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ORIGINAL ARTICLE

The clinical significance of serum N-terminal pro-brain natriuretic peptide in systemic sclerosis patients

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Abstract We evaluated the clinical significance of serum N-terminal pro-brain natriuretic peptide (NT-proBNP) level in systemic sclerosis (SSc). We studied 45 SSc patients (30 with limited and 15 with diffuse cutaneous SSc) of mean age ± SD 47.1±12.9 years, mean duration of disease 10.2± 6.0 years, and 45 age- and sex-matched healthy controls. Pulmonary artery pressure was measured by echocardiography. Lung involvement was evaluated by pulmonary function testing and by using high-resolution computed tomography scores. Serum NT-proBNP levels were measured using a sandwich electrochemiluminescent immunoassay. Serum NT-proBNP levels were significantly higher in patients with SSc compared to healthy controls. When the patients were divided into clinical subsets, serum NT-proBNP was higher in diffuse SSc than in limited SSc. Serum NT-proBNP levels were found to be positively correlated with age, skin thickness score, and systolic pulmonary artery pressure and negatively correlated with percentage of carbon monoxide diffusion capacity (DLco). Multivariate analysis showed that serum NT-proBNP levels were positively correlated with age (p=0.010), skin thickness score (p=0.000), and blood pressure (p=0.021) and negatively correlated with %DLco (p=0.016). Fifty-seven percent of the variation in log (proBNP) can be explained by the multivariate model (R^2=0.57). Serum NT-proBNP levels were higher in SSc patients (particularly the diffuse subset) than in healthy controls and were found to be correlated with skin thickness and %DLco. We conclude that serum NT-proBNP may be a biologic marker of skin fibrosis and pulmonary vascular involvement in SSc.

Keywords DLco · NT-proBNP · Skin thickness · Systemic sclerosis

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Introduction

Systemic sclerosis (SSc) is an autoimmune disease characterized by fibrotic changes in peripheral and visceral vasculatures. The clinical characteristics of SSc are Raynaud's phenomenon, a progressive fibrosis of skin, lung, heart, and of the gastrointestinal and renal systems.

The pathogenetic mechanisms of SSc are unclear but may be associated with several cytokines and growth factors. Most deaths from SSc are due to cardiopulmonary involvement, and pulmonary artery hypertension (PAH) is widely recognized to be a major complication of both limited and diffuse SSc [1]. Therefore, the early detection and treatment of PAH are essential.

N-terminal pro-brain natriuretic peptide (NT-proBNP) and BNP have been used as diagnostic and prognostic markers in cardiovascular disease [2], heart failure [3], pulmonary thromboembolism [4], and acute dyspnea [5]. Functionally, NT-proBNP causes vasodilation, diuresis, renin–angiotensin production inhibition, and vascular growth inhibition [6]. Moreover, NT-proBNP has been reported to be a biologic marker of early PAH in SSc patients [7–10].

In this paper, we measured serum NT-proBNP levels in SSc patients and investigated relationships between serum NT-proBNP levels and clinical features.

Materials and methods

Patients We evaluated 48 SSc patients who visited the Rheumatology Clinic at Seoul National University Hospital from February 2004 to June 2004. SSc was diagnosed according to the criteria of the American College of Rheumatology [11] and was classified as limited or diffuse cutaneous according to the criteria of LeRoy et al. [12]. Three patients with pulmonary artery hypertension (PAH) were excluded in the analysis. As the significance of BNP in patients with pulmonary hypertension has already been assessed, we evaluated the significance of this biological marker only in SSc patients who did not have pulmonary hypertension.

Forty-five age- and sex-matched healthy controls were also enrolled. Interviews, physical examinations, and blood samplings were performed after obtaining informed consent. Modified Rodnan skin thickness scores were determined by one rheumatologist (YS).

Sera were centrifuged at 3,000×*g* for 10 min within an hour of collection and stored at −70°C. Thirty-two patients (71%) took aspirin, 31 (68%) calcium channel blocker, 23 (51%) D-penicillamine, 9 (20%) angiotensin-converting enzyme inhibitor, and 7 (15%) dipyridamole.

Laboratory tests included a complete blood cell count, erythrocyte sedimentation rate (ESR), serum C-reactive protein and creatinine, chest radiography, transthoracic echocardiography (TTE), pulmonary function testing (PFT), and high resolution computed tomography (HRCT).

Cardiac evaluation Left ventricular ejection fractions (LVEF) and systolic pulmonary artery pressures (sPAPs) were estimated by TTE. PAH was defined as a sPAP> 40 mmHg. Blood pressure (BP) was categorized into four groups, normal (systolic BP<120 mmHg and diastolic BP< 80 mmHg), prehypertension (120–139 or 80–89 mmHg), stage I hypertension (140–159 or 90–99 mmHg), or stage 2 hypertension (≥160 or ≥100 mmHg) [13].

Pulmonary evaluation Pulmonary involvement was assessed by chest radiography, HRCT, and PFT variables, including forced vital capacity (FVC), the carbon monoxide diffusion capacity (DLco), and total lung capacity (TLC). One radiologist reviewed HRCT images (n=37) in a blind manner. HRCT scores were calculated by summing ground glass opacity (GGO) severity, honeycomb extent, and reticular opacity scores [14–15]. GGO severity scores ranged from 2 to 7. These were defined as the sum of GGO intensity score (degree of lung hyperattenuation, 1: low, 2: moderate, 3: high) and GGO extent score (%lung with GGO involvement, 1: less than 25%, 2: from 25% less than 50%, 3: from 50% less than 75%, 4: from 75% through 100%). Honeycomb extent score and reticular opacity score (traction bronchiectasis with fibrotic band) were determined based on lung involvement percent (1: less than 25%, 2: from 25% less than 50%, 3: from 50% less than 75%, 4: from 75% through 100%).

Assay for serum NT-proBNP Serum NT-proBNP concentrations were determined using an Elecsys sandwich electrochemiluminescent immunoassay using a modular analytics E 170 unit (Roche Diagnostics, Basel, Switzerland). The analytic range extended from 5 to 35,000 pg/ml, the intra-assay coefficient of variation (CV) was 1.0% for a concentration of 166 pg/ml and 0.7% for a concentration of 4,267 pg/ml, and the interassay CV was 3.2 and 3.3%, respectively. No detectable cross-reactivity was observed with atrial natriuretic peptide (ANP), NT-proANP, or BNP [16–17].

Statistical analysis The Mann–Whitney U test was used to compare the means of clinical variables in the different groups. Serum NT-proBNP levels were compared between groups using the Kruskal–Wallis test and the post hoc Mann–Whitney U test. Spearman's rank correlation coefficient method was employed to evaluate the correlation between NT-proBNP concentration and the other continuous variables. Multivariate linear regression was performed after adjusting for age, hypertension, and myocardial infarction. Values of P <0.05 were considered statistically significant, and SPSS version 10.0 was used throughout.

Results

We evaluated 45 patients with SSc (30 with limited cutaneous SSc and 15 with diffuse cutaneous SSc). Mean age and duration of disease (±SD) was 47.1±12.9 and 10.2± 6.0 years, respectively. Nine patients had hypertension (limited 6, diffuse 3), one patient had myocardial infarction (diffuse cutaneous SSc; Table 1).

Table 1 Demographic characteristics of SSc patients and healthy controls

	SSc patients (n=45)	Control group (n=45)
Age (years, mean ± SD)	47.1±12.9	47.4±12.9
Female/male	40/5	40/5
Limited/diffuse	30/15	
Disease duration (years, mean ± SD)	10.2±6.0	
Comorbid disease		
Hypertension	9	
Myocardial infarction	1	

The clinical characteristics of the two SSc cutaneous subsets are summarized in Table 2. The mean modified Rodnan skin thickness score was 10.2±9.0 in SSc (limited vs diffuse; 5.7±2.9 vs 19.1±10.5, p=0.000). HRCT scores were determined to evaluate the severity of lung involvement. No differences in %FVC, %DLco, %TLC, or HRCT scores (except GGO severity score, p=0.018) were observed between the limited and diffuse subsets (Table 2). When HRCT scores were compared to PFT variables, we found that GGO severity scores were negatively correlated with % TLC (p=0.004) and %DLco (p=0.005). Honeycomb extent scores were negatively correlated with %DLco (p=0.002). Reticular opacity scores were negatively correlated with % TLC (p=0.024) and %DLco (p=0.016). There were no significant clinical differences according to gender or autoantibody positivity (anticentromere or antitopoisomerase I antibody) in our study (data not shown).

The ESR was increased (mean ± SD, 32.5±27.2 mm/h), but liver and renal function were normal in SSc patients. None of the 45 patients had PAH, and no relation was observed between serum NT-proBNP concentration and medication.

Serum concentrations of NT-proBNP and clinical correlations

Serum NT-proBNP was significantly higher in patients with SSc than in normal controls (152.1±273.0 vs 58.5± 69.7 pg/ml, p=0.031). When patients were separated into diffuse and limited subsets, serum NT-proBNP levels were found to be significantly elevated in the diffuse subset (259.2±416.6 vs 98.6±142.9 pg/ml, p=0.021; Fig. 1). When serum NT-proBNP concentrations were analyzed in total SSc patients according to clinical features, using Spearman's correlation coefficients, they were found to correlate with age, skin thickness score, sPAP, %DLco, and serum creatinine (Table 3). Correlations, although statistically significant, are generally weak (coefficient <0.4) and may not have biological significance. Multivariate analysis showed that serum NT-proBNP levels were positively correlated with age (p=0.010), skin thickness score (p=0.000), and blood pressure (p=0.021) and negatively correlated with %DLco (p=0.016; Table 4). Fifty-seven percent of the variation in log (proBNP) can be explained by the multivariate model (R^2=0.57).

Discussion

SSc is a generalized connective tissue disorder that affects skin and internal organs. Its clinical features are Raynaud's phenomenon and progressive fibrosis of the skin, lung, heart, gastrointestinal tract, and kidney. The skin and internal organ

Table 2 Clinical characteristics of SSc patients

	Total SSc (N=45)	Limited SSc (n=30)	Diffuse SSc (n=15)	p value[a]
Skin thickness score[b]	10.2±9.0	5.7±2.9	19.1±10.5	0.000
Systolic PAP (mmHg)	29.9±5.0	29.5±5.8	30.5±3.8	0.894
LVEF	60.1±5.5	60.7±5.4	59.0±5.7	0.632
FVC (%)	77.6±15.1	80.8±13.6	71.5±16.5	0.096
DLco (%)	66.4±18.5	65.6±18.4	67.9±19.3	0.554
TLC (%)	89.7±14.0	90.1±15.2	88.6±10.9	0.942
GGO severity score	2.8±1.1	3.1±1.1	2.2±1.9	0.018
Honeycomb extent score	1.3±0.7	1.3±0.7	1.3±0.8	0.550
Reticular opacity score	1.3±0.5	1.4±0.6	1.1±0.3	0.146
ESR (mm/h)	32.5±27.2	33.4±26.5	30.9±29.3	0.639
Serum creatinine (mg/dl)	0.9±0.6	1.0±0.7	0.8±0.2	0.658

Data are shown as mean ± SD.

SSc Systemic sclerosis, *PAP* pulmonary artery pressure, *LVEF* left ventricular ejection fraction, *FVC* forced vital capacity, *DLco* carbon monoxide diffusion capacity, *TLC* total lung capacity, *GGO* ground glass opacity

[a] Limited vs diffuse, Mann–Whitney U test

[b] Modified Rodnan skin thickness score

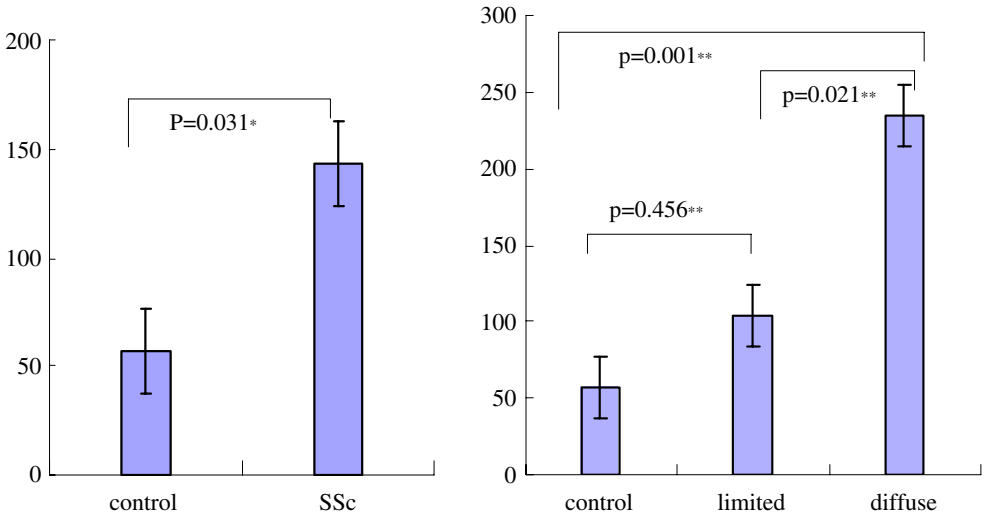

Fig. 1 Serum NT-proBNP in patients with SSc

* Mann-Whitney U test, **Kruskall-Wallis test (p=0.004) and post-hoc Mann-Whitney U test. SSc=systemic sclerosis.

involvements account for much of the morbidity and mortality associated with this disease. The major cause of death in SSc is due to cardiopulmonary involvement, e.g., interstitial lung disease and PAH [18]. Although SSc patients with renal involvement show high morbidity, outcomes have improved significantly since the introduction of angiotensin-converting enzyme inhibitors [19].

NT-proBNP is a hormone released into blood with BNP, and like BNP, NT-proBNP is known to be a biologic marker of cardiovascular disease (i.e., congestive heart failure, acute myocardial infarction, and PAH), acute dyspnea, and pulmonary thromboembolism [2–5]. Serum NT-proBNP levels increase in response to volume expansion and pressure overload of the heart. Moreover, NT-proBNP plays a role as physiological antagonist of the effects of angiotensin II on vascular tone, aldosterone secretion, renal tubule sodium reabsorption, and vascular cell growth [6], and therefore, NT-proBNP has been used as diagnostic and prognostic marker in cardiopulmonary diseases [16, 20].

A recent study reported that serum NT-proBNP levels are elevated in SSc patients with PAH, and suggested that NT-proBNP may be a biologic marker of early PAH in SSc patients [7]. Nine of ten PAH patients had an elevated NT-proBNP level at baseline, but after calcium channel blocker treatment, only two of the ten had a high level. Elevated NT-proBNP levels were found to identify SSc patients with PAH with high sensitivity and specificity (90 and 90.3%, respectively). Moreover, Mukerjee et al. [8] reported that serum NT-proBNP may be a useful diagnostic tool for the detection of pulmonary hypertension in SSc with a high specificity and a high negative predictive value.

We investigated the clinical significance of serum NT-proBNP level in SSc. Our data showed that serum NT-proBNP concentration was increased in patients with SSc, particularly diffuse subset.

Pulmonary function testing is a sensitive means of detecting lung disease in scleroderma [21]. The most

Table 3 Correlations between serum NT-proBNP and clinical features in patients with SSc

	Correlation coefficient	*p* value
Age (years)	0.406	0.006
Disease duration (years)	0.160	0.294
Skin thickness score	0.354	0.017
Systolic PAP (mmHg)	0.386	0.039
FVC (%)	−0.233	0.142
DLco (%)	−0.363	0.020
TLC (%)	−0.104	0.592
GGO severity score	−0.117	0.490
Honeycomb extent score	0.288	0.083
Reticular opacity score	−0.048	0.776
Serum creatinine (mg/dl)	0.335	0.026

By Spearman's correlation coefficient method
PAP Pulmonary artery pressure, *FVC* forced vital capacity, *DLco* carbon monoxide diffusion capacity, *TLC* total lung capacity, *GGO* ground glass opacity

Table 4 Multivariate analysis of correlations between serum NT-proBNP and clinical features in SSc

	Regression coefficient	*p* value
Age (years)	0.028	0.010
Skin thickness score	0.057	0.000
DLco (%)	−0.017	0.016
Blood pressure	0.353	0.021

Confounding variables including age, hypertension, and myocardial infarction were adjusted.

common changes in pulmonary function test results are caused by either a reduced diffusing capacity (DLco) or a reduction in lung volume (FVC) [22]. In our study, most patients (63%) showed a restrictive pattern. HRCT is a highly sensitive technique for detecting changes in the lung parenchyma. Ooi et al. [14] reported that HRCT findings correlate with interstitial lung disease in SSc. Moreover, qualitative HRCT is used to evaluate inflammation (using GGO severity scores) and fibrosis (reticular opacity and honeycomb extent scores); the former is importantly related to diffusion capacity and the latter with lung volume. We determined HRCT scores from GGO severity score, honeycomb extent score, and reticular opacity score. By univariate analysis, indices and their summation showed no correlation.

Echocardiography has been used as a screening test for pulmonary arterial hypertension in SSc [9, 20]. A %FVC/% DLco ratio of >1.4 was reported to be an excellent predictor of isolated pulmonary hypertension [23]. In our study, we grouped patients without PAH (n=29) as %FVC/%DLco ratio >1.4 or ≤1.4. The mean serum NT-proBNP concentration tended to be higher in the >1.4 group (p=0.059, data not shown), which suggests that serum NT-proBNP is correlated with pulmonary vasculature involvement rather than interstitial lung disease per se. Although TTE is an excellent tool for cardiac function evaluations, it is cruder than cardiac catheterization in terms of measuring PAP, and this may contribute to our result.

Moreover, serum NT-proBNP levels were found to be positively correlated with clinical variables including age, skin thickness scores, and sPAP and to be negatively correlated with %DLco. It has been reported that age, hypertension, and myocardial infarction affect serum NT-proBNP levels [24–25]. In the present study, we performed multivariate linear regression after adjusting for these three confounding variables and then found that NT-proBNP levels were positively correlated with age, skin thickness score, and blood pressure and negatively correlated with %DLco.

BNP and NT-proBNP are reported to be produced by cardiac myocyte and cardiac fibroblast [6, 26–27], and we found higher BNP immunoreactivities in the epithelial keratinocytes of SSc patients than in those of normal individuals (unpublished observation).

Our study has limitation. Because of its cross-sectional case control nature, we were unable to show NT-proBNP level changes with respect to clinical course.

In conclusion, serum NT-proBNP levels were elevated in SSc patients, particularly in those with diffuse cutaneous subset, and serum NT-proBNP levels were found to be positively correlated with age, skin thickness score, and blood pressure and to be negatively correlated with %DLco. A further large-scale clinical investigation is warranted.

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