical variables	Group 1 (SSc) 42 pts	Group 2 (Controls) 42 pts	p
Age (years)	50 ± 14	49 ± 13	ns
Female gender (n,%)	40 (95%)	40 (95%)	ns
Systemic arterial hypertension (n,%)	10 (24%)	16 (38%)	ns
Diabetes (n,%)	0 (0%)	1 (2%)	ns
History of Smoking (n,%)	3 (7%)	6 (14%)	ns
Limited form n (%)	35 (83%)		
Diffuse form n (%)	7 (17%)		
Scl-70 antibodies n(%)	13 (31%)		
DLCO (%)	80.6 ± 23.2		
NTpro-BNP (pg/ml)	122 ± 135		

SSc: systemic sclerosis

DLCO: diffusing capacity of carbon monoxide.

NTpro-BNP: N-terminal B-type natriuretic peptide

Table 4 Echocardiographic data

Echo variables	Group 1 (SSc) 42 pts	Group 2 (Controls) 42 pts	*p*
EF (%)	63.1±4	66.1±4	ns
LA indexed volumes(ml/m^2)	24.9±5.3	24.7±4.4	ns
E/A	1.1±0.4	1.3±0.4	ns
E/e'	**7.6±2.4**	**6.5±1.7**	**p<0.05**
PASP(mmHg)	24.1±8	21±7	ns
sec $\varepsilon_{pos\ peak}$ (%)	**18.4±4**	**21.4±7.6**	**p<0.05**
$\varepsilon_{neg\ peak}$ (%)	12.9±2	13.6±3	ns
$\varepsilon_{pos\ peak}$ (%)	**31.3±4.2**	**35±7.6**	**p<0.01**

$\varepsilon_{pos\ peak:}$ positive peak left atrial longitudinal strain; sec $\varepsilon_{pos\ peak:}$ second positive peak left atrial longitudinal strain; $\varepsilon_{neg\ peak}$ – negative peak left atrial longitudinal strain; A: mitral inflow late pulsatile Doppler wave; E: mitral inflow early pulsatile Doppler wave; e': early diastolic mitral annular velocity; EF: ejection fraction; LA: left atrium; PASP: pulmonary artery systolic pressure; SSc: systemic sclerosis.

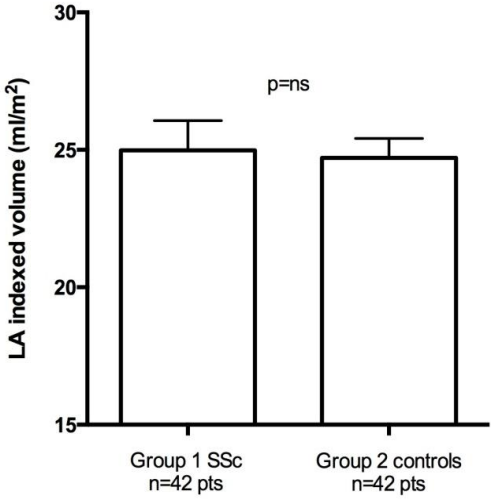

Figure 7 Differences in the indexed left atrial volume between SSc and control patients.

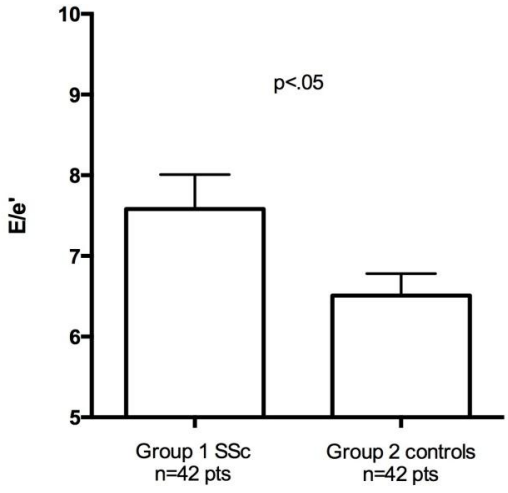

Figure 8 Differences in E/e' between SSc and control patients.

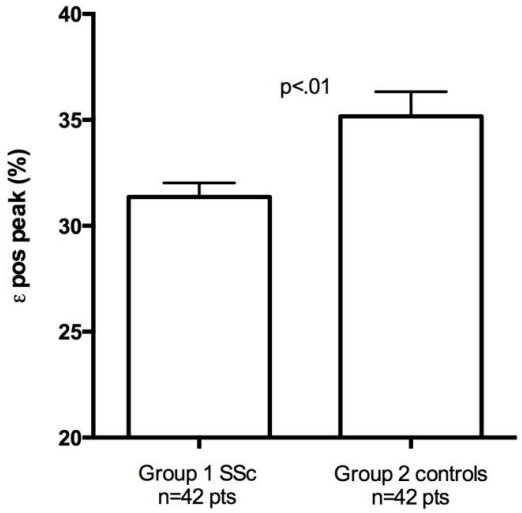

Figure 9 Differences in peak positive LA longitudinal strain ($\varepsilon_{pos\ peak}$) between SSc and control patients.

5.2. Results of the exercise echo study

From 220 patients, 22 were excluded: 13 for inadequate echocardiographic image quality, 5 for inability to perform physical activity, 3 for previously unknown cardiac conditions, and 1 for resting dyspnea (NYHA class III). In thirteen patients, tricuspid regurgitation signal was present, and measurement of TRV was not measurable both at rest and/or at peak stress (feasibility 93%), and 21 patients were excluded because resting PASP was high, above 40 mmHg (Figure 5). Specification of the remaining 164 patients is shown in Table 6.

Male, n (%)	14 (8.5)
Age (y)	58 ± 13
NYHA class II, n (%)	57 (35)
Years since diagnosis	11 ± 9
Diabetes mellitus, n (%)	6 (4)
Hypertension, n (%)	39 (24)
Diffuse form, n (%)	41 (25)
Scl-70 antibodies, n (%))	41 (25
NT-proBNP (ng/L)	144 ± 160
FEV1/VC (%)	94 ± 13
DLCO (%)	70 ± 19

Table 6 Clinical characteristics of patients

During stress test no major, serious complications occurred. No patients had ischemic positive test considering ECG and echo criteria.

Exercise echo results

Among the 164 patients who underwent EDE study, 69 (42%) patients showed a PASP at peak exercise ≥50 mmHg. Mean peak TRV was 332 cm/s (range 185–533 cm/s).

Changes in PASP characteristics from rest to peak exercise for individual patients are shown in Figure 10.

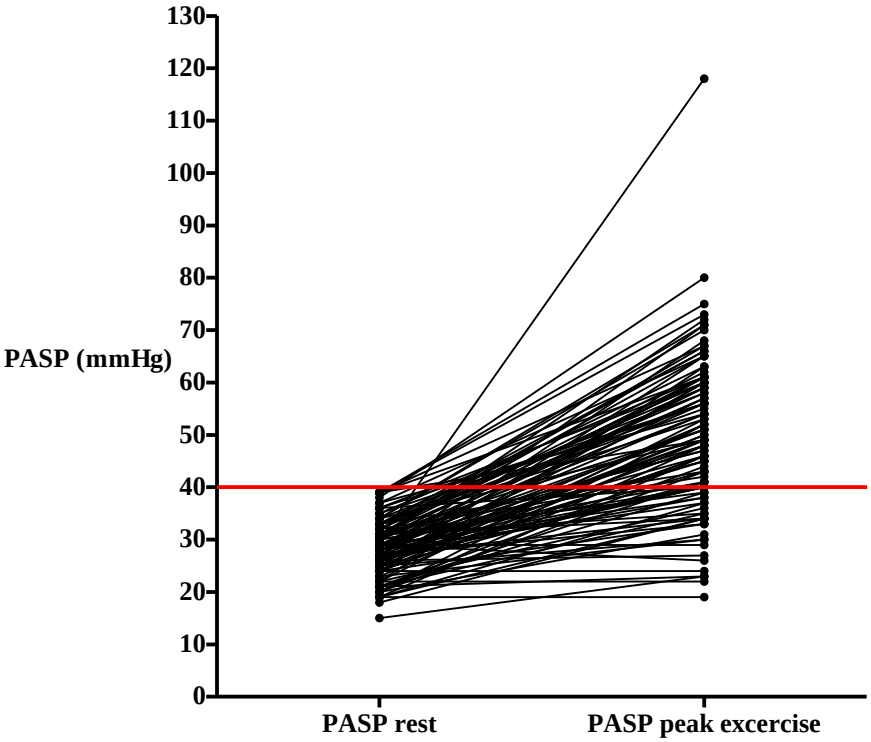

Figure 10 Changes in PASP measurements from rest to peak exercise for each individual patient.

Table 7 represents the different characteristics between patients with peak PASP < 50 and ≥50 mmHg. Univariate analysis showed that age, presence of ILD, values of resting PASP, and both right and left diastolic dysfunctions were predictors of PASP elevation over ≥50 mmHg (Table 8). According to observation, peak PASP was higher in patients older than 50 years (52 ± 14 mmHg vs. 43 ± 8 mmHg, p< .001), with LV E/e′ >8 (59 ± 17 mmHg vs. 48 ± 13 mmHg, p< .01), with RV E′/A′ <0.8

(52 ± 11 mmHg vs 44 ± 9 mmHg, p< .001), and with ILD (54 ± 14 mmHg vs. 48 ± 12 mmHg, p< .05).

	Peak PASP<50 mmHg (n = 95)	**Peak PASP≥50 mmHg (n = 69)**	**p value**
Ejection fraction (%)	65± 6	66 ± 6	NS
Wall motion score index	1 ± 1	1 ± 1	NS
LV end-diastolic volume/BSA (mL/m2)	51 ± 11	52 ± 11	NS
LV end-systolic volume/BSA (mL/m2)	16 ± 7	16 ± 6	NS
Left atrium volume/BSA (mL/m2)	24 ± 8	27 ± 12	NS
E/e′	**6.6 ± 2.0**	**7.5 ± 2.6**	**<.05**
TAPSE (mm)	22 ± 4	21 ± 3	NS
RV TDI peak systolic velocity	14 ± 4	14 ± 3	NS
Acceleration time (ms)	**124 ± 29**	**106 ± 34**	**<.01**
RV E′/A′	**0.96 ± 0.35**	**0.72 ± 0.16**	**<.001**
CI (L min−1 m−2)	2.5 ± 0.6	2.7 ± 0.8	NS
PCWP (mm Hg)	**10 ± 2**	**11 ± 3**	**<.05**
PVR (WU)	1.7± 0.4	1.7 ± 0.3	NS

Table 7 Differences in resting echocardiographic data between patients with peak PASP <50 mmHg and those with peak PASP ≥50 mmHg.

	Peak PASP<50 mmHg (n = 95)	Peak PASP≥50 mmHg (n = 69)	p value
TAPSE (mm)	26 ± 4	26 ± 4	NS
RV TDI peak systolic velocity	21 ± 6	22 ± 7	NS
Acceleration time (ms)	**98 ± 22**	**85 ± 27**	**<.05**
PCWP (mmHg)	**10 ± 2**	**11 ± 3**	**<.05**
PVR (WU)	**2.0 ± 0.7**	**2.3± 0.6**	**<.05**
Systolic arterial pressure (mmHg)	**157 ± 24**	**168 ± 20**	**<.01**
Heart rate	**138 ± 21**	**129 ± 16**	**<.01**
Oxygen saturation (%)	97 ± 4	97 ± 3	NS
Test performance (W)	79 ± 26	72 ± 24	NS
Test duration (min)	5.8 ± 2.0	5.4 ± 1.8	NS
mPAP/CO (mm Hg L−1 min−1)	**3.1 ± 2.7**	**6.5 ± 5.5**	**<.001**

Table 8 Differences in stress echocardiographic data between patients with peak PASP <50 mmHg and those with peak PASP ≥50 mmHg.

	Univariate analysis HR (95% CI) p value		Multivariate analysis HR (95% CI) p value	
Age (y)	**1.06 (1.03-1.09)**	**<.001**	1.03(0.94-1.12)	NS
Raynaud's phenomenon	0.56(0.23-1.7)	NS		
Diffuse form	1.24 (0.61-2.5)	NS		
ILD	**2.3(1.01-5.3)**	**<.05**	0.7 (0.9-5.4)	NS
DLCO (%)	0.99 (0.97-1.01)	NS		
Resting PASP (mm Hg)	**1.2 (1.1-1.3)**	**<.001**	**1.2 (1.1-1.3)**	**<.05**
E/e′	**1.19 (1.01-1.4)**	**<.05**	0.9 (0.5-1.8)	NS
RV E′/A'	**0.03 (0.003-0.36)**	**<.01**	**0.25 (0.002-0.4)**	**<.01**
Peak CI (L min−1 m−2)	1.1 (0.86-1.5)	NS		

Table 9 Univariate and multivariate analysis to predict peak PASP ≥50 mmHg.

In a subgroup of 89 patients, noninvasive hemodynamic parameters were also measured (PVR, CO). Feasibility of the acquisition was 95% at rest and 73% at peak stress. Peak PVR ≥3 WU was present in 11% of patients with peak PASP ≥50 mmHg, which represents about 5% of the total population. Univariate analysis showed that none of the parameters predictors of peak PASP ≥50 mmHg were predictors of PVR ≥3 WU (Table 9). Pressure/flow relationship in this subgroup is shown in Figure 11. The mPAP/CO ratio was 4.9 ± 4.7 mmHg L−1 min−1. The mPAP/CO ratio did not correlate with age, LV diastolic dysfunction, or ILD but correlated with RV diastolic dysfunction (expressed by TDI E′/A′, R = −0.33, p< .05).

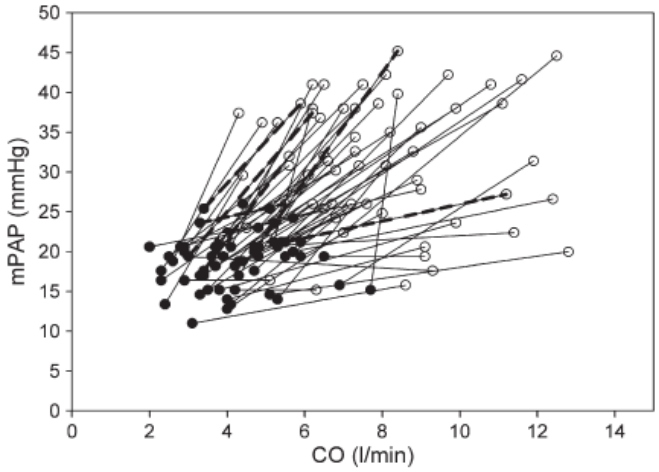

Figure 11 Pressure/Flow relationship at rest and at peak stress. Patients with peak PVR ≥3 WU are highlighted by dotted lines.

5.3. Results of the follow up study

Mean follow-up time between the two EDE was 21 ± 12 months, during which two deaths occurred. Seven patients (19%) developed resting PASP increase (mean time follow-up 22 ± 15 months), which may suggest that the previous abnormal response to exercise in these patients predicted subclinical PAH. Twenty-four patients (65%) showed the same behavior as at a previous examination, 2 patients (7%) had died, and 4 patients (11%) did not confirm the exercise-induced PASP increase, with 3 patients having started calcium-antagonist therapy. The figurative results are shown in Table 12.

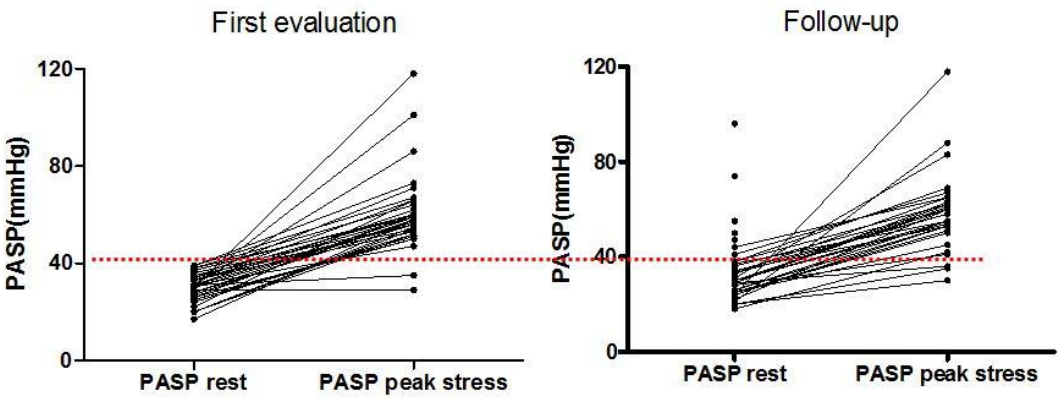

Figure 12 PASP characteristics of 37 patients during follow up stress echocardiography study.

6. Discussion

6.1. Discussion of the LA study

Our results suggest that SSc patients with normal LV systolic function and without any significant cardiac abnormalities have reduced LA STE values compared to the control group. STE may be a non-invasive, feasible method to assess early LA dysfunction in SSc patients. Such cardiac mechanic alterations can be detected in the absence of changes in LA size.

Pathophysiological mechanisms of LA dysfunction in SSc

LA is a reservoir, a conduit and a pump, which plays an important role in modulating LV filling [79] [80]. According to the Frank-Starling law, LA pump function increases in the presence of mild LV diastolic dysfunction and then significantly decreases when LV diastolic dysfunction progresses to moderate or severe degrees [80] [79] [81]. Global LA function parameters such as LA dimensions, area and volume are good prognostic markers for predicting LV diastolic dysfunction [82] [83]. However, an impairment of these parameters may appear late in the course of the disease. STE provides early, detailed information about LA mechanics. Several studies have demonstrated the usefulness of STE in assessing LA function [84] [85] [86] [87], Inaba Y et al. have shown that LA strain rate correlate with age, LA size and diastolic dysfunction in patients with atrial fibrillation [85]. LA strain rate was also an accurate predictor of recovery of LA contractility after cardioversion [86], and LA ε pos peak was a significant predictor of postoperative atrial fibrillation in patients undergoing aortic valve replacement [87].

In SSc, the myocardium is often characterized by patchy fibrosis, secondary to both repeated ischemia and/or immuno-inflammatory damage, inexorably leading to diastolic dysfunction. Episodes of ischemia in SSc are usually not due to epicardial coronary artery stenosis, but rather to microvascular dysfunction[35] since characteristic SSc vascular lesions result in major impairment of the microcirculation [9]. In addition to these fixed abnormalities, vasospasm of the small coronary arteries or arterioles may play a significant role in the early myocardial alterations in SSc [36]. Diastolic dysfunction is frequent in SSc [37] [38] [39] and is correlated with the duration of the disease[40], whereas systolic dysfunction is present in only a minority of SSc

patients [41]. Myocardial fibrosis in SSc has been reported in 50–80% of necropsy series [88 89 90 91 92 93 94], however, establishing the diagnosis of myocardial fibrosis can be challenging by non-invasive imaging. Up-to-now MRI has been considered to be the non-invasive gold standard imaging technique to assess myocardial fibrosis. Previous studies have observed high percentages of myocardial fibrosis at MRI through detection of late gadolinium enhancement, although with different prevalence [88 89 90 91 92 93 94] (95–101). In a recent study, Ntusi et al. [95] **have** found that although biventricular size and global function are preserved, there is impairment in the peak systolic circumferential and peak diastolic strain rate of the LV, which inversely correlates with diffuse myocardial fibrosis indices at MRI.

Clinical implications

Characterization of the LA has important prognostic implications [96 97]. LA enlargement is known to be associated with increased mortality in the general population [83]. Impaired LA function might also be an important predictor for the development of non-valvular atrial fibrillation or other supraventricular arrhythmias [98], which are not infrequent in SSc patients. In our population, no patients had atrial fibrillation or other significant supraventricular arrhytmia. It would be interesting to see whether LA dysfunction can predict the development of supraventricular arrhythmia in this population. In our study, the LA reservoir (ε pos peak) and conduit (sec ε pos peak) function are impaired compared to controls. We may suspect that myocardial involvement and changes in the ECM may lead to early dysfunction of LA, even before the LA starts to dilate, since there was no difference between LA volume in the two groups. Early alternations of LA function can also reflect LV involvement in SSc. Myocardial fibrosis and consequent LV diastolic dysfunction leads to the decrease of LA reservoir and conduit function and to the increase of LA pump function (Frank-Starling law). The clinical meaning of an early assessment of LA mechanism in SSc by STE is still undetermined, and follow-up studies with a larger number of patients are needed to evaluate whether this finding has any prognostic implication. However, the use of a non-invasive, non-ionizing, and relatively simple method that lets us track LA function characteristics over time is intriguing. Speckle tracking allows a very sensitive estimation and monitoring of LA mechanism that may help us understand the progressive steps in the ongoing pathophysiological process leading to overt myocardial dysfunction. STE measurement has been demonstrated to correlate also with the extent of LA fibrosis

and remodeling [99]. This new echocardiographic analysis of LA function might also be employed as a biomarker in therapeutic trials to assess efficacy in serial evaluations and follow-up of new therapeutic options.

Comparison with previous studies

To the best of our knowledge, this is the first study evaluating LA function in SSc by STE strain analysis. Several reports have previously described subclinical LV and RV abnormalities in SSc patients, using TDI parameters and strain rate imaging[38 39, 100 101 102 103 104 105]. Mele et al. have shown that TDI-derived myocardial systolic deformation indices, based on strain and strain rate analysis and E/e' ratio, are valuable approaches to detecting cardiac involvement in asymptomatic SSc patients [39]. D'Andrea et al. have further confirmed that STE imaging can detect both RV and LV early myocardial involvement in SSc, as well as coronary flow reserve and brachial artery flow-mediated dilatation, as signs of early vascular impairment[103]. In another study, Shattke et al. have employed isovolumetric acceleration, a novel tissue Doppler parameter, to detect early RV systolic impairment in SSc patients without PAH [101]. Kepez et al. have found that SSc patients without PAH and overt clinical cardiac involvement have reduced myocardial strain and strain rate, despite normal 2D, conventional Doppler and TDI parameters [100] . Interestingly, Yiu et al. have revealed that subtle LV dysfunction, still assessed by STE strain analysis, is related to lower functional capacity and rhythm disturbances in patients with SSc [104]. Recently, Spethmann et al. have highlighted that LV systolic impairment, as assessed by strain imaging, is primarily due to alterations in the basal LV segments in SSc patients with preserved LVEF [105]. All previous studies on speckle tracking in SSc patients have never addressed LA function or dimensions, because they focus on LV or RV function. It is true that assessment of both left and right ventricular dysfunction is the main target of cardiac assessment and represents the final result of all potential pathophysiological impairments. However, many of these alterations can be intercepted earlier by means of changes of the LA region mechanical characteristics and function. A few previous studies have evaluated LA characteristics in SSc patients: Dimitroulas et al. have shown that LA volume may be a useful non-invasive marker for the prediction of elevated PAP in these patients [106]. Impairment of electromechanical LA functions, including a prolonged intra-interatrial electromechanical delay and higher P-wave dispersion have also been

found in SSc patients, compared to a control group, confirming the increased risk of these patients for developing supraventricular arrhythmias [106].

Study limitations

Some limitations of the present study should be highlighted. The study population was limited in size, since SSc is a relatively rare disease. Additionally, we excluded patients with signs of overt cardiac involvement, which could have affected strain and strain rate measurements.

Therefore, our results cannot be extended to patients with heart failure or other concomitant cardiac disease. Finally, the study population was relatively old. It is well known that the occurrence of diastolic dysfunction increases with age. However, age was not different in the two groups, and interestingly, we found significant correlation between ε pos peak and age only in the control group and not in SSc patients. A main limitation was the lack of normal cut-off values for strain imaging. However, two studies report LA strain values in a healthy population, which can be considered as reference values [70 107 108].

Although very promising, a current significant limitation of strain imaging is inter-vendor variability; thus, up-to-date, strain analysis is still confined to the research field and has not yet been implemented in routine clinical practice

6.2. Discussion of the EDE study

Exercise-induced increase in PASP is frequent in patients with SSc and seems to be related to many factors affecting diastolic LV and RV function, whereas exercise induced increase in PVR is less frequent and not related to these parameters. Non-invasive hemodynamic evaluation by EDE has an established role in valvular heart disease, diastolic heart failure. Exercise induced PAH is still a poorly understood entity in patients at risk for developing PAH. It is controversial whether it could be considered an early preclinical functional phase, inexorably leading to resting PAH [53]. The recent American and European Guidelines reflect this conflict: the definition of exercise-induced PAH, always present in the past guidelines, has been abandoned because it is “not supported by enough published data”. However, exercise echocardiography is “supported by an objective interest and should be used in a

research setting". Actually, in the past few years, many reports on the use of exercise test both with RHC and EDE in different diseases[109], [110] (78–81,102,103) [111], [112] [113] [114] [115] athletes[75], or healthy volunteers have been published [116]. There are concordant data from different study groups about a significant percentage of patients with SSc with an abnormal increase of PASP at peak exercise [111] [112] [117]. However, there is consensus that the prevalence of apparently inappropriate pulmonary pressure response to exercise is too high, occurring in about 50% patients, whereas the incidence of PAH in this population is only about 5% to 10%. Our multicenter study with quite large population confirms these previous reports. Because peak PASP is also determined by the physiological increase in pulmonary output[75] [118], normalizing PASP for CI may reduce the incidence of false-positive results. Abnormal increase in PASP can be secondary to pulmonary abnormalities[119], therefore, careful history taking and other investigations should be obtained to identify the presence of ILD. Pulmonary artery systolic pressure is also influenced by the diastolic feature of LV - high LV filling pressures, which may be consequent to LV; thus, all parameters affecting LV diastolic function, such as age [120] [121] **[122]** or systemic arterial hypertension, may potentially influence peak PASP. The increase in PCWP with exercise is remarkable in healthy older subjects and may be responsible for PAP values >40 mmHg[122]. Similarly in our population, both resting and peak PASPs were significantly higher in patients older than 50 years. Cardiac involvement, LV myocardial fibrosis, which is not infrequent in SSc plays a role and determines diastolic dysfunction. All these significant influences may explain why peak PASP does not necessarily represent the best parameter to identify the subgroup of patients likely to develop resting PAH. When we consider a parameter less affected by other conditions such as PVR, the number of patients with an abnormal increase in PVR at peak stress is significantly lower, accounting for about 5% of the initial study group, a percentage consistent with the reported prevalence of hemodynamically proven PAH in large cohorts of patients with SSc [46]**.** Pulmonary involvement may also lead to an increase in PVR. In our population, the presence of ILD did not predict high peak PVR, probably because the degree of pulmonary involvement was never severe, and because this information is somehow part of RV diastolic function evaluation. However, in patients with significant ILD, PASP and PVR should not be considered as reliable indices of early PAH because the increase in both parameters may be caused by different pathophysiological basis. Our data are similar to a recently

published article of D'Alto M at al [74]**.** In this work, the authors underline that caution is necessary in interpreting the results of EDE in patients with SSc because an abnormal pulmonary vascular reserve might be related to the presence of mild to moderate ILD or to subtle signs of LV diastolic dysfunction. Moreover, they underline that the RV E'/A' ratio was lower in patients with SSc than in control subjects. Our data show that resting RV E'/A' ratio is a determinant of exercise-induced increase in PASP, although it could be debated whether this early alteration in the diastolic function is more likely to be the cause or the consequence of exercise-induced increase in PASP. Clinical implications of the present work are of importance because it exhorts to a more conscious use of EDE in patients at high risk for developing PAH. It is now quite clear that peak PASP is not enough to characterize these patients and that a more thorough hemodynamic evaluation, together with an accurate history taking and clinical assessment, is mandatory. RHC remains the reference standard for clinicians to set up the diagnosis and establish the indication to start appropriate, PAH specific therapies, but the procedure is unfortunately not without morbidity and cannot be routinely proposed. Our study outlines that heterogeneous hemodynamic mechanisms may underlie the same phenotypic markers of exercise-induced PASP increase. Diastolic dysfunction caused by primary myocardial involvement or other determinants and primary inappropriate increase in pulmonary resistance have different underlying causes, heterogeneous hemodynamic mechanisms and conceivably different therapeutic and prognostic correlates. The different mechanisms may coexist and overlap in the individual patient and also have a variable role in different stages of the disease. Some limitations of the present study should be highlighted. The cutoff value of 50 mmHg to identify an abnormal exercise-induced PASP is arbitrary because no established cutoff exists. We chose this value to be as specific as possible to identify patients with an abnormal vascular response. It seems based on previous studies that there might be a bimodal distribution of exercise-induced PASP, with a second peak at about 50 mmHg that is more likely to identify patients reacting to exercise in a different and possibly pathological way [74 115] (76,110**).** Non-invasive measurement of PVR is an indirect evaluation, and although a good correlation has been shown with invasive values [123 124] Doppler-derived PVR is not universally acknowledged as a reliable non-invasive surrogate for PVR. Moreover, previous studies have already used non-invasive PVR evaluation at peak exercise, [119 125] but the formula has been

validated only at rest [71]. Inferior vena cava diameter and collapsibility index to estimate right atrial pressure were not reassessed at peak exercise, possibly leading to PASP underestimation.

6.3. Discussion of the follow-up study

Although exercise testing (including EDE) has not been included to the recent guidelines about PAH, our preliminary results suggest that exercise stress echocardiography may play a role in the diagnostic evaluation of PAH in patients with SSc. In the pulmonary vasculature, more than 70% of the vascular bed is lost before the pressure reaches the pathological level at rest[126]. The exact mechanisms of increased PAP during exercise are unknown. However, they are likely to be due, at least in part, to the early stages of endothelial dysfunction and vascular remodeling in the pulmonary circulation that may have little initial effect at rest but become more evident during exercise. From a pathophysiological point of view, pulmonary artery systolic pressure under physical exercise depends mainly on the inability of the SSc pulmonary vascular bed to dilate and consequently would reflect the peculiar stiff vascular system characteristic of SSc[127]. From a clinical point of view, our data suggest that this inability, when exceeding a defined value, is predictive for the development of symptomatic PH during the follow-up. With EDE the identification of patients susceptible to future PAH has already been studied. However, the data are conflicting. Data from the UK national registry of CTD-PAH showed that ~20% of SSc patients with exercise during right heart catheterization (mean PAP >30 mmHg) progressed to PAH requiring advanced therapy within 3 years[49]. Moreover, Steen et al.[114] have demonstrated that EDE unmasked PAH in a substantial number of scleroderma population at increased risk for PAH (DLCO<60% predictive, forced vital capacity (FVC)/DLCO>1.6 and estimated systolic PAP 30–50 mmHg). In this study, 44% of the patients had an increase of >20 mmHg in right ventricular systolic pressure on echocardiography during exercise. These patients then underwent RHC and 62% were found to have elevated pressures. Dehnert C et al. have studied individuals susceptible to high altitude pulmonary edema in normal sea level using hypoxia and EDE in combination. Their study demonstrates that EDE in normoxia is sufficient to detect individuals susceptible to high altitude pulmonary edema[128]. Additionally, Gruenig et al.[115] have shown that exercise elevation of pulmonary

pressures is more common among first degree relatives of individuals with familial PAH, and in some probands progression to overt PAH has been observed.

In contrast, a study by Codullo et al.[129] using EDE and follow-up in a low-risk scleroderma population (asymptomatic without overt PAH; tricuspid regurgitation velocity <3 m/s-[1]) have found that only one in 19 patients developed PAH requiring advanced therapy when using an increase in systolic PAP of ≥18 mmHg after exercise as the criteria [74]. However, this study has analyzed a different patient population (low risk, versus intermediate and high risk in our study), and the conclusion has been drawn after the analysis of a very small patient population.

7. New observations

1. 2D speckle-tracking echocardiography is a sensitive tool to assess impairment of LA mechanics, which is detectable in the absence of changes in LA size and volume, and may represent an early sign of cardiac involvement in patients with SSc.

2. Exercise-induced increase in PASP occurs in almost half of the patients with SSc with normal resting PASP. Peak exercise PASP is affected by age, ILD, and right and LV diastolic dysfunction, and only in 5% of the patients, it is associated with an increase in PVR during exercise, suggesting heterogeneity of the mechanisms underlying exercise-induced PAH in SSc.

3. EDE is a feasible test to track serial changes over time in PASP in patients at high risk of developing PAH. Exercise-induced PASP increase is frequently associated with poor outcome or development of overt resting PASP increase.

8. References

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