

N-TproBNP as Biomarker in Systemic Sclerosis

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Abstract Systemic sclerosis (SSc) is a connective tissue disorder characterized by tissue fibrosis affecting the skin and internal organs, fibroproliferative vasculopathy, and autoimmune activation. SSc still heralds a poor prognosis with significant morbidity and mortality. Early detection of organ involvement is critical as currently available treatments are most effective when started early. Many candidate biomarkers have been investigated in the past two decades. However, despite the enormous efforts, no accurate tool to predict the pattern of organ involvement and to assess disease activity has been yet identified. The N-terminal fragment of probrain natriuretic peptide (N-TproBNP) is a neurohormone released by ventricular myocytes in response to pressure overload. N-TproBNP is highly relevant for diagnosis, prognosis, and prediction of pulmonary arterial hypertension in SSc. Moreover, several studies support its potential benefit for cardiac assessment of scleroderma patients. Conversely, the role of N-TproBNP as surrogate marker of pulmonary fibrosis and skin involvement is much less clear. We provide an extensive review of the studies that have previously investigated the role of N-TproBNP as candidate biomarker in scleroderma manifestations,

presenting also the findings of a recent study we conducted in a cohort of 87 SSc patients.

Keywords N-TproBNP · Systemic sclerosis · Biomarker · Pulmonary arterial hypertension · Interstitial lung disease

Introduction

Systemic sclerosis (SSc) is a chronic systemic autoimmune disorder characterized by skin and internal organ fibrosis, small vessel vasculopathy, and immune activation. SSc can present with a wide spectrum of clinical manifestations, heralding a poor prognosis with significant morbidity and mortality rate. Even though considerable progresses have been achieved in the management of specific organ involvements, no convincing disease-modifying therapy has been yet identified. Immunosuppressive agents have been shown to exert some beneficial effects, although associated with adverse safety profiles. It is thus advisable to reserve such therapeutic interventions to patients with severe and active disease. Moreover, it has been shown that pharmacologic treatment is most effective when started early on disease course, preferably during the initial inflammatory stage. Therefore, the availability of screening tools to early identify patients with severe and active organ involvement is highly desirable.

The etiopathogenesis of scleroderma is still not fully elucidated. It has been postulated that a vascular damage may be the triggering event leading to immune activation and fibroblast activation [1]. Several soluble immune mediators, as well as molecules related to extracellular matrix (ECM) remodeling, have all been assessed as scleroderma biomarkers. However, none of these molecules is currently used in everyday clinical practice.

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To date, the only biomarker routinely measured in the assessment of scleroderma patients is the serum N-terminal fragment of probrain natriuretic peptide (N-TproBNP). In fact, N-TproBNP displays an established role in the diagnosis and the follow-up of SSc-related pulmonary arterial hypertension (PAH). In addition, N-TproBNP has also been investigated in the context of other scleroderma complications. In the present paper, we will provide an extensive review of the available literature about the role of N-TproBNP as candidate biomarker of specific SSc-related clinical manifestations and report data from our cohort.

N-TproBNP: General Physiology and Clinical use

BNP belongs to the wide family of natriuretic peptides (NPs), which consists of molecules sharing a 17-amino acid ring structure. Beside BNP, NP family enlists also atrial, C-type, D-type, and V-type natriuretic peptides and urodilatin. NPs influence cardiovascular homeostasis regulating intravascular volumes through three main cell receptors (NPRs). NPR-A and NPR-B are guanylyl cyclase-linked receptors that induce cyclic guanosine monophosphate as intracellular messenger, while NPR-C exerts mainly a clearance function [2].

BNP is a neurohormone released from both right and left ventricular myocytes and fibroblasts in response to pressure overload and increased myocardial pressure. Located on chromosome 1, BNP gene expression is induced within 1 h in response to cardiac stretch. It is synthesized as a preprohormone of 134 amino acids then processed to a 108-amino acid precursor and to a 26-amino acid signal peptide. The 108-amino acid prohormone, termed proBNP, is afterwards split by protease furin into the biologically active 32-amino acid BNP and the N-terminal part, named N-TproBNP [3]. BNP action antagonizes the renin–angiotensin–aldosterone system, promoting vasodilation and natriuresis and increasing glomerular filtration rate [4]. Besides these endocrine effects, BNP exerts also an autocrine/paracrine action modulating cardiac and vascular myocyte growth, smooth muscle cell apoptosis and proliferation, fibroblast proliferation and ECM deposition, endothelial proliferation, and vascular smooth muscle contractility. The circulating half-life of BNP is 23 min, whereas N-TproBNP displays a much longer half-life (60–120 min) [5]. These characteristics are of relevance for the use as a diagnostic tool, so explaining why N-TproBNP is preferred to BNP as biomarker in clinical practice.

N-TproBNP has been mainly characterized as diagnostic biomarker in patients with heart failure (HF). A single BNP or N-TproBNP measurement has been recommended by the American College of Emergency Physicians to improve the diagnostic accuracy of HF in patients presenting with acute

dyspnea, allowing to discriminate between dyspnea of cardiac and respiratory origin. However, a recent meta-analysis could not confirm such a statement [6, 7].

In addition, N-TproBNP levels display a prognostic value in patients with chronic HF since correlate with HF severity and sudden death risk [8–11]. Since N-TproBNP gives a noninvasive determination of ventricular filling pressures, it is an extremely useful tool in optimizing therapeutic options for HF management [12].

N-TproBNP has been assessed mostly in the context of left ventricular dysfunction (LVSD), being closely related to left ventricular ejection fraction (LVEF). More recently, interest has grown about N-TproBNP in patients with right ventricular systolic dysfunction (RVSD). N-TproBNP levels have been shown to be raised in patients with right ventricle (RV) pressure overload due to primary pulmonary arterial hypertension and thromboembolism [2]. In addition, N-TproBNP is increased in several other cardiologic conditions: cardiac fibrosis, hypertrophy, infiltrative cardiopathies, valvular abnormalities, ischemic diseases, rhythm disturbances, pericardial diseases, and congenital abnormalities like shunts and stenotic lesions [13].

N-TproBNP plasma levels can be raised even in several noncardiologic conditions such as anemia, hyperbilirubinemia, and increased plasma levels of neurohormones as renin and norepinephrine [14–16]. Moreover, N-TproBNP levels decline with use of drugs such as angiotensin-converting enzyme inhibitors, sartans, calcium antagonists, loop diuretics, and spironolactone.

Besides the active uptake mediated by NPR-C, NPs undergo elimination through hydrolytic cleavage by neutral endopeptidases and renal excretion. Extrarenal excretion of N-TproBNP is markedly lower than that of BNP, so explaining why renal dysfunction influences N-TproBNP to a greater extent than BNP levels. Nevertheless, N-TproBNP has been shown to be a powerful diagnostic and prognostic tool for HF even in patients with renal impairment, provided renal function is accounted for appropriately [17].

Although other biological variables were found to affect N-TproBNP levels (age of >65 years, body mass index, gender [females display higher values], and ethnic group [African-American and Hispanic subjects display higher plasma levels]), its diagnostic utility is still accepted [18].

N-TproBNP Plasma Levels and Scleroderma-related PAH

PAH is a disease of the small arteries of pulmonary vasculature, leading to increased pulmonary vascular resistance (PVR) resulting ultimately in RV failure and premature death. The diagnosis of PAH relies on the evidence of an increased mean pulmonary artery pressure (>25 mmHg) at right heart catheterization and a PVR greater than three

Wood units with a pulmonary capillary wedge pressure of less than 15 mmHg [19].

PAH exerts a strong impact on SSc patients' survival with 40 % mortality at 1 year for those with advanced disease. The prevalence of PAH in SSc is still a matter of debate because of the different definitions and diagnostic techniques employed. However, one of the largest studies estimated a prevalence around 10–12 % [20]. Patients with the limited subset are more likely to develop PAH, although approximately 13–25 % of patients with PAH have diffuse SSc [20, 21]. SSc-PAH develops within 10–15 years from disease onset [22]. A given autoantibody profile has been associated with a PAH-increased risk; approximately 20 % of patients with anticentromere antibodies develop SSc-PAH, whereas over 30 % of subjects carrying anti-Th/To antibodies present PAH [23].

Appropriate tools are required to identify and treat PAH early since the prognosis can be significantly improved by disease-modifying therapies such as endothelin antagonists and prostanoids. Cardiac echocolor Doppler is a noninvasive screening test and is pivotal in PAH diagnostic process. Indeed, echocardiography is recommended on a yearly basis in SSc patients to screen for PAH [24]. In addition, patients with PAH usually present decreased lung diffusion capacity for carbon monoxide (DL_{CO}). Therefore, pulmonary function tests (PFTs) may help in identifying patients with PAH. Conversely, the 6-min walk test (6MWT) has been shown to be predictive of survival in primary PAH, but not in patients with SSc-related PAH [25, 26]. Unfortunately, the combination of the echocardiographic estimate of pulmonary artery systolic pressure (sPAP) and DL_{CO} have been shown to perform well only in the diagnosis of advanced SSc-related PAH, when the tricuspid gradient is above 45 mmHg. On the other hand, values of sPAP below 25 mmHg can rule out PAH [27]. However, both echocardiography and PFTs present some limitations being the former highly operator-dependent and the latter insufficiently sensitive to exclude SSc-PAH. Hence, additional screening tools are necessary.

Several studies have evaluated N-TproBNP as an independent marker for SSc-related PAH. Scleroderma patients with PAH have been found to display higher N-TproBNP plasma levels compared to those without. Moreover, several investigators have reported strong correlation between N-TproBNP levels and other hemodynamic variables [20, 28–32].

N-TproBNP displayed a sensitivity of 56 % and a specificity of 95 % in predicting PAH in SSc patients at a cutoff value of 395 pg/ml [31]. Other investigators reported a sensitivity of 100 % and a specificity of 72.3 %, with a positive predictive value of 39.1 % and a negative predictive value (NPV) of 100 %, at a lower cutoff (157.8 pg/ml) [33].

As a whole, this finding strongly supports the usefulness of N-TproBNP as a screening tool for PAH in scleroderma, particularly in patients with sPAP values detected in the

echocardiography gray zone of sensitivity (ranging between 25 and 45 mmHg).

Several authors have prospectively demonstrated that N-TproBNP levels also display a prognostic value for PAH development in SSc patients [34–36] and are related to PAH severity and survival [31, 37]. Lastly, a significant decrease in N-TproBNP levels has been documented in patients on bosentan and sildenafil [38–40], suggesting that it may be used for monitoring response to treatment. Importantly, patients, whose N-TproBNP levels increased ten times while on therapy, had a threefold increased risk of death [31].

The European Society of Cardiology suggests N-TproBNP sampling at baseline and every 6 months in the follow-up of PAH patients or every 3 months after initiation or change in therapy and in case of clinical worsening [19]. However, it should be noted that an elevation in N-TproBNP does not differentiate between pre- and postcapillary PAH and a normal level does not exclude PAH. Table 1 reports the details of all the above mentioned studies.

N-TproBNP Plasma Levels and Cardiac involvement in Scleroderma

Cardiac involvement is common in SSc, although it is often clinically occult. Its prevalence depends on the sensitivity of the diagnostic tools, ranging from 10 to 75 % [41]. Patients with the diffuse subset and a rapid skin thickening rate are at higher risk of developing heart involvement [42]. Anti-topoisomerase I antibodies have been associated with conduction disturbances, while other studies have shown that anti- U_3 RNP-positive patients are at higher risk of myocardial involvement [43–45]. The clinical spectrum of scleroderma heart disease can be polymorphic, including myocarditis, myocardial fibrosis, restrictive cardiomyopathy, systolic and/or diastolic dysfunction, cardiac failure, valvular regurgitation, coronary artery disease, conduction system abnormalities, arrhythmias, and pericardial disease [46].

Recently, the prevalence and the clinical meaning of LVSD and RVSD in scleroderma have been much debated [47, 48]. Even diastolic dysfunction in SSc is a controversial matter, as distinction between pathological findings and age-related changes is difficult [49]. It has been suggested that tissue Doppler (TD) echocardiography may help in detecting such abnormalities [50, 51].

Cardiac involvement is a poor prognostic factor for mortality, making its early diagnosis a critical issue [52]. Myocardial tissue biopsy is the gold standard for myocardial involvement detection, but it is limited to severe cases of indeterminate origin being an invasive procedure. Cardiac magnetic resonance imaging (MRI) is increasingly employed as a noninvasive quantitative and morphological

Table 1 Details of the studies investigating N-TproBNP plasma levels in SSc-related PAH

Reference	Type of study	Patients number	PAH (<i>p</i> value)	sPAP <i>p</i> (<i>r</i>) value	TRPG <i>p</i> (<i>r</i>) value	6MWT <i>p</i> (<i>r</i>) value	PVR <i>p</i> (<i>r</i>) value	mPAP <i>p</i> (<i>r</i>) value	RV diameter <i>p</i> (<i>r</i>) value
[31]	Prospective	109 SSc				<0.0001 (−0.46)	<0.0001 (0.81)	<0.0001 (0.62)	
[28]	Prospective	45 SSc	0.0007	0.003 (0.73)		0.02 (−0.60)			
[29]	Prospective	45 SSc		0.039 (0.386)					
[35]	Prospective	101 SSc	<0.005						
[30]	Prospective	51 SSc+31 controls			0.03 (0.33)	<0.001 (−0.5)			0.001 (0.5)
[54]	Prospective	69 SSc		0.001					
[33]	Prospective	119 SSc+20 controls			<0.001 (0.001)				
[36]	Prospective	66 SSc	0.012						
[32]	Prospective	25 SSc+25 controls		<0.001 (0.93)					

PAH pulmonary arterial hypertension, sPAP systolic pulmonary artery pressure, TRPG tricuspid regurgitation pressure gradient, 6MWT 6-min walk test, PVR pulmonary vascular resistance, mPAP mean pulmonary artery pressure, RV right ventricle

evaluation of myocardium fibrosis. However, it is expensive and not always available. On the other hand, tools available in the routine practice (i.e., clinical evaluation, ECG, and echocardiography) are not sensitive enough for allowing a prompt diagnosis at a preclinical stage [53]. Therefore, additional diagnostic screening tools are necessary.

Several studies have shown that patients with cardiac involvement display higher N-TproBNP levels compared to scleroderma controls without heart disease [54, 55]. In particular, Allanore and colleagues [54] demonstrated that N-TproBNP identified patients with depressed myocardial contractility as detected by echocardiography enhanced by pulsed TD measurements. A N-TproBNP cutoff concentration of 125 pg/ml has been shown to offer a sensitivity of 92 % and a specificity of 71 % in the detection of depressed myocardial contractility, while the same cutoff value presented a sensitivity of 94 % and a specificity of 78 % in the detection of overall cardiac involvement, with a NPV of 97.6 % [54]. N-TproBNP levels have been also shown to be related to RV diastolic function evaluated by TD echocardiography and to New York Heart Association functional classes in another prospective study [33, 54, 56].

Altogether, these findings strongly support the use of N-TproBNP measurement as a first-line tool in primary care setting for cardiac assessment of SSc [34]. Table 2

summarizes the main studies regarding the association between N-TproBNP plasma levels and cardiac involvement in scleroderma.

N-TproBNP Plasma Levels and Lung Involvement in Scleroderma

Interstitial Lung Disease (ILD) is the second most common visceral complication in SSc, being more frequent among patients with diffuse than limited subset [57, 58]. ILD is the leading cause of mortality in SSc, with up to 30 % of deaths directly attributable to lung fibrosis [59]. The autoantibody profile is the best predictor of ILD development: anti-topoisomerase I, anti-U₁₁/U₁₂RNP, anti-Pm/Scl, and anti-Th/To antibodies are associated with an increased risk, whereas anticentromere antibodies confer relative protection from SSc-associated pulmonary fibrosis [60]. However, the autoantibody specificity does not influence the severity of lung fibrosis [61]. Typically, SSc-ILD patients display a restrictive defect (total lung capacity [TLC] and/or forced vital capacity [FVC]; <80 %) associated with altered DL_{CO} (<75 %). As the evidence of a restrictive pattern often precedes symptoms or changes in chest radiography, PFTs are the most commonly used screening tools, while chest

Table 2 Details of the studies on the association between N-TproBNP levels and SSc-related cardiac involvement

Reference	Type of study	Patients number	Overall cardiac involvement (<i>p</i> value)	Depressed myocardium contractility (<i>p</i> value)	NYHA class (<i>p</i> value)	LA diameter (<i>p</i> value)
[54]	Prospective	69 SSc	<0.001	<0.001		
[33]	Prospective	119 SSc+20 controls		0.004	0.0012	0.019
[56]	Prospective	52 SSc+25 controls				0.0001

NYHA New York Heart Association, LA left atrium

Table 3 Details of the studies investigating N-TproBNP plasma levels in SSc-related lung involvement

Reference	Type of study	Patients number	DL _{CO}	FVC	TLC	GGO severity score	Honeycomb extent score	Reticular opacity score
			<i>p</i> (<i>r</i>) value	<i>p</i> (<i>r</i>) value	<i>p</i> (<i>r</i>) value	<i>p</i> (<i>r</i>) value	<i>p</i> (<i>r</i>) value	<i>p</i> (<i>r</i>) value
[29]	Prospective	45 SSc	0.02 (0.36)	0.14 (−0.23)	0.59 (−0.10)	0.49 (−0.12)	0.08 (0.29)	0.78 (−0.05)
[71]	Prospective	16 SSc	0.68 (0.11)					
[33]	Prospective	119 SSc+20 controls	0.03 (−0.20)	0.12 (−0.14)				0.22

DL_{CO} diffusion capacity for carbon monoxide, FVC forced vital capacity, TLC total lung capacity, GGO ground-glass opacities

high-resolution computed tomography (HRCT) is used for assessing the extent of lung fibrosis and disease progression. Measurement of arterial oxygen saturation during the 6MWT is an additional tool for monitoring the lung disease progression and the response to therapy. Cyclophosphamide (CYC) has been shown to prevent further decline in lung function, improving lung function parameters and survival in few studies, but only patients with severe lung disease apparently can benefit from CYC treatment [62, 63]. Therefore, the key question is to identify biomarkers useful for selecting SSc-ILD patients who might benefit from CYC, as only 30 % of patients with abnormal PFTs develop progressive lung fibrosis, and only 16 % presents severe disease (defined as FVC of <55 %) [61].

In this regard, several molecules have been assessed as potential ILD biomarkers [64–66]. N-TproBNP has also been evaluated in relation to lung function parameters, although conflicting results have been reported. Neither FVC nor TLC has been found to be associated with N-TproBNP levels [29, 33, 38]. DL_{CO} has been identified as the parameter with the strongest relation to N-TproBNP; few studies have described a negative correlation, although this finding has not been confirmed in another series [28, 29, 33]. N-TproBNP levels and radiographic fibrotic scores have been investigated, but no significant correlation was found [29]. A significant inverse correlation between N-TproBNP and the 6MWT was reported with conflicting results [28, 30, 55]. However, the clinical significance of such an association is questionable because of the variables due to musculoskeletal dysfunction or other comorbidities in SSc that may affect the reliability of 6MWT. Table 3 reports the main studies addressing the

association between N-TproBNP plasma levels and scleroderma lung involvement.

N-TproBNP Plasma Levels and SSc Skin Involvement

Skin thickening is a particular feature of scleroderma, being the major classification criterion [67]. The degree of cutaneous involvement has also been significantly correlated with the severity of internal organ involvement, with skin changes and cutaneous inflammation predicting poor prognosis and increased disability [68].

The most widely used tool to quantify dermal skin thickness in SSc is the modified Rodnan skin score (mRSS) [69]. Although validated in several studies, mRSS displays a poor reproducibility being not sensitive enough for detecting small, but clinically meaningful changes. Imaging techniques, as 20 MHz ultrasound evaluation and MRI of the skin, offer high specificity and reproducibility, but have considerable costs. Some devices, as the durometer and the plicometer, have been proposed in the assessment of skin thickening but none is routinely used [70].

Several molecules have been assessed as candidate biomarkers for skin involvement, reflecting the urgent need to identify sensitive and reproducible markers. Few studies have assessed the association of N-TproBNP levels with mRSS. The majority of the studies reported a significant correlation between the two parameters, even if others described conflicting results [29, 32, 33, 55, 71]. A positive correlation between N-TproBNP serum levels and the histopathological skin thickness score was also found [32]. Table 4 summarizes all the data reported in the literature.

Table 4 Details of the studies investigating N-TproBNP plasma levels in SSc-related cutaneous involvement

	Reference	Year	Type of study	Patients number	mRSS	Histopathological skin thickness score
					<i>p</i> (<i>r</i>) value	<i>p</i> (<i>r</i>) value
	[55]	2006	Case control	40 SSc+40 controls	NS	
	[29]	2008	Prospective	45 SSc	0.000 (0.028)	
	[71]	2009	Prospective	16 SSc	0.017 (0.354)	
	[33]	2010	Prospective	119 SSc+20 controls	0.037 (0.19)	
	[32]	2011	Prospective	25 SSc+25 controls	<0.001 (0.93)	<0.001 (0.92)

mRSS modified Rodnan skin score, NS not significant

Table 5 Clinical characteristics of our SSc cohort

	SSc patients (<i>n</i> =87)	lcSSc patients (<i>n</i> =45)	dcSSc patients (<i>n</i> =42)
N-TproBNP (pg/ml)	98 (82–116)	110 (93–119)	87 (74–100)
Age (years)	60 (50–68)	62 (44–66)	56 (46–68)
Disease duration (months)	89 (29–156)	109 (27–161)	77 (30–144)
mRSS	6.5 (4–12)	4 (3–8)	12 (7–19)
sPAP (mmHg)	30 (26–32)	30 (26–32)	30 (29–35)
FVC (% predicted)	98 (82–116)	110 (93–119)	87 (74–100)
FEV ₁ (% predicted)	99 (83–111)	106 (97–118)	90 (72–103)
DL _{CO} (% predicted)	70 (58–81)	71 (63–79)	67 (46–81)
LVEF (%)	65 (60–68)	65 (61–68)	65 (60–68)
Creatinine (mg/dl)	0.8 (0.7–0.9)	0.8 (0.7–0.9)	0.8 (0.7–1)

All patients had a sPAP <36 mmHg. Values are expressed as median (interquartile range)

SSc systemic sclerosis, dcSSc diffuse cutaneous systemic sclerosis, lcSSc limited cutaneous systemic sclerosis, mRSS modified Rodnan skin score, sPAP systolic pulmonary artery pressure, FVC forced vital capacity, FEV₁ forced expiratory volume in the first second, DL_{CO} diffusion capacity for carbon monoxide, LVEF left ventricle ejection fraction

N-TproBNP Plasma Levels in Our SSc Cohort

Eighty-seven scleroderma patients were enrolled in our center to assess the role of N-TproBNP as biomarker. All patients fulfilled the American College of Rheumatology classification criteria for SSc and were categorized into limited and diffuse cutaneous SSc [67, 72]. Their demographic and clinical data are listed in Table 5.

All patients had a sPAP below 35 mmHg on echocardiogram and normal renal function (creatinine of <1.3 mg/dl). Clinically significant ILD was defined as FVC or DL_{CO} of <55 % of predicted values or a 15 % decline from baseline in FVC or DL_{CO}, with evidence of fibrosis on HRCT. Cardiac involvement was defined as hemodynamically significant arrhythmias, pericardial effusion, or congestive heart failure, requiring specific treatment. Heart failure due to LVSD was defined as a LVEF below 0.40, as determined by echocardiogram. N-TproBNP plasma levels were measured by the Roche Modular Analytics E170 immunoassay.

Twenty-seven patients (31 %) presented heart involvement in our scleroderma cohort. Details of the cardiac manifestations are presented in Table 6. In line with most of the

data in the literature, N-TproBNP was significantly increased in this group compared to patients without cardiac involvement ($p=0.0003$, 95 % CI 57–232; Fig. 1).

Serum levels of N-TproBNP were also predictive for cardiac involvement at the regression analysis ($p=0.001$). A cutoff of 130 pg/ml gave a specificity of 70 %, a sensitivity of 74 %, and a NPV of 85 % (area under ROC curve 0.745). Moreover, N-TproBNP levels higher than 130 pg/ml were strongly predictive for heart involvement (odds ratio=7). The regression model remained statistical significant even after correction for LVEF, age, and creatinine. As expected, we observed a negative association between N-TproBNP levels and LVEF ($r=-0.266$; $p=0.002$; Fig. 2). We could also confirm the previously described positive association between N-TproBNP and systolic arterial blood pressure ($r=0.1895$; $p=0.008$), without any relationship of N-TproBNP with diastolic blood pressure ($r=0.052$; $p=0.3136$) [33]. Patients with

Table 6 Cardiac involvement in our cohort of scleroderma patients

Cardiac manifestations	Number of patients (%)
Hemodynamically significant arrhythmias	6 (7)
Severe cardiac conduction disturbances	13 (15)
LVEF	2 (2)
Severe heart valve disorders	3 (3)
Acute coronary syndrome	2 (2)
Pericarditis	1 (1)

LVEF left ventricle ejection fraction

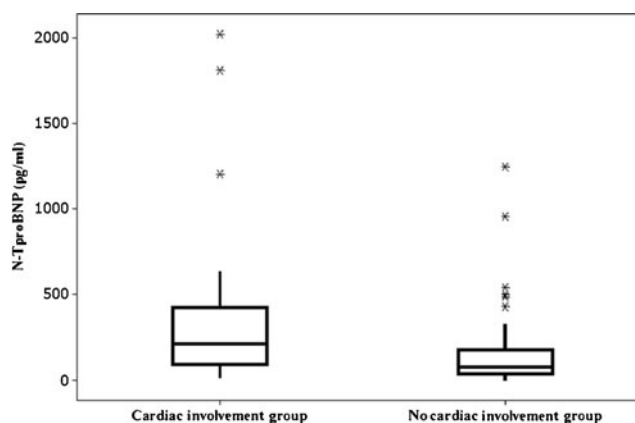


Fig. 1 Box plot of N-TproBNP in SSc patients with cardiac involvement ($n=27$) and patients without ($n=60$). Median of N-TproBNP values in the group with cardiac involvement, 211.70 pg/ml; in the group without cardiac involvement, 70.65 pg/ml ($p=0.0003$)

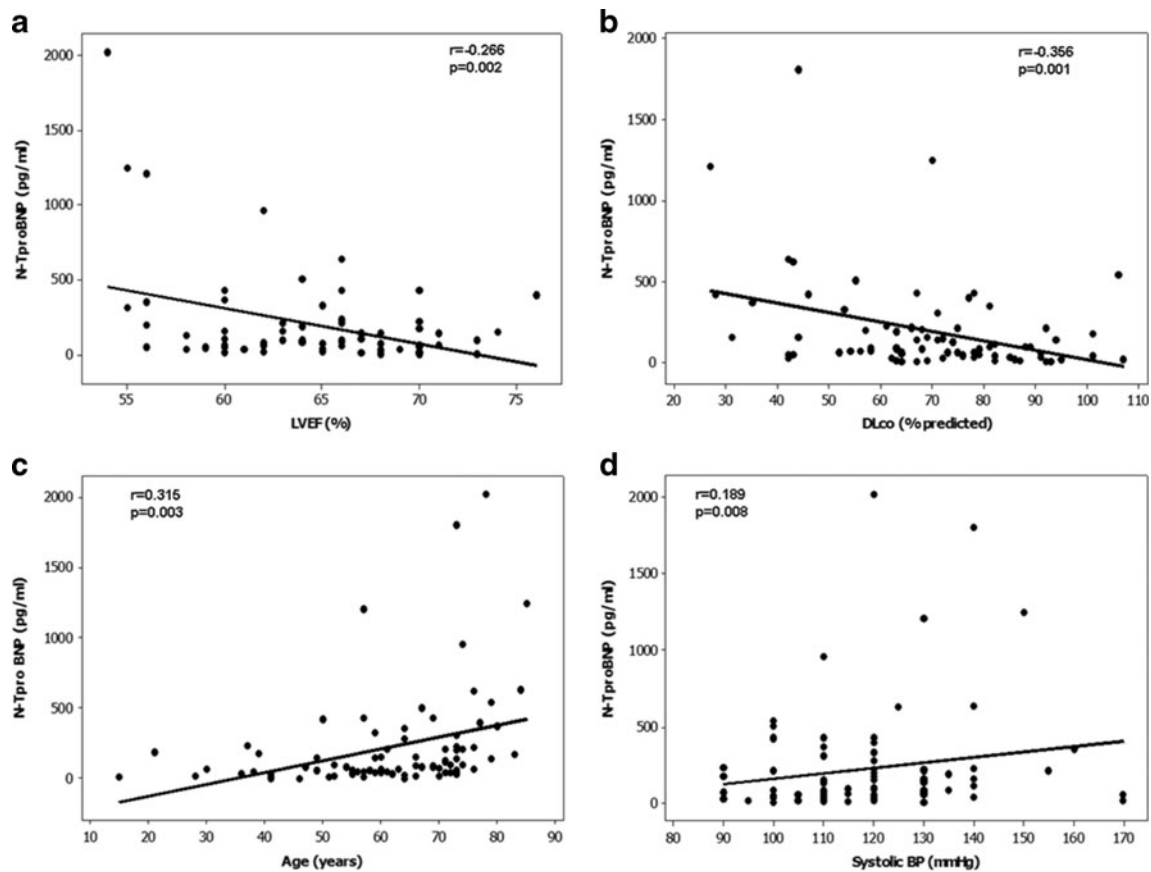


Fig. 2 Graphs showing the correlations between N-TroBNP and left ventricle ejection fraction (a), diffusion capacity for carbon monoxide (b), age (c), and systolic blood pressure (d)

ILD ($n=11$; 13 %) did not display increased N-TroBNP levels ($p=0.115$).

However, we observed weak, but statistically significant correlations between N-TroBNP levels and pulmonary function parameters such as FVC ($r=-0.3$; $p=0.007$) and forced expiratory volume in 1 s ($r=-0.368$; $p=0.001$). We also found a negative correlation with DL_{CO} ($r=-0.356$; $p=0.001$; Fig. 2) and DL_{CO} corrected for alveolar volume (DL_{CO}/VA) ($r=-0.276$; $p=0.016$). As a whole, these data are in agreement with those previously reported in the literature [28, 29, 33].

A trend towards statistical significance for the association of N-TroBNP with arthritis (0.0584) and digital ulcers (0.0923) was found, but not with other scleroderma complications such as subcutaneous calcinosis (0.461) or sicca syndrome (0.587). In our cohort, patients with longer disease duration presented higher serum N-TroBNP levels ($r=0.287$; $p=0.0008$); this finding is not supported by previous studies [29, 33]. We did not find any difference in N-TroBNP levels between the two disease subsets ($p=0.1207$), in agreement with other authors [33, 55]. To note, Choi and colleagues [29] described higher levels among diffuse patients. N-TroBNP and mRSS were not correlated in our cohort ($r=0.1422$; $p=0.1899$), and we

could not detect any association with the autoantibody profile ($p=0.2$). Noteworthy, Oravec [33] described an inverse correlation between serum N-TroBNP and anti-topoisomerase I antibodies, while patients carrying anti-centromere antibodies displayed lower levels. Lastly, we confirmed the previously reported positive association between N-TroBNP levels and age ($r=0.315$; $p=0.003$; Fig. 2) and the negative one with hemoglobin levels ($r=-0.293$; $p=0.059$) [15, 16, 29, 33, 71].

Conclusions

SSc displays a progressive course with heterogeneous presentation and unpredictable outcome. The extent of cutaneous involvement, the rate of progression, and the pattern of organ involvement are the main determinants of morbidity and mortality. These features are, however, not easily predictable. The identification of reliable biomarkers to early predict the pattern of organ involvement and to monitor disease progression is therefore compelling.

An ideal biomarker should be highly sensitive and specific, reproducible, and feasible. It should reflect the disease pathogenesis, activity, and response to therapy. Many

candidates have been identified in the past two decades; however, fully validated measures are still lacking. To date, the autoantibody profile remains the most useful and validated biomarker in SSc, being used both for diagnosis and disease classification. Novel approaches have been applied: genome-wide association studies, gene expression profiling, and proteomics techniques have all been carried out in the attempt of identifying new potential biomarkers. Many candidate molecules have been proposed, but despite all these efforts, fully validated biomarkers are still lacking.

N-TproBNP fulfils many of the characteristics for an ideal biomarker: it is easily detectable by noninvasive procedure, reproducible, and not expensive. To date, N-TproBNP is an established biomarker in scleroderma PAH, where it exerts both a diagnostic and prognostic role. N-TproBNP levels above threefold the upper limits of normal are highly suggestive of PAH, and their modifications, while on therapy, give clinically useful information on the treatment response. Besides this finding, previous studies have enlightened that N-TproBNP is a promising marker of cardiac involvement in SSc patients.

Our data support the potential function of N-TproBNP in the cardiac assessment of scleroderma patients. In fact, N-TproBNP accurately detected patients with scleroderma-related cardiac involvement in our cohort. At a cutoff value of 130 pg/ml, the assay provided a sensibility of 74 % with a specificity of 70 %. Hence, serum N-TproBNP assays may be used as first-line tool in the detection of scleroderma-related cardiac involvement and for a better risk stratification of SSc patients regarding their cardiac involvement. Our data suggest that patients with N-TproBNP plasma concentration above the threshold value of 130 pg/ml are candidates for additional cardiac investigation.

N-TproBNP has been investigated in other SSc-related visceral complications. Unfortunately, most of the studies are rather small, with limited numbers of patients enrolled. Conflicting results have been reported about the role of N-TproBNP in some of these manifestations, such as cutaneous and lung involvement. Moreover, it is difficult to compare different studies because of the variety of inclusion criteria applied and the heterogeneity of investigated parameters. It is, therefore, complicated to draw definitive conclusions about the role of N-TproBNP as biomarker of skin involvement and ILD in SSc. A lack of association between N-TproBNP levels and skin or lung involvement was also found in our cohort.

Ongoing trials, such as the DETECT study, are attempting to better define the screening process in SSc-PAH, and new guidelines should be established to achieve a better accuracy in PAH diagnosis. Prospective studies are needed to further evaluate N-TproBNP as a detection tool in scleroderma cardiac involvement [73]. Future studies will hopefully lead to the identification of novel biomarkers exerting a strong impact on SSc management and patient outcomes.

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