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Original Article

Impact of myocardial fibrosis on left ventricular diastolic function in patients with systemic sclerosis

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Abstract: Introduction: Patients with systemic sclerosis have a high prevalence of myocardial fibrosis, which has a potential negative impact on cardiac function. Diastolic dysfunction is a frequent echocardiographic finding in patients with scleroderma. The aim of this study was to evaluate the possible influence of myocardial fibrosis on the diastolic function of the left ventricle in patients with systemic sclerosis. Material and methods: Twenty seven patients with diffuse or limited cutaneous scleroderma underwent transthoracic echocardiography using Doppler and tissue Doppler parameters for diastolic function assessment and cardiac delayed-enhancement magnetic resonance imaging (DE-MRI) for myocardial fibrosis identification and characterization. Results: The prevalence of diastolic dysfunction was 22.2% and of myocardial fibrosis 81.4%. All patients with diastolic dysfunction had impaired relaxation of the left ventricle. There was a statistically significant correlation between the presence of diastolic dysfunction and the number of left ventricular segments affected by myocardial fibrosis ($r = 0.405$, $P = 0.036$), and between the amplitude of Am wave and the presence of endocardial fibrosis. When compared according to the presence or absence of diastolic dysfunction, patients with diastolic dysfunction had a significantly higher number of LV segments affected by myocardial fibrosis (4.25 ± 3.25 vs. 3 ± 3, $P = 0.044$). The presence of an increased E/Em ratio (value > 6) had a sensitivity of 32% and a specificity of 100% in predicting the presence of myocardial fibrosis at DE-MRI (area under the curve = 0.689). Conclusion: Myocardial fibrosis is frequently encountered among scleroderma patients. Its presence is associated with diastolic dysfunction of the left ventricle.

Keywords: Scleroderma, diastolic dysfunction, myocardial fibrosis, DE-MRI, echocardiography

Introduction

The most important trait of systemic sclerosis is fibrosis. It may involve all the major organs and systems, including the heart [1-4]. The development of myocardial fibrosis is believed to be secondary to repeated episodes of small coronary arteries vasospasm, leading to microvascular damage, with subsequent fibroblast proliferation [5-7]. Identification of myocardial fibrosis is important, since patients with cardiac involvement have a worse prognosis [8].

Based on histopathological studies, the prevalence of myocardial fibrosis in scleroderma patients can reach up to 70% [9]. This finding was later on confirmed by studies using delayed-enhancement magnetic resonance imaging (DE-MRI), that reported a similar prevalence of myocardial fibrosis, of up to 66% [10-19]. As a result, DE-MRI has turned out to be a non-invasive, highly sensitive tool for identification of myocardial fibrosis in systemic sclerosis patients.

Diastolic dysfunction is a frequent echocardiographic finding in patients with scleroderma [20-23]. A complete assessment of diastolic function using Doppler and tissue Doppler parameters is important, since LV diastolic dysfunction is a marker of increased mortality in systemic sclerosis patients [20]. Factors inde-

pendently associated with LV diastolic dysfunction include disease duration, age, coronary artery disease and systemic hypertension. However, a large nationwide multicentric cohort study has raised concerns about the possible existence of LV diastolic dysfunction in the absence of any other cardiopulmonary diseases, suggesting a specific cardiac involvement in scleroderma patients [24]. Since no diagnostic tool was used in the above-mentioned study for the identification of myocardial fibrosis, the relationship between myocardial fibrosis (presence and characteristics) and the diastolic dysfunction of the left ventricle remains incompletely described.

The aim of this study was to evaluate the possible influence of myocardial fibrosis diagnosed by DE-MRI on the diastolic function of the left ventricle in patients with systemic sclerosis.

Material and methods

Patient population and data collection

Thirty six patients with a diagnosis of systemic sclerosis, either the diffuse cutaneous or the limited cutaneous subtype entered the study. All patients gave informed consent before entering the study. These patients were randomly selected from a larger cohort of 134 patients with systemic sclerosis, evaluated in our center between October 2011 and February 2015.

All patients underwent a complete physical exam, blood sample testing, 12-lead ECG recording, transthoracic Doppler echocardiography, spirometry, a standard chest X-ray and, when considered appropriate, a high resolution computer tomography (CT) for pulmonary fibrosis identification. Subsequently, this group of 36 patients underwent cardiac MRI. Delayed enhancement MR (DE-MRI) imaging with gadolinium administration was performed in 33 patients and high-quality images were obtained in 30 patients.

Exclusion criteria were conditions that could affect the diastolic function of the heart: signs and symptoms of overt heart failure, significant left-sided valve disease (moderate or severe mitral or aortic valve stenosis or regurgitation), left ventricular hypertrophy, prior myocardial infarction, pericardial effusion (moderate or large) and chronic kidney disease. As a result, 3 patients were further excluded from the study: one with rheumatic moderate aortic and moderate mitral stenosis, one with moderate pericardial effusion and one with dilative cardiomyopathy and severe mitral regurgitation, reducing the final population to 27 patients.

The study protocol was approved by the Ethics Committee of "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania.

Clinical data were collected from the patient's clinical records. The cardio-vascular evaluation was performed by a full time cardiologist. MRI images were interpreted by a single physician with experience in cardiac MRI.

Evaluation of patients

Laboratory analyses: Blood sample testing included a complete blood count, erythrocyte sedimentation rate (ESR), renal function tests (blood urea nitrogen, creatinine), electrolites (Na^+, K^+, Ca^{2+}, Cl^-), glycemia, lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides), uric acid, coagulation parameters (activated partial thromboplastin time, Quick time, INR), hepatic tests (GOT, GPT, alkaline phosphatase, gamma-glutamyl transferase, bilirubin, total protein levels and albumin) and immunological tests (serum complement levels, C3, C4, circulating immune complexes, IgA, IgG, IgM, rheumatoid factor, antinuclear antibodies and anti-Scl 70 antibodies).

12 lead ECG: The 12 lead ECGs were recorded using an Esaote P8000 electrocardiograph, with an ECG amplifier sensitivity of 10 mm/mV, at a speed of 25 mm/s. All ECGs were carefully screened for the presence of supraventricular or ventricular arrhythmias, conduction disorders, QRS axis deviations, signs of atrial or ventricular hypertrophy, myocardial ischemia, low QRS voltage, prolonged QRS and QTc duration.

Transthoracic Doppler echocardiography: Echocardiographic examinations were performed using an Esaote MyLab™ X-View 50 system, with a 7.5-10 MHz transducer. Image acquisition was done using standard bidimensional mode, M-mode, Doppler and color Doppler mode. The assessed parameters were: chambers' size and volume, wall thickness, ejection fraction of the left ventricle, mitral flow pattern,

systolic function of the right ventricle, global and regional motion abnormalities, systolic, mean and diastolic pulmonary aterial pressure (sPAP, mPAP, dPAP), the presence of valve disease (stenosis and regurgitations) and the presence of pericardial effusion.

Parameters used for the evaluation of the diastolic function of the left ventricle were recorded from the apical four-chamber view as follows: E wave amplitude (m/s) and A wave amplitude (m/s) using the pulsed-wave Doppler technique, with the volume sample placed at the tip of the mitral valve; E/A ratio, calculated by dividing the value of E wave to the value of the A wave; E wave deceleration time (EDT) (ms), measured from the peak to the end of the E wave, at the intersection with the baseline; myocardial E wave velocity (Em lat) (m/s) recorded during the early phase of diastole and myocardial A wave velocity (Am lat) (m/s) recorded during the late phase of diastole, measured using the tissue Doppler technique, with the caliper placed at the lateral wall of the mitral ring; Em/Am ratio and E/Em ratio, calculated by dividing the value of Em to Am, and E to Em, respectively. The E/Em ratio was used to assess left-sided filling pressures. Normal values for these parameters were considered according to the recommendations of the American Society of Echocardiography [25], taking into account the influence of age on these values. An E/Em ratio < 8 was considered normal, E/Em ratio > 15 was considered increased, and between 8 and 15 a "grey zone" was considered present.

Normal diastolic function of the left ventricle was defined by the presence of a normal E and A wave amplitude, E/A ratio > 1, normal EDT, Em/Am > 1 and normal E/Em ratio. Patients with an E/A < 1 but with Em/Am > 1 and normal EDT were also considered to have normal diastolic function.

Based on the above mentioned parameters, 3 types of diastolic dysfunction were defined: impaired relaxation, pseudo-normal and restrictive filling. Impaired relaxation was characterized by an E/A ratio < 1, Em/Am < 1 and EDT > 240 ms. Pseudo-normal filling was characterized by an E/A ratio > 1, Em/Am ratio < 1 and EDT > 240 ms. Restrictive filling was characterized by E/A ratio > 2, Em/Am < 1 (reversible form) and EDT < 140 ms or Em/Am > 1 (irreversible form), increased E/Em ratio and EDT < 140 ms.

Mild pulmonary hypertension was defined as a sPAP between 35-49 mmHg, moderate when between 50-69 mmHg and severe when ≥ 70 mmHg.

Cardiac MRI: The aquisition of MR images was performed using a 1.5 T MR scanner (Intera, Philips, Best, The Netherlands) in the 4 axis of the heart: long axis (LA), short axis (SA), apical 4 chamber (4CH) and aortic root plane (AR). T2-weighted imaging was performed with double-inversion prepulse black blood STIR sequences in SA and A4C views. Cine images were acquired in the SA, 4CH and AR planes.

The assessed parameters were: chamber size and wall thickness (mm), the systolic function of the LV-including the ejection fraction (EF%), the end-diastolic and end-systolic volumes of the LV (ml), stroke volume (ml), cardiac output (l/min)-LV myocardial mass (g and g/m^2) and the presence of pericardial effusion (small, moderate, large).

After administering a bolus of 0.1 mmol/kg of gadolinium, perfusion at rest was evaluated.

The assessment of the presence of myocardial fibrosis was carried out using delayed enhancement MR (DE-MRI) in 3 axes (SA, LA, 4CH), after injecting a second bolus of 0.1 mmol/kg of gadolinium. When present, fibrosis was assessed in terms of distribution and pattern. The distribution was evaluated using the standard segmentation of the left ventricle (16 segments), according to the Standardized Myocardial Segmentation and Nomenclature for Tomographic Imaging of the Heart [26]. In terms of pattern, the types of fibrosis described were: focal, multifocal, linear and diffuse.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS Inc. Chicago, Illinois) version 20. Descriptive statistics were used to summarize the characteristics of patients. Shapiro-Wilk test was used to assess normality for all continuous variables. For normally distributed data, results are expressed as mean ± standard deviation (SD) and for non-normally distributed variables,

Tabel 1. General characteristics of scleroderma patients

Patients' characteristics	Diffuse cutaneous scleroderma (n=14)	Limited cutaneous scleroderma (n=13)	Total (n=27)
Gender, female n (%)	12 (85.7)	12 (92.3)	24 (88.8)
Mean age (years)	47.9 ± 12.4	49.5 ± 9.6	48.7 ± 10.9
Disease characteristics			
Skin score, median (range)	18 (3-35)	3 (0-21)	(0-35)**
Onset of Raynaud's phenomenon (years)	2 ± 13	10 ± 7	6 ± 12.5
Onset of non-Raynaud's phenomenon (years)	2 ± 4.5	7 ± 3.5	4 ± 6.62
Auto-antibodies, n (%)			
ANA positive	13 (92.8)	13 (100)	26 (96.3)
Anti Scl70 positive	11 (78.5)	3 (23)	14 (51.8)*
Comorbidities: n (%)			
Arterial Hypertension	1 (7.1)	1 (7.6)	2 (7.4)
Dyslipidemia	4 (28.5)	5 (38.4)	9 (33.3)
Anemia	5 (35.7)	1 (7.6)	6 (22.2)
Thyroid dysfunction	4 (28.5)	1 (7.6)	5 (18.5)
Pulmonary evaluation: n (%)			
Pulmonary fibrosis	10 (71.4)	6 (46.1)	16 (59.5)
Abnormal spirometry results			
Obstructive pattern	7 (50)	3 (23)	10 (37)
Restrictive pattern	3 (21.4)	1 (7.6)	4 (14.8)
ECG			
Sinus rhythm	14 (100)	13 (100)	27 (100)
Heart rate	78 ± 9.6	73.2 ± 10.3	75.4 ± 10.1
LVH	1 (7.1)	0 (0)	1 (3.7)
PAC	2 (14.2)	2 (15.3)	4 (14.8)
PVC	3 (21.4)	0 (0)	3 (11.1)
Conduction disorders	6 (42.8)	3 (23)	9 (33.3)
Ischemia	408.1 ± 19	394.2 ± 23.5	401.4 ± 22
QTc			
Echocardiography			
Left Ventricular Ejection Fraction	59.7 ± 6.8	63.7 ± 5.6	61.6 ± 6.5
Left Atrial Dilation	1 (7.1)	2 (15.3)	3 (11.1)
Right Ventricular Dilation (mild)	2 (14.2)	0 (0)	2 (7.4)
Pulmonary Hypertension: n (%)	4 (28.5)	7 (53.8)	11 (40.7)
Mild	2 (14.2)	6 (46.1)	8 (29.6)
Moderate	1 (7.1)	1 (7.6)	2 (7.4)
Severe	1 (7.1)	0 (0)	1 (3.7)
Pericardial Effusion, small: n (%)	1 (7.1)	1 (7.6)	2 (7.4)
Cardiovascular medication: n (%)			
Beta blockers	1 (7.1)	0 (0)	1 (3.7)
ACE inhibitors/ARBs	2 (14.2)	2 (15.3)	4 (14.8)
Calcium Channel Blockers	4 (28.5)	4 (30.7)	8 (29.6)
PAH-specific medication	1 (7.1)	0 (0)	1 (3.7)

ACE Inhibitors = Angiotensin Converting Enzyme Inhibitors; ANA = Antinuclear antibodies; Anti SCL70 = anti topoisomerase I; ARBs = Angiotensin Receptor Blockers; LVH = Left Ventricular Hypertrophy; PAH = Pulmonary arterial Hypertension; * - $P < 0.05$; ** - $P < 0.01$ when comparing patients with diffuse cutaneous scleroderma with patients with limited cutaneaous scleroderma.

Table 2. Echocardiographic findings of parameters characterizing the diastolic function of the left ventricle in scleroderma patients with vs. without diastolic dysfunction

	Diastolic dysfunction present (n=6)	Diastolic dysfunction absent (n=21)	Total (n=27)
E (m/s)	0.6 ± 0.26	0.77 ± 0.2	0.75 ± 0.19
A (m/s)	0.74 ± 0.21	0.67 ± 0.19	0.69 ± 0.19
E/A	0.83 ± 0.12	1.22 ± 0.25	1.07 ± 0.42**
EDT (ms)	204.3 ± 51.6	200.8 ± 43.1	201.68 ± 44.19
Em lat (m/s)	0.10 ± 0.02	0.15 ± 0.02	0.14 ± 0.03**
Am lat (m/s)	0.13 ± 0.02	0.12 ± 0.03	0.12 ± 0.03
Em lat/Am lat	0.75 ± 0.22	1.08 ± 0.55	1.06 ± 0.27**
E/Em lat	6.45 ± 3	6 ± 1.42	5.1 ± 1.1

E = transmitral E wave; A = transmitral A wave; EDT = Deceleration time of the E wave; Em lat = myocardial E wave of the lateral mitral annulus; Am lat = myocardial A wave of the lateral mitral annulus; LV = left ventricle. * - P < 0.05; ** - P < 0.01 when comparing scleroderma patients with and without diastolic dysfunction.

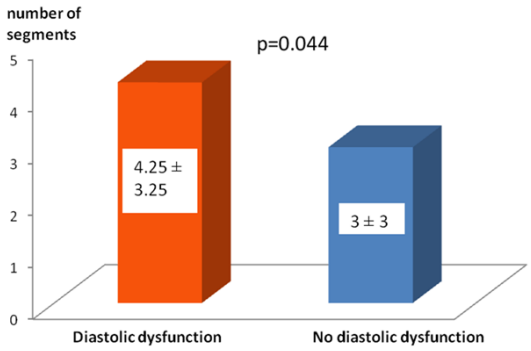

Figure 1. Comparison between the mean number of left ventricular segments affected by myocardial fibrosis detected by cardiac DE-MRI according to the presence or absence of diastolic dysfunction. Red column = scleroderma patients with diastolic dysfunction; blue column = scleroderma patients without diastolic dysfunction.

by median ± interquartile range. Categorical variables are presented as counts and proportions (%).

The chi square test was used to compare categorical variables in the scleroderma population of patients. T-test for independent samples or Wilcoxon-Mann-Whitney U test was used to compare normally distributed, respectively non-normally distributed variables.

Depending on the distribution of data (normal/non-normal), Pearson or Spearman's correlation coefficients were used to assess the relationship between different echocardiographic and MRI characteristics.

Receiver operating characteristic (ROC) curves were used to analyze the accuracy of echocardiographic diastolic function parameters in predicting the existence of myocardial fibrosis at DE-MRI.

A *P* value of < 0.05 was considered statistically significant.

Results

General characteristics of the patients

When compared according to the scleroderma subtype, patients with the diffuse cutaneous subtype had a higher average skin score (18 ± 10.5 vs. 3 ± 7, P = 0.006) and a higher percentage of Anti Scl70 positive tests (78.5% vs. 23%, P = 0.004) compared to patients with the limited cutaneous subtype. There were no other significant differences in what concerns the mean age, gender distribution, symptoms onset, presence of comorbidities, pulmonary evaluation tests, ECG findings, echocardiographic parameters and use of cardiovascular medication between patients with diffuse cutaneous and limited cutaneous scleroderma.

The general characteristics of the patients are presented in **Table 1**.

Left ventricular diastolic function determined by transthoracic echocardiography

The prevalence of diastolic dysfunction in the studied population was 22.2% (n = 6 patients). All patients with diastolic dysfunction had impaired relaxation of the left ventricle. There were no patients with "pseudonormal" or "restrictive filling" patterns.

There were no statistically significant differences in what concerns the echocardiographic parameters characterizing the left ventricular diastolic function between patients with diffuse and limited cutaneous scleroderma. However, when compared according to the presence or absence of diastolic function, patients with left ventricular diastolic dysfunction had a significantly lower E/A ratio (0.83 ± 0.12 vs. 1.22 ± 0.25, P = 0.00004), lower Em values (0.1 ± 0.002 vs. 0.15 ± 0.02, P = 0.00024) and lower

Table 3. MRI findings in scleroderma patients according to the presence or absence of left ventricular diastolic dysfunction

Parameter	Scleroderma patients with diastolic dysfunction (n=6)	Scleroderma patients without diastolic dysfunction (n=21)	Total (n=27)
LVEF (%)	56.5 ± 17.6	58.1 ± 6	57.7 ± 9.4
LV EDV (ml)	128.3 ± 29.4	128.7 ± 27.5	128.6 ± 27.3
LV ESV (ml)	57.9 ± 33.6	53.5 ± 14.7	54.5 ± 19.7
Stroke Volume (ml)	70.6 ± 21	75.2 ± 17.1	74.8 ± 17.8
Cardiac Output (l/min)	5.4 ± 2	5.6 ± 1.2	5.6 ± 1.3
Myocardial Mass (g)	94.6 ± 21.4	80 ± 13.6	83.4 ± 16.4
Myocardial Mass indexed (g/m^2)	52 ± 9.1	49.8 ± 6.8	50.3 ± 7.2
Fibrosis present	6 (100)	16 (76.2)	22 (81.4)
Fibrosis localization			
Epicardial	2 (33.3)	9 (42.8)	11 (40.7)
Mid-wall	5 (83.3)	13 (61.9)	18 (66.6)
Endocardial	1 (16.6)	4 (19)	5 (18.5)
Number of segments involved	4.25 (3-11)	3 (0-7)	4 (0-11)*
Topography			
Basal segments affected, n (%)	14 (29.1)	25 (14.8)	39 (18)*
Middle third segments affected, n (%)	16 (33.3)	23 (13.7)	39 (18)**
Apical segments affected, n (%)	5 (10.4)	14 (8.3)	19 (8.8)
Pattern of fibrosis			
Focal	2 (33.3)	9 (42.8)	11 (40.7)
Multifocal	2 (33.3)	4 (19)	6 (22.2)
Linear	2 (33.3)	7 (33.3)	9 (33.3)
Diffuse	2 (33.3)	2 (9.5)	4 (18.5)

LVEF = Left Ventricular Ejection Fraction; LV EDV = Left Ventricular End-Diastolic Volume; LV ESV = Left Ventricular End-Systolic Volume. * - P < 0.05; ** - P < 0.01 when comparing patients with diffuse cutaneous scleroderma with patients with limited cutaneaous scleroderma.

Em/Am ratio (0.75 ± 0.22 vs. 1.08 ± 0.55) compared to patients with normal left ventricular diastolic function. There was also a trend towards a higher E/Em ratio in patients with diastolic dysfunction, but this did not reach statistical significance (P = 0.09) (**Table 2**).

Cardiac MRI findings in scleroderma patients

The prevalence of myocardial fibrosis in the overall population was high, 81.4% of patients (n = 22) having at list one segment of the left ventricle involved. Fibrosis involved most frequently the middle layer of the myocardium (66.6% of patients), affecting mostly the basal segments (18%) and the middle third segments (18%) of the left ventricle. The most frequently encountered pattern of fibrosis was focal (40.7% of patients), followed by the linear (33.3%), multifocal (22.2%) and diffuse (18.5%).

There were no statistically significant differences in what concerns the presence of myocardial fibrosis between patients with diffuse cutaneous scleroderma and patients with limited cutaneous scleroderma (85.7% vs. 76.9%, P = 0.55). However, patients with the diffuse cutaneous subtype had a lower LV ejection fraction compared to patients with limited cutaneous scleroderma (53.8 ± 8.8 vs. 61.9 ± 8.4, P = 0.02), as well as higher left ventricular end-systolic volumes (61.8 ± 21 vs. 46.6 ± 15.3, P = 0.04). There were no other significant differences between these 2 subgroups of patients in what concerns the different myocardial fibrosis features.

When compared according to the presence or absence of diastolic dysfunction of the left ventricle, patients with diastolic dysfunction had a significantly higher number of LV segments

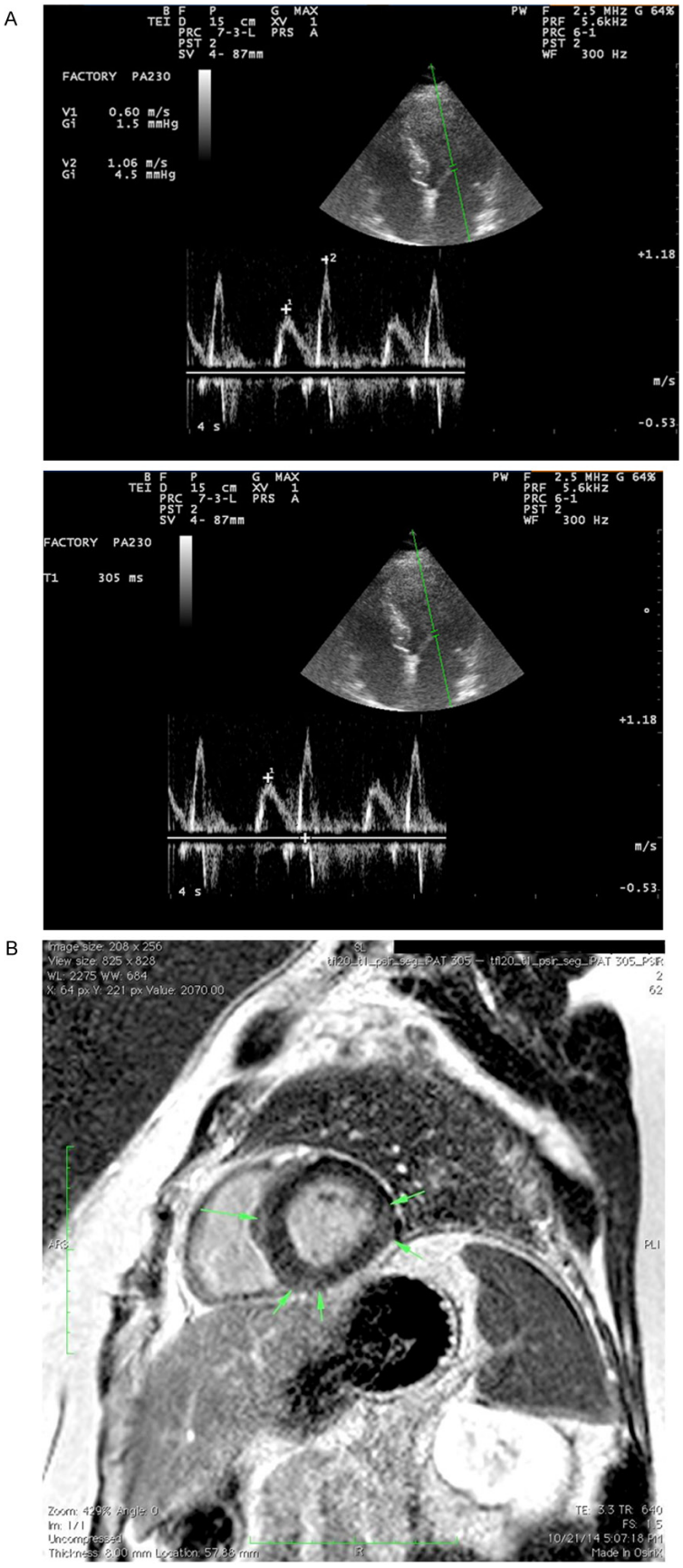

Figure 2. Transthoracic echocardiographic images (A) and cardiac DE-MRI (B) of a patient with limited scleroderma. The echocardiographic images show the amplitude of the E wave (m/s) and A wave (m/s), with a decreased E/A ratio of 0.56 (first echocardiographic image) and prolonged E deceleration time (second echocardiographic image), compatible with the presence of diastolic dysfunction (impaired relaxation of the left ventricle). The Em/Am ratio in this patient was 0.45 (not shown). The DE-MR image (lower panel) shows the presence of extensive intra-mural fibrosis (green arrows).

affected by myocardial fibrosis (4.25 ± 3.25 vs. 3 ± 3, P = 0.044) (**Figure 1**), as well as a higher number of basal and middle-third segments involved (29.1% vs. 14.8%, P = 0.023), (33.3% vs. 13.7%, P = 0.0018) (**Table 3**).

Relationship between left ventricular diastolic dysfunction (echocardiography) and myocardial fibrosis (DE-MRI)

In the present studied population, there was a statistically significant correlation between the presence of diastolic dysfunction and the extent of myocardial fibrosis (r = 0.405, P = 0.036), and between the amplitude of Am wave and the presence of endocardial fibrosis (r = -0.407, P = 0.043). The presence of an increased E/Em ratio (value > 6) had a sensitivity of 32% and a specificity of 100% in predicting the presence of myocardial fibrosis at DE-MRI (area under the curve = 0.689).

In patients with diffuse cutaneous scleroderma, there was a significant correlation between the presence of diastolic dysfunction and the

presence of multifocal (r = 0.679, P = 0.008) and diffuse fibrosis (r = 0.679, P = 0.008), between the presence of pulmonary hypertension and the presence of linear fibrosis (r = 0.589, P = 0.034), between the value of EDT and the presence of epicardial fibrosis (r = 0.584, P = 0.036) and a negative correlation between the values of the inter-ventricular septum, posterior wall of the LV, LV end-systolic and end-diastolic diameter and the presence of endocardial fibrosis (r = -0.704, P = 0.005; r = -0.584, P = 0.028; r = -0.674, P = 0.008; r = -0.563, P = 0.036).

In patients with limited cutaneous scleroderma, there was a positive correlation between the amplitude of the E wave and the number of LV segments affected by myocardial fibrosis (r = 0.593, P = 0.033), the presence of diffuse fibrosis (r = 0.570, P = 0.042) and a negative correlation with the LV mass (r = -0.674, P = 0.012). Significant correlations were also found between the EDT and the presence of diffuse fibrosis (r = 0.570, P = 0.042), the thickness of left ventricular posterior wall and the presence of endocardial fibrosis (r = 0.628, P = 0.022) and between the amplitude of the A wave and the presence of endocardial fibrosis (r = 0.571, P = 0.042).

There were no other significant correlation between LV diastolic function parameters and MRI findings.

A transthoracic echocardiographic image and a cardiac DE-MRI image of a patient with limited scleroderma is showed in **Figure 2**.

Discussion

The present study assesses the relationship between myocardial fibrosis and diastolic dysfunction in patients with scleroderma. The main findings of our research can be summarized as follows: 1) diastolic dysfunction is a relatively common finding among scleroderma patients 2) patients with scleroderma have a high prevalence of myocardial fibrosis 3) patients with left ventricular diastolic dysfunction have a significantly lower E/A ratio, lower Em values and Em/Am ratio compared to patients with normal left ventricular diastolic function. 4) there is a correlation between the extent and type of myocardial fibrosis and the presence of diastolic dysfunction in this population of patients. These findings merit further discussion.

The prevalence of diastolic dysfunction in previous studies was reported to be around 20%: 16% in the study of Muangchan et al [27], 17% in the study of de Groote et al [24], 23% in the study of Hinchcliff et al [20]. Therefore, our prevalence of 22% is concordant with the existing data.

We found no statistically significant differences in echocardiographic left ventricular diastolic function parameters between patients with diffuse and limited cutaneous scleroderma.

In the present study, patients with left ventricular diastolic dysfunction had a significantly lower E/A ratio compared to patients with normal LV diastolic function. This finding has been described before [23, 28, 29]. However, the E/A ratio is known to be age-dependant, with a tendency to progression to a sub-unitary E/A ratio in senior patients. Since the patients with diastolic dysfunction from the present study were significantly older than the ones with normal diastolic function, no conclusion about the different E/A ratio between these 2 subgroups can be drawn from the present study.

There are studies in the literature suggesting that data obtained with tissue Doppler imaging parameters might be more accurate for the assessment of diastolic function in systemic sclerosis patient [29, 30]. In our study, patients with diastolic dysfunction had lower Em values and a lower Em/Am ratio compared to patients with normal left ventricular diastolic function, which are concordant to the results of other authors [22, 29]. However, Gullulu et all [29] demonstrated that despite using age-based cut-offs for lateral tissue Doppler Em velocity, reduced Em was still strongly associated with age; therefore the lower Em values in the diastolic dysfunction group from our study need to be interpreted with caution. The E/Em ratio might be a better parameter that characterizes diastolic function by estimating left-sided filling pressures [30], but we found similar E/Em values in patients with and without diastolic dysfunction. The explanation might be that all patients with diastolic dysfunction from our study had mild diastolic dysfunction (impaired relaxation of the left ventricle) and even though the E/Em ratio was slightly higher in these patients (6.5 ± 5.5 vs. 5.1 ± 4.9), the difference did not reach statistical significance. However, an increased value of E/Em > 6 had a perfect

specificity for the presence of myocardial fibrosis at DE-MRI.

In what concerns myocardial fibrosis, its reported prevalence in scleroderma patients varies according to the diagnostic test used, generally being between 15 and 66% in studies using DE-MRI [10-13, 16, 17] (with the exception of one study performed on a very small population of patients, n = 10, reporting a prevalence of 100% [31]) and up to 70% in studies using histopathological confirmation [9]. We found a prevalence of myocardial fibrosis of 81.4%. This difference in the reported prevalence between these studies might be related to the different characteristics of the populations described, such as disease duration, scleroderma subtype [19], auto-immune antibodies, anti-fibrotic medication used and the presence of commorbidities. The most common localization of fibrosis was mid-wall, affecting mainly the basal and middle-third segments of the left ventricle, which is in concordance with previously published data [10, 11, 16], and the predominant pattern of fibrosis was focal, followed by the linear pattern. This finding is only slightly different from the previously described characteristic pattern of myocardial fibrosis in scleroderma patients, "mid-wall and linear" [10, 12, 16].

We found no difference in the prevalence of myocardial fibrosis according to the scleroderma subtype (diffuse cutaneous vs. limited cutaneous), a finding reported by other authors as well [10, 16], nor in the extent and pattern of fibrosis in these 2 subgroups. However, when compared according to the presence or absence of diastolic dysfunction of the left ventricle, patients with diastolic dysfunction had a significantly higher number of LV segments affected by myocardial fibrosis, as well as a higher number of basal and middle-third segments involved. Moreover, we found a significant correlation between the presence of diastolic dysfunction and the extent of myocardial fibrosis, which represents, in our opinion, the most important finding of the study.

There is one large study in the literature conducted on scleroderma patients that raised concerns about the possible existence of LV diastolic dysfunction in the absence of any other cardio-pulmonary disease, suggesting a specific cardiac involvement in this population. In their nationwide multicentric cohort study performed on 570 patients, De Groote et al [24] found in a small subgroup of scleroderma patients with no cardiac or pulmonary diseases the presence of severe diastolic dysfunction, suggesting that this might be due to a primary cardiac involvement. However, no diagnostic tool was used in this study for the identification of myocardial fibrosis; therefore no direct correlation between myocardial fibrosis and diastolic dysfunction of the left ventricle could be established. Even though in our study patients had only a mild form of diastolic dysfunction ("impaired relaxation" compared to "restrictive filling" in De Groote's study), the correlation reached statistical significance. Due to the small number of patients included in this study, this association needs future confirmation.

Another study performed on systemic sclerosis patients [32] tried to evaluate the diastolic function using gated SPECT and to test if diastolic dysfunction is an early sign of cardiac complications, which can exist independent of other significant cardiac involvement. The authors proved that significant diastolic abnormalities existed even in patients with normal perfusion and normal systolic function, an argument supporting the idea that diastolic dysfunction in scleroderma patients might be the result of a primary cardiac involvement.

The amount and type of fibrosis needed to induce diastolic dysfunction remains unknown. Rodriguez-Reyna et al [19] showed in their study that even though the prevalence of myocardial fibrosis in their population of scleroderma patients was 45%, the amount of fibrosis-affected myocardium of the LV was only 4%. However, patients with myocardial fibrosis had more perfusion abnormalities compared to the ones without myocardial fibrosis.

Another question that still requires an answer is whether certain patterns (focal/multifocal/linear/diffuse) and localizations (epicardial/intramural/endocardial) of myocardial fibrosis are more important than others in the development of LV diastolic dysfunction. For example, in the present study, there was a significant correlation between the amplitude of Am wave and the presence of endocardial fibrosis. Furthermore, in patients with diffuse cutaneous scleroderma, diastolic dysfunction correlated with the presence of multifocal and diffuse fibrosis, but not with the focal and linear pat-

tern; the value of EDT correlated with the presence of epicardial fibrosis. In patients with limited cutaneous scleroderma, the amplitude of the E wave and the EDT correlated with the presence of diffuse fibrosis, and the amplitude of the A wave correlated with the presence of endocardial fibrosis. Due to the small-sample population included in this study, this question remains unanswered.

Limits of the study

The present study has several important limitations. The most important one is the small number of patients involved. Had a larger number of patients with scleroderma been included, the study of the relationship between myocardial fibrosis and the diastolic dysfunction of the left ventricle might have yielded different results.

Secondly, the only form of diastolic dysfunction found among scleroderma patients included in the present study was the impaired relaxation type. Inclusion of patients with "pseudonormal" and "restrictive filling" patterns might have changed the relationship between myocardial fibrosis and the diastolic dysfunction of the left ventricle.

Myocardial fibrosis is frequently encountered among scleroderma patients. Its presence can be detected and its topography and pattern of distribution can be described using DE-MRI. There are no significant differences in the prevalence of myocardial fibrosis and diastolic dysfunction between the 2 scleroderma subtypes. The presence of myocardial fibrosis negatively influences the diastolic function of the left ventricle, in both types of the disease. The presence of an increased E/Em at echocardiography is highly specific for the presence of myocardial fibrosis. Future studies on larger populations are needed to better characterize the relationship between myocardial fibrosis and the diastolic function of the left ventricle in patients with scleroderma.

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Disclosure of conflict of interest

None.

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