Early Detection of Cardiac Involvement in Systemic Sclerosis Assessed by Tissue-Doppler Echocardiography: Relationship with Neurohormonal Activation and Endothelial Dysfunction

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ABSTRACT. ***Objective.*** Cardiopulmonary complications are common in patients with systemic sclerosis (SSc). We assessed cardiac involvement in patients with SSc using echocardiography and investigated the association of N-terminal pro-brain natriuretic peptide (NT-proBNP) and asymmetric dimethylarginine (ADMA) with echocardiographic measures of myocardial function in sera of patients with SSc who had no symptoms of heart failure.

Methods. We prospectively studied 52 patients with SSc (mean age 55.7 ± 10.1 yrs, 51 women), with conventional and tissue-Doppler echocardiography. Plasma NT-proBNP and ADMA levels were measured in all patients. Data were compared with those obtained from 25 healthy controls comparable for age and sex.

Results. Patients with SSc had impaired left ventricular (LV) and right ventricular diastolic function expressed by inverted ratio of peak early to peak late transmitral (Mit E/A) and transtricuspid velocity and increased left atrial diameter compared with controls. Peak systolic mitral lateral annular motion velocity and peak early diastolic mitral lateral annular motion velocity (LV Em) were lower, while LV E/Em ratio was higher, in patients with SSc compared to controls. ADMA was significantly related with LV Em and E/Em ratio. NT-proBNP was associated with Mit E, Mit E/A ratio and mitral deceleration time. Significant correlation was also observed between NT-proBNP and ADMA levels.

Conclusion. Depressed cardiac function is common, even in asymptomatic patients with SSc. NT-proBNP and ADMA are significantly correlated with echocardiographic abnormalities, providing a potent link for cardiac function, neuroendocrine derangement, and endothelial dysfunction in patients with SSc who have cardiac disease. (First Release March 1 2010; J Rheumatol 2010; 37:993–9; doi:10.3899/jrheum.090931)

Key Indexing Terms:
SCLERODERMA ECHOCARDIOGRAPHY CARDIOVASCULAR DISEASES

Systemic sclerosis (SSc) is a multisystem connective tissue disease characterized by microvascular injury and abnormalities of the immune system, leading to fibrosis of the skin and internal organs. Patients with SSc may have cardiac involvement, an important prognostic factor[1]. Pericarditis, rhythm disturbances, conduction defects, and systolic and diastolic cardiac dysfunction are the major manifestations of cardiac involvement in scleroderma[2]. Pulmonary arterial hypertension, which occurs in 10%–15% of patients with SSc[3], is widely recognized as a major cardiac complication of SSc and is associated with a worse prognosis than idiopathic pulmonary hypertension[4]. Subtle symptoms and physical findings of cardiac involvement can be missed and consequently a patient may present with advanced signs of heart failure and an ominous prognosis. Thus, early detection of cardiac abnormalities is important.

Transthoracic Doppler echocardiography, together with clinical evaluation, have been suggested for routine cardiac assessment in SSc[5]. But recent data show that these indices may not allow a prompt diagnosis at a preclinical stage and the use of specific therapy for the disease, at the time when

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it is most likely to be effective[6]. Tissue-Doppler echocardiography (TDE) is a modern echocardiographic method that provides accurate and reproducible presentation of regional and global left ventricular (LV) and right ventricular (RV) function. Recent studies have explored the role of TDE in the assessment of heart involvement in patients with SSc[7,8].

Blood tests such as N-terminal pro-brain natriuretic peptide (NT-proBNP) and asymmetric dimethylarginine (ADMA) may be useful tools in the assessment of patients with cardiac involvement. NT-proBNP is a sensitive marker of elevated ventricular wall stress and has been described as a marker of both heart involvement[9] and pulmonary arterial hypertension in patients with SSc[10]. ADMA, an endogenous inhibitor of nitric oxide (NO) synthase, is a biochemical marker of endothelial dysfunction, which represents a novel risk factor of cardiovascular disease and mortality[11]. In the context of SSc, ADMA is increased in patients with the diffuse cutaneous subtype of disease[12] and in those with pulmonary arterial hypertension[13].

Our aim was to examine the regional and global myocardial function in patients with SSc by using conventional echocardiography and pulsed-wave TDE and to investigate the relationships between echocardiographic and biochemical abnormalities.

MATERIALS AND METHODS

The study included 52 patients with SSc as defined by the revised ACR criteria[14] who visited our center for followup care. Patients were selected after exclusion of hypertrophic, dilated or restrictive cardiomyopathy, atherosclerotic or coronary artery heart disease, systolic heart failure [LV ejection fraction (LVEF) < 55%], any left-sided or right-sided valvular disease more than moderate in severity, atrial fibrillation, medical history of diabetes mellitus, dyslipidemia, smoking or hypertension, or renal failure determined by serum creatinine levels > 1.2 mg/dl. Moreover, patients with pulmonary hypertension were excluded from the study. The demographic and clinical characteristics of the patients are detailed in Table 1.

The study received ethical approval from the scientific committee of the Aristotle University of Thessaloniki and patients provided written informed consent.

Patients with SSc were compared to 25 healthy age and sex-matched controls referred to our cardiology department for regular cardiac status determination. The controls had no evidence of present or past heart disease or risk factors predisposing them to endothelial dysfunction such as smoking, dyslipidemias, diabetes mellitus, or arterial hypertension.

Blood samples and NT-proBNP and ADMA measurement. In all patients, blood samples were drawn and analyzed for routine laboratory measurements, including Westergren erythrocyte sedimentation rate, C-reactive protein, and homocysteine levels, on the same day as the echocardiography examination.

Plasma NT-proBNP concentration was measured using a commercial enzyme immunoassay kit (Biomedica GmbH, Wien, Austria). The kit uses an immunoaffinity purified polyclonal antibody specific for proBNP (8-29) that is attached to the plastic surface of a 96-well microtiter plate and a horseradish peroxidase labeled peptide (8-29) as a tracer. The detection limit of the assay is 5 fmol/ml. The intraassay variation for a concentration of 100 fmol/ml was estimated to be 7.5%.

Concentration of ADMA was measured in serum samples using a commercial enzyme immunoassay ELISA kit (DLD Diagnostika, Hamburg, Germany). The kit uses an immunoaffinity, a highly specific and sensitive rabbit anti-ADMA antibody. The ADMA concentrations obtained and the performance of the ELISA have been found to be consistent with other widely applied methods used to quantify ADMA, such as gas chromatography–mass spectrometry and liquid chromatography–mass spectrometry[12].

Table 1. Demographic and clinical characteristics of the patients.

Characteristic	Patients, n = 52
Age, yrs	55.7 ± 10.1
Women/men	51/1
Duration of the disease, yrs	11 (0.7–27)
Limited/diffuse SSc	28/23
No. with Raynaud's phenomenon	46
No. with pulmonary fibrosis	19
No. with esophagus insult	31
No. with digital ulcers	21
Creatinine, mg/dl	0.8 ± 0.2
Homocysteine, μmol/l	11.4 ± 4.9
FVC, % predicted	88.1 ± 23.1
FEV1, % predicted	90.2 ± 25.0
DLCO, % predicted	74.0 ± 16.5
ESR, mm/h	16.0 ± 4.5
CRP, mg/ml	0.4 ± 0.1
No. with antinuclear antibodies	47
No. with antitopoisomerase I antibodies	18
No. with anticentromere antibodies	21
Prednisolone treatment (< 10 mg/d)	38

FVC: forced vital capacity; FEV1: forced expiratory volume in 1 second; DLCO: diffusing capacity for carbon monoxide; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein.

Echocardiography. All echocardiographic examinations were performed by an experienced echocardiographer blinded to clinical data using a commercial ultrasound system (Vivid 7, Vingmed, GE, Oslo, Norway). Echocardiography was performed with the participants in the partial left decubitus position.

Two-dimensional and color-flow Doppler images were obtained from parasternal and apical (4-chamber and 2-chamber) long-axis views. Measurements were done according to the guidelines of the American Society of Echocardiography[15] and LVEF was estimated by Simpson's method. The estimated pulmonary arterial systolic pressure was calculated as the sum of the transtricuspid gradient and the estimated right atrial pressure. Right atrial pressure was estimated using the diameter of the inferior vena cava and the response to changes in respiration[16]. Pulmonary hypertension was defined as a systolic pulmonary artery pressure (sPAP) value ≥ 45 mm Hg[17].

The transmitral and transtricuspid diastolic flow tracings were obtained from the apical 4-chamber view by using pulsed Doppler echocardiography with the sample volume sited at the tips of the mitral and tricuspid leaflets. Peak early transmitral (Mit E) and early transtricuspid (Tr E) filling velocity, peak late transmitral (Mit A), and late transtricuspid (Tr A) filling velocity and their ratio (Mit E/A, Tr E/A, respectively) were recorded. Pulsed wave tissue-Doppler myocardial motion velocities were measured in the apical 4-chamber view from the lateral mitral annular sites and the tricuspid annulus at the RV wall. Pulsed TDE was characterized by a myocardial systolic wave (Sm) and 2 diastolic waves — early diastolic (Em) and late diastolic. Several cardiac cycles were evaluated and the best 3 consecutive ones were analyzed and averaged. Deceleration time of peak early transmitral velocity (Mit DT), isovolumic contraction time, and isovolumic relaxation time derived by TDE were also obtained. Those time intervals were required for calculation of the myocardial performance index (MPI)[18].

LV Sm, Em, and E/Em ratio measurements were used to identify patients with more severe LV involvement according to previously applied cutoff values[7]: depressed LV systolic function was defined as Sm < 0.075 m/s and impaired LV diastolic function as Em < 0.1 m/s or E/Em ratio > 15 (determinant of increasing LV filling pressures).

Statistical analysis. Variables are presented as mean ± SD, apart from NT-proBNP and duration of disease, which are expressed as median (25th–75th percentile). Categorical data are presented as absolute value, and comparisons were tested by Fisher's exact test. Clinical and biological characteristics between patients with SSc and healthy controls were compared using the Student's t test or the nonparametric (Mann–Whitney U) test as appropriate. NT-proBNP was log-transformed to achieve normality before correlations and Bonferroni posthoc analysis. Pearson's rank correlation analysis was used to explore linear relationships between echocardiographic and biochemical indices of the patients in the study. Differences between the 3 groups (patients with SSc with and without systolic dysfunction and controls) were compared using 1-way ANOVA with Bonferroni posthoc analysis.

The statistical software used was SPSS 12.0 for Windows. A value of $p < 0.05$ was considered significant.

RESULTS

Echocardiographic findings in patients with SSc and controls are shown in Table 2. Heart rate and diastolic and systolic blood pressure of patients with SSc were similar to those of healthy controls.

Mit E/A ratio was lower in patients with SSc (1.01 ± 0.16) than in controls (1.52 ± 0.17; $p < 0.0001$). The inver-

Table 2. Echocardiographic and biochemical measurements in control and scleroderma groups.

	Controls, n = 25	Patients with SSc, n = 52	p
HR	73.7 ± 9.5	76.6 ± 10.2	NS
SBP, mm Hg	143.6 ± 2.7	145.5 ± 2.4	NS
DBP, mm Hg	85.8 ± 1.8	86.3 ± 2.0	NS
NT-proBNP, fmol/l	256.3 (195.3–315.3)	382.7 (198.8–430.8)	NS
ADMA, μmol/l	0.25 ± 0.13	0.34 ± 0.18	NS
LA, mm	31.9 ± 3.2	36.2 ± 5.4	0.0001
LVEF, %	68.6 ± 5.8	71.2 ± 7.9	NS
Mit E, m/s	0.81 ± 0.11	0.79 ± 0.87	NS
Mit A, m/s	0.53 ± 0.09	0.79 ± 0.03	< 0.0001
Mit E/A	1.52 ± 0.17	1.01 ± 0.16	< 0.0001
Tr E, m/s	0.60 ± 0.90	0.52 ± 0.11	0.04
Tr A, m/s	0.41 ± 0.05	0.51 ± 0.16	0.008
Tr E/A	1.47 ± 0.16	1.11 ± 0.33	< 0.0001
sPAP, mm Hg	24.2 ± 2.3	39.2 ± 5.8	< 0.0001
MV-DT, ms	169.5 ± 14.8	183.4 ± 42.3	NS
LV (lateral mitral annular site)			
Sm, m/s	0.10 ± 0.02	0.07 ± 0.02	< 0.0001
Em, m/s	0.10 ± 0.01	0.07 ± 0.01	< 0.0001
Am, m/s	0.10 ± 0.01	0.09 ± 0.01	NS
E/Em	5.9 ± 0.6	11.3 ± 2.3	< 0.0001
IVRT-LV, m/s	71.3 ± 9.9	74.7 ± 13.7	NS
IVCT-LV, m/s	53.2 ± 7.9	54.7 ± 12.4	NS
MPI	0.31 ± 0.04	0.31 ± 0.06	NS
No. with Sm < 0.075	0	15	0.0016
No. with Em < 0.1 or E/Em > 15	0	24	< 0.0001
RV			
Sm, m/s	0.10 ± 0.02	0.11 ± 0.02	NS
Em, m/s	0.13 ± 0.02	0.08 ± 0.03	0.006
Am, m/s	0.12 ± 0.01	0.19 ± 0.01	NS
E/Em	6.1 ± 1.5	10.9 ± 2.1	0.002
IVRT-RV, m/s	67.2 ± 12.1	71.0 ± 14.5	NS
IVCT-RV, m/s	71.8 ± 11.2	65.6 ± 13.8	NS
MPI	0.24 ± 0.07	0.26 ± 0.04	NS

HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; NT-proBNP: N-terminal pro brain natriuretic peptide; ADMA: asymmetrical dimethylarginine; LA: diameter of left atrium; LVEF: left ventricular ejection fraction; Mit E: peak velocity of early diastolic mitral flow; Mit A: peak velocity of late diastolic mitral flow; DT: deceleration time; sPAP: systolic pulmonary arterial pressure; LV: left ventricular; RV: right ventricular; Sm: peak systolic myocardial velocity derived by pulsed wave Doppler tissue; Em: peak early diastolic myocardial velocity derived by pulsed wave Doppler tissue; AM: peak late myocardial velocity derived by pulsed wave Doppler tissue; IVCT: isovolumic contraction time; IVRT: isovolumic relaxation time; MPI: myocardial performance index; MV: mitral valve; NS: nonsignificant.

sion of Mit E/A ratio seemed to depend mainly on the significant increase of peak Mit A in patients with SSc ($p < 0.001$), associated with a slight but not statistically significant decrease of peak Mit E. Patients with SSc had a larger left atrial diameter and a higher sPAP compared with controls. Tr E/A ratio was significantly decreased in patients with SSc (1.11 ± 0.33) compared to controls (1.47 ± 0.16; $p < 0.0001$), because of a significant decrease of peak Tr E ($p = 0.04$) and an increase of peak Tr A ($p = 0.008$).

Pulsed TDE relieved lower LV Sm (0.07 ± 0.02 m/s) compared to healthy controls (0.10 ± 0.02 m/s; $p < 0.0001$), indicating impaired LV systolic function. LV Em was lower in patients with SSc (0.07 ± 0.01 m/s), compared to controls (0.10 ± 0.01 m/s; $p < 0.001$). LV filling pressure, estimated from E/Em ratio, was higher in patients with SSc (11.3 ± 2.3 compared to 5.9 ± 0.6; $p < 0.0001$).

Using the TDE indices, impaired LV systolic function was detected in 15 patients and diastolic dysfunction in 24. Patients with reduced LV systolic performance had higher NT-proBNP concentrations [330.1 fmol/ml (263.9–556.1)] compared with patients with SSc who have normal LV systolic function [259 fmol/ml (164.7–347.3)] and controls [256.3 fmol/ml (195.3–315.3); $p = 0.001$; Figure 1].

ADMA was significantly correlated with LV diastolic function, as assessed by LV Em ($p = 0.03$, $r = 0.29$; Figure 2), and LV E/Em ($p = 0.03$, $r = 0.29$). NT-proBNP was associated with peak Mit E ($p = 0.04$, $r = 0.28$), Mit E/A ratio ($p = 0.005$, $r = 0.44$), and Mit DT ($p = 0.047$, $r = –0.28$). Significant correlation was also shown between NT-proBNP and ADMA levels ($p = 0.023$, $r = 0.27$; Figure 3).

Nonsignificant correlations were detected between epidemiological (age, sex, disease duration), clinical and functional pulmonary measurements, and serological profile and echocardiographic indices (data not shown).

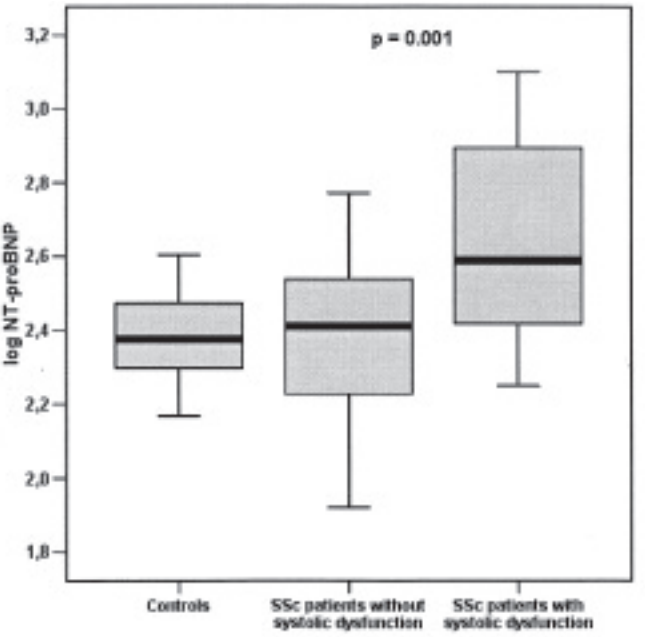

Figure 1. Values of N-terminal pro-brain natriuretic peptide (NT-proBNP); boxes indicate the mean and 25th–75th percentiles in patients with SSc and systolic cardiac dysfunction compared with patients with SSc who do not have systolic dysfunction, and with controls.

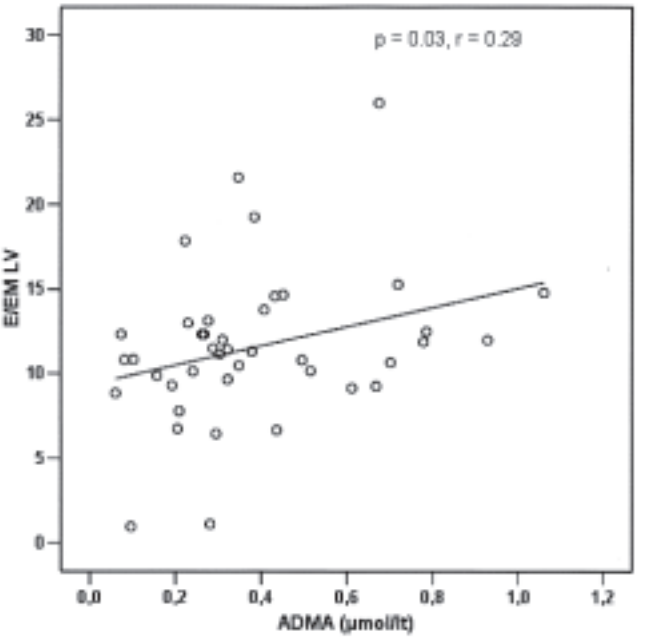

Figure 2. Correlation between asymmetric dimethylarginine (ADMA) and E/Em ratio of left ventricular diastolic function in patients with SSc.

DISCUSSION

We demonstrated that subclinical LV and RV impairment is common in patients with SSc who have not already demonstrated cardiac involvement. Correlations between echocardiographic measurements and biochemical markers were also an interesting finding of our study.

Reduced cardiac performance due to myocardial fibrosis is usual in patients with SSc and results in abnormal LV function, although most studies have shown a low prevalence of reduced LVEF[19-21]. A recent study of the European League Against Rheumatism Scleroderma Trial and Research group database of 7073 patients showed that the prevalence of LV dysfunction — defined as LVEF below 55% — assessed by conventional echocardiography was only 5.4%[22]. In our study, indicators of LV systolic function such as Sm and MPI were seriously impaired in patients with SSc, reinforcing the hypothesis that LV systolic abnormalities are present even if LVEF values are in the normal range. These data are in agreement with a recent study that assessed cardiac involvement in 100 consecutive patients with SSc using TDE[7]. As the proportion of deaths due to SSc heart disease has not changed significantly during the last decades[23], the low sensitivity of routinely measured LVEF mandates the systemic implementation of additional echocardiographic modalities, including TDE, in order to adequately investigate patients with SSc.

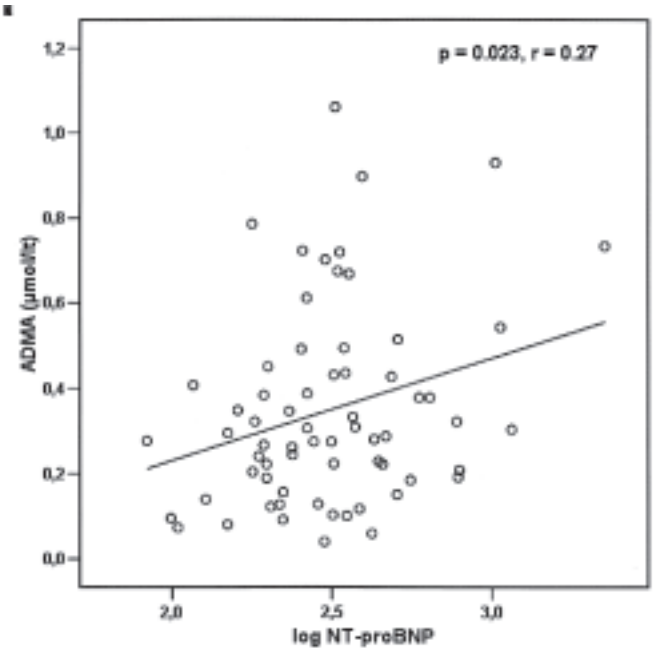

Figure 3. Correlation between asymmetric dimethylarginine (ADMA) and N-terminal pro-brain natriuretic peptide (NT-proBNP) in patients with SSc.

The diastolic changes observed in patients with SSc, mainly in the relaxation phase, resulted in an increase of left atrial contribution to filling (peak A) and a decrease of early diastolic filling (peak E). There is controversy in the literature about the cause of LV diastolic dysfunction in SSc; it may be specific to SSc or secondary to such factors as age, heart rate, or elevated blood pressure. We took particular care with these measurements by matching our healthy control subjects with patients with SSc. As a result, LV diastolic dysfunction, assessed by Mit E/A ratio, Em, and E/Em ratio, was independent of other risk factors. Our results are in accord with studies of patients with SSc who do not have clinical manifestations of heart disease[7,8]. These findings suggest that SSc is characterized by LV and RV filling abnormalities and support the hypothesis that diastolic dysfunction reflects global, intrinsic, myocardial involvement.

Moreover, RV dysfunction has been detected in patients with SSc even when LV function is normal[24,25]. Isolated abnormal RV function may suggest a latent pulmonary hypertension in this group of patients, as revealed by stress echocardiography and TDE[26].

ADMA, an inhibitor of NO synthesis, is a novel marker of endothelial dysfunction and predictor of cardiovascular events in patients with coronary artery disease[27] and peripheral artery disease[28]. Endothelial dysfunction and activation is one of the major events in SSc, resulting in vascular remodeling and fibrosis of skin and internal organs. We found close correlations between echocardiographic measurements of LV diastolic dysfunction and ADMA. Recently, such associations were established in patients with chronic heart failure[29]. This new finding may imply a link between the NO/ADMA pathway and cardiac involvement in SSc. Longterm blockade of NO synthesis in rats plays a key role in the pathogenesis of cardiac fibrosis by enhancing gene expression and protein production of transforming growth factor ß (TGF-ß) by angiotensin II type 1 receptors[30]. Since TGF-ß is one of the most significant profibrotic cytokines involved in pathogenesis of SSc, the results of our study may lend support to the potent etiopathogenetic role of abnormal NO metabolism in SSc cardiac involvement. It is possible that the inhibition of NO synthesis by ADMA may contribute to myocardial fibrosis and the intramyocardial coronary vessel involvement typical of SSc heart disease.

NT-proBNP is a well established biochemical marker of cardiac dysfunction and has been associated with echocardiographic measurements of RV overload in patients with SSc and pulmonary hypertension[31,32]. NT-proBNP levels are also correlated with hemodynamics[33] and prognosis[34] and have been identified as independent predictors of the occurrence of pulmonary hypertension in patients with SSc[35]. In a recent study, NT-proBNP reliably detected the presence of cardiac involvement in 69 patients with SSc[9], while depressed LV or RV contractility (measured by TDE) and increased sPAP (measured by conventional echocardiography) were independent predictors of NT-proBNP concentrations. As a result, serial NT-proBNP measurements added onto clinical and echocardiographic findings seem to be a promising assessment approach in the care and followup of patients with SSc, meriting further scrutiny.

The close relationship between NT-proBNP and ADMA, which has already been established in patients with chronic heart failure[36], suggests that ADMA may be a useful additional biochemical tool in assessing patients with SSc at high risk for cardiac disease. This is the first study that explores the relationship between ADMA and cardiac involvement in SSc. Additional investigations are warranted to confirm these findings in patients with and without clinical evidence of heart dysfunction.

Our study has some limitations, including the lack of data from invasive studies correlating hemodynamic measurements with TDE measurements. However, this study was designed to show the potent value of easily performed surveys, such as TDE and blood tests, in investigating patients with SSc to detect cardiac involvement before the onset of symptoms. We used ADMA values for assessing endothelial dysfunction. Whether other noninvasive methods of endothelial dysfunction, such as peripheral artery or coronary artery reserve, are associated with cardiac involvement merits further evaluation. Finally, we chose not to include in the study protocol patients with known heart failure and reduced LVEF and that could explain the lack of correlation between ADMA and systolic function as expressed by LV Sm values.

Our results suggest that LV and RV dysfunction in

patients with SSc can be assessed early in the course of the disease by using TDE. NT-proBNP and ADMA are significantly correlated with echocardiographic abnormalities, linking cardiac function, neuroendocrine derangement, and endothelial dysfunction in patients with SSc who have cardiac disease.

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