# The role of asymmetric dimethylarginine alone and in combination with N-terminal pro-B-type natriuretic peptide as a screening biomarker for systemic sclerosis-related pulmonary arterial hypertension: a case control study

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*For funding and competing interests see page S-134.*

**ABSTRACT**

**Objective.** *Asymmetric dimethylarginine (ADMA) is a novel biomarker of endothelial cell dysfunction. In this proof of concept study, we sought to evaluate the role of ADMA as a screening biomarker for incident systemic sclerosis-related pulmonary arterial hypertension (SSc-PAH).*

**Methods.** *ADMA levels were measured using high performance liquid chromatography in 15 consecutive treatment-naive patients with newly-diagnosed SSc-PAH and compared with 30 SSc-controls without PAH. Logistic regression models were used to evaluate the independent association of ADMA with PAH. The optimal cut-point of ADMA for SSc-PAH screening was determined. NT-proBNP levels were previously measured in the same patients and the optimal cut-point of NT-proBNP of ≥210ng/mL was coupled with the optimal cut-point of ADMA to create a screening model that combined the two biomarkers.*

**Results.** *The PAH group had significantly higher mean ADMA levels than the control group (0.76±0.14 μM versus 0.59±0.07 μM; p<0.0001). ADMA levels remained significantly associated with PAH after the adjustment for specific disease characteristics, cardiovascular risk factors and other SSc-related vascular complications (all p<0.01). An ADMA level ≥0.7 μM had a sensitivity of 86.7%, specificity of 90.0% and AUC of 0.86 for diagnosing PAH. A screening model that combined an NT-proBNP ≥210ng/mL and/or ADMA ≥0.7 ng/mL resulted in a sensitivity of 100% and specificity of 90% for the detection of SSc-PAH.*

**Conclusion.** *In this small study, use of ADMA in combination with NT-proBNP produced excellent sensitivity and specificity for the non-invasive identification of SSc-PAH. The role of ADMA as a screening biomarker for SSc-PAH merits further evaluation.*

## Introduction

Systemic sclerosis (SSc) is a heterogeneous, multisystem connective tissue disease characterised by autoantibodies, vasculopathy and fibrosis affecting the skin and a number of internal organs. Systemic sclerosis related pulmonary arterial hypertension (SSc-PAH) is a major cause of morbidity in this disease, conferring a significantly increased risk of death and accounting for approximately 30% of SSc related mortality (1-3). In this context, screening for PAH has emerged as an important consideration in the optimal management of patients with SSc (4). The rationale behind screening for SSc-PAH is based on the early identification of an aggressive but treatable complication in an at risk population, allowing prompt initiation of therapies that appear to offer significant clinical benefit. A recent French study showed higher 3-, 5- and 8-yr survival rates in patients identified by screening, compared with patients diagnosed during routine care, where symptoms and/or signs directed further investigation (81%, 73% and 64% vs. 31%, 25% and 17%, respectively) (5).

Right heart catheterisation (RHC) is necessary for making a definitive diagnosis of SSc-PAH but is not feasible for general screening as it is invasive. Instead, current international guidelines recommend regular trans-thoracic echocardiography (TTE), with or without diffusing capacity for carbon monoxide (DLCO), for screening in order to

help identify high-risk individuals who should undergo RHC (6-8). However, current screening tests perform better when PAH is more advanced (9) as the high capacitance physiology of the pulmonary microcirculation means that it is only after >50% of the pulmonary vasculature is obstructed that the mean pulmonary artery pressure rises (10). Therefore, interest is turning to alternative strategies such as incorporating blood biomarkers reflecting the pathophysiology, pathogenesis or genetics of the condition, to assist in the risk stratification of SSc patients leading to the earlier detection of PAH.

In case control and cohort studies, we have previously evaluated the role of serum N-terminal pro-B-type natriuretic peptide (NT-proBNP), released as a result of myocardial stress, in screening for SSc-PAH and found a level ≥ 210ng/mL to have optimal properties for diagnosing PAH, with a sensitivity of 93.3%, specificity of 100% and AUC of 0.94 (11, 12).

Asymmetrical dimethylarginine (ADMA, or $N^G$-$N^G$-Dimethyl-L-arginine) is also a potential biomarker of interest in pulmonary hypertension (13). It is the principal endogenous inhibitor of nitric oxide synthase (NOS) and results in impaired NO production (see Figure 1) (14, 15). ADMA also directly affects endothelial cell gene expression including bone morphogenetic protein (BMP), which has been associated with idiopathic PAH (16). Monomethylarginine (MMA) and symmetrical dimethyarginine (SDMA) are released alongside ADMA in the process of arginine methylation, but only ADMA and MMA are thought to directly inhibit NOS. In SSc, Dimitroualis *et al*. reported significantly higher mean ADMA levels in SSc patients with a systolic pulmonary artery pressure on transthoracic echocardiography ($sPAP_{TTE}$) ≥40 mmHg compared with SSc patients with a $sPAP_{TTE}$ <40mmHg; however, the lack of RHC in this study was a major limitation (17). Elevated ADMA levels have also been reported in other types of pulmonary hypertension including idiopathic PAH (iPAH), chronic thromboembolic pulmonary hypertension (CTEPH), congenital heart disease related PH and PAH secondary to sickle cell disease, suggesting that ADMA may be a biomarker of endothelial cell dysfunction in pulmonary hypertension (18-21).

The goal of this study was to evaluate the relationship between serum ADMA levels in SSc patients with and without PAH confirmed by RHC, and to determine the clinical utility of ADMA alone and in combination with NT-proBNP as a screening biomarker for incident SSc-PAH.

## Patients and methods

*Study design and population*

In this case-control study, sera of SSc patients with and without PAH were collected, analysed and compared. All patients fulfilled ACR criteria for SSc (22). All clinical parameters, TTE and pulmonary function tests (PFTs) including DLCO were assessed within three months of the serum collection and prospectively recorded in the Australian Scleroderma Cohort Study (ASCS) database as previously described (23). The ASCS is approved by the human research ethics committees of the 13 participating Australian centres, and patients provide written informed consent at recruitment.

Group 1 comprised patients with SSc-PAH and included 15 consecutive patients with a new diagnosis of RHC confirmed PAH based on a mean pulmonary artery pressure (mPAP) ≥25mmHg and pulmonary capillary wedge pressure (PCWP) ≤15mmHg. These patients had no more than minor changes of interstitial lung disease (ILD) on high-resolution CT lung (HRCT).

Group 2 (n=30) were SSc-controls who had no evidence of cardiopulmonary complications, based on $sPAP_{TTE}$ <30mmHg, normal myocardial function on TTE, DLCO corrected for haemoglobin ($DLCO_{corr}$) >70% predicted, (forced expiratory volume in one second/forced vital capacity (FEV1/FVC) % predicted >0.7, no ILD on HRCT (and in those without an HRCT, FVC ≥80% predicted), and World Health Organisation Functional Class (WHO-FC) I or II.

Exclusion criteria for both groups included the presence of abnormal left ventricular systolic or diastolic function for age measured at TTE, abnormal left atrial size, an unrecordable tricuspid regurgitant Doppler signal, and estimated glomerular filtration rate (eGFR) <30ml/min.

*Cardiac and pulmonary assessments*

Left ventricular systolic and diastolic function was determined by 2-dimensional TTE performed within three months of collection of serum. The $sPAP_{TTE}$ was measured at rest, based on peak velocity of the tricuspid regurgitant jet and estimation of right atrial pressure of 5–10 mmHg based on the diameter and respiratory variation of the inferior vena cava. TTE was performed only at tertiary centres for SSc assessment. Pulmonary involvement was assessed by PFT and/or HRCT within 3 months of serum collection. HRCT were reported as no, mild, moderate or severe ILD by a radiologist. All $DLCO_{corr}$ (ml/mmHg/min) values are reported as % predicted values, corrected for haemoglobin. All FEV1 (litres), FVC (litres) and $FVC/DLCO_{corr}$ values are reported as percentage predicted for sex, race and height (24).

*Serum samples*

All sera were collected from patients within three months of their annual clinical assessment and cardiopulmonary investigations. All PAH patients (Group 1) had serum collected for ADMA measurement at the time of their RHC and prior to the commencement of pulmonary vasodilator therapy. Blood samples were collected at rest into tubes containing EDTA. Samples were centrifuged and stored at -80°C until used.

*Measurement of ADMA, SDMA and L-arginine levels*

ADMA, SDMA and L-arginine levels were determined by High Performance Liquid Chromatography (HPLC) with Solid Phase Extraction (SPE) at the Cardiology Unit, The Queen Elizabeth Hospital, University of Adelaide, using an established, published method (25). HPLC with SPE is one of the most accessible methods for ADMA determination, and very high accuracy can be

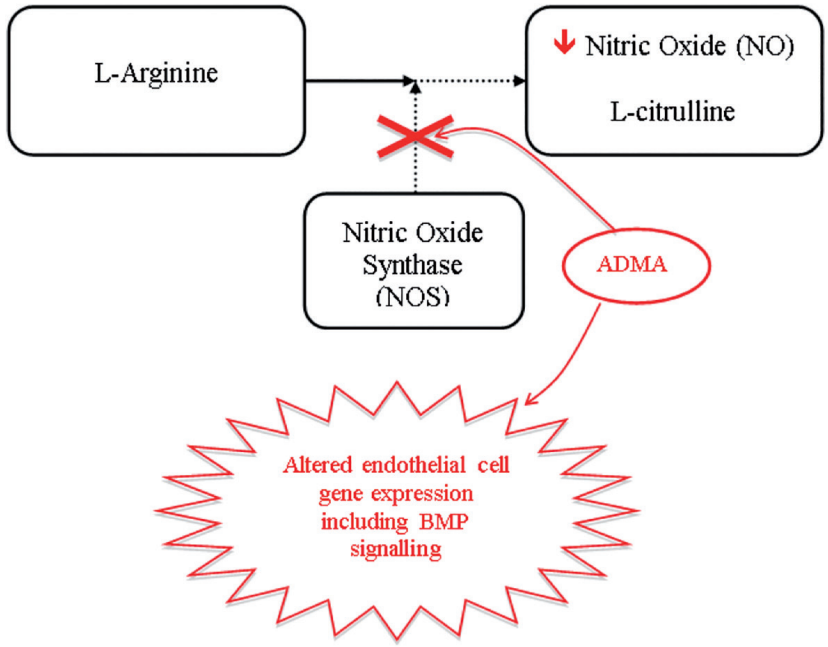


**Fig. 1.** NO metabolism and the effects of ADMA
NOS converts L-Arginine to NO and L-citrulline. ADMA is the principal inhibitor of all NOS isoforms. ADMA (along with SDMA and MMA) is produced as a result of degradation of methylated proteins, and cleared predominantly by the action of dimethylarginine dimethylaminohydrolase (DDAH). ADMA competes with arginine and blocks NO production by acting as a false substrate for NOS. ADMA also influences endothelial cell gene expression, in particular BMP signalling.
NOS: nitric oxide synthase; NO: nitric oxide; ADMA: asymmetric dimethlyarginine; SDMA: symmetrical dimethylarginine; MMA:monomethylarginine; BMP:bone morphogenetic protein.

achieved with standardised, validated techniques (26). Briefly, ADMA and SDMA were extracted from serum by strong cation exchange (SCX), which was followed by fluorescent derivatisation. The resultant highly stable fluorescent derivatives were then processed by HPLC, which achieved an excellent separation of the arginine metabolites from internal standard and endogenous serum components. The interassay variability for ADMA/SDMA was 6%, and 8% for arginine (n=17 sets). The % recovery in spiked samples for ADMA/SDMA was 90%, and 76% for arginine. The limit of detection for ADMA/SDMA is 0.1 μM.

*Statistical analysis*

In this case-control study design, ADMA, SDMA and L-arginine levels in patients with RHC-proven SSc-PAH and SSc-controls were compared using the student t-test after appropriate transformations were performed to satisfy the assumptions of normality and homogeneity of variance. Logistic regression models were used to explore the independent association of ADMA with PAH, after the adjustment for each covariate in individual models. Important correlations of ADMA and SDMA were assessed using Spearman's rank correlation coefficient (rho). Receiver operator characteristic (ROC) curve analysis was used to determine the optimal cut-points that maximised desired test properties, namely sensitivity and negative predictive value. Based on ROC curve analysis, combination biomarker models were tested using contingency tables. All statistical analyses were performed using STATA 12.1 (Statacorp, College Station, TX, USA).

## Results

*Characteristics of the patient population*

ADMA levels were evaluated in 45 SSc patients, consisting of 15 treatment-naïve SSc patients with PAH (group 1; SSc-PAH) and 30 SSc patients with no PAH (group 2; SSc-controls). Demographic, clinical and investigative characteristics of these patients are summarised in Tables I and II.

*Comparison of ADMA levels: SSc-PAH vs. SSc-controls*

ADMA levels were compared between groups (Fig. 2). The PAH group had significantly higher mean ADMA levels than the control group (0.76±0.14 μM *vs.* 0.59±0.07 μM; $p<0.0001$). Serum SDMA levels were also shown to be significantly higher in SSc-PAH (0.79±0.26 μM *vs.* 0.46±0.07; $p<0.0001$) (Fig. 3). L-Arginine levels were significantly lower in SSc-PAH *versus* SSc-controls (97.28±27.40 vs. 117.45±26.70μM, $p=0.017$). A significantly lower L-arginine to ADMA ratio was noted in SSc-PAH ($p<0.0001$).

*Adjusting ADMA levels for the influence of covariates*

Individual logistic regression models were used to evaluate the independent association of ADMA with PAH, after the adjustment for each of the covariates in turn. Accordingly, due to a limited sample size, each model contained only two variables, ADMA level and the covariate. Covariates included clinical characteristics, co-morbidities, and vascular complications (see Table III). As older age and longer disease duration have been associated with PAH, and were available in the study cohort, the effect of these variables was adjusted for using logistic regression. ADMA was associated with PAH (OR =1.14, 95%CI: 1.03-1.25, $p=0.008$) after adjustment for age (OR =1.12, 95%CI: 1.01-1.24, $p=0.031$). Similarly, ADMA was associated with PAH (OR =1.16, 95%CI: 1.05-1.27, $p=0.003$) after adjustment for disease duration (OR 1.09, 95%CI: 0.98-1.20, v=0.104). ADMA levels remained significantly associated with PAH after adjustment for cardiovascular risk factors (including hypertension, hypercholesterolaemia, diabetes, current or previous smoking), vascular complications (including Raynaud's phenomenon and digital ulcers) as well as calcium channel blocker usage (see Table III). ADMA levels were also significantly associated with PAH after adjustment for disease subtype, modified Rodnan skin score and body mass index.

The independent associations of SDMA with PAH, after adjustment for covariates were similarly significant (Table IV).

*Correlations of ADMA with RHC parameters in SSc-PAH*

The correlations of ADMA levels with RHC parameters were evaluated in SSc-PAH patients. There was no significant correlation of ADMA level with mPAP (rho =0.12, *p*=0.661), PVR (rho =0.30, *p*=0.341) or mRAP (rho =0.10, *p*=0.739). Similarly, SDMA did not correlate with mPAP (rho =-0.23, *p*=0.41), PVR (rho =0.13, *p*=0.68) or mRAP (rho =-0.004, *p*=0.99).

*ROC curve analysis*

As can be seen in Table V, an ADMA level ≥0.694 μM for PAH *versus* controls, had a sensitivity of 86.7% (95%CI: 58.4-97.7%), specificity of 90.0% (95%CI: 72.3-97.4%), with an AUC of 0.86 (95%CI: 0.7-1.0) for diagnosing PAH. On the other hand, an SDMA level ≥0.621μM had a sensitivity of 80.0% and specificity of 100%, with an AUC of 88% for diagnosing PAH (95%CI: 74-100%).

*Screening model and properties*

The optimal biomarker cut-points derived from ROC curve analysis were used to form screening models for SSc-PAH *versus* SSc-controls (see Table V). The goal of developing these composite biomarker models was to maximise the sensitivity (*i.e.* eliminate false negatives), as would be required of a screening model, whilst maintaining specificity. As can be seen in Table V, the screening model that combined an NT-proBNP ≥210 ng/mL (as determined in our previous study (11)) and/or ADMA ≥0.7 ng/mL resulted in a sensitivity of 100%, which was higher than either biomarker in isolation; the specificity of this model was 90%.

**Table I.** Comparison of clinical characteristics between groups.

| Characteristics | Group 1 SSc-PAH (mean ± SD) | Group 2 SSc-controls (mean ± SD) | *p*-value |
|---|---|---|---|
| Number (n) | 15 | 30 | N/A |
| Age at onset (y) | 44.5 ± 12.9 | 40.6 ± 13.2 | 0.355 |
| Age at study (y) | 62.1 ± 10.9 | 48.7 ± 10.1 | <0.0001 |
| Dis. duration (y) | 19.2 ± 12.4 | 7.8 ± 7.2 | 0.0011 |
| Female, n (%) | 12 (80) | 30 (100) | 0.011 |
| Male, n (%) | 3 (20) | 0 | |
| Limited (n) | 13 | 23 | 0.429 |
| Diffuse (n) | 2 | 7 | |
| ANA, n | 14 | 29 | 0.160 |
| Anti-Scl70, n | 1 | 5 | 0.333 |
| Anti-cent, n | 7 | 16 | 0.592 |
| MRSS | 10.4 ± 12.1 | 7.6 ± 7.5 | 0.432 |
| WHO FC | | | |
| -1 | 0 | 25 | <0.0001 |
| -2 | 2 | 5 | |
| -3 | 11 | 0 | |
| -4 | 2 | 0 | |

Dis. duration: disease duration; PAH: pulmonary arterial hypertension; ILD: interstitial lung disease; ANA: Anti-nuclear antibody; Anti-Scl70: anti- topoisomerase-1 antibody; Anti-cent: anti-centromere antibody; MRSS: modified Rodnan skin score; WHO FC: World Health Organisation functional class.

**Table II.** Comparison of investigation parameters between groups.

| Investigations | Group 1 PAH | Group 2 Controls | *p*-value |
|---|---|---|---|
| TTE parameters | | | |
| TR vel (m/s) | 3.8 ± 0.7 | 2.20 ± 0.2 | <0.0001 |
| sPAP (mmHg) | 65.8 ± 27.3 | 26.3 ± 2.6 | <0.0001 |
| RHC results | | | |
| mPAP(mmHg) | 40.2 ± 12.5 | N/A | N/A |
| mRAP (mmHg) | 10.1 ± 3.1 | N/A | N/A |
| PVR (wood units) | 6.2 ± 3.4 | N/A | N/A |
| PFT | | | |
| FVC (% pred) | 75.5 ± 24.3 | 102.8 ± 13.4 | <0.0005 |
| $DLCO_{corr}$ | 45.6 ± 11.7 | 86.8 ± 13.0 | <0.0001 |
| $FVC/DLCO_{corr}$ | 1.76 ± 0.38 | 1.20 ± 0.20 | <0.0001 |
| 6MWD (m) | 337 ± 100 | 520 ± 48.6 | <0.0001 |
| C-reactive protein | 8.6 ± 9.7 | 5.9 ± 10.0 | 0.379 |
| ESR | 24.7 ± 13.3 | 12.4 ± 14.5 | 0.0094 |

TRV: tricuspid regurgitant velocity; sPAP: systolic pulmonary artery pressure; mPAP: mean pulmonary artery pressure; mRAP: mean right atrial pressure; PVR: pulmonary vascular resistance; FVC: forced vital capacity (% predicted); DLCO: diffusion capacity of lung for carbon monoxide (% predicted); 6MWD: six minute walk distance; m: metres; Erythrocyte Sedimentation Rate (ESR); PAH: pulmonary arterial hypertension.

## Discussion

The results of this study suggest that serum ADMA levels may serve as an important screening biomarker for SSc-PAH. Additionally, a composite biomarker screening algorithm, using NT-proBNP in combination with ADMA, may achieve an excellent sensitivity (and also specificity) for the identification of SSc-PAH.

Serum ADMA levels, measured by HPLC, were shown to be significantly higher in SSc-PAH than SSc-controls, even after adjusting for a number of the systemic vascular and fibrotic complications that characterise SSc. This is in line with the work of Kielstein *et al*. and Pulamsetti *et al*., who showed higher levels of ADMA in iPAH patients compared to healthy controls (18, 19). Our study overcomes some of the limitations of the previous study in SSc patients which reported higher ADMA levels in SSc patients with an $sPAP_{TTE}$ ≥40mmHg compared with those with a $sPAP_{TTE}$ < 40mmHg but did not confirm the presence or absence of PAH with RHC. This concern was further highlighted by the unexpectedly similar DLCO % predicted among cases and controls in that study (61±21 vs. 65±22, *p*=NS), which is unusual given that SSc-PAH is usually associated with a markedly decreased DLCO compared to SSc controls (17). In the wider literature, 'normal' ADMA levels

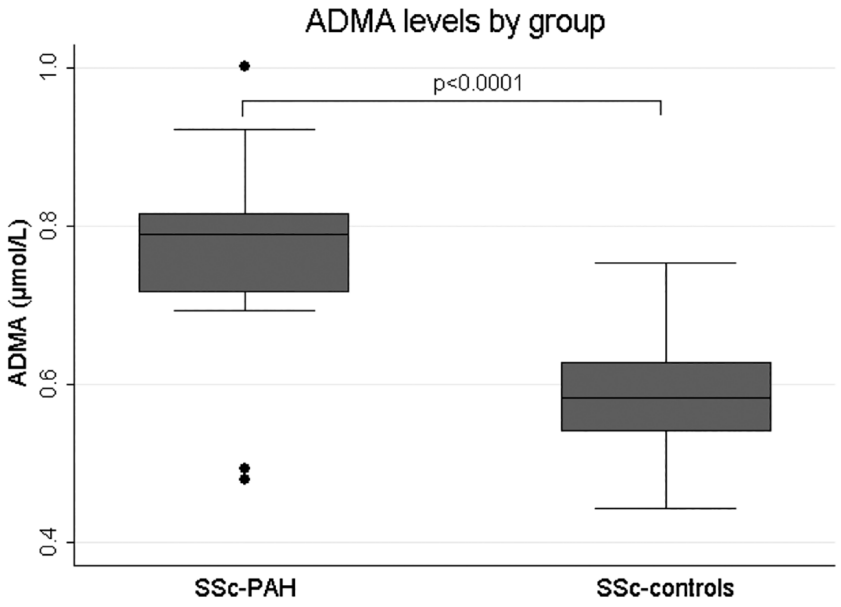


**Fig. 2.** Comparison of ADMA levels in SSc-PAH *versus* SSc-controls.
ADMA: asymmetric dimethylarginine; SSc: systemic sclerosis; PAH: pulmonary arterial hypertension.

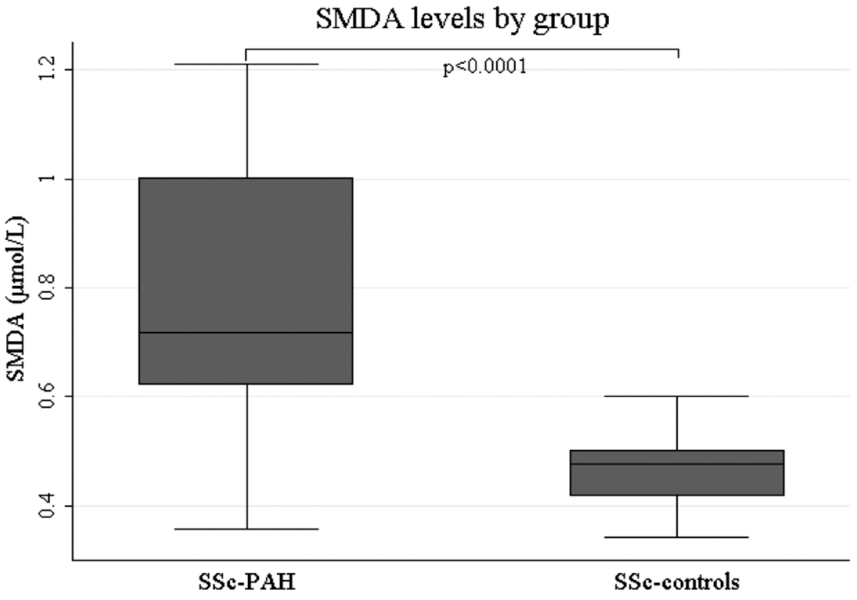


**Fig. 3.** Comparison of SDMA levels in SSc-PAH *versus* SSc-controls.
SSc: systemic sclerosis; PAH: pulmonary arterial hypertension. SDMA: symmetrical dimethylarginine.

in healthy subjects have been reported to lie within a narrow range of 0.4 - 0.6 µM, with relatively small elevations of ADMA associated with adverse outcomes such as acute coronary events and mortality in patients with coronary artery disease, organ failure and haemodialysis (26-30). In this study, the mean ADMA concentration of 0.76±0.14 µM in SSc-PAH was clearly outside the published normal range and significantly above the ADMA levels seen in SSc patients without PAH. The ROC curve analysis also gave an optimal ADMA cutpoint of ≥0.7 µM for the identification of SSc-PAH. Taken together, these results suggest that elevated ADMA levels may be associated with SSc-PAH.

ADMA levels were not found to correlate with specific RHC haemodynamics (mPAP, mRAP or PVR). This is not surprising when we consider that measured serum levels of ADMA are very much below the 10µM that would be required to directly affect NO production (15). Instead, it seems more likely that ADMA may act as a 'surrogate' marker of intracellular changes in methylarginine metabolism, with small changes in serum ADMA reflecting greater changes in intracellular ADMA concentrations (31). ADMA is also known to directly influence endothelial cell gene expression, including BMP signalling, which has been associated with iPAH (16). In a population of 57 iPAH patients, Kielstein *et al*. showed a mild but significant correlation of ADMA with mRAP (r =0.39, $p<0.0003$), with both ADMA and mRAP independently predicting survival (18). Kielstein *et al*. directly sampled mixed venous blood from the pulmonary arterial vascular bed at RHC, and this may have enabled the more accurate correlation of ADMA levels Alternately, it may reflect inherent differences in SSc-PAH vs. iPAH, or simply the greater sample size of that study. Nonetheless, an abnormal ADMA level presents an attractive non-invasive biomarker for SSc-PAH, and further studies are required.

In this study, serum measurement of ADMA with a cut-point of 0.7 µM achieved a very good sensitivity and specificity for detection of PAH (86.7% and 90.0%, respectively). However, a high sensitivity is crucial in screening for SSc-PAH, and in order to improve this further, ADMA was combined with NT-proBNP. NT-proBNP is a 76 amino-acid polypeptide that is released by cardiac myocytes in response increased ventricular wall stress, as typically occurs with volume overload and ventricular contractile dysfunction (32). NTproBNP is a well-studied candidate biomarker for SSc-PAH, demonstrating utility in the screening, diagnosis, and prognosis of SSc-PAH (23, 33, 34). One of factors impeding the wider use of NT-proBNP in the screening and diagnosis of SSc-PAH has been the lack of sufficiently high sensitivity as a 'stand-alone' test (35, 36). In order to improve the sensitivity of these biomarkers, ADMA and NT-proBNP were combined. This 'biomarker-based' model achieved an

**Table III.** Independent associations of ADMA with PAH, after adjustment for covariates.

| Covariate | OR of ADMA for PAH | 95% CI | *p*-value |
|---|---|---|---|
| Age (y) | 1.14 | 1.03, 1.25 | 0.008 |
| Disease duration (y) | 1.16 | 1.05, 1.27 | 0.003 |
| Disease subtype | 1.18 | 1.07, 1.30 | 0.001 |
| Hypertension | 1.16 | 1.06, 1.28 | 0.002 |
| High Cholesterol | 1.15 | 1.04, 1.26 | 0.004 |
| Smoking status | 1.17 | 1.06, 1.29 | 0.002 |
| MRSS | 1.17 | 1.06, 1.29 | 0.001 |
| BMI | 1.15 | 1.04, 1.26 | 0.006 |
| Raynaud's disease | 1.18 | 1.07, 1.29 | 0.001 |
| Digital ulceration | 1.19 | 1.07, 1.32 | 0.001 |
| Calcium channel blocker use | 1.19 | 1.07-1.31 | 0.001 |

*OR are reported per 0.01μM increase in ADMA. In each logistic regression model, there were only two independent variables: ADMA and each of the covariates in turn.
ADMA: asymmetric dimethylarginine; PAH: pulmonary arterial hypertension; OR: odds ratio; CI: confidence interval; y: years; MRSS: modified Rodnan skin score; BMI: body mass index.

**Table IV.** Independent associations of SDMA with PAH, after adjustment for covariates.

| Covariate | OR of SDMA for PAH | 95% CI | *p*-value |
|---|---|---|---|
| Age (y) | 1.12 | 1.01, 1.25 | 0.025 |
| Disease duration (y) | 1.16 | 1.04, 1.29 | 0.008 |
| Disease subtype | 1.17 | 1.06, 1.30 | 0.001 |
| Hypertension | 1.17 | 1.05, 1.31 | 0.005 |
| High cholesterol | 1.16 | 1.05, 1.29 | 0.005 |
| Smoking status | 1.15 | 1.05, 1.28 | 0.004 |
| MRSS | 1.17 | 1.06, 1.29 | 0.002 |
| BMI | 1.16 | 1.04, 1.29 | 0.007 |
| Raynaud's disease | 1.17 | 1.06, 1.29 | 0.002 |
| Digital ulceration | 1.19 | 1.06, 1.32 | 0.002 |
| Calcium channel blocker use | 1.18 | 1.06, 1.31 | 0.004 |

*OR are reported per 0.01μM increase in SDMA. In each logistic regression model, there were only two independent variables: SDMA and each of the covariates in turn.
SDMA: symmetric dimethylarginine; PAH: pulmonary arterial hypertension; OR: odds ratio; CI: confidence interval; y: years; MRSS: modified Rodnan skin score; BMI: body mass index.

excellent sensitivity and specificity (100% and 90%, respectively) for the detection of SSc-PAH. Thus, it may be possible to screen for SSc-PAH using a non-invasive combination biomarker model using NT-proBNP coupled with ADMA. This finding clearly needs to be validated in a larger prospective cohort of SSc-PAH.

Interestingly, raised SDMA levels were found in SSc-PAH. While the role of SDMA in endothelial dysfunction is uncertain, it has been hypothesised that SDMA may indirectly inhibit NO synthesis by interfering with arginine uptake through the inhibition of the human cationic amino acid transporter hCAT-2B (37). Raised SDMA levels have also been previously reported in iPAH (19). While SDMA performed well in this study as a stand-alone biomarker the sensitivity of SDMA for the detection of PAH was less than that of ADMA; furthermore, SDMA did not improve the performance of NTproBNP. More studies are required to confirm these findings, and the possible role SDMA may have as a biomarker for PAH.

This study has a number of strengths. Firstly, PAH was defined according to internationally accepted RHC criteria. Secondly, ADMA levels were assayed in newly diagnosed SSc-PAH patients, before the commencement of advanced pulmonary vasodilator therapies. Thirdly, accurate and validated techniques of ADMA measurement were employed using HPLC. Lastly, clinically relevant ADMA cut-points were determined in isolation, and in combination with NT-proBNP, which may enable clinicians to non-invasively identify patients who should undergo further testing for PAH.

This study has some important limitations. The study was small and had an observational case-control design. Patients with significant left ventricular and renal dysfunction were excluded, as both of these conditions have been associated with raised ADMA levels. Therefore, larger studies inclusive of these population subsets would provide further information about the strengths and limitations of ADMA in identifying patients with SSc-PAH. Furthermore, prospective, sequential sampling of ADMA levels, particularly in patients developing PAH, would help better understand the utility of ADMA levels in at risk individuals, as well as providing valuable information about the stability of ADMA over time and the response to therapy.

In summary, this study demonstrates the association of increased ADMA levels with SSc-PAH and the potential utility of ADMA in the screening and diagnosis of SSc-PAH. This has important clinical implications as non-invasive, biomarker-based screening models may enable the convenient and accurate identification of SSc patients who require further diagnostic evaluation for the presence of PAH.

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**Table V.** ROC analysis for PAH compared to controls.

| Variable | Optimal cutpoint | Sensitivity (95% CI) | Specificity (95% CI) | +LR | -LR | AUC |
|---|---|---|---|---|---|---|
| ADMA (μM) | ≥0.7 | 86.7% (58.4%, 97.7%) | 90.0% (72.3%-97.4%) | 8.7 (2.9-25.8) | 0.15 (0.04-0.54) | 0.86 (0.70, 1.0) |
| SDMA (μM) | ≥0.621 | 73.3% (44.8%, 91.1%) | 100.0% (85.6%, 99.7%) | - | 0.27 (0.12-0.62) | 0.88 (0.74-1.0) |
| ADMA ≥0.7 &/or SDMA ≥0.621 | - | 86.7% (58.4, 97.7%) | 90% (72.3%, 97.4%) | 8.7 (2.9-25.8) | 0.15 (0.04, 0.54) | - |
| SDMA ≥0.621 &/or NTproBNP ≥210 | - | 93.3% (66.0%, 99.7%) | 100% (85.9%, 99.7%) | - | 0.07 (0.01, 0.44) | - |
| ADMA ≥0.7 &/or NTproBNP ≥210 | - | 100% (74.7%, 99.4%) | 90% (72.3%, 97.4%) | 10.0 (3.4-29.3) | - | - |

ADMA: asymmetric dimethylarginine; SDMA: symmetrical dimethylarginine (SDMA); NT-proBNP: N-terminal pro-B-type natriuretic peptide; CI: confidence interval; LR: likelihood ratio; AUC: area under the curve.

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## Competing interests

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