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High alveolar concentration of nitric oxide is associated with alveolitis in scleroderma

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ABSTRACT

Alveolar concentration of nitric oxide (C_ANO) is a non invasive prognostic marker of systemic sclerosis (SSc) lung disease. There is, however, as yet no direct evidence showing concomitant increase of C_ANO and the presence of inflammatory cells in alveoli. We have therefore measured C_ANO and performed broncho-alveolar lavage (BAL) in SSc patients. Exhaled NO was measured, by the means of two different models, the two-compartment model (2CM) and the trumpet model with axial diffusion (TMAD), in 22 SSc patients and compared with 15 healthy controls. BAL was performed in all SSc patients. Alveolitis was defined as lymphocytes >14%, polymorphonuclears >4%, or eosinophils >3% on cell count in BAL fluid. Comparisons of C_ANO levels were made between SSc patients with, and without, alveolitis. Levels of C_ANO were significantly higher in SSc patients as compared with controls ($p < 0.001$). Median C_ANO was significantly higher in SSc patients with alveolitis as compared with SSc patients without alveolitis (8.4 ppb; 1st and 3rd interquartile range: 6.0–10.5 vs 3.3 ppb; 2.2–3.5; $p = 0.004$ for 2CM and 5.4 ppb; 3.2–9.2 vs 3.2 ppb; 1.4–3.3, $p = 0.02$ for TMAD), while bronchial airway output of NO ($J'awNO$, $p = 0.19$), and fractional exhaled NO (F_ENO, $p = 0.12$) were comparable. C_ANO was consistently high in SSc patients with alveolitis irrespective of the methods chosen (TMAD or 2CM). Our findings showed that increased C_ANO was associated with alveolitis in patients with SSc. We submit that C_ANO could be used as a reliable non-invasive surrogate biomarker of alveolitis in scleroderma lung disease.

Introduction

Systemic sclerosis (SSc) is characterized by endothelial and auto-immune dysfunction, and excessive skin and organ fibrosis. Interstitial lung disease (ILD) is a fearful complication of SSc as it markedly worsens the prognosis of the disease. It is thought that repeated episodes of alveolitis pave the way to uncontrolled lung repair processes, and ultimately cause lung fibrosis. Detecting alveolitis in SSc patients is therefore of crucial importance. Currently, broncho-alveolar lavage (BAL) performed by bronchoscopy is the only, and invasive, means to detect alveolitis in SSc patients with ILD [1,2]. Increased rates of different mononuclear cells including polymorphonuclear leukocytes (PMN), eosinophilic (EOS) leucocytes, or lymphocytes observed in BAL fluid reflects accumulation of inflammatory cells in lung interstitial tissue and alveolar spaces [2].

In the inflammatory lung parenchyma, activation of immune system releases several pro-inflammatory cytokines and stimulates gene transcription of the inducible isoform of nitric oxide synthase (iNOS). This in turn causes the release of large amounts of nitric oxide (NO) in lung tissue, with a significant portion that can be detected in the exhaled air [3]. It was reported that elevated level of fractional exhaled NO (F_{ENO}), reflecting the whole NO production from proximal airways and the alveolar space, was associated with alveolitis in interstitial lung disease associated with systemic sclerosis (SSc) [4–6]. Recent technical progress in the measurement of exhaled NO and advanced knowledge in the dynamic of NO exchanges in the lung [7–10] allow us to separate the distal bronchial and alveolar NO output from the tracheal and proximal bronchi NO output.

The two-compartment model (2CM) described by Tsoukias et al. [8] subdivided exhaled NO into alveolar concentration of NO (C_ANO) and conducting airway flux of NO ($J'awNO$). The trumpet model with axial diffusion (TMAD), a more sophisticated model, takes in account the anatomical ramification of bronchial tree described by Weibel [11] and axial diffusion of NO, and avoids overestimation of C_ANO by the 2CM [9,10]. Recently, we [12] and other [13] have shown that alveolar concentration of nitric oxide (C_ANO) is a valuable non invasive means to assess the severity and

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prognosis of ILD in patients with SSc [14]. However, the clinical importance of this new tool needs to be clarified. Indeed, evidence supporting a direct relationship between C_ANO level and the underlying alveolar inflammation is not yet available. We hypothesized that C_ANO may reflect alveolitis in scleroderma lung disease. We have therefore investigated whether high C_ANO, measured according to 2CM and TMAD, truly reflects alveolitis documented by BAL.

Methods

Patients

Study design

This present study was conducted from May 2008 and 2010, in the department of Internal Medicine, Hospital Saint Antoine, Paris. Patients who met the below criteria were eligible for the study.

Inclusion criteria. Patients were considered for inclusion if they were older than 18 years and had a diagnosis of SSc and its subsets according to the American College of Rheumatology criteria [15], and Leroy's criteria [16], respectively. Patients with SSc were eligible, irrespective of the level of forced vital capacity (FVC), and diffusing lung capacity for carbon monoxide (DLCO). Patients with ILD diagnosed by chest high-resolution computed tomography (HRCT) or treated with immunosuppressive agents were also eligible. Importantly, Patients with SSc were eligible if BAL was indicated for the following reasons: expansion of ILD on HRCT, and suspicion of lung infection or lung cancer.

Exclusion criteria. Exclusion criteria were the presence of recent airway upper tract infection, or pneumonia confirmed by the presence of bacterial, viral or fungal agents associated with alveolar inflammation documented by BAL fluid analysis. Patients with other systemic infection in the last three months were also excluded. Patients with IPF or ILD associated with connective tissue disease other than SSc were excluded.

Data collection

The study was approved by the local ethics committee (Comité de la Protection des Personnes d'Ile de France V, n°08757). After written informed consent, all patients underwent 6-min walk test lung high-resolution computed tomography (HRCT), echocardiogram, pulmonary function tests (PFTs) [17], partitioned exhaled NO measurements and bronchoscopy with BAL.

Interstitial lung disease. Interstitial lung disease was considered present if pulmonary HRCT demonstrated compatible changes in reticular or air space opacities according to the ATS/ERS Consensus [18].

Extensive lung disease. We defined the "extensive lung disease" according to the prognostic composite index described by Goh et al. [19] using morphological extent of ILD on HRCT and the reduced lung volumes resulting from scleroderma lung fibrosis.

Pulmonary hypertension. To estimate systolic pulmonary artery pressure (sPAP), the maximal transtricuspid pressure gradient was calculated using simplified Bernouilli equation [18]. 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. If patients with SSc had sPAP estimated by echocardiogram more than 40 mmHg, a right heart catheterization was performed to determine hemodynamically the mean pulmonary artery pressure that defines the pulmonary hypertension according to the expert consensus 2009 [21].

Partitioned exhaled NO measurements. Partitioned exhaled NO measurements was performed using two models, previously described as two-compartment model (2CM) and trumpet model with axial diffusion (TMAD), within the week before BAL.

Partitioned exhaled NO measurement by two-compartment model. Fractioned exhaled NO (F_{ENO}) was measured using a chemiluminescent analyzer (EndoNO 8000®, SERES, Aix-en-Provence, France), according to the American Thoracic Society/European Respiratory Society recommendations [22]. After full inspiration from room air with ambient NO levels less than 20 part per billion (ppb), the subject exhaled against a positive pressure that was constantly kept between 5 cm H_2O (lower limit) and 20 cm H_2O (upper limit) to generate exhalation flow rates (V'_E) of 100, 150 and 200 ml/s. For each V'_E, the elimination rate of NO (V'_{NO}) was calculated ($V'_{NO} = -V'_E \cdot F_{ENO}$). F_{ENO} is inversely related to V'_E, whereas V'_{NO} varies directly as a function of V'_E. At the flow rate >50 ml/s, the latter relationship is linear and can be expressed as $V'_{NO} = V'_E \cdot F_{ENO} = -CA_{NO} \cdot V'_E + J'awNO$ [8]. For each patient, the R^2 values of the relationship between F_{ENO} and V'_E were calculated.

Partitioned exhaled NO measurement by trumpet model with axial diffusion. For each patients and healthy controls, we estimated the $J'awNO$ and the CA_{NO} according to the model described by Condorrelli et al. [10] that takes account the trumpet shape of airway tree and the axial diffusion of NO and simplifies the mathematical equations of the dynamic exchange of NO in the respiratory system, we used the linear relationship for the elimination rate of NO ($V'NO$, pl/s) as function exhaled flow (V') in a range to 100 ml/s < V' < 250 ml/s:

$$V'NO = (CANO_{(\text{trumpet model})} + J'awNO_{(\text{trumpet model})} \times 0.00078)V + J'awNO/1.7$$

The slope of this linear relationship S and the intercept I was obtained by plotting the $V'NO$ vs V' in previous defined range. Otherwise S and I are the $CANO_{(\text{two-compartment model})}$ and $J'awNO_{(\text{two-compartment model})}$, respectively.where

$$J'awNO_{(\text{trumpet model})} = 1.7 \times I$$

and

$$CANO_{(\text{trumpet model})} = S - I(0.00078(s/ml)/0.57) = S - I/740(ml/s)$$

Broncho-alveolar lavage. Broncho-alveolar lavage was done as described elsewhere [2,23]. After saline instillation, BAL fluids were obtained from both lungs by fiber optic bronchoscope that was performed after the partitioned exhaled NO measurement within the week. BAL differential cells count was done on 200 cells in slide stained by with a modified Giemsa stain. Bacterial and fungal cultures were performed in a sample of BAL fluid and were sterile.

Alveolar inflammation. Alveolar inflammation in interstitial lung disease associated with SSc was defined according to the criteria of alveolitis on BAL cells counts previously reported [24,25]. BAL fluid was considered to be active alveolitis when any one of the following criteria was met: rate of lymphocytes >14%, neutrophils >4%, or eosinophil leukocytes >3% on cell count [24,25].

All healthy volunteers had normal chest X-ray, normal left ventricular output and systolic pulmonary artery pressure less than 30 mmHg [20]. They had no history of asthma and the IRB approval was obtained from the local ethic committee (CPP d'Ile de France V, n°08757)". All parameters of patients were compared with those from 15 healthy volunteers (46.0 years; 41.5–51.0) who had C_ANO measurements. Bronchoscopy was not performed on healthy volunteer for ethical reasons.