Can exhaled nitric oxide differentiate causes of pulmonary fibrosis?

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Summary
Background: Interstitial lung diseases (ILD) comprise a heterogeneous group of disorders, and when diagnosed at the stage of pulmonary fibrosis, the underlying lung disease can sometimes be difficult to identify. The aim of the present study was to determine whether there are differences in FENO (fraction of exhaled nitric oxide) between different subtypes of fibrotic ILD.

Methods: Sixty-one patients, with honeycombing on computed tomography (CT) scan, and whose FENO levels had been measured during chronic dyspnoea evaluation, were divided into four groups based on pulmonary fibrosis aetiology: idiopathic pulmonary fibrosis (IPF), chronic hypersensitivity pneumonitis (HP), connective tissue disease-associated ILD disorders (CTD-ILD), drug-induced pneumonia. The FENO values of each group were compared and CT scan features were analysed to identify the mechanisms involved in FENO change.

Results: The median FENO value of patients with chronic HP was 51 ppb (IQR 36–74), higher than that of the other groups (22 ppb (IQR 17–30) in IPF, 19 ppb (IQR 17–21) in drug-induced pneumonia, and 25 ppb (IQR 17–37) for CTD-ILD; P = 0.008). At the cut-off value of 41 ppb, the optimal sensitivity and specificity to diagnose HP with FENO were respectively 0.86 and 0.74.

Abbreviations: CANO, alveolar concentration of exhaled NO; DIP, drug induced pneumonia; FENO, fractional exhaled nitric oxide; FEF 25–75, 25–75% Forced Expiratory Flow; FEV1, Forced Expiratory Volume in 1 s; GGO, ground-glass opacities; HP, hypersensitivity pneumonitis; IQR, interquartile range; ILD, interstitial lung disease; CTD-ILD, connective tissue disease-associated ILD disorders; IPF, idiopathic pulmonary fibrosis; J’awNO, conducting airway flux; SSC, systemic sclerosis; TLC, Total Lung Capacity; VC, vital capacity.

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Introduction

Interstitial lung diseases (ILD) comprise a group of heterogeneous disorders which can lead to pulmonary fibrosis with a poor prognosis [1,2]. ILD can be idiopathic, or the consequence of an underlying disease which is sometimes difficult to identify when the diagnosis is made at the stage of fibrosis, characterized by honeycombing on computed tomography (CT) scan. However, it is important to identify the underlying lung disease, as it may influence survival or treatment. For example, the survival rate for systemic sclerosis with ILD is better than for other connective tissue diseases with ILD, and the prognosis for non-specific interstitial pneumonia (NSIP) is much better than for idiopathic fibrosis (IPF) [3,4]. In terms of treatment, oral corticosteroid may be used in hypersensitivity pneumonitis (HP), a cause of ILD, but it has little or no effect on IPF [1,5,6]. Furthermore, the diagnosis of HP in the context of ILD is crucial, so that any contact with the antigen responsible for the disease can be avoided [6].

Patient care could therefore be improved by identifying a marker to differentiate causes of honeycombing. In HP, IgG precipitin to the antigen may be missed in the early stages of the disease. Thoracic CT may help to differentiate between causes of honeycombing [7]. Lobular areas of decreased attenuation or centrilobular nodules are mostly observed in HP. Conversely, basal predominance of honeycombing is mostly seen in IPF [7]. However, CT scan features are not specific and surgical lung biopsy is sometimes required to establish the diagnosis [7,8].

Nitric oxide (NO) is produced by airway epithelial cells in the respiratory tract, and by airway and circulatory endothelial cells in both large and peripheral airways. Fractional exhaled nitric oxide (FE\textsubscript{NO}) is commonly used as a marker of airway inflammation in asthma to determine the optimal dose of inhaled corticosteroid [9,10]. There have been few studies of FE\textsubscript{NO} in ILD, and they have mostly concerned systemic sclerosis and had conflicting results [11,12]. Furthermore, to the best of our knowledge, no study has focused on FE\textsubscript{NO} as a marker of pulmonary fibrosis aetiology.

The aim of the present study was to determine whether there are differences in FE\textsubscript{NO} between different subtypes of fibrotic ILD. We carried out a retrospective analysis of FE\textsubscript{NO} data of patients presenting with dyspnoea and honeycombing on CT. Patients were divided into four groups: chronic HP, IPF, connective tissue disease-associated ILD disorders (CTD-ILD), and drug-induced pneumonia. FE\textsubscript{NO} values were compared between groups, and CT scan features were analysed to investigate the relationship between anatomic lesion and increase in FE\textsubscript{NO}.

Sample and methods

Study sample

The files of patients with pulmonary fibrosis attending the respiratory medicine department of Tours University Hospital between June 2009 and June 2012 were reviewed. Inclusion criteria were honeycombing on CT scan and measurement of fractional exhaled nitric oxide (FE\textsubscript{NO}) as part of chronic dyspnoea management (n = 74). Time to onset of dyspnoea must be superior to 3 months. Patients with a recent increase of dyspnoea were excluded. Honeycombing was defined as subpleural clustered cystic airspaces with thick fibrous walls, as described in the glossary of the Fleischner Society [13]. Patients were excluded if they had symptoms of asthma as defined by GINA, or post-bronchodilator improvement of FEV\textsubscript{1} on pulmonary function tests [14]. Patients were also excluded if they had no clear aetiology of pulmonary fibrosis and if surgical biopsy was not available (n = 13).

Causes of pulmonary fibrosis were divided into four groups: chronic hypersensitivity pneumonitis (HP), idiopathic pulmonary fibrosis (IPF), connective tissue disease with lung involvement (CTD-ILD), and drug-induced pneumonia (DIP). Diagnosis of IPF was based on ATS/ERS criteria [1]. Diagnosis of chronic HP was based on the criteria established by Lacasse et al. or histological features [15–17]. CTD-ILD was identified on the basis of criteria described in the literature [18–20]. The pharmacological criteria proposed by Edwards et al. were used to diagnose drug-induced pneumonia [21]. Precipitins were negative for all patients in the IPF, CTD-ILD and DIP groups and none of them had allergen exposure. Diagnosis was made by two lung specialists who established a consensus. They were blinded to FE\textsubscript{NO} values and CT quantification of lesions performed by radiologists. Connective tissue diseases were confirmed by a rheumatologist or an internal medicine physician.

Data concerning age, sex, smoking habits, pulmonary function tests, lactate dehydrogenase serum level (LDH), blood eosinophilia, oxygen treatment and immunosuppressive treatment (including corticosteroid treatment) were collected to rule out potential confounding factors of FE\textsubscript{NO} values. The study was approved by the Institutional Review Board of the French society for respiratory medicine — Société de Pneumologie de Langue Française — (CEPRO# 2011-028). All patients gave their informed consent for inclusion in this study.

Pulmonary function tests

Lung function tests were performed using Sensormedics Vmax Encore plethysmography (Carefusion\textsuperscript{®}; San Diego,
CA). Forced Vital Capacity (FVC), Total Lung Capacity (TLC), Forced Expiratory Volume in one second (FEV1), and 25–75% Forced Expiratory Flow (FEF 25–75) values were expressed as percentages of predicted values according to gender, weight and age. Measurements were performed following ATS/ERS recommendations [22]. The predicted values were those of the ERS [23]. FENO values were obtained using an electrochemical device (Hypair FeNO, Medisoft®; Sorinnes, Belgium) following ATS/ERS recommendations [24]. Repeated and reproducible exhalations were performed until at least two NO plateau values differing by less than 10% were obtained. Flow rate exhalation was 50 ml/s. The FENO value retained was the mean of two reproducible measurements. In patients using oxygen, measurements were performed 30 min after stopping oxygen. FENO was expressed in parts per billion (ppb). FENO values were obtained 12 h at least after tobacco consumption.

Thoracic computed tomography (CT)

Thoracic CT scans were performed on all patients using a 64-slice Philips CT Scanner (Philips® DA Best, Netherlands). CT scans were evaluated by two thoracic radiologists blinded to the patients’ clinical and functional details, in accordance with previously published criteria [7]. The two observers easily established a consensus about the quantification of lesions. In brief, areas of ground-glass opacities (GGO) were classified as involving less than 25%, 25–50%, or more than 50% of the lung parenchyma. The extent of lobular areas with decreased attenuation was evaluated by counting the number of secondary lobules with decreased attenuation on all inspiratory images, and classified in two categories: class 1 (up to four lobules and involving less than four lobes), and class 2 (five or more lobules in more than four lobes, the lingula being considered as a separate lobe). Lobular areas with decreased attenuation were focal zones with parenchymal decreased attenuation associated with decreased vascularity [13]. Areas of consolidation or centrilobular nodules were also identified.

Statistical analysis

Continuous data were described with medians and interquartile ranges (IQR). Categorical data were expressed as percentages. Continuous data for each pulmonary fibrosis group were compared using the Kruskall–Wallis test. Fisher exact tests were used to compare category data for each pulmonary fibrosis group. The Mann–Whitney test was used to compare FENO values with CT scan parameters. Statistical significance was defined as a p-value <0.05. Receiver operator characteristics (ROC) curves and area under curve (AUC) were used to assess FENO as a potential marker of chronic HP. Sensitivity and specificity of FENO for chronic HP diagnosis were also defined.

Results

Pulmonary fibrosis causes

Data from 61 patients with pulmonary fibrosis were analysed. Causes of pulmonary fibrosis were divided into four groups. Eighteen patients (29.%) had IPF, 17 with UIP pattern on CT scan and 1 with an inconsistent pattern on CT scan but with a histological UIP pattern on surgical lung biopsy. Thirteen patients (21%) had chronic HP, including six with farmer’s lung, and four with bird-breeder’s lung. For the other three patients in this group, there was no clear evidence of exposure, and diagnosis was based on histological features. CTD-ILD was identified in 22 patients (36%) including four with rheumatoid arthritis, three with Sjögren syndrome, three with dermatomyositis, two with systemic sclerosis and one with mixed connective tissue disease. This group included nine patients with signs of undifferentiated connective tissue disease. Among the eight patients (13%) with drug-induced pneumonia, amiodarone was suggested to be involved in pulmonary fibrosis in four cases, and ergot alkaloid, simvastatin, bleomycin and indapamide in one case each.

Clinical and functional characteristics

The characteristics of patients with pulmonary fibrosis are summarized in Table 1. Clinical, biological, and functional parameters were similar in all groups. There was no statistical difference for age, sex ratio, blood eosinophilia, or LDH (Table 1). Patients with drug-induced pneumonia had a median age of 80 years (IQR 71–84) which was older than the other patients but the difference was not statistically significant (p = 0.79). In smokers, cigarette consumption was lower in chronic HP than in the other groups, but the difference was not statistically significant (p = 0.03). Time of smoking cessation was significantly longer in chronic HP patients than in others (p = 0.14). Median FVC and TLC values were lower in patients with chronic HP (68% (IQR 54–90) and 62% (IQR 53–83) of predicted values respectively) than other groups, but the difference was not statistically significant (p = 0.26 and p = 0.21 respectively). Immunosuppressive treatments are detailed in Table 1. In the chronic HP group, one patient received rapamycin for chronic kidney disease. The number of patients receiving immunosuppressive treatment was lower in the IPF group (22%), but the difference was not statistically significant (p = 0.11). The number of patients treated with steroids and the steroid dosage were similar between groups (p = 0.28 and p = 0.74, respectively). BAL cell count was higher in chronic HP patients, but there was only a tendency (p = 0.09).

FENO values in pulmonary fibrosis

FENO values were higher in patients with chronic HP than in those with other causes (p = 0.008) (Fig. 1). Patients with chronic HP had a median FENO value of 51 ppb (IQR 36–74) compared with 22 ppb (IQR 17–30) in IPF, 19 ppb (IQR 17–21) in drug-induced pneumonia, and 25 ppb (IQR 17–37) for CTD-ILD. ROC curve analysis showed that FENO values are a good marker for predicting hypersensitivity pneumonitis (Fig. 2). At the cut-off value of 41 ppb, the optimal sensitivity and specificity were respectively 76.9% and 85.4%.
There was no correlation between PFT findings and FENO in chronic HP patients (data not shown). FENO values were similar in patients with or without immunosuppressive treatment (27 ppb (IQR 17–39) versus 23 ppb (IQR 17.5–43.5) respectively, \( p = 0.97 \)), and also in patients with or without steroids (28 ppb (IQR 17–43) versus 25 ppb (IQR 18.5–43.5) respectively \( p = 0.93 \)). Steroid dosages were not correlated with FENO values.

\( \text{FENO values and CT scan parameters} \)

CT scan parameters of bronchiolar or alveolar features were evaluated to investigate the increase in FENO values in chronic HP (Supplementary Data). Values were significantly higher in patients with extensive lobular areas with decreased attenuation (class 2) than in those in class 1 (Fig. 3A). The median FENO value in patients in class 2 was 47 ppb (IQR 37–62), compared to 22 ppb (IQR 17–32) in patients in class 1 \( (p = 0.0002) \). The extent of ground-glass opacities was not statistically correlated with FENO values, as shown in Fig. 3B. Median FENO values were 25 ppb (IQR 17–44), 34 ppb (IQR 19–47), and 19 ppb (16–24) in patients with less than 25%, 25–50%, and more than 50% of GGO respectively \( (p = 0.19) \). FENO values were not correlated with bronchiolar nodules or consolidation \( (p = 0.24 \text{ and } p = 0.73 \text{ respectively}) \) (Fig. 3C, D). Median FENO values were 30 ppb (IQR 19–49) and 23 ppb (IQR 17–34) in patients with and without centrilobular nodules respectively, and 26 ppb (IQR 13–56) and 25 ppb (IQR 19–43) in patients with and without consolidation respectively. Other features were analysed on CT scan, such as fibrosis localization, fibrosis severity, subpleural sparing, but none of them were correlated with FENO values (data not shown).

\( \text{Discussion} \)

In our study, patients with chronic HP had significantly higher FENO values than those in the groups of other aetiologies of pulmonary fibrosis \( (p = 0.008) \). This suggests that FENO could help identify chronic HP as the origin of pulmonary fibrosis in patients with honeycombing on CT scan.
FENO values were higher in patients with extensive lobular areas with decreased attenuation, indicating that FENO may be a marker of bronchiolar damage in HP.

Clinical and functional parameters were similar between all groups of pulmonary fibrosis except for smoking status. We found that the percentage of smokers was lower in patients with chronic HP than in the other groups, but the difference was not statistically significant. Moreover, time of smoking cessation was significantly longer in chronic HP patients than in others ($p = 0.03$). It has been well documented that active smoking decreases FENO [25–27]. However, there were only two active smokers in our cohort and excluding these two patients from the statistical analysis did not alter our results. Several studies have assessed FENO values in ex-smokers with conflicting results. One study showed that FENO decreases in ex-smokers compared to healthy subjects (17.7 versus 22.8 ppb, respectively) [25]. Two studies showed opposite results. In these studies, ex-smokers and healthy subjects had similar FENO values (17.1 versus 16.9 ppb respectively in one study, and 18.4 versus 17.5 ppb respectively in the other) [26,27]. If ex-smoking status modifies FENO, the proportion of decrease is very low compared to the difference observed between pulmonary fibrosis groups in our study. Smoking status does not explain the considerable increase in FENO in chronic HP patients in our study.

Exhaled nitric oxide has only been evaluated in patients with HP in one previous study [28]. The authors evaluated alveolar concentration of NO and bronchial NO flux but not FENO. They analysed exhaled alveolar and bronchiolar NO in 40 patients with asthma, 17 with “alveolitis”, and 57 healthy control subjects. Patients with “alveolitis” included seven farmers with HP and 10 patients with IPF who were compared with asthmatic patients and controls. Bronchial NO flux was higher in asthma patients than in the “alveolitis” group (including HP and IPF) and healthy controls. Alveolar NO concentration was higher in “alveolitis” patients than in the asthma and control groups. In that study, HP and IPF were assessed together and no conclusion can be drawn regarding exhaled NO and HP or IPF independently. However, the two diseases have different mechanisms and treatment. For this reason, we analysed FENO dividing causes of honeycombing into four groups of pulmonary fibrosis: chronic HP, IPF, drug-induced pneumonia, and CTD-ILD. In our study, we chose to assess FENO and not alveolar concentration of NO or bronchial NO flux, because only FENO is available in most lung function test labs.

In ILD, exhaled nitric oxide has mainly been assessed in systemic sclerosis (SSC) with lung involvement. However, evaluation was usually based on alveolar concentration of exhaled NO (CANO) and conducting airway flux ($J'awNO$) rather than on FENO. Only one study evaluated FENO in SSC, and no increase in concentration was found [29]. With regard to CANO and $J'awNO$, findings are conflicting, possibly because there is no standardized method of measuring exhaled NO and no precise definition of ILD features on CT scan; one study reported no difference between SSC with and without ILD [26], four studies reported higher CANO values in SSC with than without ILD [11,30–32], and two studies reported lower CANO values in SSC without than those with ILD [12,33]. In another study, exhaled NO levels were found to be higher in IPF than in SSC patients, which were in turn higher than in controls [34]. In that study, levels were evaluated from the exhaled NO peak. Exhaled NO has also been assessed in asbestosis; FENO values were higher in asbestosis patients than in healthy subjects [35].

CT scan is considered as a useful tool to classify ILD in terms of histopathological alteration and even aetiology. However, at the stage of fibrosis characterized by honeycombing, it is more difficult to identify specific features.
FENO appears to be a promising marker to identify chronic HP in patients with pulmonary fibrosis. At the cut-off of 41 ppb, FENO can be used to diagnose chronic HP with a sensitivity and specificity of 76.9% and 85.4%, respectively. In a study conducted by Silva et al., the sensitivity and specificity of CT scan features enabling the diagnosis of chronic HP were 78% and 88%, respectively, which is similar to data obtained with FENO in our study[7]. However, the data of the CT scan study were obtained with experienced radiologists. FENO can be used with a calibrating device as a marker by all physicians.

The mechanism of FENO increase in HP patients is unknown. The main hypothesis, based on what happens in asthma, is bronchiolar disease[9]. Histologically, HP is characterized by a triad comprising chronic interstitial pneumonia with peribronchiolar accentuation, bronchiolitis, and non-caseating granulomas [16,17]. The inflammatory process involved in HP could be responsible for the increase in the inducible nitric oxide synthase produced by alveolar macrophages [9]. This enzyme produces nitric oxide which has direct toxic effects on cells and can be found in the patient’s exhaled breath. To understand the mechanisms involved in FENO increase, we examined our patients’ CT scans. We evaluated lobular areas of decreased attenuation, which is reported to be significantly more frequent in HP patients than in patients with IPF and NSIP [7]. Patients with lobular areas of decreased attenuation in class 2 (five or more lobules in more than four lobes) had statistically higher FENO values than patients in class 1 (p = 0.0002). The lobular areas of decreased attenuation are presumed to be secondary to small airway obstruction due to cellular bronchiolitis [7]. This data is therefore consistent with the hypothesis that FENO increase results from bronchiolar disease. The number of patients with expiratory CT scan was too small to assess air trapping, which may be a marker of bronchiolar disease. Bronchiolar disease is also observed in connective tissue lung disease, and some patients in this group did indeed have increased FENO concentrations. However, the difference between the FENO values in connective tissue disease and HP was statistically significant.

In conclusion, FENO concentration is higher in chronic HP than in other causes of pulmonary fibrosis, which could help diagnose chronic HP in ILD with honeycombing on CT scan. The mechanisms involved in FENO increase is unknown, but may involve bronchiolar disease, as suggested by extensive lobular areas of decreased attenuation in patients with high FENO values. Further studies are needed to confirm these results and understand the mechanism of increased FENO in chronic HP. Other markers should be assessed, such as sputum eosinophilia or air trapping on CT scan. Steroid treatment should also be assessed to determine whether FENO could be a predictive marker of steroid sensitivity, as demonstrated in asthma.

**Author contribution**

L. Guilleminault MD: data collection, writing the manuscript.  
A. Saint-Hilaire MD: CT scan interpretation.  
O. Favelle MD: CT scan interpretation.  
A. Caille MD: Statistical analysis.  
E. Boissinot MD: lung function test interpretation.  
AC Henriet MD: data collection.  
P. Diot MD, PhD: proofreading.  
S. Marchand-Adam MD, PhD: proofreading.

**Figure 3** Comparisons of FENO values with CT scan parameters. A) Lobular areas with decreased attenuation in class 2 were associated with high FENO values, while class 1 was associated with low FENO values. B) Extent of ground-glass opacities, C) consolidation, and D) centrilobular nodules were not correlated with high FENO values. (*: p = 0.0002).
Conflict of interest

No conflict of interest exists for the author or co-authors.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.rmed.2013.07.007.

References


