Disproportionate elevation of N-terminal pro-brain natriuretic peptide in scleroderma-related pulmonary hypertension

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ABSTRACT: N-terminal pro-brain natriuretic peptide (NT-proBNP) is a marker of neurohormonal activation that is useful in the diagnosis and prognosis of various forms of pulmonary arterial hypertension (PAH). We sought to characterise and compare NT-proBNP in a cohort of PAH related to systemic sclerosis (PAH-SSc) and idiopathic PAH (IPAH) patients.

NT-proBNP levels, collected from PAH-SSc and IPAH patients followed prospectively, were compared and correlated with haemodynamic variables. Cox proportional hazard models were created to assess the predictive value of NT-proBNP.

98 patients (55 PAH-SSc, 43 IPAH) were included. Haemodynamics were similar, except for lower mean pulmonary arterial pressure in PAH-SSc. NT-proBNP levels were significantly higher in PAH-SSc ($3,419 \pm 3,784$ versus $1,393 \pm 1,633$ pg·mL^{-1}; $p < 0.01$) and were more closely related to haemodynamics in PAH-SSc than IPAH. 28 patients died. NT-proBNP predicted survival (hazard ratio (HR) 3.18; $p < 0.01$) in the overall cohort; however, when stratified by group, predicted survival only in PAH-SSc (HR 3.07, $p < 0.01$ versus 2.02, $p = 0.29$ in IPAH).

This is the first description showing NT-proBNP levels are 1) significantly higher in PAH-SSc than IPAH despite less severe haemodynamic perturbations, and 2) stronger predictors of survival in PAH-SSc, suggesting that neurohormonal regulation may differ between PAH-SSc and IPAH. Future studies to define pertinent mechanisms are warranted.

KEYWORDS: Prognostic markers, pulmonary hypertension, scleroderma

Pulmonary arterial hypertension (PAH) is a chronic disease of the pulmonary vasculature that leads to right heart failure and death. PAH affects ~8–14% of patients with underlying systemic sclerosis (SSc) and is associated with a worse prognosis as compared to patients with other forms of PAH [1–5]. The reasons for these differences in survival are unclear, but may be related to several factors.

Direct cardiac involvement in PAH-SSc may account for the differences in survival. Patients with PAH-SSc may be more likely to have small vessel disease than patients with idiopathic PAH (IPAH) [6]. Myocardial fibrosis in PAH-SSc may lead to cardiac dysfunction and conduction abnormalities [7]. Left heart abnormalities, such as left ventricular hypertrophy and left atrial

dilatation, are common in PAH-SSc [8]. Similarly, we have demonstrated that non-systolic dysfunction may be more prevalent in PAH-SSc than IPAH [5]. Other investigators have demonstrated a higher proportion of arrhythmias in PAH related to connective tissue disease than in IPAH [9]. Furthermore, large vessel pulmonary vascular disease related to scleroderma may increase the effective load on the right ventricle (RV) and lead to a more rapid decline in RV function [10].

Neurohormonal activation (NHA) in response to progressive right heart failure in PAH may also contribute to disease progression and adverse outcomes in PAH. Although known to be central to the pathogenesis of heart failure due to left heart disease, little is known about the role of

NHA in PAH. NHA may be especially important in PAH-SSc. We have recently demonstrated a robust correlation between hyponatraemia, a marker of neurohormonal activation and right ventricular dysfunction in a cohort of PAH patients, and have shown its strong association with survival [11]. Interestingly, in this mixed cohort, PAH-SSc patients were more likely to be hyponatraemic than other PAH patients despite similar haemodynamics, suggesting possible differences in NHA. Furthermore, in a separate cohort of patients with similar haemodynamics, we have demonstrated higher serum catecholamine levels in PAH-SSc compared with IPAH, suggesting differential NHA between these two groups [12].

N-terminal pro-brain natriuretic peptide (NT-proBNP) is a pro-hormone, secreted by the myocardium in response to various stimuli, including mechanical stretch and hypoxia, along with neurohormonal factors such as endothelin, catecholamines and tumour necrosis factor. Over the past several years, NT-proBNP has been studied in various populations of patients with PAH and found to be useful in diagnosing PAH in patients with scleroderma and predicting survival in both PAH-SSc and IPAH [13, 14]. However, there are limited data comparing NT-proBNP levels, correlation with clinical and haemodynamic variables, and prognostic value between PAH-SSc and IPAH. In this study, we sought to characterise and compare NT-proBNP, a marker of NHA, in a cohort of PAH-SSc and IPAH patients.

METHODS

Data were collected prospectively from the Johns Hopkins Pulmonary Hypertension Program, which maintains a registry of all patients evaluated. The Institutional Review Board (The Johns Hopkins Institutional Review Board, Baltimore, MD, USA) approved the registry and this specific analysis; written consent was obtained from all patients. Routine collection of NT-proBNP on patients presenting for right heart catheterisation (RHC) began in 2005 at our institution. Consecutive outpatients with a diagnosis of IPAH, PAH related to anorexigen use or PAH-SSc confirmed by RHC, for whom serum NT-proBNP levels were obtained within 1 week of RHC were included and followed prospectively. Although PAH related to anorexigen use is classified as associated PAH according to consensus statements, we included these patients in our IPAH group given the similarities in clinical presentation, pathobiology and outcomes [15, 16]. Patients in whom PAH therapy had been initiated prior to evaluation at our centre were excluded.

Limited and diffuse scleroderma was defined as previously described [17]. PAH was defined as a mean pulmonary artery pressure ($\bar{P}$pa) of >25 mmHg, pulmonary capillary wedge pressure (PCWP) ≤15 mmHg, and pulmonary vascular resistance (PVR) >3 Wood units, in the absence of other known causes of pulmonary hypertension [18, 19]. Pulmonary function test (PFT), 6-min walk test (6MWT), computed tomography of the chest and echocardiography closest to the initial RHC were reviewed and recorded. Reference formulae were used to calculate percentage predicted values for all PFT data [20, 21]. Patients with significant obstructive lung disease, defined as a forced expiratory volume in 1 s ($FEV1$)/forced vital capacity ratio (FVC) of <0.7 accompanied by radiographic evidence of emphysema, were excluded [22]. Patients were

classified as having interstitial lung disease (ILD) if they had a total lung capacity (TLC) <70% predicted combined with moderate-to-severe fibrosis (grade 3–4) on high-resolution computed tomography (HRCT) [23]. Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) study equation [24].

Serum for NT-proBNP levels was collected within 1 week prior to RHC. Samples were centrifuged within 60 min of collection and stored at -70°C. Measurements were performed on an Elecsys 2010 instrument (Roche Diagnostics, Basel, Switzerland) using a sandwich immunoassay. The reported coefficient of variation for this assay is 0.9–2.2% [25].

Survival was ascertained by reviewing the medical record, phone contact, as well as the Social Security Death Index.

Statistical analysis

Continuous variables were summarised by mean±SD or median (range) and compared using a unpaired t-test or the Wilcoxon rank-sum test where appropriate. Categorical variables were compared using the Chi-squared statistic. NT-proBNP levels were log-transformed to achieve normal distribution. Pearson's correlation coefficient was used to analyse the relationship between NT-proBNP and clinical and haemodynamic parameters. Multivariable linear regression models were created to assess the relationship between NT-proBNP levels and multiple explanatory variables. A two-tailed p-value with a significance level of 0.05 was used to detect statistically significant differences between groups. Factors associated with transplant-free survival were ascertained by univariable Cox proportional hazard models. Variables found to be significant in univariable analysis (p≤0.10), shown previously to predict survival in PAH, and potential confounders of the relationship between NT-proBNP and survival were incorporated into multivariable models. Variables that were found to be collinear by variance inflation factor testing in multiple linear regression (pulmonary vascular resistance (PVR) and pulmonary arterial oxygen saturation (Sa,O₂)) were excluded from Cox multivariable analyses. The proportional hazards assumption was examined for all covariates using a continuous time-varying predictor and generalised linear regression of scaled Schoenfeld residuals on functions of time. Time-to-event analyses were performed using the Kaplan–Meier product limit estimator. Subjects who underwent organ transplantation or died from causes unrelated to underlying cardiovascular diseases were censored. All analyses were performed using Stata version 9.0 (StataCorp., College Station, TX, USA).

RESULTS

Patient characteristics

Between January 2005 and August 2008, 123 patients, who had undergone RHC and had NT-proBNP collected, were considered for analysis. 10 patients were excluded because the NT-proBNP was collected more than 1 week from the RHC date. 12 were excluded because their TLC was <70% pred and had significant fibrosis on HRCT chest scan, and thus they were not considered to have PAH according to consensus guidelines [19]. Three patients were excluded because their initial RHC was performed at an outside institution.

The demographic and clinical characteristics of the 98 subjects included in this cohort are summarised in table 1. Overall, the majority of subjects were white females. More than half (55 (56%) out of 98) had PAH-SSc; the remainder had IPAH (n=38) and PAH related to anorexigen use (n=5). Subjects with IPAH had significantly greater body mass index (BMI) compared with PAH-SSc and achieved a greater distance on 6MWT. There were no differences in New York Heart Association (NYHA) functional classification assessed at time of RHC. The mean eGFR indicated stage II disease by MDRD classification (mean eGFR 60–89 mL·min^{-1}·1.73 m^{-2}) and did not differ between the PAH and PAH-SSc groups. PFTs at baseline revealed significantly reduced percentage predicted FVC, FEV1, TLC, and diffusing capacity of the lung for carbon monoxide ($D_{L,CO}$) in the PAH-SSc group compared to the IPAH group. 53 of the subjects were on PAH-specific therapy at the index RHC. More PAH-SSc than IPAH subjects were on PAH-specific therapy at index RHC. 46 out of the 55 PAH-SSc subjects received low-dose (<60 mg daily) dihydropyridine-type calcium channel blockers (DTCCB) for the treatment of Raynaud phenomenon. None of the IPAH subjects received DTCCB therapy. None of the PAH-SSc subjects received immunosuppressive therapy during the course of the study. On average, PAH-SSc patients had a shorter duration of pulmonary hypertension and a shorter duration of disease prior to collection of NT-proBNP.

Echocardiography

58 of the subjects (21 IPAH, 37 PAH-SSc) had echocardiography within 3 months of RHC and were included for

TABLE 1	Demographic and haemodynamic characteristics			
	Overall	PAH-SSc	IPAH	p-value
Subjects n	98	55	43	
Age yrs	55 ± 14	57 ± 14	53 ± 13	0.14
Sex female	83 (83)	49 (89)	34 (77)	0.11
Race white	64 (65)	39 (71)	25 (57)	0.15
BMI kg·m^{-2}	28 ± 7	26 ± 6	29 ± 6	<0.01
NYHA I+II/III+IV %	41/59	34/66	50/50	0.23
6MWD m	357 ± 131	320 ± 114	395 ± 140	<0.01
eGFR mL·min^{-1}·1.73 m^{-2}	82 ± 31	83 ± 37	81 ± 22	0.81
NT-proBNP	2518 ± 3176	3419 ± 3784	1393 ± 1633	<0.01
NT-proBNP log10	2.98 ± 0.71	3.17 ± 0.66	2.75 ± 0.70	<0.01
Disease duration days	1164 ± 839	942 ± 641	1440 ± 973	<0.01
Disease duration prior to proBNP days	455 ± 685	273 ± 495	681 ± 818	<0.01
DTCCB received	46 (47)	46 (84)	0 (0)	<0.01
PAH-specific treatment	45 (46)	22 (40)	23 (53)	0.30
ERA	24 (24)	12 (22)	12 (27)	0.53
PDE5I	23 (23)	14 (25)	9 (20)	0.56
Inhaled iloprost	1 (1)	1 (2)	0 (0)	0.92
Prostacyclin	13 (13)	4 (7)	9 (20)	0.06
FVC % pred	75 ± 21	72 ± 15	80 ± 19	0.05
FEV1 % pred	72 ± 20	69 ± 20	78 ± 18	0.03
FEV1/FVC % pred	76 ± 14	75 ± 18	79 ± 6	0.14
TLC % pred	81 ± 16	77 ± 16	87 ± 20	0.04
$D_{L,CO}$ % pred	58 ± 21	49 ± 17	71 ± 20	<0.01
f_C bpm	82 ± 14	85 ± 13	77 ± 15	<0.01
P_{ra} mmHg	9 ± 6	9 ± 6	10 ± 6	0.29
$\bar{P}_{pa}$ mmHg	45 ± 14	41 ± 13	48 ± 14	<0.01
Cardiac index L·min^{-1}·m^{-2}	2.5 ± 0.8	2.5 ± 0.9	2.5 ± 0.8	0.79
PCWP mmHg	11 ± 4	10 ± 4	12 ± 3	0.33
PVR Wood units	8 ± 5	8 ± 5	9 ± 5	0.67
PA sat %	65 ± 8	65 ± 9	66 ± 8	0.74
SV mL·beat^{-1}	58 ± 24	51 ± 18	65 ± 27	<0.01
Deaths	28 (29)	20 (36)	8 (19)	0.05

Data are presented as mean±SD or n (%), unless otherwise stated. PAH-SSc: pulmonary arterial hypertension related to scleroderma; IPAH: idiopathic pulmonary arterial hypertension; BMI: body mass index; NYHA: New York Heart Association functional class; 6MWD: 6-min walk distance; eGFR: estimated glomerular filtration rate; NT-proBNP: N-terminal pro-brain natriuretic peptide; DTCCB: dihydropyridine-type calcium channel blocker; ERA: endothelin receptor antagonist; PDE5I: phosphodiesterase-type 5 inhibitor; FVC: forced vital capacity; % pred: % predicted; FEV1: forced expiratory volume in 1 s; TLC: total lung capacity; $D_{L,CO}$: diffusing capacity of the lung for carbon monoxide; f_C: cardiac frequency; P_{ra}: right atrial pressure; $\bar{P}_{pa}$: mean pulmonary artery pressure; PCWP: pulmonary capillary wedge pressure; PVR: pulmonary vascular resistance; PA sat: pulmonary artery saturation; SV: stroke volume.

TABLE 2	Echocardiographic assessment of left heart function in subjects with echocardiography within 3 months of N-terminal pro-brain natriuretic peptide collection		
Parameter	**IPAH**	**PAH-SSc**	**p-value**
Left atrial dilation	8/21 (38)	12/36 (33)	0.72
Left ventricular hypertrophy	14/21 (3)	31/29 (9)	0.17
Left ventricular ejection fraction	57 (21)	60 (36)	0.46
Non-systolic dysfunction	4/18 (22)	7/29 (24)	0.88

Data are presented as n/N total (%) or mean % (n), unless otherwise stated. IPAH: idiopathic pulmonary arterial hypertension; PAH-SSc: pulmonary arterial hypertension related to scleroderma.

FIGURE 1. Box and whisker plots of the N-terminal pro-brain natriuretic peptide (NT-proBNP) levels measured in idiopathic pulmonary arterial hypertension (IPAH) and pulmonary arterial hypertension related to systemic sclerodosis (PAH-SSc) patients. Boxes represent interquartile range (IQR) and horizontal lines are the medians (808.5 pg·mL^{-1} and 1,846 pg·mL^{-1} for IPAH and PAH-SSc, respectively). Whiskers represent the closest value within 1.5 times of the IQR. Dots represent individual measurements exceeding the values represented by the whiskers.

analysis. As shown in table 2, there were no differences in left heart function between PAH-SSc and IPAH as assessed by left atrial size, left ventricular hypertrophy, left ventricular ejection fraction, and presence of non-systolic dysfunction.
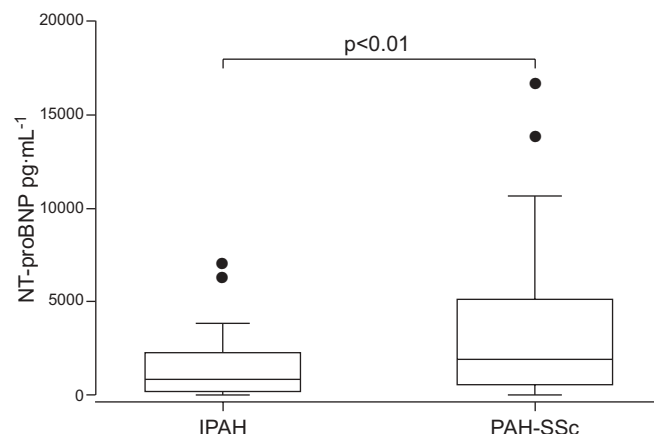
Haemodynamics

Haemodynamic measurements obtained at RHC revealed moderate-to-severe PAH overall. Although the mean right arterial pressure ($\bar{P}$ra) was similar between groups, the $\bar{P}$pa was significantly higher in the IPAH group, suggesting more severe haemodynamic impairment. However, neither the cardiac index nor the PVR differed between groups. The Sa,O$_2$ was also similar between groups.

The NT-proBNP levels were significantly higher in the PAH-SSc group (fig. 1); this relationship persisted when the NT-proBNP levels were log-transformed. Overall, significant correlations between the log of NT-proBNP levels and various clinical and haemodynamic parameters were found. However, these correlations were stronger in the PAH-SSc group, especially for cardiac index (r= -0.58, p<0.01 for PAH-SSc versus r= -0.46, p<0.01 for IPAH), PVR (r=0.54, p<0.01 for PAH-SSc versus r=0.41, p<0.01 for IPAH), and Sa,O$_2$ (r= -0.60, p<0.01 for PAH-SSc versus r= -0.41, p<0.01 for IPAH) (fig. 2). Similar correlations between groups were found for other parameters, such as 6MWD (r= -0.42, p<0.01 for PAH-SSc versus r= -0.27, p=0.09 for IPAH) and right atrial pressure (Pra) (r=0.46, p<0.01 for PAH-SSc versus r=0.31, p=0.04 for IPAH). In multivariable linear regression models, NT-proBNP remained significantly greater in the PAH-SSc group when controlling for cardiac index, BMI, treatment status and renal function.

When examining only treatment-naïve subjects, PAH-SSc subjects demonstrated significantly higher NT-proBNP levels than IPAH subjects despite similar haemodynamics (table 3); this relationship also persisted in multiple linear regression models.

Follow-up and survival

Subjects were followed for a minimum of 3 months following index RHC. All patients received PAH specific therapy during the follow-up period. The median follow-up time was 2 yrs (720 days, range 96–1,359 days) and did not differ significantly between groups. Overall, 28 subjects died (20 PAH-SSc, eight

IPAH) during the course of the study. Two subjects were censored from the survival analysis; one who died as a result of a motor vehicle accident and one who underwent lung transplantation. 21 of the deaths were related to progressive right heart failure, six related to sudden cardiac death, and one related to gastrointestinal haemorrhage. No subjects were lost to follow-up. The 1-, 2- and 3-yr survival estimates for the whole cohort were 88% (95% CI 80–93%), 74% (95% CI 63–82%) and 70% (95% CI 58–79%), respectively. The 1-, 2- and 3-yr survival estimates were poorer in the PAH-SSc group compared with the IPAH group (87% (95% CI 75–94%), 64% (95% CI 48–75%), 64% (95% CI 48–75%), versus 91% (95% CI 77–96%), 88% (95% CI 73–95%) and 78% (95% CI 57–89%), respectively, log-rank p=0.04).

In univariable analysis of the entire cohort, NT-proBNP was found to predict survival; for every 10-fold change in NT-proBNP level, there was a more than three-fold increased risk of death (HR 3.18, 95% CI 1.60–6.34; p<0.01) (table 4). This relationship persisted when adjusted for diagnostic group (IPAH versus PAH-SSc), treatment status, renal function (i.e. eGFR), BMI and haemodynamics in separate multivariable models. However, when similar analyses were performed in the IPAH and PAH-SSc cohorts separately, NT-proBNP predicted survival in the PAH-SSc group only (HR 3.07, 95% CI 1.35–7.00; p<0.01) and not in the PAH group (HR 2.02, 95% CI 0.55–7.52; p=0.29). When adjusting for treatment status, renal function, BMI and haemodynamics in separate multivariable models, NT-proBNP remained strongly associated with survival in the PAH-SSc group (table 5). Furthermore, when restricted to only treatment-naïve PAH-SSc patients (n=33), NT-proBNP was an even stronger predictor of survival (HR 4.83, 95% CI 1.14–20.41, p=0.03).

DISCUSSION

To our knowledge, this is the largest study of NT-proBNP in patients with PAH and the first to compare NT-proBNP levels
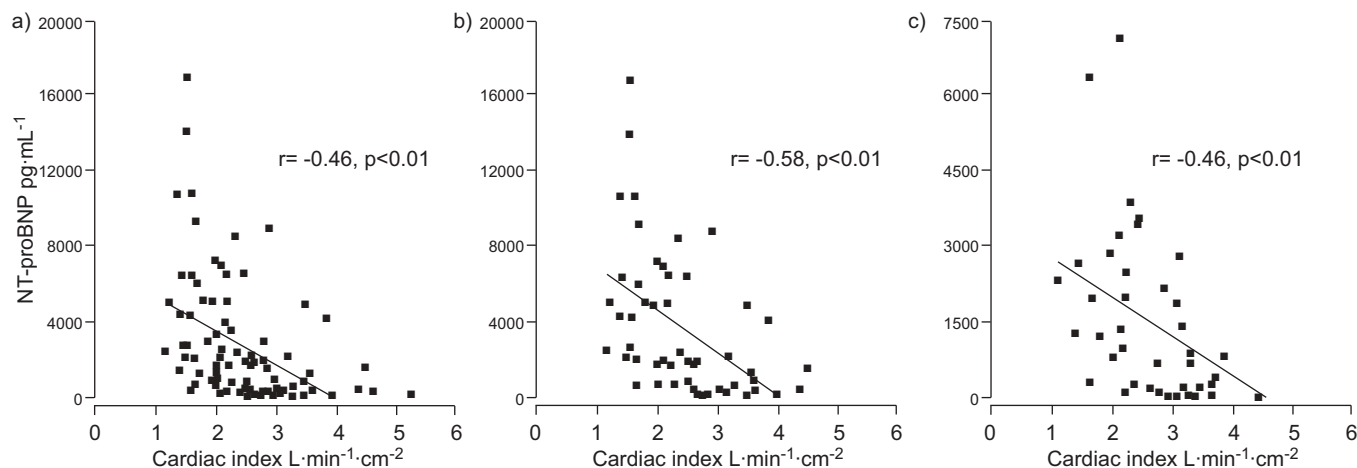
FIGURE 2. Correlations between N-terminal pro-brain natriuretic peptide (NT-proBNP) and cardiac index in a) the overall cohort, b) pulmonary arterial hypertension related to systemic scleroderma patients and c) idiopathic pulmonary arterial hypertension patients.

between patients with PAH-SSc and IPAH. In this study, we found that NT-proBNP levels were significantly higher and correlated more strongly with various demographic and haemodynamic parameters in PAH-SSc compared with IPAH patients, despite similar haemodynamic parameters between the two groups. Importantly, these differences persisted when controlling for potential confounders, such as renal function and treatment. We also found that NT-proBNP levels were stronger predictors of survival in PAH-SSc than IPAH patients.

The utility of NT-proBNP in the assessment of PAH has been examined in several recent studies. FIJALKOWSKA et al. [14] found strong correlations between NT-proBNP and echocardiographic variables, such as pulmonary artery acceleration time, and with haemodynamic parameters, such as cardiac index and pulmonary artery saturation in a heterogeneous cohort of pulmonary hypertension patients that included 36 patients with IPAH. SOUZA et al. [26] found a strong relationship between NT-proBNP and PVR. Similarly, BLYTH et al. [27] defined a threshold value of NT-proBNP that reliably detected right ventricular systolic dysfunction as assessed by cardiac magnetic resonance imaging in a cohort of 25 patients (11 IPAH, eight PAH related to connective tissue disease and six chronic thromboembolic disease). Several investigators have demonstrated the responsiveness of NT-proBNP to specific PAH therapies, including sildenafil [28] and bosentan [29, 30], and have found associations between decreased NT-proBNP and improved RV function in response to various PAH therapies [31].

As several investigators have shown previously, NT-proBNP has prognostic value in various forms of pulmonary hypertension. A normalised ratio of NT-proBNP >2.5, calculated by dividing the measured value of NT-proBNP by age- and sex-adjusted normal values, conferred a >10-fold increased risk of death in a heterogeneous population of 118 pulmonary hypertension patients, including 66 PAH subjects [32]. Similarly, baseline NT-proBNP levels >1,400 pg·mL^{-1} were associated with an eight-fold increased risk of death in a cohort of 36 IPAH patients [14]. Investigators at the Royal Free Hospital (London, UK) have suggested the usefulness of NT-proBNP in the diagnosis, assessment, and prognosis of PAH related to scleroderma. In a

cohort of 109 patients with scleroderma, 68 of whom had PAH-SSc, NT-proBNP levels >395 pg·mL^{-1} were found to have a high positive predictive value for the diagnosis of PAH-SSc, confirming their previous findings from a smaller cohort [13, 33] Furthermore, the investigators found every order of magnitude increase in NT-proBNP level on baseline assessment was associated with a four-fold increased risk of death. Importantly, this relationship was also noted in patients whose NT-proBNP increased despite PAH-specific therapy. Patients whose NT-proBNP levels increased 10-fold while on therapy had a three-fold increased risk of death, suggesting the potential role of NT-proBNP as a marker for response to therapy.

In the present study, we sought to evaluate NT-proBNP in a cohort of PAH patients to characterise its association with disease type, in order to compare its relationship with clinical and haemodynamic parameters, and to assess its prognostic value between IPAH and PAH-SSc. Our results suggest that NT-proBNP levels are significantly higher in PAH-SSc compared with IPAH despite similar haemodynamic measurements. In fact, $\bar{P}$pa was lower in the PAH-SSc group compared with the IPAH group, whereas cardiac index was similar, suggesting less severe haemodynamic perturbation. NT-proBNP levels were consistently higher in the PAH-SSc group, even when controlling for other clinical variables such as age, sex, BMI and renal function, all of which have been shown to affect NT-proBNP levels [32, 34, 35]. These differences persisted when adjusting for treatment status and when examining only subjects who were treatment-naïve at enrolment. Furthermore, NT-proBNP levels in PAH-SSc were more closely correlated with haemodynamics than in IPAH. Importantly, NT-proBNP was significantly associated with survival only in the PAH-SSc group. Although haemodynamics were similar in both groups, 34 (62%) out of 55 of the PAH-SSc cohort had peptide levels >1,400 pg·mL^{-1} compared with 16 (36%) out of 43 of the IPAH cohort (p=0.01); this threshold was identified as a marker of poor prognosis in a prior study [14]. Furthermore, the relationship between NT-proBNP and survival remained significant when controlling for other clinical variables known to affect NT-proBNP levels and for treatment status.

TABLE 3	Demographic and haemodynamic characteristics of treatment-naïve subjects			
	Overall	**PAH-SSc**	**IPAH**	**p-value**
Subjects n	53	33	20	
Age yrs	57 ± 13	58 ± 13	56 ± 14	0.69
Sex female	43 (83)	28 (85)	15 (80)	0.59
Race white	32 (62)	23 (70)	9 (47)	0.11
BMI kg·m^{-2}	28 ± 7	26 ± 7	30 ± 7	0.06
NYHA I+II/III+IV %	40/60	36/64	47/53	0.44
6MWD m	329 ± 134	308 ± 112	359 ± 159	0.24
eGFR	84 ± 37	88 ± 42	77 ± 26	0.29
NT-proBNP	2496 ± 3060	3200 ± 3552	1275 ± 1271	0.02
NT-proBNP log10	3.03 ± 0.7	3.18 ± 0.63	2.76 ± 0.64	0.02
FVC % pred	75 ± 21	70 ± 21	82 ± 19	0.05
FEV1 % pred	72 ± 21	67 ± 21	80 ± 18	0.04
FEV1/FVC % pred	76 ± 15	74 ± 18	78 ± 6	0.46
TLC % pred	80 ± 22	76 ± 21	87 ± 21	0.13
$D_{L,CO}$ % pred	60 ± 21	51 ± 15	78 ± 20	<0.01
f_C bpm	82 ± 15	86 ± 12	73 ± 14	<0.01
P_{ra} mmHg	9 ± 6	9 ± 5	9 ± 7	0.77
$\bar{P}_{pa}$ mmHg	41 ± 14	40 ± 12	44 ± 14	0.21
Cardiac index L·min^{-1}·m^{-2}	2.6 ± 0.8	2.5 ± 0.9	2.6 ± 0.8	0.22
PCWP mmHg	11 ± 4	11 ± 4	12 ± 3	0.90
PVR Wood units	8 ± 6	8 ± 5	8 ± 6	0.85
PA sat %	65 ± 8	64 ± 9	67 ± 8	0.22
SV mL·beat^{-1}	59 ± 25	52 ± 19	72 ± 27	<0.01
Deaths	14 (26)	10 (30)	4 (20)	0.42

Data are presented as mean $\pm$ SD or n (%), unless otherwise stated. PAH-SSc: pulmonary arterial hypertension related to scleroderma; IPAH: idiopathic pulmonary arterial hypertension; BMI: body mass index; NYHA: New York Heart Association functional class; 6MWD: 6-min walk distance; eGFR: estimated glomerular filtration rate; NT-proBNP: N-terminal pro-brain natriuretic peptide; FVC: forced vital capacity; % pred: % predicted; FEV1: forced expiratory volume in 1 s; TLC: total lung capacity; $D_{L,CO}$: diffusing capacity of the lung for carbon monoxide; f_C: cardiac frequency; P_{ra}: right atrial pressure; $\bar{P}_{pa}$: mean pulmonary artery pressure; PCWP: pulmonary capillary wedge pressure; PVR: pulmonary vascular resistance; PA sat: pulmonary artery saturation; SV: stroke volume.

There are several possible explanations for the difference in NT-proBNP levels between PAH and PAH-SSc despite similar haemodynamics. A recent study by OVERBEEK et al. [36] described the relationship between mean ventricular pressure and stroke volume comparing PAH-SSc and IPAH. Although both groups had similar P_{ra} and cardiac index, PAH-SSc patients demonstrated lower stroke volumes for any given mean RV pressure, suggesting impaired RV contractility. In the current cohort, the right ventricular stroke work index was significantly lower in the PAH-SSc group compared with the IPAH group (13.1 ± 5.3 versus 17.0 ± 8.6 g·m^{-2}; p<0.01), suggesting RV maladaptation in PAH-SSc for a comparable cardiac load. Similarly, we have demonstrated impaired RV systolic function in PAH-SSc compared to IPAH as assessed by the tricuspid annular plane systolic excursion despite similar cardiac index by RHC [37]. Impaired contractility in PAH-SSc may be related to more extensive myocardial fibrosis from direct cardiac involvement of scleroderma. Although there are sparse data on RV fibrosis in PAH-SSc patients, FERNANDES et al. [7] demonstrated abnormal collagen deposition in RV biopsies of 15 out of 16 of scleroderma patients without evidence of left or right heart disease, suggesting that myocardial fibrosis precedes the development of clinical cardiac involvement. We have recently collected RV biopsies on seven PAH-SSc patients [38] and found significantly more myocardial fibrosis in these patients when compared with seven IPAH patients and control subjects (H.C. Champion, personal communication). As myocardial fibrosis can stimulate production of NT-proBNP, it is possible that differences in the degree of myocardial fibrosis between PAH-SSc and PAH contributed to differences in NT-proBNP levels in our study [39].

RV wall stress is another important determinant of NT-proBNP release [40]. In PAH, both systolic and diastolic components contribute to overall RV wall stress: low stroke volume increases systolic wall stress while high end-diastolic volume (EDV) is a main determinant of diastolic wall stress [41]. As shown previously, stroke volume (SV) decreases more in response to increased $\bar{P}_{pa}$ in PAH-SSc than IPAH, which may explain why PAH-SSc patients experience right heart failure earlier in the course of disease [36]. Consistent with this, we found significantly reduced SV in PAH-SSc compared to IPAH in our cohort, despite similar cardiac index (table 1). RV diastolic dysfunction is common in both IPAH and PAH-SSc as demonstrated in several echocardiographic studies [42–45]. Thus, it is likely that 1) RV wall stress was higher in the PAH-SSc group due to both systolic (lower stroke volume) and diastolic (higher EDV) dysfunction, though this cannot be definitively determined without cardiac MRI or pressure-volume loop measurements; and 2) increased RV wall stress in PAH-SSc compared to PAH contributed to the increased NT-proBNP levels seen in this group. Left ventricular dysfunction (LVD), both systolic and non-systolic in nature, can contribute to elevations in NT-proBNP [46]. LVD may be more prevalent in PAH-SSc than IPAH [5]. However, we found no differences in the prevalence of LVD by echocardiography between groups in this cohort. Furthermore, there were no differences in NT-proBNP levels between subjects with and without LVD, both in the overall cohort and within the PAH-SSc and IPAH sub-groups, suggesting that differences in NT-proBNP levels were unlikely to be affected by left ventricular function.

Patients with SSc have underlying autonomic dysfunction as evidenced by impaired cardiovascular reflexes and sympathetic skin responses [47, 48]. Prominent clinical features such as Raynaud's phenomenon and gastrointestinal dysmotility are likely at least in part related to disturbances in the autonomic system [49, 50]. As the autonomic nervous system is integrated with NHA, it is possible that up-regulation of the neurohormonal axis in PAH-SSc compared to PAH in response to cardiac stress may explain the differences in NT-proBNP levels between these groups. Neurohormones, in particular catecholamines, angiotensin II and endothelin, may enhance secretion of NT-proBNP through paracrine and endocrine mechanisms [51]. Furthermore, neurohormones may contribute to myocardial fibrosis and cause direct myocyte damage, which could impair myocardial contractility [52, 53]. Although we did not routinely collect serum for measurement of catecholamines, angiotensin II, or endothelin in this cohort, we have previously demonstrated increased serum

TABLE 4   Univariable Cox proportional hazard ratio (HR) models

Predictor	Overall cohort HR (95% CI; p-value)	PAH-SSc HR (95% CI; p-value)	IPAH HR (95% CI; p-value)
Age	1.00 (0.97–1.03; 0.90)	1.01 (0.97–1.04; 0.78)	0.97 (0.91–1.03; 0.28)
Race	1.31 (0.60–2.86; 0.49)	2.92 (1.14–7.45; 0.03)	0.48 (0.09–2.49; 0.38)
Sex	0.79 (0.27–2.31; 0.67)	1.02 (0.24–4.42; 0.98)	0.45 (0.08–2.45; 0.36)
BMI	0.93 (0.86–0.99; 0.04)	0.92 (0.84–1.00; 0.08)	0.98 (0.86–1.11; 0.71)
NYHA	2.58 (1.47–4.49; <0.01)	2.76 (1.37–5.52; <0.01)	1.95 (0.73–5.17; 0.18)
6MWD	0.99 (0.99–1.00; 0.01)	0.99 (0.99–1.00; 0.17)	0.99 (0.99–1.00; 0.05)
eGFR	1.00 (0.98–1.01; 0.60)	0.99 (0.98–1.01; 0.69)	0.99 (0.96–1.03; 0.78)
Treatment status	0.97 (0.36–2.07; 0.95)	1.52 (0.62–3.66; 0.35)	0.48 (0.10–2.17; 0.34)
Disease duration	0.99 (0.99–0.99; <0.01)	0.99 (0.99–0.99; <0.01)	0.99 (0.99–0.99; 0.03)
Duration prior to proBNP	0.99 (0.99–1.00; 0.38)	1.00 (0.99–1.00; 0.56)	0.99 (0.99–1.00; 0.31)
P_{ra}	1.07 (1.00–1.15; 0.04)	1.05 (0.98–1.14; 0.15)	1.12 (0.99–1.28; 0.08)
$\bar{P}_{pa}$	1.02 (0.99–1.05; 0.11)	1.03 (0.99–1.07; 0.07)	1.03 (0.98–1.09; 0.21)
Cardiac index	0.32 (0.17–0.63; <0.01)	0.32 (0.15–0.67; <0.01)	0.42 (0.11–0.57; 0.01)
PCWP	0.94 (0.85–1.06; 0.36)	0.96 (0.86–1.07; 0.41)	0.95 (0.75–1.20; 0.67)
PVR	1.16 (1.09–1.24; <0.01)	1.17 (1.09–1.27; <0.01)	1.18 (1.06–1.33; <0.01)
PA sat	0.93 (0.89–0.97; <0.01)	0.92 (0.88–0.96; <0.01)	0.96 (0.87–1.04; 0.30)
NT-proBNP log10	3.18 (1.60–6.33; <0.01)	3.07 (1.35–7.00; <0.01)	2.02 (0.55–7.52; 0.29)

PAH-SSc: pulmonary arterial hypertension related to scleroderma; IPAH: idiopathic pulmonary arterial hypertension; BMI: body mass index; NYHA: New York Heart Association functional class; 6MWD: 6-min walk distance; eGFR: estimated glomerular filtration rate; proBNP: pro-brain natriuretic peptide; P_{ra}: right atrial pressure; $\bar{P}_{pa}$: mean pulmonary artery pressure; PCWP: pulmonary capillary wedge pressure; PVR: pulmonary vascular resistance; PA sat: pulmonary artery saturation; NT-proBNP: N-terminal proBNP.

levels of epinephrine and norepinephrine in PAH-SSc compared with PAH in a separate cohort, despite similar haemodynamics [38]. Endothelin levels may be higher in PAH-SSc than PAH due to impaired clearance in the pulmonary vasculature, thus contributing to higher NT-proBNP levels [54]. Additionally, hyponatraemia, a marker of neurohormonal activation in heart failure states, strongly predicts survival in PAH and may be more common in PAH-SSc than other forms of PAH despite similar haemodynamics [11]. In the current cohort, although, the groups had a similar mean cardiac index, the PAH-SSc group had a higher resting heart rate, which also may reflect enhanced NHA. Thus, it is possible that underlying differences in neurohormonal activation between PAH-SSc and IPAH contribute to the differences in NT-proBNP expression. Although hypoxia induces

peptide release independent of atrial or ventricular stretch, both in patients with cardiac disease and in normal subjects [55, 56], there were no differences in pulmonary artery saturation between PAH-SSc and IPAH in this cohort. Thus, it is unlikely that local hypoxia explains the difference in NT-proBNP levels.

This study confirms previous observations about the prognostic significance of NT-proBNP in PAH-SSc, but offers several advantages over the prior study [13]. First, the vast majority of patients in that study were on PAH-specific therapy at the time of enrolment (51 out of 68); less than half were on therapy in the current study. Importantly, our study found the relationship between NT-proBNP and survival in both the PAH-SSc cohort overall and in the treatment-naïve

TABLE 5   Multivariable Cox proportional hazard ratio (HR) models

	Overall cohort HR (95% CI; p-value)	PAH-SSc HR (95% CI; p-value)	IPAH HR (95% CI; p-value)
Log NT-proBNP	3.18 (1.60–6.33; <0.01)	3.07 (1.35–7.00; 0.01)	2.02 (0.55–7.52; 0.29)
Adjusted for			
Diagnosis	2.78 (1.37–5.64; <0.01)		
NYHA	2.60 (1.27–5.32; <0.01)	2.81 (1.22–6.44; 0.01)	
P_{ra}	2.95 (1.44–6.16; <0.01)	3.00 (1.21–7.42; 0.02)	
Cardiac index	2.08 (0.95–4.59; 0.07)	1.65 (0.60–4.56; 0.33)	
Treatment status	3.18 (1.60–6.32; <0.01)	2.93 (1.29–6.65; 0.01)	
eGFR	3.71 (1.77–7.77; <0.01)	3.80 (1.55–9.36; <0.01)	
BMI	2.86 (1.43–5.71; <0.01)	2.85 (1.22–6.63; 0.02)	

PAH-SSc: pulmonary arterial hypertension related to scleroderma; IPAH: idiopathic pulmonary arterial hypertension; NT-proBNP: N-terminal pro-brain natriuretic peptide; NYHA: New York Heart Association functional class; P_{ra}: right atrial pressure; eGFR: estimated glomerular filtration rate; BMI: body mass index.

cohort. Secondly, in the previous study, NT-proBNP was collected up to 6 months prior to RHC, introducing significantly more variability to the relationship between serum levels and 1) RHC parameters and 2) survival. The current study only included subjects whose NT-proBNP was collected within 1 week of RHC. Thirdly, >30% of the subjects included in the previous study had significant ILD, which we and others have now shown to portend a poorer prognosis in scleroderma-related pulmonary hypertension [57, 58], and thus may have biased their survival analysis [59].

However, the lack of association between NT-proBNP and survival in IPAH in this cohort was an unexpected finding. This may be related to the size of the cohort or the effect of PAH-specific therapies on NT-proBNP. However, to our knowledge, only one prior study has examined the prognostic value of NT-proBNP in an IPAH population; this study included fewer patients (n=36), all of whom were on PAH-specific therapy, and reported a similar number of deaths (n=9) [14]. In the current study, NT-proBNP was not associated with survival in the overall IPAH population or in the treatment-naïve IPAH population. Although reasons for the difference between the current study and the study by FIJALKOWSKA et al. [14] are unclear, it is possible that high NT-proBNP levels despite PAH-specific therapy portend a poor prognosis (as shown in PAH-SSc [13]), and not necessarily baseline values prior to therapy. Thus, although widely accepted as a prognostic marker in IPAH, there are few data to support this practice, particularly for patients not receiving PAH-specific therapy.

There are several limitations to this study. First, all subjects were referrals to our pulmonary hypertension programme and thus selection bias towards inclusion of those patients who were most ill is likely. However, as both groups were as likely to be subject to referral bias, the relationship between these two groups is probably valid. Secondly, survival estimates may be influenced by lead-time bias given that half of the cohort received PAH-specific therapy at the time of NT-proBNP collection; patients with established disease may have been more likely to die during follow-up than newly diagnosed patients. Similarly, although PAH-specific therapy may influence NT-proBNP levels, PAH-SSc subjects had significantly higher NT-proBNP levels than IPAH subjects, suggesting that our comparison may actually underestimate the true difference between groups. DTCCBs may also impact NT-proBNP levels in SSc as it tends to lower NT-proBNP [60]. However, as only PAH-SSc subjects in our cohort received DTCCBs, the NT-proBNP levels in our PAH-SSc subjects may actually be higher than reported and the difference between groups may be underestimated. Thirdly, although patients with significant ILD based upon a combination of PFT and HRCT findings were excluded from the cohort, it is possible that ILD contributed to the pulmonary vascular disease noted in our SSc population. However, the impact of ILD upon NT-proBNP is unknown and it is unlikely that pulmonary fibrosis alone causes up-regulation of NT-proBNP [61]. Importantly, as discussed above, there were no differences in pulmonary artery saturation between groups, thus minimising the chance that local hypoxia related to ILD contributed to the differences in NT-proBNP. Similarly, patients with scleroderma may be more likely to have renal insufficiency related to the underlying disease, which could have influenced survival. However, the association between NT-proBNP and survival persisted in the PAH-SSc group when controlling for eGFR. Finally, it is possible that factors for which we have not accounted may confound the association between NT-proBNP and survival.

In conclusion, in this cohort of patients with PAH, NT-proBNP levels were significantly higher in PAH-SSc subjects compared to IPAH subjects despite similar haemodynamics, suggesting differences in response to cardiac load. Furthermore, NT-proBNP was a strong predictor of survival only in the PAH-SSc group, further emphasising the role of this noninvasive marker in the evaluation of patients with PAH-SSc. Although the reasons for the differences in levels and in predictive value of NT-proBNP by disease-type remain unclear, underlying variations in cardiac function and neurohormonal response to haemodynamic perturbations between PAH-SSc and IPAH may exist. Future studies are needed to confirm these findings and to elucidate underlying mechanisms.

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