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Significance of plasma N-terminal pro-brain natriuretic peptide in patients with systemic sclerosis-related pulmonary arterial hypertension

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KEYWORDS
N-Terminal proBNP;
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Cardiac catheterisation;
Right ventricular dysfunction

Summary *Objectives:* A single centre pilot study to investigate the role of the plasma N-terminal pro-brain natriuretic peptide (N-T proBNP) assay to risk stratify patients with suspected pulmonary arterial hypertension (PAH) from a background SSc population.

Methods: Out of 49 SSc patients, 23 had and 26 did not have PAH. Right ventricular haemodynamic variables, six-minute walk test and plasma N-T proBNP levels were recorded from patients catheterised for suspected PAH (23 with PAH and 11/26 without PAH).

Results: Mean value of N-T proBNP for SSc patients with PAH was 3365 (standard error 1095) pg/ml compared to 347 (174) pg/ml for patients without PAH. There was a statistically significant correlation ($P<0.05$) between N-T proBNP values and (i) mean pulmonary artery pressure ($r = 0.53$), (ii) right ventricular end diastolic pressure ($r = 0.59$) and (iii) pulmonary vascular resistance ($r = 0.49$). Receiver operator characteristic curve analysis showed that a cut-off value of 395.34 pg/ml had a sensitivity of 0.69 and specificity of 1.0.

Conclusions: N-T proBNP estimation in systemic sclerosis-related pulmonary hypertension is a potentially useful diagnostic tool with a high specificity and negative predictive value. This test has the potential to have an important role in risk stratification and monitoring of therapy in the future.

Introduction

Brain natriuretic peptide (BNP) is a member of the natriuretic peptide family, which consists of three molecules that share significant amino acid sequence homologies and a looped motif. BNP was first isolated from porcine brain but is produced mostly from the ventricular myocardium.[1] Secretion is triggered from the cardiomyocyte, where the BNP is initially synthesised in the pre–pro-form. After cleavage of a signal sequence, the pro-form is synthesised as a 108 amino acid precursor and

Abbreviations: NT-proBNP, N-terminal pro-brain natriuretic peptide; mean PAP, mean pulmonary artery pressure; RVEDP, right ventricular end diastolic pressure; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; ROC, receiver operating characteristic; SE, standard error; SScPAH, systemic sclerosis-related pulmonary arterial hypertension; RV, right ventricle

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appears in the circulation in two forms—a 76 amino acid peptide N-terminal pro-brain natriuretic peptide (N-T proBNP) molecule and the 32 amino acid physiologically active BNP peptide. Elevated plasma levels are found in patients with hypertensive left ventricular dysfunction, acute myocardial infarction and unstable angina.[2,3] Based on these findings, the Breathing Not Properly Multinational Study Investigators[4] performed a large prospective multicentre study and showed BNP to be a useful screening test for congestive cardiac failure. As with ANP, raised plasma BNP levels promote both natriuresis and diuresis. They also inhibit the renin–angiotensin–aldosterone system and facilitate pulmonary vasodilatation.[5] In contrast to studies in left ventricular dysfunction, there have been few studies on BNP levels in patients with right ventricular dysfunction in the background of pulmonary vascular disease. Amongst patients with primary pulmonary hypertension (PPH), Nagaya et al.[6,7] have shown that plasma BNP levels increase in proportion to the degree of right ventricular dysfunction. These authors measured plasma BNP levels using a BNP ELISA assay in 53 patients with PPH at diagnostic catheterisation, with a follow-up period of 18 months. Kaplan–Meyer analysis showed a wide variation in survival, with patients with higher plasma N-T proBNP levels having a worse survival. Multivariate analysis confirmed BNP to be an independent predictor of mortality in this condition.

Although right-heart catheterisation is the gold standard for quantifying right ventricular dysfunction, the expertise required to accurately perform this investigation is not readily available in many units. In addition, right-heart cardiac catheterisation carries a serious complication rate of approximately one in 2000. Hence, it would be useful if a non-invasive blood test could identify patients at an increased risk of developing pulmonary arterial hypertension (PAH) related to SSc, so that they could be selected for invasive investigation and managed as outlined in the recently published guidelines by Gibbs.[8]

The role of BNP in SSc patients has never been previously investigated. The majority of SSc patients are believed to suffer from subclinical cardiac fibrosis,[9] which is difficult to identify at its prefibrotic ''inflammatory'' stage. Hence, the existence of this presumed low-level inflammatory activity predisposing to fibrosis has been difficult to demonstrate. Thus, BNP levels could be elevated in the SSc population at large, diminishing its value as a discriminative test in the setting of sclerosis-related pulmonary arterial hypertension (SScPAH). Yet, this is also the population with the highest incidence of PAH, and the only population in which annual screening is considered essential.[10] Current non-invasive screening techniques (echocardiography and pulmonary function tests) lack sensitivity and specificity[11] and are not readily accessible in all centres. The availability of a reliable blood test to screen for PAH in the SSc population would significantly improve patient care.

Newer assays have been recently developed that accurately measure N-T proBNP.[12] The clinical suitability of this assay is superior to those that have previously measured the active plasma BNP. In contrast to the 32 amino acid-active BNP, this form of the secreted peptide is highly stable in plasma at room temperature for up to 72 h and can be stored for >5 days at a refrigerator temperature of 4–8°C. Additionally, there is no variation of levels detected by this assay which is dependent on either circadian rhythm or the position of the test person (lying/sitting position or after stress exposure). This makes the N-T proBNP an ideal assay in the clinical setting with a high internal accuracy.[13] Hence, given that the test can be performed under routine laboratory conditions, it would be important to evaluate its usefulness in patients with SSc in general and PAH in particular.

Aims

The aim of our pilot study were first to determine whether circulating plasma N-T proBNP levels would discriminate between patients with and without PAH in the setting of SSc. Second, to determine whether absolute N-T proBNP levels in SSc patients with PAH correlated with haemodynamic and functional (the six-minute walk test (SMWT))[14] indices of disease severity.

Method

Population

Forty-nine patients with SSc as defined by the revised ACR criteria[15] were enrolled in the pilot study. Out of 49 patients, 23 had SScPAH confirmed on cardiac catheter as defined by the modified NIH criteria.[16] Twenty-six patients had SSc but no evidence of PAH on lung function tests and echocardiography. Of these 26 SSc patients, 11 underwent cardiac catheterisation for investigation of breathlessness—PAH was excluded in all. Patients with PAH had a mean age of 57 years (range 34–80 years). The control subjects had a

Table 1 Characteristics of 49 SSc patients undergoing N-T proBNP estimation, cardiac catheterisation and functional (SMWT) evaluation.

Category ($N = 49$)	Patients with SScPAH ($N = 23$)	SSc patients without PAH ($N = 26$)
Age with range	57 (34–80) years	56 (27–78) years
M:F	2:21	7:19
Autoantibody profile	ACA + 18, ANA + 4, Scl 70 + 1	ACA + 19, ANA + 5, Scl 70 + 2
LcSSc:DcSSc	21:2	23:3
Patients with pulmonary fibrosis (on HRCT)	0	1
% predicted DLCO (mean ± 2 SD)	56.7 ± 3.2	72 ± 4.7
MPAP (mmHg)	44.5 ± 13.5	19.8 ± 4.1[a]
MRAP (mmHg)	7.7 ± 4.8	2.1 ± 1.2
MAP (mmHg)	93.9 ± 15.6	98 ± 11.2
PVR	687 ± 564	102 ± 14
SVR	1713 ± 606	1842 ± 532
CI	2.6 ± 1.4	4.1 ± 1.2
SVO_2	63.9 ± 10.7	85.2 ± 11.0

% predicted DLCO in mmol/min/kPa, calculated assuming Hb = 13.5 mg/ml. MRAP = mean right atrial pressure in mmHg; MPAP = mean pulmonary artery pressure in mmHg; MAP = mean arterial pressure in mmHg; PVR = pulmonary vascular resistance in dyne s/cm^5; SVR = systemic vascular resistance in dyne s/cm^5; CI = cardiac index in l/min/m^2; SVO_2 = mixed venous oxygen content in ml/dl.

[a]*Note:* Haemodynamic data available from 11/26 SSc patients undergoing cardiac catheterisation.

mean age of 56 years (range 27–78 years). The haemodynamic and lung function characteristics of the 23 patients with PAH and 26 controls is shown in Table 1. Patients with significant renal disease were excluded from the study (creatinine >1.5 mg/dl) as plasma BNP levels have previously been reported to be elevated in chronic renal failure.[17] SMWTs were performed on all patients found to have PAH within 1 month of the N-T proBNP estimation. We compared N-T proBNP values with the patient's functional state as measured by the SMWT.

Screening and diagnostic programme for PAH

The Royal Free Hospital, London is a national referral centre for patients with SSc. Seven hundred and ninety four patients are under active follow-up. All patients are screened on an annual basis for evidence of PAH. Patients with an increase in breathlessness are evaluated invasively unless there has been clear progression in lung fibrosis both radiologically and on lung function testing. All are screened by standardised echocardiographic evaluation of the right ventricle and tricuspid gradient (using the Sonitron CFM 800 machine, Oslo, Norway), and by lung function assessment. Patients with tricuspid gradient >35 mmHg, or evidence of right ventricular strain, and those with a 20% reduction in DLCO compared to the previous value, or a DLCO <40% with no lung fibrosis on HRCT are evaluated by right-heart catheterisation irrespective of the presence of symptoms. Breathless patients not found to have PAH on right-heart catheter underwent left-heart catheterisation to investigate for a cause of their symptoms. Hence, 11 out of 26 SSc patients who were chosen as control subjects underwent left- and right-heart cardiac catheterisation as they were clinically found to have an increase in their level of breathlessness.

N-T proBNP measurement

Approval was obtained from the hospital ethics committee. Each patient gave signed informed consent allowing measurement of plasma N-T proBNP levels. A 10 ml whole blood sample was drawn from each patient into a heparinised (EDTA) plastic tube. This was transported at room temperature to the chemical pathology department and the assay was performed within 4 h of blood sampling. The plasma N-T proBNP level was measured with the Elecsys Systems E170 proBNP assay kit by two of the above authors (P.A. and D.N.). The assay calculated the N-T proBNP concentration of each sample in values of pg/ml. Expected value for women at age <50 years was 153 pg/ml, 50–65 years were 334 pg/ml, for men at age <50 was 88 pg/ml and 50–65 years was 227 pg/ml.

Haemodynamic studies

Diagnostic right-heart catheterisation was performed on 34 SSc patients (23 patients with SSc PAH and 11 controls with SSc but no PAH) within 3 months of N-T proBNP estimation. Baseline haemodynamic variables were measured, including mean pulmonary arterial pressure (mean PAP), pulmonary capillary wedge pressure (PCWP), right ventricular end diastolic pressure (RVEDP) and cardiac output by the thermodilution method. Pulmonary vascular resistance (PVR) was calculated by the formula (mean PAP−PCWP) × 80/cardiac output. All patients with pulmonary hypertension underwent vasodilator testing.

Statistics

The Stat Direct[18] statistics package and Microsoft Excel was used for statistical calculations. Mean values of BNP were expressed along with standard error (SE) of the mean and compared between patients with PAH and controls. Linear regression analysis was used to determine correlation between plasma BNP levels and mean PAP, RVEDP as well as PVR.

The receiver operating characteristic (ROC) curve method of analysis was used to assess the diagnostic value of BNP in patients with PAH compared to controls.

Results

Correlation of plasma N-T proBNP levels with haemodynamic indices

Mean value of N-T proBNP (SE) for patients with PAH was 3365 (1095) pg/ml compared to 347 (1 7 4) pg/ml for those without PAH (Fig. 1). This indicates that plasma N-T proBNP levels are slightly elevated in SSc patients (controls) but they are elevated to a significantly higher magnitude in patients with PAH. Amongst the latter, there was a significant positive correlation between plasma N-T proBNP levels and mPAPs (Fig. 2) ($r^2 = 0.28$, $P < 0.05$). It was notable that the four patients with significant PAH with mPAP of 60 mmHg on cardiac catheter had a wide variation in their N-T proBNP values between 1500–13,000 pg/ml. A similar correlation was found between RVEDP (Fig. 3) and plasma N-T proBNP levels. Similarly, there was a significant correlation between PVR and N-T proBNP levels (PVR in dynes/s^5, normal range up to 140. Correlation coefficient $r = 0.49$ ($r^2 = 0.24$, $P < 0.05$), SE = 0.87). All correlations were statistically significant with $P < 0.05$.

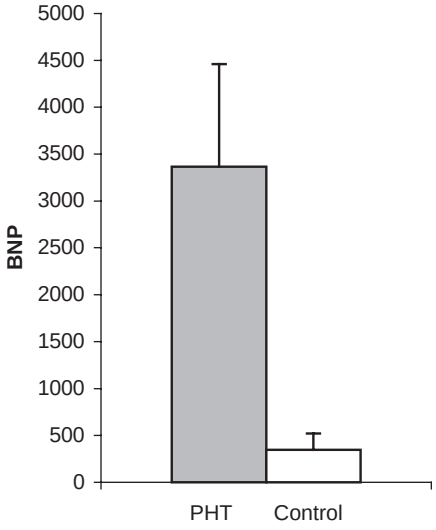

Figure 1 Mean N-T proBNP values (pg/ml) with SE (bars) comparing PAH and control SSc patients.

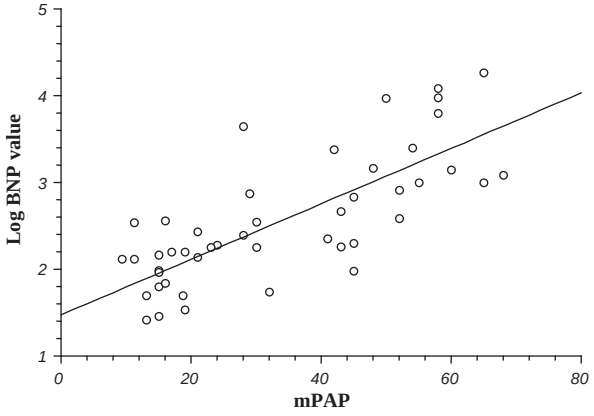

Figure 2 Correlation between N-T proBNP values (pg/ml) and mean PAP (mmHg) on cardiac catheter. Correlation coefficient $r = 0.53$ ($r^2 = 0.28$, $P < 0.05$), SE = 26.8. Note: BNP log scale used due to wide variation in values.

Correlation of plasma N-T proBNP levels with functional state

The comparison between the patient's functional state as measured by the SMWT showed that patients with increased severity of disease as reflected by decreased exercise capacity had higher plasma N-T proBNP levels (Fig. 4).

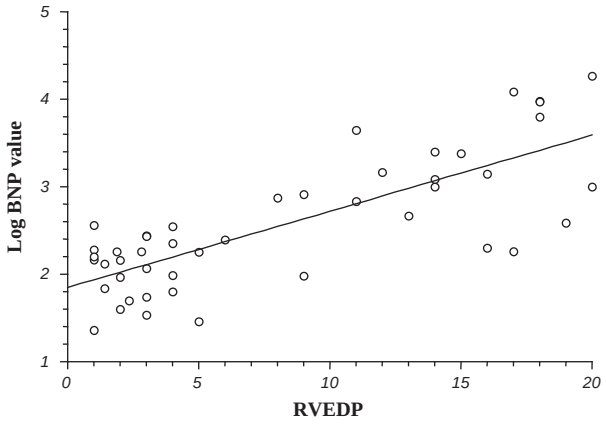

Figure 3 Correlation between N-T proBNP values (pg/ml) and RVEDP in mmHg. Correlation coefficient $r = 0.59$ ($r^2 = 0.35$, $P < 0.05$), SE = 68. Note: BNP log scale used due to wide variation in values.

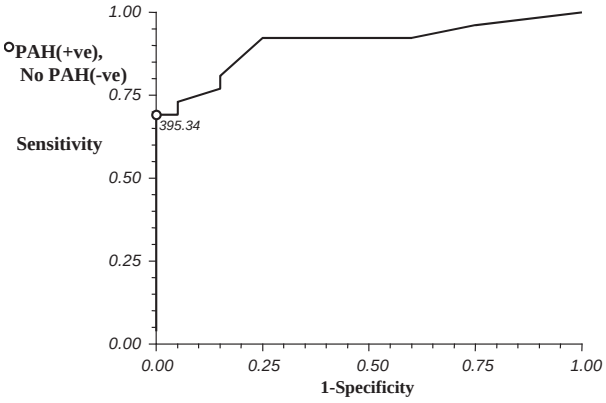

Figure 5 ROC curve for N-T proBNP in SSc patients in the diagnosis of pulmonary hypertension. True-positive rates and false-positive rates are plotted to obtain a cut-off value of 395.34 pg/ml.

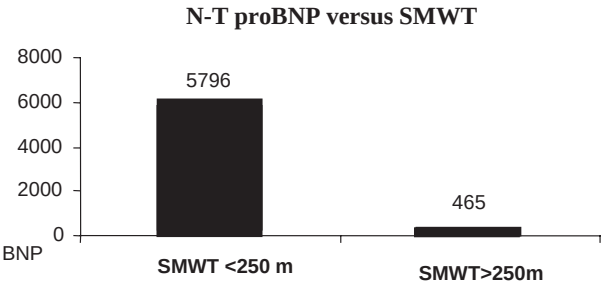

Figure 4 Comparison of plasma N-T proBNP levels (pg/ml) in 23 SSc patients with catheter-diagnosed PAH with (a) SMWT < 250 m ($n = 14$) and (b) SMWT > 250 m ($n = 9$). Mean BNP value in pg/ml given above bars for each group.

Ascertainment of the cut-off value for N-T proBNP that predicts mPAP of 25 mmHg on cardiac catheter (i.e., mPAP diagnostic of PAH by NIH criteria)

Fig. 5 shows the ROC curve for N-T proBNP in SSc patients with PAH compared to controls. A cut-off value of 395.34 pg/ml gave a test sensitivity of 0.69 and specificity of 1.0. The high specificity of this test implied that all patients with level of less than 395 pg/ml did not have PAH. The area under the ROC curve by Wilcoxon's estimate (95% confidence interval) was 0.91. This suggested a good negative predictive value for the assay.

Discussion

Our pilot study demonstrated a positive correlation between haemodynamic parameters of RV dysfunction and plasma N-T proBNP levels in 23 SSc patients with PAH. There was a significant correlation between N-T proBNP levels and the severity of PAH as judged by elevations in mPAP, PVR and RVEDP on cardiac catheter. In addition, we found that SScPAH patients with severe functional impairment, with a SMWT < 250 m (corresponding to modified NYHA grade III/IV dyspnoea) had a 10-fold increase in levels compared to patients with SMWT > 250 m (corresponding to NYHA dyspnoea grades II/III). Our findings concur with those of Nagaya et al,[6,7] who correlated plasma BNP levels with mPAPs and functional capacity in PPH patients. These authors used an ELISA assay for plasma BNP, not the N-terminal propeptide ligand, and suggested that raised levels are associated with a poor prognosis in PPH. Interestingly, the same authors[19] showed a correlation with the severity of PAH in 34 patients with chronic thromboembolic disease, with levels falling when the RV impairment was rectified after thromboendarterectomy.

As haemodynamic indices of RV dysfunction in SScPAH are known to predict survival,[20] plasma N-T proBNP levels in SScPAH may have a similar prognostic role. This is supported by the finding that our patients with high N-T proBNP levels had severe limitation of their functional capacity as measured by the SMWT. Longitudinal observations from our actively followed up cohort of SScPAH patients indicate that some SSc patients with markedly elevated mean pulmonary artery pressures clinically do well, and some with less elevated pressures succumb. The scatter distribution of plasma N-T proBNP levels raises the exciting possibility that those with greater RV dysfunction but similar mPAPs may have higher levels of N-T proBNP. This was intimated in our group of four

patients with mPAP >60 mmHg who had a wide variation in their N-T proBNP levels. On closer study of the clinical characteristics of this subgroup, the two patients with high levels had a SMWT of <200 m (NYHA grade IV symptoms). None of these patients had concomitant left ventricular dysfunction or pulmonary fibrosis on HRCT. In the future, it may be possible to risk stratify individuals with SScPAH, with patients with elevated plasma N-T proBNP levels comprising a readily identifiable poor prognostic group. Currently, SMWTs and repeated invasive haemodynamic studies are used to serially assess therapeutic response and disease progression. The former procedure is cumbersome and the latter functional test prone to day-to-day fluctuations. If plasma N-T proBNP levels have an even equivalent prognostic accuracy, then our ability to monitor patients and adjust therapy with SScPAH will be significantly improved.

Along with transforming growth factor β and endothelin-1, BNP has been implicated in the pathogenesis of cardiac fibrosis.[21,22] We found elevated levels of this peptide in the background SSc population, and markedly elevated levels in the presence of PAH. The mean level in the SSc control patients was 347 pg/ml and 3365 pg/ml in patients with PAH. The values for the upper range of normal suggested by the manufacturers of the NT-proBNP Elecsys assay, which will be recommended as a cut-off to screen for patients with heart failure in Europe and USA in the near future (Dr. B Agrawal, Roche, UK, personal communication) are 93 pg/ml for males and 144 pg/ml for females. The finding that N-T proBNP levels were 2.5 times the normal upper limit in the non-PAH SSc patients strengthens the suggestion that BNP acts directly on the myocardium to inhibit myocyte hypertrophy and fibrosis. The finding that the levels are elevated in the control SSc patients implies that either the normal ranges for N-T proBNP do not apply in this population, or that there may be a subclinical activation of N-T proBNP production occurring in SSc patients without the stimulus of pressure loading of the right ventricle. The former scenario is interesting given that natriuretic peptide receptors (NPRs) have been found to have an important role in collagen synthesis and inhibition within cardiac fibroblasts and myocytes.[23,24] There are three classes of NPRs, with BNP activity mediated predominantly through NPR A and C.[25] NPR-A knockout mice demonstrate cardiac hypertrophy and LV dilatation, and this response has been found to be reduced by chronic treatment with enalapril, frusemide and losartan.[26] It has also been shown that within the context of RV hypertrophy, NPRs are downregulated within the endocardium as a modulatory measure.[27] Enhancement of natriuretic peptide activity may be achieved through the inhibition of metabolism by neutral endopeptidase. Hence, endopeptidase inhibitors such as omapatrilat or candoxatril[28] may be beneficial by arresting cardiac hypertrophy. Similarly, the recombinant B-type natriuretic peptides such as Nesiritide[29] may have therapeutic potential in slowing progression of RV dysfunction amongst SSc patients by inhibition of cardiac fibroblast proliferation and collagen deposition, which is known to occur in this disease.

Limitations

This was a small pilot study, where only limited conclusions can be drawn due to the lack of statistical power. Not all control subjects with SSc underwent cardiac catheterisation and hence PAH may have been missed in these non-catheterised patients. However, echocardiography, DLCO and clinical assessment including CXRs and ECG were all normal in these individuals, diminishing the possibility that they had subclinical PAH. Due to the limitation of the design, it is not known whether a single elevated N-T proBNP value is predictive of survival in patients with right ventricular dysfunction in the future. In order to investigate this further, one would be required to follow-up SScPAH patients with varying N-T proBNP levels but similar mPAPs on catheterisation serially over a period of time.

Conclusions

Plasma N-terminal pro-brain natriuretic peptide levels significantly correlated with haemodynamic measures of disease severity in systemic sclerosis-related pulmonary hypertension (SScPAH). Although the levels of this peptide were elevated twice the upper range of normal in the background SSc population, this test has a good negative predictive value, suggesting that patients with a brain natriuretic peptide (BNP) value below a certain cut-off value are highly unlikely to have pulmonary arterial hypertension. The assay used in this study had good internal validity and was feasible for use in busy clinical settings. Measurement of this peptide may provide important prognostic information and help guide therapy in SScPAH patients. Our results justify a larger future study to confirm the above findings and further investigate the prognostic role of BNP in SScPAH.

Uncited references

[30,31]

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