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Diagnostic value of exhaled nitric oxide to detect interstitial lung disease in systemic sclerosis

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Abstract. *Background and aim:* Increased alveolar concentration of nitric oxide (CA_{NO}) is related to the severity of interstitial lung disease (ILD) in systemic sclerosis (SSc). However, cut-off levels of CA_{NO} to rule out, or to rule in, the presence of ILD in individual patients are unknown. We aimed to assess the validity of CA_{NO} for the diagnosis of ILD in SSc and to determine the thresholds of CA_{NO} that can be used in clinical practice to predict the likelihood of ILD in SSc. *Methods:* Lung HRCT scan, PFTs and partitioned exhaled NO measurements were performed in 65 consecutive SSc patients. ILD was diagnosed on pulmonary HRCT according to the presence of ground glass or reticular opacities. Diagnostic performance of CA_{NO} for ILD diagnosis was assessed using ROC curves. *Results:* 38 out of 65 SSc patients had ILD. CA_{NO}, at a cut-off level of 4.3 ppb, had a sensitivity and specificity for the diagnosis of ILD of 87% (95% CI: 77 to 99) and 59% (95% CI: 41 to 78), respectively. The same cut-off level of CA_{NO} could detect impairment of gas exchange with a sensitivity and specificity of 78% (95% CI: 67 to 90) and 73% (95% CI: 46 to 99), respectively. Moreover, ILD could be ruled in (positive predictive value > 95%) when $CA_{NO} \geq 10.8$ ppb, and ruled out C_{ANO} values ≤ 3.8 ppb (negative predictive value > 95%). *Conclusion:* CA_{NO} could be a valid non-invasive biological marker of ILD in SSc, and be of use in clinical practice. *(Sarcoidosis Vasc Diffuse Lung Dis 2009; 26: 32-38)*

Key words: systemic sclerosis, exhaled nitric oxide, interstitial lung disease, diagnostic value

Introduction

Systemic sclerosis (SSc) is a connective tissue disease characterized by progressive and excessive fibrosis processes involving skin and numerous organs, including the lungs (1). Interstitial lung disease (ILD) occurs in 25 to 33% of patients with SSc, resulting in restrictive pulmonary function and reduced gas lung transfer (2). The loss of lung volume is associated with increased morbidity whilst becoming the main cause of mortality in patients with SSc (3). High resolution computed tomography (HRCT) of the lung is the most sensitive method to diagnose ILD and to assess its extent. It is possible to detect structural interstitial abnormality by lung HRCT early in the course of the disease, before lung function impairment takes place. The physiopathology of ILD associated with SSc remains, however, unclear. Immune activation and alveolar inflammation, leading to activation of the inducible isoform of nitric oxide synthase (iNOS) (4) and the release of

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several pro-inflammatory cytokines and a high amount of nitric oxide (NO), probably play a central role in scleroderma lung disease. Measurement of NO in exhaled air from patients with SSc showed an increased amount of NO produced by airway conduct and lung tissue (5-7). Partitioning exhaled NO into alveolar and conducting airway compartments has shown that alveolar concentration of NO (CA_{NO}) was significantly higher in SSc patients with ILD as compared with SSc patients without ILD and healthy controls (8). Moreover, we have shown that CA_{NO} levels were related to the extent and the severity of SSc-associated ILD suggesting that CA_{NO} is a marker of alveolar inflammation that potentially could be of use for the diagnosis of ILD in SSc (9). However, cutoff levels of CA_{NO} to rule out, or to rule in, the presence of interstitial lung disease (ILD) in individual patients with SSc have not yet been investigated. The aim of this prospective study was therefore to assess the validity of CA_{NO} as a non-invasive marker for the diagnosis of ILD in SSc and to determine the thresholds of CA_{NO} that can be used in clinical practice to predict, or to exclude, the likelihood of ILD in SSc patients.

Methods

Subjects

Between November 2004 and March 2006, 69 consecutive patients with SSc were eligible for the study. Thirty eight were new and consecutive patients whose results have never been reported. Results from the remaining 27 patients have been published, in part, in a recent preliminary report (9). Four patients were excluded as they were unable to perform expiratory maneuvers allowing exhaled NO measurement (3 patients for concomitant cough and 1 patient for air leak due to her inability to tightly close her lips around the mouth piece). Consequently, 65 patients (58 women, 7 men, mean ± SD 54.8 ± 11.3 years), fulfilling the American College of Rheumatology criteria of SSc (10), were included in this prospective study at Saint-Antoine Hospital, academic referral centre of SSc in France. Data from 38 consecutive patients were newly added to those 27 SSc patients previously reported (9). The mean duration of disease (defined as the number of years after the first symptom attributable to SSc) was 10.8 ± 10.4 years. Twenty three patients had diffuse form and 42 had limited form of SSc according to LeRoy classification subset (11). Fifteen patients were current smokers, 13 were treated by low (less than 10 mg/day) doses of corticosteroids; among them, 3 took immunosuppressive therapy, but none took NO donors. The local ethics committee approved the study, and after giving their informed consent, all patients underwent pulmonary function tests (PFTs), echocardiogram, pulmonary HRCT and exhaled NO measurements.

Pulmonary high resolution computed tomography

Interstitial lung disease was considered present if pulmonary HRCT, which was considered as the gold standard test (12), demonstrated compatible changes in reticular or air space opacities. These changes included ground glass attenuation, defined as a hazy increase of lung parenchyma attenuation, and reticular fibrosis, defined as lobular septal thickening and subpleural honeycomb change.

Echocardiogram

All patients underwent echocardiogram (Vivid® 7 G.E medical systems, Norway). To estimate systolic pulmonary artery pressure (sPAP), the maximal transtricuspid pressure gradient was calculated using simplified Bernouilli equation (13). To calculate right ventricular systolic pressure, estimated as equal to sPAP, 10 mmHg, as an estimate of right atrial pressure, was added to the pressure gradient. Pulmonary hypertension was defined by a right ventricular systolic pressure of > 40 mmHg on echocardiogram.

Lung function measurement

Pulmonary function tests (PFT) (total lung capacity (TLC), forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), carbon monoxide lung diffusion (DLCO), alveolar volume (V_A) and blood gas measurements were performed (MasterScreen® Body, VIASYS Healthcare GmbH, Hoechberg, Germany) according to the American Thoracic Society/European Respiratory Society rec-

ommendations on clinical pulmonary function testing (14). All parameters were expressed as percentage of predicted values.

Partitioned exhaled NO measurement

NO was measured using a chemoluminescent analyzer (EndoNO 8000®, SERES, Aix-en-Provence, France), according to validated method for the online measurement of the exhaled NO concentration (FE_{NO}) in adults (15). The sensitivity of NO analyzer is less than 0.5 ppb. After full inspiration from room air with ambient NO levels less than 20 part per billion (ppb), the subject exhaled against positive pressure that was constantly kept between 5 cmH_20 (lower limit) and 20 cmH_2O (upper limit) to generate exhalation flow rates (V'_E) of 50, 100, 150 and 200 ml/s ($FE_{NO50-200}$). For each measurement, prolonged (more than 6 seconds) exhalation was required at each flow rate and we retained values of NO plateau that lasted more than 3 sec for subsequent analysis as recommended by ATS/ ERS guideline[15]. For each V'_E, the elimination rate of NO (V'_{NO}) was calculated ($V'_{NO} = V'_E \bullet FE_{NO}$) (16, 17). FE_{NO} was inversely related to V'_E, whereas V'_{NO} varied directly as a function of V'_E. At the flow rate > 50 ml/s, the latter relationship was linear and could be expressed as $V'_{NO} = V'_E \bullet FE_{NO} = CA_{NO} \bullet V'_E + J'aw_{NO}$ [16, 17]. $J'aw_{NO}$ is the maximal conducting airway flux. For each flow rate (50, 100, 150 and 200 ml/s), two measurements was performed and a minimal of 6 valid measurements were required to derive the R^2 values of the relationship between Fe_{NO} and V'_E.

Statistic analysis

We used descriptive statistics to summarize the data. Groups were compared using Student's t tests, Wilcoxon tests or chi-2 tests as appropriate. Spearman's rank order correlation coefficients were computed to examine relationship between quantitative variables.

We assessed the diagnostic performance of CA_{NO} for ILD diagnosis (positive if lung HRCT ground glass score equal or higher than 1 and negative if score equal to 0) by using receiver operating characteristic curves, formed by plotting sensitivity on the y axis and 1-specificity on the x axis for all possible cut-off values of the test. The overall discriminatory ability of the test was shown by the area under the curves. The best cut-off levels for diagnosis of ILD were further evaluated versus impairment of lung gas exchange defined as DLCO less than 80% of predicted value.

We also calculated likelihood ratios as a measure of the extent to which the pre-test odds are altered by the test results (values >1 increase the odds; values <1 decrease the odds). Using the grey zone approach proposed by Coste et al. (18), we determined the two cut-off values ensuring critical posttest probabilities of 0.95 and 0.05: CA_{NO} up-level and CA_{NO} low-level respectively. If CA_{NO} level from SSc patient was higher than the CA_{NO} upper-level threshold, ILD could be considered definitely positive on lung HRCT and if CA_{NO} level from SSc patient was less than the CA_{NO} lower-level threshold, lung HRCT could be considered definitely normal.

Results

Demographic characteristics, PFTs' parameters, pulmonary HRCT score and C_{ANO} results are given in table 1. Among 65 SSc patients, 38 had ILD defined by presence of ground glass attenuation or septal thickness on pulmonary HRCT. Characteristics of patients with and without ILD are listed in Table 1, showing that TLC, FVC and DLCO were significantly lower in SSc patients with ILD as compared with those with normal pulmonary CT scan. The mean level of CA_{NO} from SSc patients with ILD (10.2 ± 7.6 ppb) was significantly higher than that from SSc patients without ILD (4.6 ± 3.3 ppb; $p<0.0001$). The mean level of CA_{NO} from smokers patients (n=15, 6.8 ± 6.3 ppb) was not significantly different from that from non smokers (n=50, 6.9 ± 6.0 ppb; p=0.94). Similarly, the mean levels of CA_{NO} did not significantly differ between patients treated with low doses of corticosteroids or immunosuppressive therapy (n=13, 8.0 ± 5.8 ppb) and untreated patients (n=52, 6.6 ± 6.0 ppb, p=0.41). The mean level of J'aw NO was significantly lower in SSc patient with ILD (7.0 ± 31.8 nl/min) as compared with SSc patients without ILD (25.0 ± 28.0 nl/min, p=0.007), but fractional exhaled NO concentration at 50 mL/s were not different between the two groups. The cutoff level of CA_{NO} at 4.3 ppb was as-

Table 1. Characteristics of the patients with systemic sclerosis

	SSc (n=65)	SSc patients with ILD (n=38)	SSc patients without ILD (n=27)	P
Age, (yrs)	54.8 ± 11.3	56.3 ± 11.2	52.8 ± 11.5	0.24
Female (%)	58 (89.2)	32 (84.2)	26 (96.3)	0.22
dSSc/lSSc	23/42	21/17	2/25	0.0001
Smokers (%)	15 (23)	9 (23.7)	6 (22.2)	0.89
Duration of disease (yrs)	10.8 ± 10.4	11.5 ± 9.8	9.8 ± 11.3	0.26
TLC (% pred)	93 ± 21	87 ± 22	102 ± 15	0.003
FVC (% pred)	92 ± 23	84 ± 22	102 ± 20	0.001
FEV_1 (% pred)	89 ± 22	82 ± 20	98 ± 20	0.002
DLCO (% pred)	61 ± 18	52 ± 14	71 ± 16	<0.0001
Systolic PAP>40 mmHg	8	7	1	0.12
C_{ANO} (ppb)	7.9 ± 6.75	10.2 ± 7.6	4.6 ± 3.3	<0.0001
$J'_{aw}NO$ (nl/min)	14.5 ± 31.4	7.0 ± 31.8	25.0 ± 28.0	0.007
R^2	0.96 ± 0.03	0.97 ± 0.03	0.96 ± 0.03	0.40
Fe NO, 50 (ppb)	16.1 ± 13.7	17.4 ± 15.7	14.1 ± 10.2	0.17
Corticosteroid treatment*		12	1	0.01

Values are means ± SD except otherwise indicated, SSc: systemic sclerosis, ILD interstitial lung disease, yrs: years, lSSc: limited SSc, dSSc: diffuse SSc, % pred: percentage of predicted value, TLC: total lung capacity, FVC: forced vital capacity, FEV_1: forced expiratory volume in one second, DLCO: diffusing lung carbon monoxide factor, VA: alveolar volume, PAP: pulmonary arterial pressure estimated by echocardiogram, NO: nitric oxide, $J'_{aw}NO$: conducting airway flux, C_{ANO}: concentration alveolar of NO, *among these 13 patients taking low dose of corticosteroids, 3 took immunosuppressive treatment. Fe NO, 50: fractional exhaled NO concentration at 50 mL/s, R^2: relationship between the elimination rate of NO and exhalation flow rate

sociated with highest combination of sensitivity and specificity, resulting in the best accuracy test for diagnosis of ILD (Tab. 2, Fig. 1): sensitivity and specificity were 87% (95% CI: 77 to 99) and 59% (95% CI: 41 to 78) respectively (area under curve (AUC) = 0.79). Moreover, the same cut-off level of CA_{NO} at 4.3 ppb could also assess with high accuracy the early impairment of lung gases exchange in SSc patients, defined as diffusing lung carbon monoxide factor (DLCO) less than 80% of predicted value (AUC=0.80). The sensitivity and specificity were 78% (95% CI: 67 to 90) and 73% (95% CI: 46 to 99), respectively. The sensitivity and specificity were both lesser for lower levels of DLCO cut off, e.g. DLCO < 60% or DLCO < 55% (AUC=0.70).

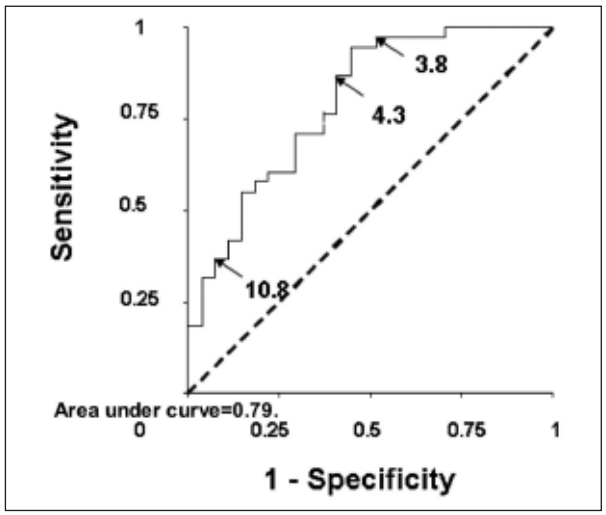

Fig. 1. ROC curve of measurement of CANO in the diagnosis of interstitial Lung disease in systemic sclerosis

The cut-off points that ensure post-test probabilities of ILD over 0.95, CA_{NO} upper-level (i.e. higher than this threshold, the post-test probability of ILD is over 0.95) and under 0.05, CA_{NO} lower-level (i.e. lower than this threshold, the post-test probability of ILD is lower than 0.05) were 10.8 ppb and 3.8 ppb, respectively (Tab. 2).

Six patients with systolic pulmonary pressure higher than 40 mmHg were included in this study, and C_{ANO} levels from all but one patient were higher than 4.3 ppb (mean ± SD: 7.6 ± 3.9 ppb). There was no difference (p=0.82) between CA_{NO} from SSc patients with systolic pulmonary pressure below and above 40 mmHg (Fig. 2).

Discussion

Alveolar inflammation can be assessed by measuring exhaled NO, using the two-compartment

Table 2. Sensitivity, specificity of the measurement of alveolar concentration of NO in the diagnosis of interstitial lung disease in patients with systemic sclerosis

CANO (ppb)	Sensitivity	Specificity	LR+	LR-
<2.0	1	0.22	1.28	0.00
>3.0	0.97	0.44	1.75	0.06
>3.8*	**0.95**	**0.56**	**2.13**	**0.09**
>4.3	*0.87*	*0.59*	*2.13*	*0.22*
>6.0	0.7	0.7	2.40	0.41
>8.0	0.50	0.85	3.37	0.58
>9.0	0.42	0.89	3.78	0.65
>10.8**	**0.32**	**0.96**	**8.52**	**0.71**
>13.5	0.18	1	∞	0.81

C_{ANO}: alveolar concentration of nitric oxide, LR+: positive likelihood ratio, LR-: negative likelihood ratio, * Threshold of C_{ANO} associated with the maximal positive diagnostic likelihood ratio (DLR+≥ 10), **Threshold of C_{ANO} associated with the minimal negative diagnostic likelihood ratio (DLR-≤ 0.1)

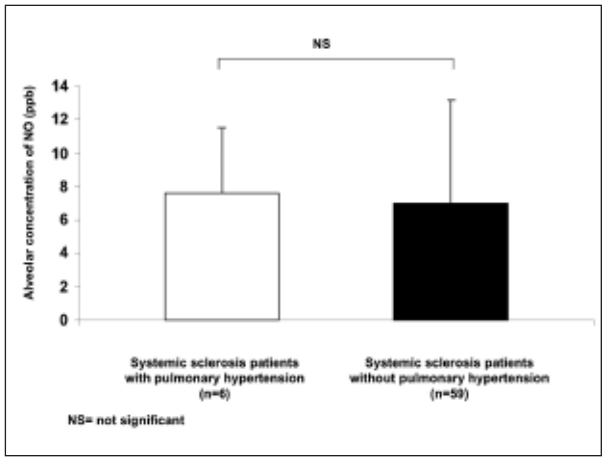

Fig. 2. Alveolar concentration from systemic sclerosis patients with and without pulmonary hypertension

model approach (16). We have recently reported that increased CA_{NO} could be used to non invasively assess lung inflammation, and that the levels of CA_{NO} are related to the severity of lung disease in SSc (9). Results from this prospective study, that included data from 38 new and successive patients added to those from 27 patients recently reported (9), have confirmed our previous observation that CA_{NO} was related to both PFTs' parameters and the extent of ILD. More importantly, the current study has set the best threshold of CA_{NO} at 4.3 ppb to detect ILD in SSc patient with sensitivity and specificity of 88% and 59%, respectively. The threshold at 4.3 ppb could discriminate early impairment of lung gas diffusion exchange and reduced DLCO in SSc patient. The sensitivity was satisfactory however the specificity was rather low indicating that the aim of CA_{NO} was not to replace HRCT in ILD diagnosis but to help to predict the likelihood of abnormal HRCT according to initial exhaled CA_{NO} values. It also determined that SSc patients with maximal positive diagnosis likelihood ratio, and minimal negative diagnosis likelihood ratio were those whose CA_{NO} was higher than 10.8 ppb, and lower than 3.8 ppb, respectively.

In early stage of ILD, alveolar inflammation could be associated with CT scan abnormality without restrictive lung impairment (19). Oxidative stress and inflammation induce the release of pro-inflammatory cytokines and iNOS activation, acute or chronic lung inflammation induces NO production by various pulmonary cells, thereby increasing exhaled NO.

Partitioning NO in the exhaled air into alveolar concentration and conducting airway flux, can differentiate distal lung alveoli from proximal conducting airways sites of inflammation (16, 17). The relationship between both DLCO impairment and the presence of ILD on lung CT scan, and CA_{NO} levels demonstrate that a cut off of CA_{NO} higher than 4.3 ppb was associated with an early and significant decrease of DLCO with acceptable sensitivity and specificity. Moreover, measurement of CA_{NO} levels for diagnosis likelihood ratios, suggested that patients whose CA_{NO} exceeded 10.8 ppb were very likely to have ILD. On the other hand, ILD can almost be excluded in patients whose CA_{NO} remained lower than 3.8 ppb, with a negative predictive value higher than 95%.

Partitioned exhaled NO measurement could be used as a screening tool to define subsets of SSc patients with low, or high, risk to have ILD in order to tailor the numbers and frequencies of follow-up visits according to the likelihood of scleroderma lung disease in individual patients. We have already demonstrated in a previous study (9) that CA_{NO} levels were increased in patients with SSc-associated ILD who were not treated by immunosuppressive or high (more than 10 mg/day) dose of corticosteroids. In this prospective study, we included consecutive SSc patients who were able to perform partitioned exhaled NO measurement. We did not exclude current smokers or SSc patients with corticosteroids or

immunosuppressive treatments in order to reflect real situations in clinical practice. Smoking or corticosteroids therapy could reduce the levels of peak, or fractional concentration of, exhaled NO (20, 21), but it is unclear whether cigarette smoke or corticosteroids also affect the levels of CA_{NO}. The results from this study, however, suggest that neither condition seems to affect NO concentration in the alveoli, at least in a small subgroup of SSc patients. FE_{NO} concentrations are generally highly sensitive to corticosteroids particularly in asthma (21), however, FE_{NO} was still high in some asthmatic patients in spite of corticosteroids inhaled or systemic treatment (22). The lack of difference in CA_{NO} levels between untreated SSc patients and patients treated with low dose of corticosteroids and/or immunosuppressive therapy might be explained by the fact that the latter, who possibly had more severe disease than the formers, might potentially have higher levels of CA_{NO} if they were untreated. This is consistent with the well established inhibitory effects of corticosteroids on NO production.

Measurement of CA_{NO} level provides a new tool to assess ILD but it can neither replace lung CT scan nor PFT's. Our results, however, could have practical implications as SSc patients whose CA_{NO} was less than 3.8 ppb without dyspnoea would unlikely to exhibit PFT's and lung CT scan abnormalities. The levels of J'aw NO were lower in SSc patient with ILD as compared with SSc patients without ILD. The low $J'aw_{NO}$ could reflect reduced NO production in the airways of SSc patients as a result from the inhibitory effects that high alveolar concentration of NO might exert on bronchial epithelial NO synthase activity (23). Alternatively, as CA_{NO} and $J'aw_{NO}$ are derived from the slope and intercept of the linear relationship between V'_E and V'_{NO}, it is conceivable that the higher the slope, the lower the intercept, based on the two compartment-model of pulmonary nitric oxide exchange dynamics (16, 17). The limitation of our results relates to the fact that the prevalence of diffuse form of SSc was higher than expected rate from large cohorts (24, 25), as diffuse form of disease was more associated with ILD, this fact suggested that our studied population could be slightly biased toward a high degree of severity. Still, this should not affect the overall validity of our conclusion related to the contribution of C_{ANO} in reflecting the presence of ILD in SSc patients.

Many factors might account for increased pulmonary vascular tone, including overproduction of vasoconstrictors and growth factors (26), inflammatory cytokines (26), or reduced synthesis of pulmonary vasodilators (27, 28). It is likely that inflammatory mechanisms play a key role in the pathogenesis of pulmonary arterial hypertension (26), and that increased CA_{NO} levels could also be observed in patients with pulmonary arterial hypertension (8, 9), as reflected by similarly high levels of CA_{NO} in SSc patients whose systolic pulmonary pressure was above or below 40 mmHg. This possibility, however, would not affect the value of CA_{NO} as a marker for diagnosis of ILD. This non-invasive test is easily performed on a regularly basis to reach to a decision to additional exams during the follow-up.

In conclusion, this study has allowed us to calculate the threshold values of CA_{NO} levels to predict the likelihood of the presence, or absence, of ILD in SSc patients. We submit that CA_{NO} might be considered as an additional noninvasive screening test of ILD in patients with SSc with acceptable levels of sensitivity and specificity. Our data highlight the potential interest of this method in the clinical management of ILD in patients with SSc.

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