Herz 2015 · 40:709–715
DOI 10.1007/s00059-014-4113-2
Received: 22 February 2014
Revised: 18 April 2014
Accepted: 27 April 2014
Published online: 19 June 2014

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Right ventricular and atrial functions in systemic sclerosis patients without pulmonary hypertension

Speckle-tracking echocardiographic study

Systemic sclerosis (SSc) is a systemic connective tissue disease characterized by chronic inflammation, fibrosis, and autoimmunity [1]. Excessive fibrosis can affect visceral organ systems such as the heart and lungs and cardiac involvement of SSc patients is one of the most important causes of death [2]. Cardiac manifestations include pulmonary hypertension (PH) and right ventricular (RV) failure secondary to PH in patients with SSc.

Although RV impairment is generally related to elevated pulmonary artery pressure (PAP), RV impairment can sometimes be observed in patients with normal PAP due to ongoing ischemic episodes related to vascular lesions [3]. Therefore, detection of RV systolic impairment before the development of PH in SSc patients might be useful in clinical follow-up. Two-dimensional (2D) speckle tracking echocardiography (STE) is a new angle-independent quantitative technique to evaluate the myocardial function by analyzing spots on the 2D grayscale ultrasound images of the myocardium [4]. In previous studies a correlation between 2D STE and conventional echocardiographic parameters was shown in detecting RV systolic dysfunction [5]. The aim of the present study was to assess the RV and right atrial (RA) functions of SSc patients without PH by using 2D STE.

Methods

Study population

In this study 49 patients with SSc followed up by the Department of Rheumatology were evaluated for enrollment between May 2012 and September 2012. The diagnosis of patients with SSc was based on the American Rheumatism Association (ARA) criteria [6]. Patients with coronary artery disease, valvular heart disease, cardiomyopathy, arrhythmias or conduction disorders, left ventricular (LV) systolic dysfunction with LV ejection fractions (LVEF) <55 %, significant pulmonary arterial hypertension (systolic pulmonary artery pressure >35 mmHg), chronic obstructive pulmonary disease, asthma, hypertension, and diabetes mellitus were excluded. After exclusion criteria were applied, 40 patients with SSc were included in the study and 40 age- and sex-matched healthy volunteers were included as a control group. The investigation complied with the principles outlined in the Declaration of Helsinki. The study was approved by the local ethics committee and all participants gave written informed consent before participating.

Standard echocardiography and 2D speckle tracking echocardiography

Conventional standard echocardiographic examination of patients was performed by using a commercially available echocardiography device (Vivid 7, GE Vingmed Ultrasound AS, Horten, Norway). Echocardiographic data were obtained with a 3.5 -MHz transducer at a depth of 16 cm in the parasternal and apical views during breath hold, stored in cine loop format from three consecutive beats, and transferred to a workstation for further offline analysis (EchoPAC 6.1; GE Vingmed Ultrasound AS). Gain settings, filters, and pulse repetitive frequency were adjusted to optimize color saturation, and a color Doppler frame scanning rate of 100–140 Hz was used for color tissue Doppler images (TDI). Cardiac dimensions were measured according to the guidelines of the American Society of Echocardiography (ASE) and LVEF was calculated by the biplane Simpson's method [7].

The LV end diastolic diameter (EDD), LV end systolic diameter (ESD), left atrial area (LAA), mitral inflow early (E wave) and late (A wave) diastolic velocities, deceleration time of early diastolic velocity and early (E' wave) and late (A' wave) diastolic annular velocities assessed at the septal mitral annulus, deceleration time (DT), and the E/E' ratio were used in the analysis. Diastolic dysfunction was de-

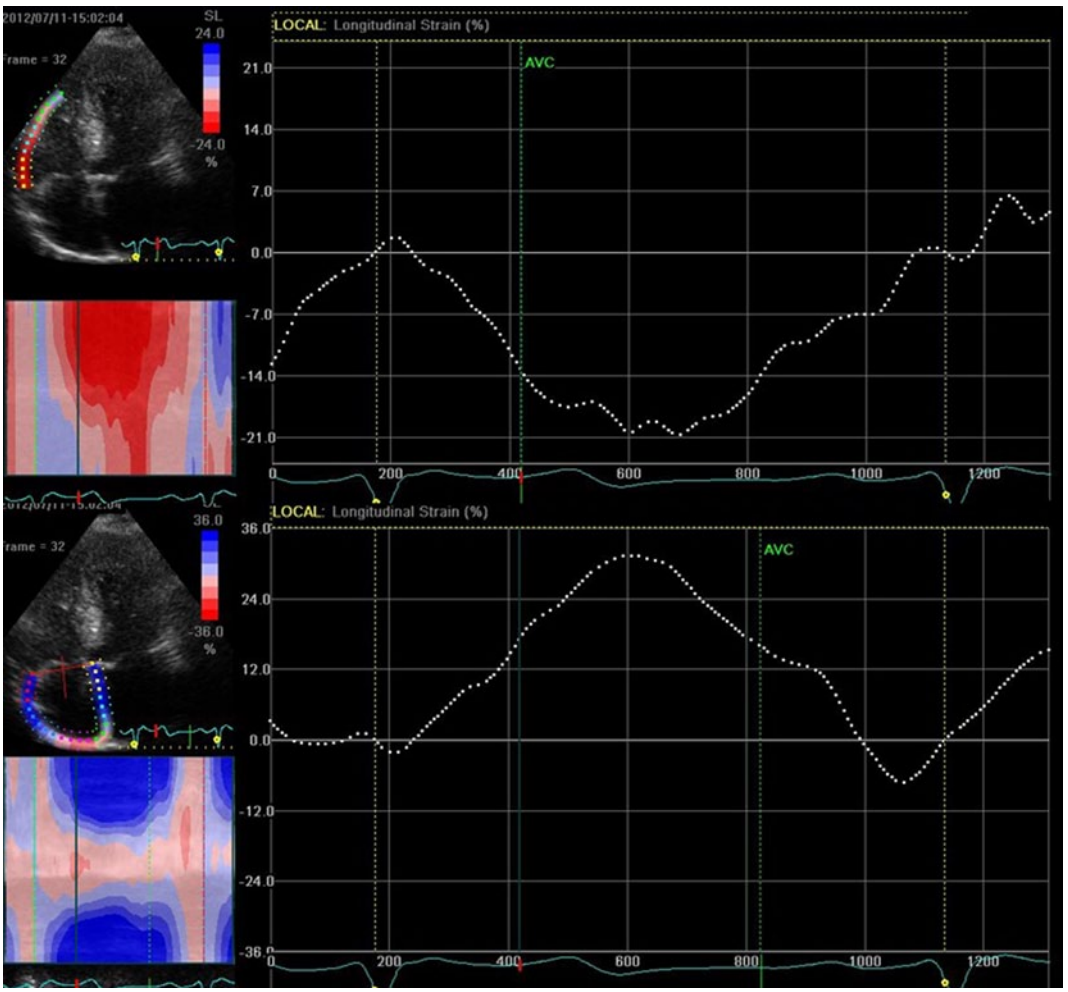

Fig. 1 ◀ Right ventricular (*upper panel*) and right atrial (*lower panel*) speckle tracking imaging in a patient with systemic sclerosis

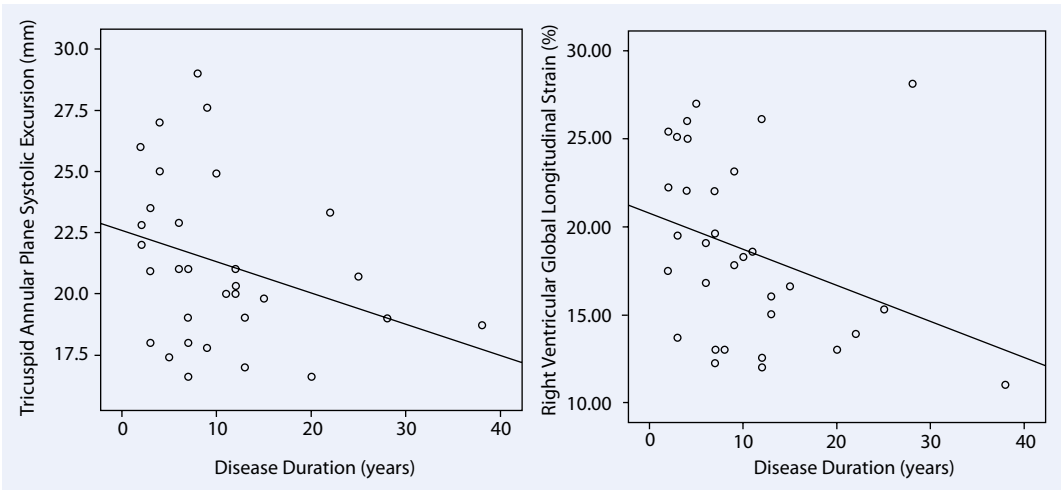

Fig. 2 ◀ Graphs showing an inverse correlation between disease duration and tricuspid annular plane systolic excursion (*left*) and right ventricular global longitudinal strain (*right*)

fined as a tissue Doppler lateral E' velocity of less than 10 cm/s. Right ventricular and atrial dimensions were measured as suggested in the ASE guidelines [4]. RV function was also evaluated according to the guidelines of the ASE by using tricuspid annular plane systolic excursion (TAPSE), maximal systolic myocardial velocity (S') measured with TDI and fractional area change (FAC). Systolic pulmonary artery pressure (sPAP) was calculated by measuring the peak systolic gradient from the peak velocity of the continuous-wave Doppler of the tricuspid regurgitation jet by using the Bernoulli equation.

The speckles, natural acoustic markers equally distributed throughout the myocardium, can be detected and tracked on the standard grayscale 2D images. Myocardial strain can be calculated by mea-

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Right ventricular and atrial functions in systemic sclerosis patients without pulmonary hypertension. Speckle-tracking echocardiographic study

Abstract

Background. Systemic sclerosis (SSc) is a systemic connective tissue disease and cardiac involvement is one of the most important causes of death. Right ventricular (RV) systolic dysfunction is a poor prognostic finding in SSc patients. Assessment of RV function has some difficulties because of its crescent shape and extensive trabeculations. Two-dimensional (2D) speckle-tracking echocardiography (STE) is an angle-independent quantitative technique to evaluate myocardial function. The aim of this study was to assess the RV and right atrial (RA) functions of SSc patients without pulmonary hypertension by using 2D STE.

Patients and methods. A total of 40 patients with SSc (mean age 48.5±11.4 years, 28 female) and 40 healthy volunteers (mean age 45.9±7.6 years, 21 female) were included in the study. All subjects underwent transthoracic echocardiography for evaluation of RV and RA functions with 2D STE.

Results. Although left ventricular systolic and diastolic functions, systolic pulmonary artery pressure (PAP), and RA measurements were similar in both groups, tricuspid annular plane systolic excursion (TAPSE) and maximum systolic myocardial velocity (S') were decreased in SSc patients. The RV free wall global longitudinal strain (GLS) of SSc patients was lower than the controls (−18.5±4.9% vs. −21.8±2.4%, p<0.001) and the RA reservoir and conduit functions were also decreased in SSc patients compared with controls (34.4±9.9% vs. 39.7±11.2%, p=0.027 and 15.0±5.7% vs. 18.7±6.4%, p=0.009, respectively). Disease duration was inversely correlated with RVGLS and TAPSE (r: −0.416, p=0.018 and r: −0.383, p=0.031, respectively).

Conclusion. The use of 2D STE can be helpful in the detection of impairment in RV and RA functions in SSc patients with normal PAP.

Keywords

Systemic sclerosis · Speckle-tracking echocardiography · Strain · Right ventricle · Right atrium

Rechtsventrikuläre und Vorhoffunktion bei Patienten mit systemischer Sklerose ohne pulmonale Hypertonie. Untersuchung mit der Speckle-Tracking-Echokardiographie

Zusammenfassung

Hintergrund. Die systemische Sklerose (SSc) ist eine systemische Bindegewebserkrankung, und die Herzbeteiligung stellt eine der Haupttodesursachen dar. Eine rechtsventrikuläre (RV) systolische Funktionseinschränkung ist ein Befund, der bei SSc-Patienten für eine schlechte Prognose steht. Die Untersuchung der RV-Funktion ist aufgrund der Halbmondform und ausgedehnter Trabekulierungen des RV schwierig. Die zweidimensionale (2-D-)Speckle-Tracking-Echokardiographie (STE) ist eine winkelunabhängige quantitative Untersuchungstechnik für die Myokardfunktion. Ziel dieser Studie war es, die Funktion des RV und des rechten Vorhofs (RA) bei SSc-Patienten ohne pulmonale Hypertonie mittels 2-D-STE zu ermitteln.

Patienten und Methoden. In die Studie wurden 40 Patienten mit SSc (Durchschnittsalter: 48,5±11,4 Jahre, 28 w) und 40 gesunde Kontrollen (Durchschnittsalter: 45,9±7,6 Jahre, 21 w) aufgenommen. Bei allen Teilnehmern wurden die RV- und RA-Funktion mittels transthorakaler Echokardiographie in Kombination mit 2-D-STE untersucht.

Ergebnisse. Die linksventrikuläre systolische und diastolische Funktion, der systolische Pulmonalarteriendruck (PAP) und die RA-Messungen waren in beiden Gruppen zwar ähnlich, aber die systolische Exkursion auf der Ebene des Trikuspidalrings (TAPSE) und die maximale systolische Myokardgeschwindigkeit (S') waren bei SSc-Patienten vermindert. Der globale longitudinale Strain (GLS) der freien RV-Wand war bei SSc-Patienten geringer als bei den Kontrollen (−18,5±4,9% vs. −21,8±2,4%; p<0,001) und auch die Reservoir- und Conduitfunktion war bei den SSc-Patienten gegenüber den Kontrollen vermindert (34,4±9,9% vs. 39,7±11,2%; p=0,027 bzw. 15,0±5,7% vs. 18,7±6,4%; p=0,009). Die Krankheitsdauer stand in inverser Korrelation mit dem RV-GLS und TAPSE (r −0,416; p=0,018 bzw. r −0,383; p=0,031).

Schlussfolgerung. Der Einsatz der 2-D-STE könnte zur Erkennung einer Einschränkung der RV- und RA-Funktion bei SSc-Patienten mit normalem PAP von Nutzen sein.

Schlüsselwörter

Systemische Sklerose · Speckle-Tracking-Echokardiographie · Strain · Rechter Ventrikel · Rechter Vorhof

suring the change of the position of the speckles within a myocardial segment along the cardiac cycle. The assessment of global radial strain (GRS) and global circumferential strain (GCS) of the LV were performed by applying 2D speckle tracking imaging to the parasternal short-axis views of the LV. The midventricular short axis of the LV was divided into six segments, and the values of GRS and GCS were derived from the average of the six segmental peak systolic strain values. The assessment of global longitudinal peak systolic strain was performed by applying 2D speckle tracking imaging to the apical four-chamber views of the LV. The LV was divided into six segments in apical view. The values of global longitudinal strain (GLS) were derived from the average of the six segmental peak systolic longitudinal strain values. The RV strain was also assessed using 2D speckle tracking imaging as previously described from the apical four-chamber view [8] and The RV basal lateral and midlateral wall endocardial borders were manually traced at the end systolic frame. The epicardial border was traced automatically by software (**Fig. 1**). If necessary, to obtain optimal tracking the region of interest (ROI) was modified by the observer. The RVGLS was derived from the average of these two segments.

For the RA speckle tracking analysis, RA-focused images in apical four-chamber view were obtained. A minimum

Tab. 1 Baseline characteristics and clinical data of the patients with scleroderma

Age (years)	48.5±11.4
Gender (male, n)	12 (30%)
Body mass index (kg/m^2)	25.8±3.9
Limited/diffuse cutaneous systemic sclerosis (n)	20/20
Time since diagnosis (years)	8.0±6.3
Time since onset of Raynaud phenomenon (years)	10.4±6.3
Raynaud's phenomenon (%)	97.1
Interstitial lung disease (%)	64.7
Joint involvement (%)	50
Digital ulcer (%)	41.2
Esophageal involvement (%)	73.5
Active disease [a] (%)	32.4
Current smoker (%)	9.4
Antinuclear antibodies (%)	97.1
Anti-SCL 70 antibodies (%)	32.4
Anticentromere antibodies (%)	11.8
DLCO (%)	72.9±17.8
CRP (mg/dl)	7.2±6.5
Sedimentation rate (mm/h)	31.4±13.5

Data are presented as mean ± standard deviation or number of patients. *DLCO* diffusing capacity for carbon monoxide, *CRP* C-reactive protein. [a] Active disease is defined as the European Scleroderma Study Group activity index score of higher than 3

Tab. 2 Comparison of left ventricular and atrial echocardiographic parameters of scleroderma patients and controls

Parameter	Scleroderma patients (n=40)	Controls (n=40)	p
LVEF (%)	65.1±4.7	64.5±4.9	0.680
LVEDD (mm)	44.2±5.4	45.6±3.7	0.180
LVESD (mm)	28.5±5.1	28±3.9	0.610
LAA (cm^2)	14.7±3.5	15±2.0	0.700
LAA/BSA	8.8±1.8	8.0±1.2	0.230
LVE (m/s)	0.74±0.13	0.71±0.11	0.290
LVA (m/s)	0.73±0.14	0.62±0.12	**<0.001**
DT (ms)	189±32	198±39	0.270
LVE' (cm/s)	11.3±3.5	12.9±3.4	0.290
E/E'	5.9±2.4	7.1±1.9	**0.020**
Diastolic dysfunction (n)	12 (30%)	7 (17.5%)	0.180
LVGLS (%)	−20.5±3.4	−20.9±2.7	0.580
LVGRS (%)	39.4±18.6	42.2±13.1	0.430
LVGCS (%)	−17.5±5.5	−18.8±4.8	0.260

LV left ventricle, *LAA* left atrial area, *EF* ejection fraction, *LVEDD* left ventricle end diastolic diameter, *LVESD* left ventricle end systolic diameter, *DT* deceleration time, *LVE* left ventricle mitral inflow early wave, *LVA* left ventricle mitral inflow atrial wave, *LVE'* left ventricle tissue Doppler peak velocity during early diastole, *E/E'* ratio of LVE to LVE', *GLS* global longitudinal strain, *GRS* global radial strain, *GCS* global circumferential strain, *BSA* body surface area

frame rate of 40 frames per second was required for the reliable operation of this program. For 2D speckle tracking strain analysis, a line was manually drawn along the RA endocardial border of the apical four-chamber view after contraction (**Fig. 1**), when the RA was at its minimum volume, using the point-and-click approach as previously described [9, 10]. The software then automatically generated additional lines near the atrial epicardium and mid-myocardial line, with the narrowest ROI. The ROI then included the entire RA myocardial wall and was adjusted for the thickness. The software generated strain curves for each atrial segment. The value of peak early and late diastolic longitudinal strains was determined as right atrial reservoir (RA Res) and conduit (RA Con) functions.

Statistical analysis

Statistical analyses were performed using the SPSS15.0 statistical package for Windows. Continuous data were expressed as mean ± standard deviation while categorical data were presented as percentages. The χ^2 test was used for comparison of categorical variables while Student's t test or the Mann–Whitney U test was used to compare parametric and nonparametric continuous variables, respectively. For correlation analysis, the Spearman test was used. Intraobserver and interobserver variability was calculated as the absolute difference between two measurements as a percentage of the mean. A value of $p<0.05$ was considered statistically significant.

Results

The 40 patients with SSc (mean age 48.5±11.4 years, 28 female) were compared with 40 healthy age- and sex-matched controls (mean age 45.9±7.6 years, 21 female, p=0.16 and p=0.10, respectively). Baseline characteristics and clinical data of SSc patients are displayed in **Tab. 1**.

LV echocardiographic parameters of the SSc patients and healthy controls are compared in **Tab. 2**. Conventional LV dimensions and EF were similar in both groups. Although LV diastolic dysfunction was observed more commonly in SSc patients than in the control group, it did not reach statistical significance (p=0.180). We also evaluated LV function by using 2D STE and LVGLS, GRS, and GCS were similar between the two groups (p>0.05).

Right ventricular and atrial dimensions and functions are shown in **Tab. 3**. The RV annulus diameter and RV inflow tract diameter were higher in SSc patients compared with controls (p=0.010 and p=0.014, respectively). Mean RAA was slightly lower in SSc patients (13 cm^2 vs. 14.2 cm^2, p=0.040), but when we normalized to body surface area (BSA), RAA/BSA was similar between the two groups (7.8±1.2 vs. 7.6±1.0, p=0.470). Although sPAP was within normal limits, the sPAP of SSc patients was slightly higher compared with the controls (24.2±5.7 vs. 19.8±6.2 mmHg, p=0.002). Evaluation of RV systolic function by TAPSE and RVS' showed that the RV function of SSc patients was decreased compared with con-

Tab. 3 Comparison of right atrial and ventricular echocardiographic parameters of the study population

Parameter	Scleroderma patients (n=40)	Controls (n=40)	p
RV inflow tract diameter (mm)	28.7±4.2	26.8±2.2	0.014
Tricuspid annulus diameter (mm)	34.1±3.8	31.3±2.5	0.010
RAA (cm^2)	13	14.2	**0.040**
RAA/BSA	7.8±1.2	7.6±1.0	0.470
FAC (%)	32.9±4.1	33.8±3.7	0.300
TAPSE (mm)	21.1±3.2	24.3±3.4	**<0.001**
RVS'(cm/s)	10.1±1.5	12.9±2.0	**<0.001**
RVE' (cm/s)	9.4±2.8	11.2±2.8	**0.006**
RVA' (cm/s)	11.3±2.4	14.4±3.8	**<0.001**
TR severity			**0.010**
– < Mild (n-%)	15 (37.5%)	25 (62.5%)	
– Mild (n-%)	25 (62.5%)	15 (37.5%)	
sPAP (mmHg)	24.2±5.7	19.8±6.2	**0.002**
RVGLS (%)	−18.5±4.9	−21.8±2.4	**<0.001**
RA res (%)	34.4±9.9	39.7±11.2	**0.027**
RA con (%)	15.0±5.7	18.7±6.4	**0.009**

RV right ventricle, *RAA* right atrial area, *TAPSE* tricuspid annular plane systolic excursion, *FAC* fractional area change, *RVS'* tissue Doppler maximal systolic myocardial velocity, *RVE'* tissue Doppler peak velocity during early diastole, *RVA'* tissue Doppler peak velocity during atrial contraction, *sPAP* systolic pulmonary artey pressure, *GLS* global longitudinal strain, *res* reservoir, *con* conduit, *TR* tricuspid regurgitation, *BSA* body surface area

Tab. 4 Comparison of right ventricular functions of scleroderma patients with and without left ventricular diastolic dysfunction

Right ventricular function	Scleroderma with diastolic dysfunction (n=12)	Scleroderma without diastolic dysfunction (n:28)	p
FAC (%)	31.5±5.2	33.5±3.4	0.163
TAPSE (mm)	21.1±2.8	21.0±3.5	0.920
RVS'(cm/s)	10.3±1.2	10.1±1.6	0.699
RVE' (cm/s)	9.8±1.5	9.2±3.2	0.442
sPAP (mmHg)	25.1±7.1	23.7±5.0	0.493
RVGLS (%)	−16.9±3.6	−19.2±5.3	0.110
RA res (%)	35.8±9.7	33.7±10.0	0.558
RA con (%)	16.0±6.4	14.6±5.5	0.510

RV right ventricle, *TAPSE* tricuspid annular plane systolic excursion, *FAC* fractional area change, *RVS'* tissue Doppler maximal systolic myocardial velocity, *RVE'* tissue Doppler peak velocity during early diastole, *sPAP* systolic pulmonary artery pressure, *GLS* global longitudinal strain, *res* reservoir, *con* conduit

trols (TAPSE 21.1±3.2 vs. 24.3±3.4 mm, p<0.01 and S' 10.1±1.5 vs. 12.9±3.0 cm/s, p<0.001), but the TAPSE and S' value of SSc patients were nearly normal compared with previous reports. The RV-FAC was similar between the two groups (p=0.300) and RVGLS was significantly decreased in SSc patients compared with the controls (RVGLS −18.5±4.9% vs −21.8±2.4%, p<0.001). The RA reservoir and conduit functions were significantly lower than the controls (RA res 34.4±9.9% vs. 39.7±11.2%, p=0.02 and RA con 15.0±5.7% vs. 18.7±6.4%, p<0.01).

To evaluate the effect of LV diastolic dysfunction on RV systolic function, we divided the SSc patients into two groups according to the presence of diastolic dysfunction, and found that RV systolic functions were similar between the two groups (**Tab. 4**). Correlation analysis was performed to evaluate associations between sPAP, RVS, and TAPSE. We did not observe any significant correlation between RVS, TAPSE, and sPAP (p>0.05 for all). We also analyzed RV function and its relation with disease duration. We demonstrated an inverse correlation between disease duration and RVGLS and TAPSE (r=−0.41, p=0.01 and r= −0.38, p=0.03, respectively; **Fig. 2**).

Discussion

In the present study we showed that RV systolic function of the SSc patients without PH was significantly decreased compared with healthy controls by using 2D STE. The RA reservoir and conduit functions were also impaired in SSc patients compared with controls.

SSc is a progressive, systemic disease characterized by inflammation, fibrosis, and autoimmunity [1]. Excessive fibrosis mainly affects the skin and visceral organ systems. In a recently published meta-analysis, the most severe organ complications related with scleroderma involved the heart, lungs, kidneys, muscles, joints, and fingers [11]. Cardiopulmonary involvement was the most important cause of death [2]. Cardiac manifestations were more common in African-Americans, and the 20-year mortality was 32% [12].

Cardiac manifestations in patients with SSc include LV diastolic and systolic dysfunctions, PH, and RV failure secondary to PH. Although RV impairment is generally related to higher PAP in patients with SSc, RV impairment may be observed in patients without PH. The possible underlying mechanism of impairment of the RV function is myocardial fibrosis secondary to repeated ischemia-reperfusion abnormalities due to vascular lesions in SSc patients [3].

Assessment of RV function in SSc patients by echocardiography has been performed in several studies, but because of the crescent shape and extensive trabeculations of the RV it has some limitations. The most commonly used conventional echocardiographic parameters to evaluated RV function are tissue Doppler-derived peak systolic velocities (S') and TAPSE [13, 14, 15] and TAPSE could also be used to predict the re-hospitalization rate of SSc patients [15]. A recently published trial on assessment of RV function by using conventional echocardiographic parameters and its correlation with EF measured by magnetic resonance imaging demonstrated that the cut-off val-

ues of RVS' and TAPSE for detection of RVEF <50% were 11 cm/s and 17 mm, respectively [14].

Two-dimensional STE is new echocardiographic technique that has been validated in the assessment of LV function by several studies [16, 17, 18, 19, 20] and was also used to evaluate the right side of the heart in various diseases [21, 22, 23, 24]. Meris et al. [5] compared normal subjects with patients who had RV dysfunction, and found that the RV global longitudinal systolic strain cut-off value of –19% was helpful in distinguishing the two groups. There was limited data about using 2D STE to evaluate RV function in SSc patients. In a previous study reported by Matias et al. [25], the RV basal free wall strain of SSc patients without PH was not different from controls whereas Schattke et al. [26] demonstrated that the RV basal and mid-free wall strain value of SSc patients without PH was lower than controls.

In our study, although RV systolic function of SSc patients without PH was decreased compared with the controls, TAPSE was not lower than previously described cut-off values and S' and RV GLS were very close to the cut-off value used to distinguish RV failure. One of the possible explanations of these results is that RV failure generally related to increased afterload [27], but in our study patients did not have PH. In addition, another reason for RV failure may be LV diastolic dysfunction but we did not observe any differences in RV function of SSc patients with and without LV diastolic dysfunction. Another reason why RV functions of SSc patients were closer to normal levels is an inadequate duration of time for the development of RV failure in SSc patients. In our study we demonstrated a negative correlation between RV function and disease duration. In patients with SSc, increased afterload is not the only reason for RV failure and SSc is a progressive disease characterized by fibrosis. The possible underlying mechanism of ventricular impairment could be myocardial fibrosis secondary to repeated ischemia-reperfusion abnormalities due to vascular lesions in SSc patients [3]. In a study comparison between SSc patients with PH and idiopathic pulmonary arterial hypertension (IPAH) it was shown that although RV afterload was similar in both disease groups, the prognosis of SSc with PH was worse than for IPAH patients [28]. This demonstrated that an intrinsic systolic dysfunction may be found in SSc patients.

Previous studies showed that LA strain and strain analysis by 2D STE correlated with Doppler parameters that display LA functions [29]. Although deterioration in LA strain values have been shown by STE in several cardiac diseases, such as paroxysmal atrial fibrillation [30], hypertrophic cardiomyopathy [31], and dilated cardiomyopathy [32], RA strain data are limited. In our study, we showed that RA reservoir and conduit parameters were significantly lower in SSc patients compared with controls. To the best of our knowledge, this is the first study to evaluate RA strain parameters in patients with SSc. Although we do not know the exact mechanism, a higher sPAP of SSc patients may be a reason.

Study limitations

Our first limitation is the small sample size. This may explain why the differences in some echocardiographic parameters were not significant. Although 2D STE is a novel echocardiographic technique known to be an angle-independent quantitative method for evaluating the myocardial function, there are some difficulties in evaluating RA functions. For example, RA myocardium is thinner than RV; therefore, the thinness of the atrium represents a possible technical limitation when evaluating the myocardial deformation by 2D STE. Despite the fact that our study had a cross-sectional design, we were not able to evaluate the prognostic value of the RA and RV functions in SSc patients without PH. Further prospective studies are needed to evaluate the prognostic value of determining right heart functions in patients with SSc. Moreover, it would be better to follow-up the patients and explore the relationship between adverse cardiac events and right heart functions in these patients.

Conclusion

Two-dimensional STE might be useful in detecting impairment in RV and RA functions in SSc patients with normal PAP. Future large-scale prospective studies are needed to clarify the right heart functions in SSc patients.

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Compliance with ethical guidelines

Conflict of interest. E. Durmus, M. Sunbul, K. Tigen, T. Kivrak, G. Ozen, I. Sari, H. Direskeneli, and Y. Basaran state that there are no conflicts of interest. All studies on humans described in the present manuscript were carried out with the approval of the responsible ethics committee and in accordance with national law and the Helsinki Declaration of 1975 (in its current, revised form). Informed consent was obtained from all patients included in studies.

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