Increased alveolar nitric oxide in early systemic sclerosis

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ABSTRACT

Objective. *Assessment of inflammatory activity in interstitial lung disease of systemic sclerosis (SSc) is difficult. Nitric oxide (NO) has gained attention in the pathogenesis of SSc. The aim of the study was to investigate alveolar NO concentration ($C_{A_{NO}}$) in SSc patients with short disease duration and to relate $C_{A_{NO}}$ to radiologic findings.*

Methods. *In a prospective study, 34 consecutive patients with disease duration of less than 2 years from onset of first non-Raynaud symptom and 26 healthy controls were enrolled. Exhaled NO was measured and $C_{A_{NO}}$ was calculated. $C_{A_{NO}}$ levels were related to the radiologic extent of pulmonary fibrosis measured as the extent of traction bronchiectasis within areas of ground glass opacities and reticulations.*

Results. *$C_{A_{NO}}$ levels were increased in patients with early SSc compared to healthy controls (3.52 (2.94–4.09) versus 2.08 (1.6–2.6); $p<0.001$). Both SSc patients with SSc-ILD (3.56 (3.04–4.73), $p<0.001$) and SSc patients without SSc-ILD (2.98 (2.68–3.98), $p<0.01$) had higher $C_{A_{NO}}$ levels compared with healthy controls (2.08 (1.6–2.6)). $C_{A_{NO}}$ levels did not differ between SSc patients without SSc-ILD and SSc patients with SSC-ILD. $C_{A_{NO}}$ levels did not correlate to the extent of pulmonary fibrosis but were associated with the extent of ground glass opacities ($r_s=0.37$, $p<0.05$) and reticulations ($r_s=0.37$, $p<0.05$) on HRCT. $C_{A_{NO}}$ levels were not correlated to lung function tests.*

Conclusion. *In patients with early SSc, alveolar NO is increased and may precede radiological changes of SSc-ILD. $C_{A_{NO}}$ may therefore be a marker of early lung involvement.*

Introduction

Interstitial lung disease (ILD) associated with systemic sclerosis (SSc) results in considerable morbidity and mortality. Therefore, assessment of the presence of SSc-ILD is crucial for identification of patients in potential need of treatment with immunosuppressive agents. Conventional pulmonary function evaluation together with high-resolution computer tomography (HRCT) analysis of the lungs is currently a standard measure of SSc-ILD (1). SSc-ILD is reflected by the presence of inflammatory cells and mediators in bronchoalveolar lavage fluid (2). Analysis of pulmonary inflammation by non-invasive methods would be preferred in clinical praxis. Nitric oxide (NO) is produced by a wide variety of cells during inflammation (3). Increased levels of NO related end products are present in the bronchoalveolar lavage fluid and increased levels of NO are present in the total exhaled air of SSc patients (4). Since measurement of total exhaled NO derived from the whole bronchial tree do not reflect the events in the peripheral small airways and alveoli, estimation of the alveolar NO concentration ($C_{A_{NO}}$) is preferred (5). Increased levels of $C_{A_{NO}}$ have been shown in patients with extensive SSc-ILD (6-8), but data on the involvement of $C_{A_{NO}}$ as an inflammatory marker in early SSc are lacking. The aim of the study was to investigate $C_{A_{NO}}$ in SSc patients with short disease duration and to relate $C_{A_{NO}}$ to radiologic findings.

Materials and methods

Patients and controls

From June 2006 to June 2009, seventy-three consecutive SSc patients were investigated. Thirty-four patients had disease duration of less than two years from non-Raynaud symptom onset and met our inclusion criteria for early disease. Twenty-nine patients fulfilled the criteria for SSc according to the American College of Rheumatology. Eighteen patients (15 female, 3 men) had limited cutaneous SSc (lcSSc) and 11 (7 female, 4 men) had skin involve-

ment proximal to elbow or knee and diffuse cutaneous SSc (dcSSc). Five female patients with a median age of 68 years and a median disease duration of 0.5 years did not fulfil the ACR criteria for SSc but fulfilled the criteria for limited SSc (lSSc) suggested by LeRoy and Medsger (9). None had received any immunomodulating medication prior to the examination. Seven patients had a daily dose of prednisolone of ≤10mg and one patient had a daily dose of prednisolone of 40mg. Twenty-six healthy non-smoking controls were enrolled. The regional ethics committee in Lund approved the study, and written informed consent was obtained from all individuals.

Clinical assessment

All clinical and laboratory data reported in this study were obtained within one week of investigation of NO measurements. Clinical characterisation was performed as previously described (10). Lung function tests included assessment of vital capacity (VC), forced expiratory volume in 1 second (FEV_1) by dry spirometry and diffusing capacity for carbon monoxide (DLCO) by single-breath test.

Radiologic analysis of HRCT images

The HRCT images were analysed by a chest radiologist (GB) who was blinded concerning the clinical data of the patients. Each patient was analysed for the extent of ground glass opacities (GGO) and reticulations (Ret), estimated in the percentage of the total lung volume with a minimum step size of 5%. Fibrosis was defined by the presence of traction bronchiectasis within areas of GGO and Ret (11) (supplementary Fig. 1). The extent of traction bronchiectasis was described and staged as non detectable, few (≤5%), intermediary (6–10%) and extensive (>10%). The HRCT images were also analysed for presence of honeycombing and emphysema.

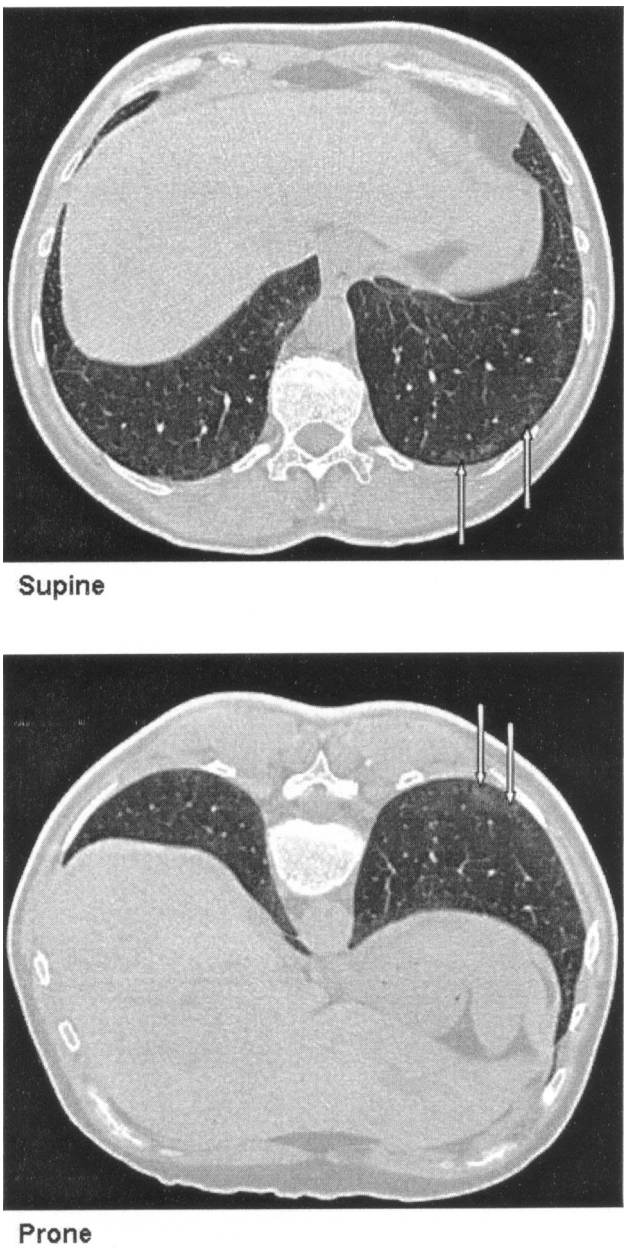
Measurement of fractional exhaled nitric oxide (FENO)

NO measurements were performed using a NIOX (Aerocrine, AB, Stockholm, Sweden) (12). Patients were comfortably seated; inhaled NO depleted ambient air, and exhaled at different flow rates (50 (giving FENO50), 100, 200 and 400ml/s) 3–4 times, depending on divergence. $C_{A_{NO}}$ and bronchial NO flux (*J'awNO*) was approximated by plotting NO-output (production of concentration and flow) against exhaled flow (at 100–400ml/s). The slope and intercept of this line approximate $C_{A_{NO}}$ and *J'awNO*, respectively (5, 12).

Statistical analyses

Data are depicted as median and interquartile range (IQR). The Mann-Whitney test was used for comparison between two groups. Spearman's (r_s) analysis was used to estimate correlations. Probability values (p) were considered significant when <0.05.

Suppl. Fig. 1.
Evaluation of the extent of fibrosis according to the presence of traction broncieactasis. The HRCT images in supine (upper) and prone (lower) position showing subtle ground glass opacities and traction bronchiectasis (signet ring sign), as marked by the arrows.



Results

The early SSc patients included in this study had significantly higher $C_{A_{NO}}$ levels compared to healthy individuals, whereas levels of bronchial NO flux (*J'awNO*) and $FE_{NO}50$ did not differ between the groups (Table I). Levels of $C_{A_{NO}}$ were not correlated to age, disease duration, modified Rodnan skin score or intake of oral cortisone. Levels of $C_{A_{NO}}$ did not differ between smokers

Table I. Demographic data[§].

	SSc (n=34)	Healthy (n=26)
Clinical data		
Female/male, no.	27/7	17/9
Age, years	59 (47–68)	53 (50–56)
Smokers, no.	3	0
lSSc/lcSSc/dcSSc, no.	5/18/11	n.d.
Duration, years	0.96 (0.5–1)	n.d.
Skin Score	6 (2–11)	n.d.
Nitric oxide measures		
CA_{NO}, ppb	3.52 (2.94–4.09) ***	2.08 (1.60–2.60)
J'awNO, nl/s	0.77 (0.62–1.31)	0.88 (0.65–1.38)
FENO50, ppb	17.8 (13.5–29.8)	18.9 (13.0–27.9)
Lung function		
VC, %p	90 (80–95)	n.d.
FEV1, %p	90 (80–99)	n.d.
TLC, %p	85 (80–91)	n.d.
DLCO, %p	72 (63–86)	n.d.
DLCO/VA, %p	62 (54–74)	n.d.
RV, %p	75 (67–83)	n.d.
PAP syst, mmHg	27 (23–31)	n.d.
Radiologic finding		
Ground glass opacities, no. (5–20/25–40/>40%)	19 (16/3/0)	n.d.
Reticulation, no. (5–20/25–40/>40%)	19 (16/3/0)	n.d.
Traction bronchiectasis, no. (≤5/6–10/>10%)	19 (7/9/3)	n.d.
Honey combing, no.	3	n.d.

[§]Data are shown as median (IQR) or as numbers (no.). CA_{NO}: alveolar NO concentration; DLCO: diffusing capacity for carbon monoxide; FENO50: fractional exhaled NO at flow 50 ml/s; J'awNO: bronchial flux of NO; PAP syst: systolic pulmonary arterial pressure; RV: residual volume; TLC: total lung capacity; VA: alveolar volume; VC: vital capacity; n.d.: not determined; ***p<0.001 *vs*. healthy.

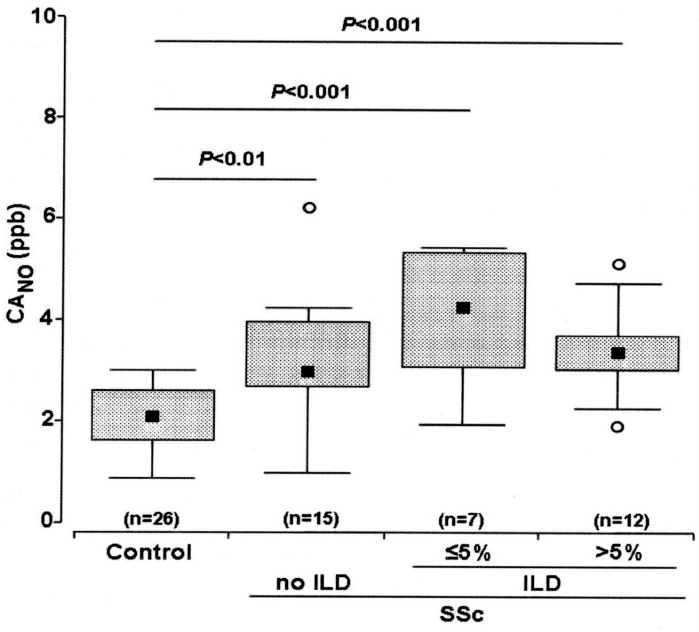


Fig. 1. Increased levels of CA_{NO} in SSc patients with short disease duration.
The SSc patients are grouped as SSc with no ILD or as SSc with ILD, *i.e*. with pulmonary fibrosis of different extent according to the presence of traction bronchiectasis; few (≤5%) and intermediary and extensive (>5%). The graph shows box plots with median, IQR and outliers.

and non-smokers, or whether (n=4) or not patients were taking inhaled corticosteroids or anti-histamines.

Figure 1 shows CA_{NO} measurements in healthy controls (n=26) and SSc patients with short disease duration (n=34) without or with different extent of pulmonary fibrosis. All patient groups had higher CA_{NO} measurements compared to healthy controls. CA_{NO} measurements did not differ between patients with SSc-ILD (3.56 (3.04–4.73), n=19) and SSc patients without (2.98 (2.68–3.98), n=15) (p=n.s.). CA_{NO} measurements did not correlate to the extent of pulmonary fibrosis as measured by traction bronchiectasis (r_s=0.18, p=n.s.).

CA_{NO} levels correlated to the extent of ground glass opacities (r_s=0.37, p<0.05) and reticulations (r_s=0.37, p<0.05) in the SSc patients (Fig. 2). CA_{NO} levels did not correlate to lung function analysis such as DLCO, VC, TLC, RV or FEV_1.

Discussion

This prospective study shows 1) that alveolar NO (CA_{NO}) is increased in SSc patients with short disease duration compared with healthy controls; 2) that CA_{NO} levels are associated with radiological findings of pulmonary inflammation in early SSc, *i.e*. ground glass opacities and reticulation; and (3) that CA_{NO} levels do not reflect radiological visible pulmonary fibrosis in early SSc.

Evaluation of the degree of inflammation in the lungs of patients with SSc is a difficult task in clinical care. Pulmonary involvement in SSc is analysed by means of radiographic measures of fibrotic changes. The radiographic picture is complemented by analysis of lung function to determine VC or DLCO. The most reliable sign of treatment indicating activity in the lung is the decline in VC and progressive fibrotic changes (13). Thus, diagnosis relies on already established damage that is to be prevented in an optimal setting. Measurement of CA_{NO} is believed to give an estimate of the peripheral inflammation, but is not a standard tool for the clinical examination of pulmonary involvement in SSc. In line with the assumption that an early inflammation precedes radio-

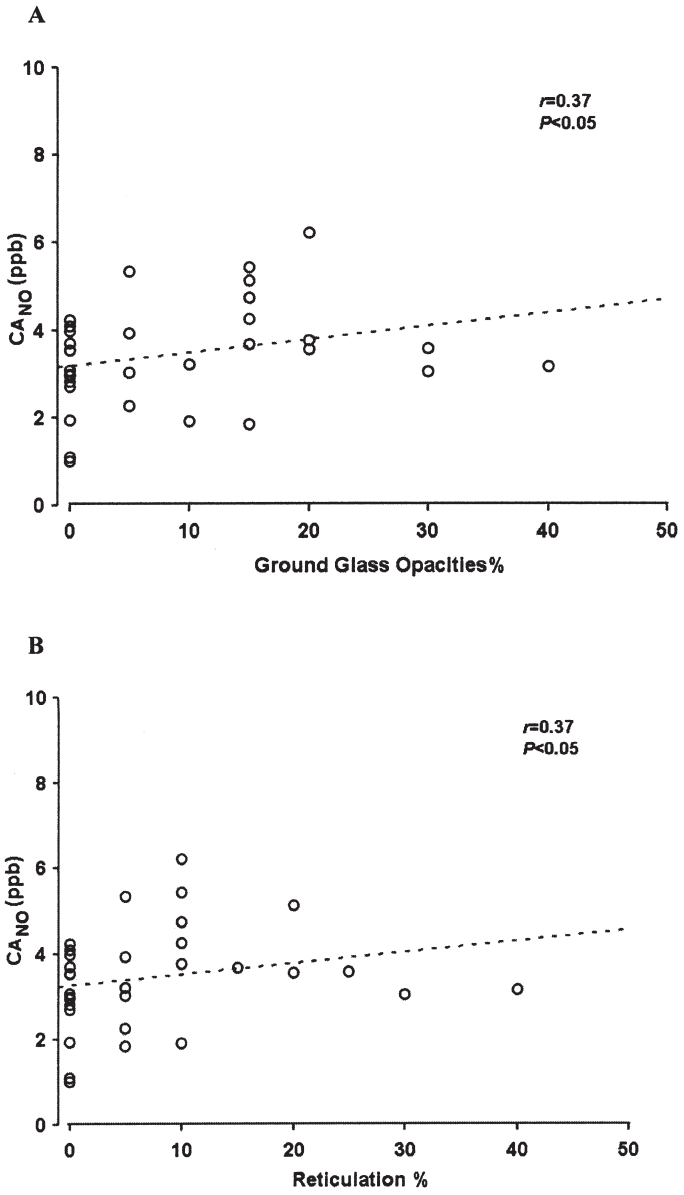


Fig. 2. Correlation of CA_{NO} with the extent of reticulation and ground glass opacities in SSc patients with short disease duration.
The correlation between CA_{NO} and ground glass opacities (**A**) and reticulation (**B**) are depicted. Ground glass opacities and reticulation are shown as percent of lung involvement. Hatched lines show the linear fit.

logical visible fibrosis development in SSc (14), we focused on SSc patients with early disease.

Patients with short disease duration either with or without SSc-ILD had higher CA_{NO} levels than healthy controls. Interestingly, CA_{NO} levels had no correlation with the degree of fibrosis that has previously been described in SSc with longer disease duration (7, 8). However, CA_{NO} levels were associated with the degree of fibrosis in our patient population with a disease duration longer than three years (data not shown), in accordance with the previous publications (7, 8).

CA_{NO} seems to reflect an inflammation in the lung independently of spirometric measurements. In SSc patients with short disease duration, we did not detect any correlation of CA_{NO} with spirometric values. CA_{NO} correlated to the radiological parameter ground glass opacities and reticulation but not to the extent of traction bronchiectasis. However, the highest CA_{NO} values could be seen in patients with 5–20 percent of ground glass opacities and reticulations (Fig. 2). This is in accordance with the finding that mild traction bronchiectasis appears to have the highest CA_{NO} values (Fig. 1). Since SSc patients without SSc-ILD have increased CA_{NO} values, it appears that inflammation measured by CA_{NO} already occurs before the radiographic findings. Thus, production of alveolar NO together with ground glass opacities and reticulation may precede the fibrotic process that is reflected by accumulation of traction bronchiectasis. A rapid fibrotic transformation may have replaced an inflammatory process already where CA_{NO} levels are no longer increased. Alternatively, these SSc patients have a rapidly progressing SSc-ILD that is independent of inflammatory activity (15). These patients might no longer be suitable for immunosuppressive treatment. Longitudinal studies will hopefully show the prognostic value of CA_{NO}measurements.

In conclusion, our study indicates that an increase in CA_{NO} may reflect pulmonary inflammation in patients with SSc with early disease independently of the presence of SSc-ILD. CA_{NO} measurements may therefore be of clinical importance for diagnosis of early peripheral lung involvement when radiologic changes are not yet present, or to select patients eligible for treatment within the population with radiologic changes. CA_{NO} measurements might have the potential to become a tool to monitor immunosuppressive therapy in SSc-ILD.

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