The impact of cardiac involvement in patients with systemic sclerosis

Ph.D. Thesis

Gyöngyvér Költő M.D.

Supervisor: András Komócsi, M.D., D.Sc.

Head of the Ph.D. program: László Czirják, M.D., DSc

University of Pécs

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Introduction

Systemic sclerosis (scleroderma, SSc) is an autoimmune connective tissue disease whose pathogenesis is characterized by three hallmarks: small vessel vasculopathy, production of autoantibodies, and fibroblast dysfunction leading to increased deposition of extracellular matrix. The clinical manifestations and the prognosis of SSc vary, with the majority of patients having skin thickening and variable involvement of internal organs including, in particular, the skin, lungs, heart, kidneys and digestive tract. Subsets of SSc can be discerned, that is limited cutaneous SSc and diffuse cutaneous SSc.

Although disease modifying therapies have demonstrated minimal efficacy in SSc, several organ-specific therapies have emerged over the past couple of decades resulting in improved survival and quality of life. The development of successful disease-modifying therapies for SSc is hindered by the heterogeneous clinical manifestations of this disease, also making early diagnosis challenging. Besides pulmonary and renal involvement cardiovascular disease is a principal determinant of mortality in SSc. In contrast to its importance, cardiac disease represents a diagnostic challenge in SSc. The reason for this is the complex nature of cardiac involvements with overlapping, non-specific symptoms. Patients with early disease are more likely to respond to targeted therapies, and irreversible organ damage may be prevented.

The aim of our research team was to properly characterize cardiac involvement in patients with systemic sclerosis, to investigate the prognostic value of the presence and overlap of different cardiac manifestations and the most important risk factors as well as to identify applicable markers, which could improve the detection and follow-up patients with cardiac involvement.

AIMS

Our goal with long term follow-up studies were the following:

1. Previously, we demonstrated in a single center, cross-sectional study the frequent coexistence of different forms of cardiac involvement in systemic sclerosis including pulmonary arterial hypertension (PAH), coronary artery disease (CAD) and microvascular dysfunction (MVD). These diverse pathologic processes affecting the heart in SSc have different prognostic significance. In our 5-year follow-up we investigated the prognostic value of the presence and overlap of different cardiac manifestations.

2. Our aim was to investigate the diagnostic and prognostic value of circulating concentrations of N-terminal fragments of A- and B-type natriuretic peptides (NT-proANP and NT-proBNP) in patients with SSc.
3. We aimed to assess the abnormalities of the diastolic function, analyze the characteristics of the disease progression, and to investigate the prognostic value of diastolic dysfunction in SSc patients.

Methods

Patients and methods

General inclusion and exclusion criteria

Consecutive patients with systemic sclerosis diagnosed in the tertiary center of the University Rheumatology and Clinical Immunology Department were recruited at the time of their regular yearly cardiological check-up. Diffuse and limited subset of SSc cases were diagnosed by the commonly used criteria, all cases complied with the recently updated classification criteria.

Each patient underwent a baseline physical examination, echocardiography, and a 6-minute walk test. Lung involvement was investigated by using chest X-ray, pulmonary function tests, and high resolution computed tomography if interstitial lung disease was suspected. Cardiac functional capacity was assessed based on the New York Heart Association (NYHA) classification. Duration of Raynaud's phenomenon (RP) at the time of the study entry was evaluated by clinical interview, while duration of SSc was determined from the time of the onset of the first SSc-related non-Raynaud symptom. Those with an ejection fraction ,<30% on echocardiography, or with known severe valvular disease were excluded. Patients with severe lung fibrosis (forced vital capacity, 50% on a pulmonary function test) were also excluded from further studies.

- Based on the criteria below selected patients underwent an extended invasive protocol comprising right heart catheterization (RHC) and coronary angiography, supplemented with thermodilution coronary flow reserve (CFR).Cardiac catheterization was initiated in the presence of abnormalities, either suggestive of PAH ("suspected PAH") or of CAD ("suspected CAD").

- Criteria for the "***suspected PAH***" included signs of right ventricular involvement on echocardiography (velocity of tricuspid regurgitation higher than 3 m/s, or consistent with 2.5-3m/s in the presence of unexplained dyspnoea, signs of right ventricular hypertrophy/dilatation, or a systolic D-sign) and effort related dyspnoea with disproportional decrease of CO diffusion capacity (DLCO) compared to the forced vital capacity (FVC/DLCO >1.8).
- Patients were included in the "***suspected CAD***" group if they complained of recent deterioration of physical status, evolving effort dyspnoea or chest pain, or fulfilled the criteria of the New York Heart Association (NYHA) functional classes III-IV, or if their 6-minute walking distance was <380m, but they did not fulfil the criteria for PAH.

Special inclusion and exclusion criteria of the 3 longitudinal studies

A. In our study analyzing the impact of the cardiac involvement on the risk of mortality 120 consecutive SSc patients classified according to conventional criteria were enrolled from January 2006 as described above.
B. In our evaluation of prognostic value of N-terminal natriuretic peptides, patients were recruited at the time of their regular yearly cardiological check-up from January to November in 2007 into a prospective trial. Patients were excluded from further investigations if they had conditions known to affect NP levels or interfere with the compliance to the screening protocol; with severly decreased systolic function (ejection fraction <30% on echocardiography), with significant renal impairment (creatinine >150μmol/L), with known severe valvular disease or with severe lung fibrosis (forced vital capacity <50%).
C. SSc patients referred for cardiologic consultation as part of their regular yearly check-up during a 3 months period in the year of 2006 were considered for inclusion to asses progression of left ventricular diastolic dysfucntion. Patients with severely impaired left ventricular systolic function (ejection fraction <30%), severely impaired right ventricular systolic function (tricuspid annular S < 9 cm/s[9]), atrial fibrillation, significant left or right sided valvular abnormalities or prosthetic valves were excluded from the study. Data from the investigation of 23 healthy volunteers (mean age 53±10 years, 17 female) without the signs or symptoms of any cardiac disease were used as control.

Follow-up

Patients were followed for 5 years after the initial investigation with yearly scheduled visits. Patients not being able to attend the visits were contacted by phone calls by health professionals and were also followed-up by their treating physicians to justify that the information gained was adequate. Medical records of deceased patients were collected and treating physicians and relatives were interviewed. Data obtained from these sources were reviewed by a team of rheumatologists and cardiologists to determine the cause of death. Death was considered as cardiovascular death when it was due to myocardial infarction, sudden death or congestive heart failure. If the date of death was not known, survival time was calculated based on the date of the last follow-up visit.

End point definitions

A. In our long term follow up of cardiac involvement we analyzed the cardiovascular event-free survival and the cardiovascular mortality of patients with pulmonary hypertension, with coronary artery disease or microvascular dysfunction groups and in the entire cohort. In addition we investigated the cardiovascular event-free survival depending on the results of cardiac catheterization (patients with one, two or three disorders).

B. The main outcome parameter of the investigation of prognostic value of natriuretic peptides study was the occurrence of symptomatic heart disease as defined by right heart catheterization proven PAH, development of left ventricular systolic dysfunction (ejection fraction <50%), non-fatal myocardial infarction (MI) or coronary revascularization (MACE-Major Adverse Cardiac Events). The primary clinical outcome measure was the composite of the above and all-cause mortality during the period of five years.

C. Evaluating the long term prognostic significance of the diastolic dysfunction we investigated echocardiographic parameters indicating the progression of diastolic dysfunction (left ventricular mass index, left atrial volume and lateral E'values). The primary outcome of the study was cardiovascular-related mortality.

Echocardiography

General echocardiographic protocol

Investigating the cardiovascular involvement on the risk of mortality and in the assessment of prognostic significance of natriuretic peptides studies due to the large patient population, routine echocardiographic measurement was performed with Aloka ProSound 5500 ultrasound equipment. Ejection fraction was measured by biplane Simpson's method. Transmitral flow was recorded from the apical 4-chamber view, while placing the sample volume at the level of the mitral valve leaflet tips. Peak of the early (E) and late (A) velocities were measured and E/A ratio was calculated. Systolic pulmonary artery pressure was estimated by using the simplified Bernoulli equation, and calculated from the peak tricuspidal regurgitation velocity ($4v^2$ plus estimated right atrial pressure according to the diameter and collapse index of the inferior vena cava).

Left ventricular diastolic function

Specific aspects were taken into account in case of assessment the progression of diastolic dysfunction. Echocardiography was performed at the baseline and repeated after five-year follow-up using the same equipment by a single investigator. All 2D and M-mode measurements were obtained. End-diastolic thickness of the septum and the posterior wall as well as the end-diastolic diameter of the left ventricle were measured from parasternal long axis view, by M-mode. Left ventricular mass was calculated according to the Devereux formula and corrected for body surface area (LVM index). In addition to the conventional echocardiographic transmitral flow measurements (peak of the early (E) and late (A) velocities, calculated E/A ratio) tissue Doppler measurements were also performed at the lateral mitral and tricuspid annulus. Peak systolic (S) and early diastolic (E') longitudinal velocities were measured. Mitral E/E' ratio was calculated. Doppler measurements were obtained from 3 consecutive beats. Biplane Simpson's method was used to measure the maximal left atrial volume as apical 4- and 2-chamber views were visualized at the time of mitral valve opening. Atrial appendage and pulmonary veins were excluded from the measurements. LA volume was corrected for body surface area (LA volume index).

Natriuretic peptide levels

Natriuretic peptide levels were determined from blood samples. Plasma fraction of the blood was separated and the samples were stored at -20°C until analysis. The plasma concentrations of NT-proANP and NT-proBNP were determined with radioimmunoassays utilizing antisera directed to NT-proANP$_{46–79}$ and NT-proBNP$_{10–29}$. The sensitivities of the assays were 60 and 40 pmol/L, respectively.

Statistical analysis

Data were analyzed with the Statistical Package for the Social Sciences software. Categorical data were expressed as frequencies and percentages; continuous data were expressed as the mean ± SD. Comparisons between groups of patients were performed using Student's t-test for continuous variables and the chi-square test or Fisher's exact test for categorical variables. Non-normally distributed variables between groups were analyzed with the Mann-Whitney test.

Kaplan-Meier analysis was used to estimate survival from the time of diagnosis to the date of last follow-up observation up to 5 years after diagnosis. Comparisons for factors were conducted by log rank test and linear trend for factor levels was assessed pooled over strata. Univariate analyses were conducted using Cox models for time-to-event analyses. The level of significance was set at $P<0.05$.

The predictive values of natriuretic peptides levels, the progression of the diastolic dysfunction characterized by echocardiographic parameters as well as the optimal cut-off points were determined by receiver operating characteristic (ROC) curve analysis.

Results

Impact of cardiac involvement on the risk of mortality

120 SSc patients were included. At the initial cross sectional phase of the study cardiac catheterization was performed in 30 cases. Follow-up time was 52.7±15.5 months. 23 deaths were registered, 14 cardiovascular approved by the multidisciplinary case review: two cases of fatal arrhythmia, one myocardial infarction and eleven deaths due to systolic heart failure. Of the non-cardiac deaths, three were caused by respiratory failure or infection, one by intracranial hemorrhage, one by severe malabsorption and one by traffic accident. Three patients died of malignancy.

During the follow-up phase 34 additional catheterizations were performed, and altogether 30% of the entire cohort underwent invasive investigation at some point in the study. Of the 15 patients with coronary lesions detected by morphological and functional assessment, eight underwent revascularization (1 bypass grafting, 7 percutaneous coronary stent implantation). During the follow-up, one patient required a non-target vessel stent implantation; while six additional patients were diagnosed with CAD in three of whom required percutaneous coronary intervention. No further cases with MVD were diagnosed. **(Figure 1.)**

Of the 14 patients with PAH at the cross sectional investigation, eight had elevated pulmonary mean pressures at rest and six on exertion. During the follow-up one of the 6 patients with SiPAH developed PAH at rest. Further two cases with PAH at rest and two cases with stress induced PAH were additionally diagnosed. According to the Dana Point definitions at the cross sectional phase 8 patient had PAH, 11 borderline and 11 normal pulmonary pressures. During the follow-up 2 new cases with PAH and 2 with borderline mPA were diagnosed. One patient with borderline progressed to PAH. **(Figure 1.)**

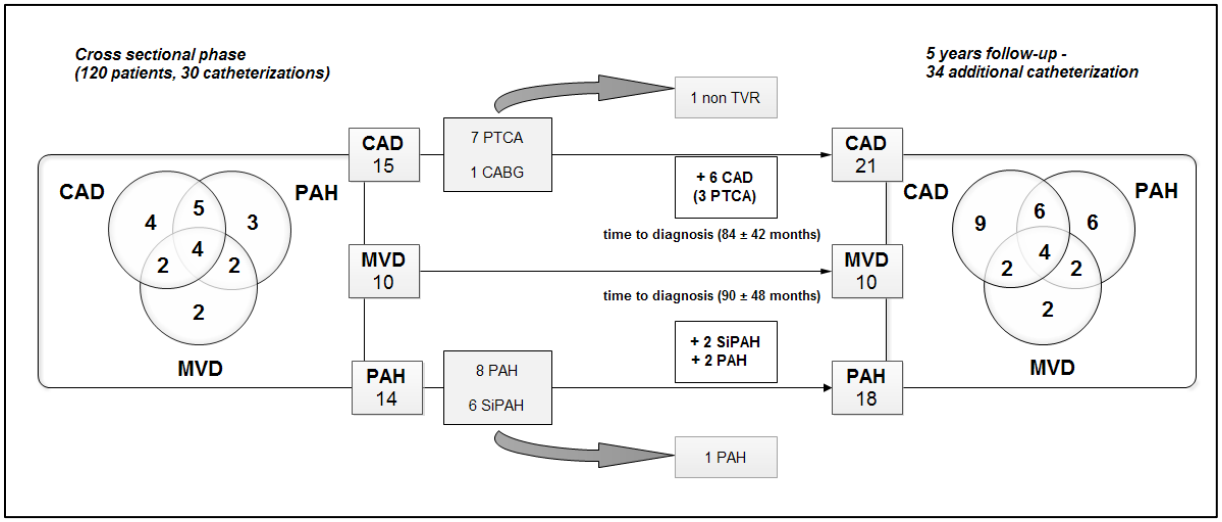

Figure 1. Flowchart showing cardiac involvement during 5 years in 120 systemic sclerosis (SSc) patients. Diagnosis of coronary artery disease (CAD), pulmonary arterial hypertension (PAH) and microvascular dysfunction was verified in 22 SSc cases with considerable overlap among the different processes. (Panel A) During the 5-years follow-up 34 additional catheterization verified CAD in six, PAH in four patients. (Panel B) Cardiac catheterization was performed in 30% of the cohort during the study. Panel C shows the distribution and overlap of diagnoses verified during the entire study. Abbreviations: PTCA; percutaneus transluminal coronary angioplasty, non TVR; non target vessel revascularization, CABG; coronary artery bypass grafting.

The 5-year Kaplan-Meier estimates of survival of the overall SSc cohort and the thirty patients referred for cardiac catheterization were 80.5±3.7% and 83.1±6.9%, respectively (p = 0.635). The cumulative rate of cardiovascular death were 12.4% and 16.9%, (p=0.42). (Figure 2.)

The frequency of cardiovascular events at 5 years was 11.1%, 33.3%, 25.0%, in patients with one, two, or three disorders, respectively. **(Figure 2.)** Patients with a combination of various abnormalities had a poorer prognosis. Cardiovascular event-free survival was significantly lower among patients with two or three disorders. (p<0.05)

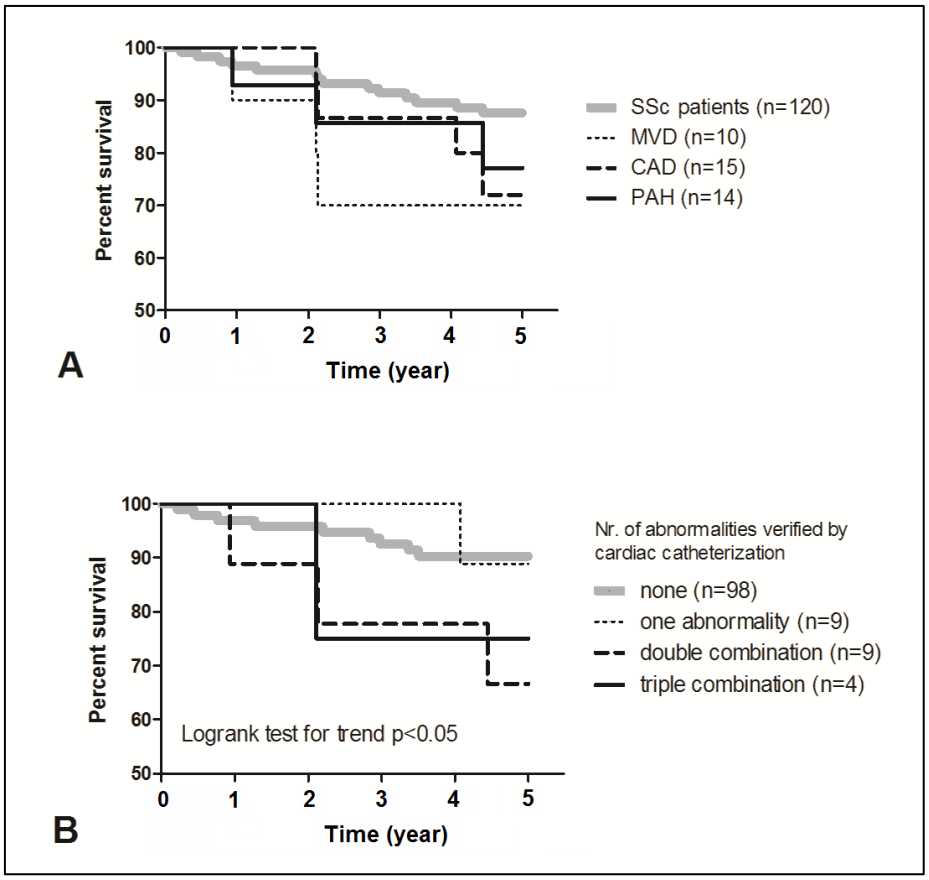

Figure. 2 Cardiovascular mortality of patients depending on the results of cardiac catheterization. A) Survival curves of patients with pulmonary hypertension (PAH) (solid line), coronary artery disease (CAD) (dashed line), or microvascular dysfunction (MVD) (dotted line) are represented. Gray line depicts the survival curve of the 120 systemic sclerosis (SSc) patients. Patients withMVD had a significantly worse survival (p <0.05).

B) Impact of the combined cardiac involvement. Gray line represents the survival curve of patients without verified cardiac involvement. Dotted line shows patients with one disorder (i.e., PAH, CAD, or MVD). Combinations of two or three types of cardiac involvement are marked with dashed and solid lines, respectively. Cases with combined cardiac involvement had a higher frequency of cardiac death (p <0.05). A significant trend for higher frequency of cardiovascular mortality was found patients with combined cardiac involvement.

Surviving proportions among PAH patients were numerically lower, however, this difference did not reach statistical significance. This finding was consistent independently from the PAH definition used. Using the modified Venice criteria [6] this difference was 78.6% vs 81.1%, p=0.844 for overall mortality and 21.4% vs 10.4%, p=0.261 for cardiovascular death compared to the data when the Dana Point definition was used: 75% vs 81.3%, p=0.91 and 25% vs 10.7%, p=0.40, respectively.

Multiple Cox proportional hazard model analyses found that the diffuse cutaneous form, presence of kidney involvement, diabetes mellitus, and the velocity of tricuspid regurgitation were independent predictors of the overall mortality. Regarding the cardiovascular mortality, diffuse cutaneous subgroup, diabetes mellitus, and velocity of tricuspid regurgitation as well as the cardiovascular functional status (NYHA) were significantly associated with worse outcome.

Prognostic value of N-terminal natriuretic peptides in systemic sclerosis

150 patients were evaluated for study inclusion. After exclusion of 6 patients (1 patient with significant aortic regurgitation, 1 with severely depressed systolic function and, 4 with significant renal impairment) 144 patients were included in the study and followed for the mean follow-up of 4.57 +/- 0.9 years. We registered 13 deaths. During the study 55 cardiac catheterization, 19 RHC and 36 coronary angiographies were performed in 28 patients from the cohort. Pulmonary arterial hypertension was diagnosed in 6 cases, these included 2 cases with known PAH and those 3 where the PAH was diagnosed during the initial check-up. Coronary intervention was performed in 11 cases (4 during the initial work-up, 5 cases during the follow-up period). Five patients were diagnosed with left ventricular systolic dysfunction.

Levels of NT-proANP and NT-proBNP were significantly higher (791.4±379.9 pmol/l versus 608.0±375.8 pmol/l $p<0.05$ and 183.1±162.6 versus 125.7±117.5 pmol/l $p<0.05$) in patients reaching any of the predefinied criteria of significant heart disease during the study. Analysis of the Kaplan-Meier event-free survival curves showed a significant trend for better outcome in patients within the lower quartiles of baseline NT-proANP and NT-proBNP concentrations. **(Figure 3. column A)** An optimal NT-proANP cut-off value of 822.5 pmol/l was suggested with a sensitivity of 56.3%, specificity of 79.5%, negative predictive value of 86.4% and NT-proBNP cut-off value of 154.5 pmol/l with a sensitivity of 50.0%, specificity of

76.8%, negative predictive value of 83.7%. **(Figure 3, column B)** An area under the curve (AUC) of 0.663±0.058 (Asymptotic significance (AS): p=0.005; 95% Asymptotic Confidence Interval (CI): 0.549-0.777) was slightly superior in case of the NT-proANP levels to NT-proBNP (AUC: 0.624±0.059, p=0.015, CI: 0.509-0.738).

Analysis of the outcome of patients after exclusion of those with known cardiac disease or diagnosed at the initial work-up of the study (prevalent cases), revealed that cases with higher NT-pro-ANP levels over the ROC defined cut-off had significantly worse event-free survival. Similar strong trend in the level of NT-proBNP was observed, however this latter differences did not reach the level of statistical significance (p=0.052). **(Figure 3, column C)**

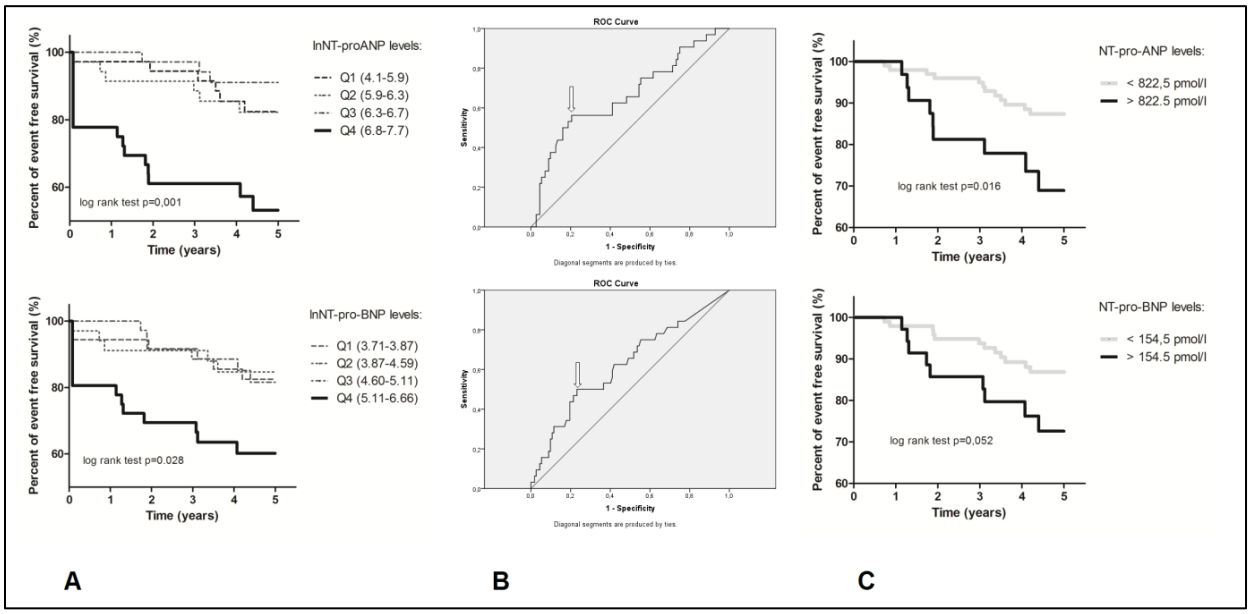

Figure 3. Prognostic values of natriuretic peptid levels for the 5-year event free survival
Column A shows Kaplan-Meier survival curves for 4 groups based on quartiles of distribution of ln natriuretic peptides. Differences in 5-year event-free survival with log rank test for trend in the level of lnNT-proANP and lnNT-proBNP were statistically significant. Q: quartile.

Column B presents the Receiver-operator characteristic (ROC) curve analyses of the predictive values of natriuretic factor levels in determining the composite endpoints. The optimal cutoff value with 56.3% sensitivity and 79.5% specificity was 822.5 pmol/l NT-proANP (arrow), and with 50.0% sensitivity and 76.8% specificity was 154.5 pmol/l NT-proBNP (arrow). Diagonal segments are produced by ties.

Column C demonstrates the prognostic values of NT-proANP and NT-proBNP measurements in systemic sclerosis. Kaplan-Meier curves of event free survival of patients who did not reach end-point during the initial visit separated significantly if patients were divided according to the ROC definied cut-off of NT-pro-ANP level.

Univariate analysis found significant correlation in 7 characteristics: elevated NT-proANP (OR: 4.17 [2.06-8.41], p<0.001), elevated NT-proBNP (OR: 2.83 [1.41-5.67], p<0.01), diffuse cutaneous subset of SSc (OR: 2.16 [1.03-4.55], p<0.05), renal involvement (OR: 8.39 [1.11-63.24], p<0.05), carbon-monoxide diffusion capacity (DLCO) (OR: 0.97 [0.95-0.99], p<0.01), and DLCO/VA (OR: 0.96 [0.94-0.99], p<0.01), and ratio of the forced vital capacity and the DLCO (FVC/DLCO) (OR: 0.97 [0.95-0.99], p<0.01). In the multivariable model elevated NT-pro-ANP level (OR: 4.062 [1.89-8.73] p<0.001) and the ratio of FVC/DLCO OR: 0.97 [0.95-0.99] p<0.01)

Left ventricular diastolic dysfunction: determinants of mortality and disease progression

Altogether 40 SSc patients were referred to our institution during the enrolment period. Six of them, however, were excluded from the study (2 severe mitral regurgitation, 1 atrial fibrillation, 1 severely reduced right ventricular function, 1 poor acoustic window, 1 refused informed consent). Finally 27 patients with limited cutaneous SSc and 7 patients with diffuse cutaneous SSc were enrolled into the study. Baseline clinical data of the 34 SSc patients are reported in **Table 1** Twenty four patients underwent right heart catheterization. In 3 patients mild form of resting PAH (mean pulmonary artery pressure 29.3±4.9 mmHg), while in 8 cases stress induced PAH was diagnosed. In patients with resting PAH sildenafil treatment was initiated. In 27 patients coronary angiography was performed. In 13 patients significant CAD was diagnosed. In 10 cases percutaneous coronary intervention was performed while one patient underwent coronary artery bypass surgery. In 2 cases coronary intervention was not feasible. Detailed description of the further co-morbidities and cardiovascular medication is outlined in **Table 1.**

	SSc patients Baseline data (n=34)	**Healthy volunteers (n=23)**	**p**
Clinical data			
Age (years)	57±12	53±10	0.091
Female gender (%)	31 (91%)	17 (74%)	0.080
BSA (m^2)	1.74±0.17	1.83±0.18	0.057
NYHA functional class I. II. III.	 4 (12%) 27 (79%) 3 (9%)	23 (100%)	**0.000**

Echocardiography			
Ejection fraction (%)	61.0±3.3	61.3±3.1	0.766
Right ventricular pressure (mmHg)	31.3±8.3	24.5±3.6	**0.000**
End diastolic diameter (mm)	47±2	48±3	0.108
LVM index (g/m^2)	109.9±22.1	96.4±12.4	**0.005**
E (cm/s)	68.3±15.8	66.1±12.9	0.592
A (cm/s)	72.0±19.7	59.7±13.7	**0.008**
E/A	1.0±0.3	1.1±0.3	0.091
Lateral S (cm/s)	8.9±2.2	11.4±2.1	**0.000**
Lateral E' (cm/s)	9.3±2.6	11.3±1.9	**0.002**
Lateral A' (cm/s)	9.7±1.4	11.5±2.3	**0.002**
E/E'	7.8±2.5	5.9±2.5	**0.001**
LA volume index (ml/m^2)	30.2±11.0	25.4±7.0	**0.048**
Stage of diastolic function - normal - impaired - pseudonormal	 13 (38%) 9 (26.5%) 12 (35.5%)	 16 (69%) 5 (22%) 2 (9%)	**0.027**
Co-morbidities			
Coronary heart disease (%)	13 (38%)		
Pulmonary arterial hypertension (%)	11 (32%)		
Hypertension (%)	17 (50%)		
Heart failure (%)	19 (56%)		

Table 1. Baseline characteristics of the SSc population and comparison with healthy subjects.

Comparison of the SSc population with the data of healthy controls

The group of SSc patients and healthy controls were matched in age, gender distribution and body surface area (**Table 1**). Left ventricular ejection fraction was preserved in all SSc patients, the longitudinal myocardial systolic velocity (S), however, was significantly lower in this group. LVM index and calculated right ventricular systolic pressure was significantly higher in SSc patients. No significant difference was found between the E/A ratio measured in the two groups. Though, myocardial early diastolic velocity (E') was significantly lower, while E/E' and LA volume index were significantly higher in SSc patients. Mild or moderate diastolic dysfunction was found in 62% of the SSc patients and in 30% of the healthy subjects.

Long term prognostic value of the diastolic dysfunction in SSc patients

During the follow-up time of 5.4±1.2 years, six SSc patients (18%) died. All of them suffered cardiovascular death (5 heart failure, 1 heart failure and consequential pneumonia). The deceased patients were significantly older, had significantly higher LVM index and LA volume index as well as significantly lower E' values than the survivors. The frequency of the higher skin score, CAD and systemic hypertension was numerically higher among the deceased patients, but the differences were statistically not significant (**Table 2**).

	No event (n=28)	**Event (n=6)**	**p**
Clinical data			
Age (years)	55±12	65±7	**0.049**
Female gender (%)	26 (93%)	5 (83%)	0.462
BSA (m^2)	1.76±0.17	1.62±0.16	0.085
LcSSc (%)	24 (86%)	3 (50%)	0.053
ANA positivity (%)	23 (82%)	6 (100%)	0.270
Skin-score	2.2±2.1	8.2±10.0	0.250
Duration of the disease (years)	12.5±6.5	11.7±11.6	0.865
NYHA functional class I. II. III.	 4 (14%) 22 (79%) 2 (7%)	 5 (83%) 1 (17%)	0.249
6MWT (m)	359±62	331±26	0.330
Echocardiography			
Ejection fraction (%)	61.0±3.3	60.7±3.6	0.788
Right ventricular pressure (mmHg)	31.2±8.8	31.7±6.4	0.911
LVM index (g/m^2)	104.0±18.1	137.4±18.5	**0.000**
E/A	1.0±0.3	0.8±0.2	0.120
Lateral S (cm/s)	9.3±2.3	7.5±1.0	0.068
Lateral E' (cm/s)	9.6±2.6	7.3±1.4	**0.043**
Lateral A' (cm/s)	9.7±1.5	9.7±0.9	0.839
E/E'	7.6±2.5	8.9±1.9	0.249
LA volume index (ml/m^2)	27.5±9.7	42.9±8.2	**0.001**

Stage of diastolic function - Normal - Impaired relaxation - Pseudonormal	 7 (25%) 9 (32%) 12 (43%)	 4 (67%) 2 (33%)	0.207
Co-morbidities			
Coronary heart disease (%)	9 (32%)	4 (67%)	0.120
Pulmonary arterial hypertension (%)	9 (32%)	2 (33%)	0.956
Hypertension (%)	13 (46%)	4 (67%)	0.375
Heart failure (%)	14 (50%)	5 (83%)	0.142
Medication			
ACE inhibitors (%)	8 (29%)	4 (67%)	0.081
Ca-channel blockers (%)	21 (75%)	5 (83%)	0.667
Beta-blockers (%)	8 (29%)	4 (67%)	0.081
Spironolactone (%)	14 (50%)	4 (67%)	0.465
Other diuretics (%)	11 (39%)	3 (50%)	0.634

Table 2. Clinical and echocardiographic data of the deceased SSc patients compared to the survivors.

In univariate Cox regression analysis, age (HR=1.08; $p<0.05$), LVM index (HR=1.07; $p<0.01$), lateral E' (HR=0.64; $p=0.05$) and LA volume index (HR=1.11; $p<0.01$) were predictors of the survival. The strong correlation between LVM index and LA volume index was proved also in our population ($r=0.63$; $p<0.0001$), thus, two different models were used for multivariable regression analysis.

In Model 1 (age, LVM index, lateral E') LVM index (HR=1.07; 95% CI of HR: 1.03-1.12; $p<0.01$) while in Model 2 (age, LA volume index, lateral E') LA volume index (HR=1.13; 95% CI of HR: 1.04-1.22; $p<0.01$) and lateral E' (HR=0.48; 95% CI of HR: 0.23-0.99; $p<0.05$) were the independent predictors of the survival.

Using ROC analysis, LVM index>112.8 g/m^2, LA volume index>35.4 ml/m^2 and lateral E'<8.5 cm/s were the best predictors of the mortality. ROC curves and Kaplan-Meier cumulative survival curves demonstrating the predictive role of these values are presented in **Figure 4.**

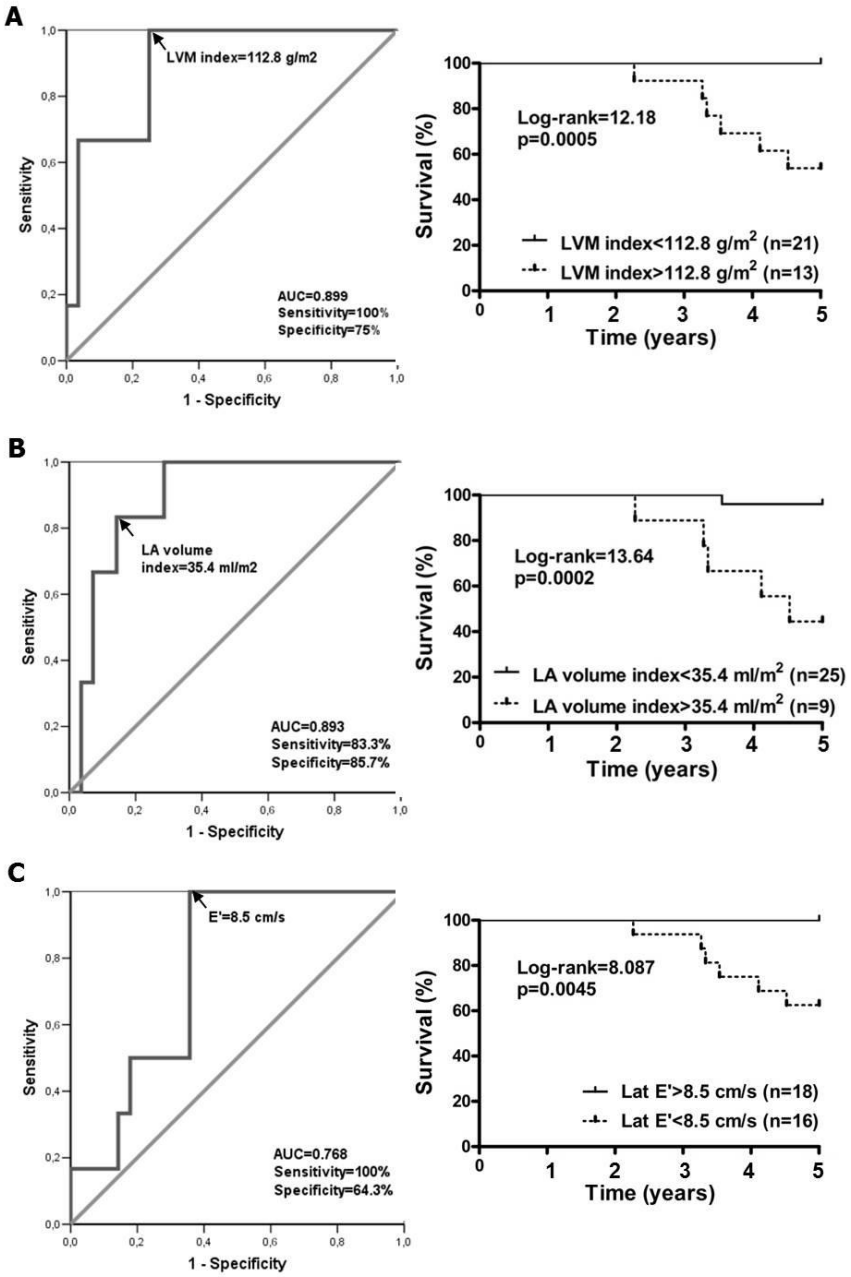

Figure 1 ROC curves and Kaplan-Meier survival curves demonstrating the diagnostic accuracy and the predictive value of LVM index **(A)**, LA volume index **(B)** and lateral E'**(C)**

Progression of the left ventricular diastolic dysfunction during the follow-up period

Although the left ventricular ejection fraction was still preserved in all of the surviving SSc patients, LA volume index and E/E' increased significantly during the follow-up period. In addition, a trend of increase in the LVM index was also observed, while the lateral E' showed

no significant changes. The rate of the mild or moderate diastolic dysfunction became significantly higher during the follow-up. In addition, the functional capacity of the patients declined significantly.

Linear regression analyses suggested that the only predictor of the disease progression was the duration of the SSc by showing a positive correlation with the increase in the LA volume index (Δ LA volume index). In addition, a negative correlation was found between the disease duration and the decrease in the lateral E' values (Δ Lateral E').

Differences in the follow-up time did not influence the disease progression. No correlation was found between the further characteristics of the SSc (ANA positivity, skin-score, elevated pulmonary artery pressure or/and CAD) and the echocardiographic parameters characterizing the left ventricular diastolic function.

Novel findings of the thesis

The goal of our studies was to follow patients with systemic sclerosis to find the most important cardiovascular risk factors and prognostic factors as well as adapt easy-to-use method for the care and follow-up of such patients. The main results of our three prospective cohort studies were the following:

A. In the prospective cohort of impact of cardiac involvement on the risk of mortality in SSc patients, coronary angiography-verified CAD cases were found to have a slightly, and those with microvascular dysfunction a significantly elevated risk for cardiovascular mortality. PAH was also associated with worse prognosis, however, this did not reach statistical significance in this cohort. These findings underline the importance of the assessment of coronary anatomy and function in symptomatic SSc patients. In addition, our data also support that early detection and specific therapy may effectively improve prognosis of scleroderma related pulmonary hypertension.
B. Natriuretic peptide levels beside their diagnostic value also have an important negative predictive value in patients with systemic sclerosis. Elevated natriuretic levels indicate a poor prognosis, so they are not only proved to have diagnostic, but also prognostic role as well. Using NT-proANP as an additional marker may help establishing early diagnosis and in the determination of the prognosis of cardiac involvement is SSc.

C. In SSc patients left ventricular diastolic dysfunction is highly prevalent and is associated with increased risk of mortality. Our data suggest that in the advanced phase of the disease the myocardial fibrotic processes burns out while the increase of the filling pressure progresses continuously. Follow-up of the progression of the diastolic dysfunction should be a part of the cardiac examination.

Conclusions

Prior to our prospective cohort studies, numerous observational studies highlighted that different visceral manifestations, especially cardiac involvement has significant impact on the outcome of SSc.

Previously, our team confirmed the well-known fact that patients with cardiopulmonary involvement in SSc are at a higher risk of death. Additionally, meta-regression of studies providing SMRs demonstrates a trend for improvement over the last decades in which the life expectancy of SSc patients approaches that of the general population. Advances in terms of better and earlier diagnosis as well as more efficient treatment of internal organ manifestations may be the most important contributing factors to the improved outcome of SSc.

Our study of impact of the cardiac involvement on the risk of mortality showed frequent coexistence of different components of cardiac involvement in symptomatic cases (i.e. pulmonary arterial hypertension, coronary artery disease and microvascular dysfunction). Microvascular dysfunction and coronary artery disease alone or in combination with pulmonary arterial hypertension significantly affected 5-year cardiovascular mortality. Importantly, these entities were not isolated and the participants with an overlap of vascular abnormalities had worse outcome with significantly higher risk of cardiovascular mortality. Our aim was to analyze the long term survival of patients with SSc investigating the prognostic role of different forms of heart involvement. Patients with cardiac manifestations may remain undiagnosed, if the progression manifests only as a slowly reducing physical capacity, and limiting effort related dyspnoea. Screening programs for SSc patients are demonstrated to improve the prognosis of cardiac involvement.

Current guidelines recommend regular screening of SSc patients to asses cardiac functional status including yearly echocardiography in order to facilitate timely diagnosis and treatment of pulmonary hypertension. In addition, there is a need for reliable biomarkers with potential to support diagnosis and define prognosis. Natriuretic peptides have emerged as important candidates for the development of diagnostic tools in cardiovascular disease.

Due to their diagnostic and prognostic relevance natriuretic peptide levels became important diagnostic tools in numerous cardiac diseases including cardiac failure and pulmonary hypertension.

Furthermore plasma natriuretic peptide concentrations are also valuable predictors of outcome. Patients with higher NP levels have worse prognosis even if cases with prevalent left ventricular dysfunction, coronary artery disease and PAH are disregarded. The elevated plasma level may be useful in selecting patients for right-heart catheterization. Elevated baseline and change in NT-proBNP levels may help identifying SSc-PAH patients with particularly adverse prognosis who may have benefit from the introduction or modification of advanced therapies. Natriuretic peptide levels beside their diagnostic value also have an important prognostic value in patients with systemic sclerosis. In our study NT-proANP was superior to NT-proBNP in prediction of prognosis that may support more extensive use of this marker.

Recurrent vasospasm, poor vasodilator reserve, focal ischemia, and recurrent ischemia-reperfusion injury, and inflammation (when present) all culminate in the same end result: myocardial fibrosis. All of these pathologic insults can lead to the varied clinical manifestations of myocardial disease in SSc, which include asymptomatic LV systolic or diastolic dysfunction, which can occur several years prior to becoming clinically evident and clinically overt heart failure.

Several earlier studies - mainly based on the Doppler analysis of the transmitral flow pattern (E/A) - suggested that left ventricular diastolic dysfunction is prevalent in SSc. TDI has been reported to be a preload independent technique. Early diastolic myocardial velocity (E’) measured at the mitral annulus using TDI is a reliable index of the left ventricular relaxation, while the ratio of the early diastolic velocity of the mitral inflow to early diastolic velocity of the mitral annulus (E/E') provides a good estimate of the actual left ventricular filling pressure.

If myocardial fibrosis progresses, diastolic compliance of the left ventricle (characterized by the mitral annular E' velocity) decreases and, even in the presence of normal ejection fraction, diastolic dysfunction or even manifest diastolic heart failure may evolve.

Multiple characteristic symptoms of SSc patients (decreased exercise capacity, dyspnoea, decompensation) are strongly related to the degree of left ventricular diastolic dysfunction. The recently used term of this phenomenon (heart failure with preserved ejection fraction -HFPEF) refers to the fact, that, though the left ventricular ejection fraction is preserved, the longitudinal systolic velocity of the left ventricle (characterized by the mitral annular S velocity) is reduced, as the sign of the subclinical impairment of the left ventricular systolic function.

Our data suggest that LA volume index is also an independent predictor of the mortality in these patients. In patients having relatively early form of scleroderma, the decline of the left ventricular relaxation (lateral E' parameter) was clear during the follow-up period, while in patients having advanced SSc the annular E' did not change or even showed a mild improvement, probably as the consequence of the random error of the tissue Doppler measurements. On average, lateral E' showed no significant changes during the follow up period. On the other hand, continuous progression of LA volume index and E/E' was found.

Our results suggest that in the more advanced phase of the SSc the decline of the left ventricular compliance slows down while the elevation of the filling pressure remains progressive. **(Figure 4.)**

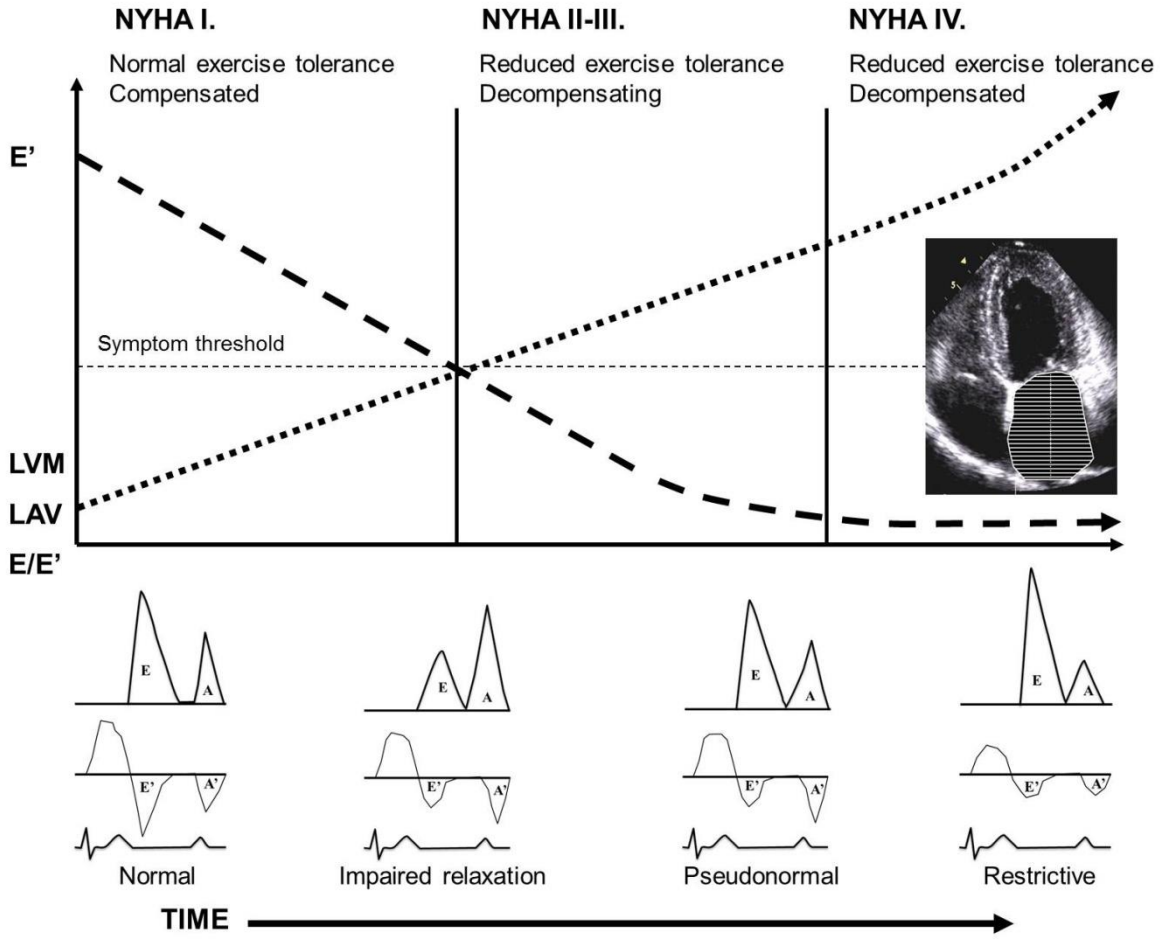

Figure 4. Schematic drawing demonstrating the progression of the diastolic dysfunction in SSc patients

Considering that mitral annular E' is thought to be the marker of generalized myocardial involvement of SSc, we assume, that the fibrotic process in the myocardium burns out in the more advanced phase of the disease, resulting in this phenomenon.

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