Association between fractional exhaled nitric oxide and clinical characteristics and outcomes in patients with subacute cough

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Abstract

Objectives: To investigate the relationships between fractional exhaled nitric oxide (FENO) and clinical characteristics and outcomes in patients with subacute cough.

Methods: Patients with subacute cough (n = 189) after upper respiratory tract infection were enrolled in this single-center prospective study, and were divided into low-FENO (<25 ppb) and high-FENO groups (≥25 ppb). Empirical therapies (without inhaled or systemic corticosteroids) were prescribed based on clinical experience and follow-up until the disease course reached 8 weeks. FENO values, cough symptom scores (CSS), and Leicester Cough Questionnaire (LCQ) scores were obtained, analyzed, and compared between two groups of patients.

Results: The low-FENO and high-FENO groups comprised 136 and 53 patients, respectively. The multiple regression analysis showed that blood eosinophil count and gender were independent factors for elevated FENO (β = 1.38, 0.25, respectively). LCQ scores, total CSS, and daytime CSS were comparable between the low-FENO and high-FENO groups. The nighttime CSS of the high-FENO group were significantly higher than that of the low-FENO group (P = .03). The CSS and LCQ score were improved in both groups but were comparable between groups after 10 days treatment.

Conclusions: Patients with subacute cough and high-FENO levels have more severe nocturnal cough than those of patients with low-FENO levels. However, FENO levels do not appear to correlate with the clinical outcomes or treatment response. The significance of FENO in the management of subacute cough needs to be further evaluated, at least in the current empirical treatment without corticosteroids.

KEYWORDS
cough, nitric oxide, eosinophil, quality of life

1 | INTRODUCTION

Cough is a common cause for consultation with pulmonary specialists in outpatient clinics. Based on duration, cough can be classified as acute (<3 weeks), subacute (3-8 weeks), or chronic (>8 weeks). For patients with cough lasting more than 3 weeks, 34.8% (184/529) were judged to have subacute cough in a prospective study. Subacute cough may be prolonged manifestations of acute cough, or an early feature of chronic cough. A
protracted subacute cough is a psychological burden and negatively affects the daily life and work of patients. For patients with subacute cough, the guidelines of the American College of Chest Physicians (ACCP) recommend that it should first be determined whether the cough is secondary to previous respiratory infection. Then, administer empirical therapy to improve symptoms, shorten the disease course, and promote quality of life.

It has been reported that fractional exhaled nitric oxide (FENO), a noninvasive marker of airway inflammation, is possibly useful for the diagnosis and management of asthma, predicting responses to corticosteroid therapy, and etiological and differential diagnosis of chronic cough. In addition, FENO may also be useful in the management of patients with subacute cough.

We hypothesized that those of patients with higher FENO levels have identifiable clinical features and benefit from specific treatment. In this prospective study, we investigated factors that may influence FENO levels in patients with subacute cough. We also compared clinical characteristics and outcomes of patients between those with the low-FENO and those with high-FENO levels.

2 | MATERIALS AND METHODS

2.1 | Subjects

The study was approved by the Ethics Committee of Beijing Chao-Yang Hospital, Capital Medical University (2014-129). All subjects provided written informed consent.

All patients with subacute cough attending outpatient clinics at Beijing Chao-Yang Hospital, Capital Medical University between August 2014 and March 2016 were enrolled in this prospective observational study. The inclusion criteria were: cough lasting 3-8 weeks as the unique or main symptom; normal chest X-ray; and aged 18-60 years (either gender).

Potential subjects were excluded if they were with the following conditions or diseases: chronic diseases of the respiratory system or any serious disease; allergic rhinitis, chronic sinusitis, or allergic urticaria; pregnant or lactating; participation in another clinical trial; or receiving corticosteroid treatment, or treatment with an angiotensin converting enzyme inhibitor (ACEI) within the previous 2 months.

2.2 | Study design

FENO (reported as parts per billion, ppb) was measured using a nitric oxide analyzer (NIOXMINO, Aerocrine, Sweden), in accordance with the standard procedure recommended by the American Thoracic Society/European Respiratory Society. During the test, subjects breathed quietly for at least 3 cycles, and exhaled fully at the last expiration. With the mouthpiece fitted to avoid leakage, the subjects inhaled nitric oxide-free air maximally via their mouths to total lung capacity and subsequently exhaled at a constant flow rate (50 mL/s) for 10 seconds.

Cough symptom scores (CSS) were used to evaluate daytime and nighttime cough symptoms; cough was rated from 0 to 3, depending on the severity of cough. The CSS reflect the patient’s cough frequency, intensity, and quality of life as affected by cough.

The Leicester Cough Questionnaire (LCQ) is a 19-item questionnaire with physical, psychological, and social domains. Each item is divided into 7 levels, ranging from “all of the time” to “none of the time,” and scored from 1 to 7 according to severity, with lower scores indicating greater severity. The total score is the sum of each item score in each domain. A higher score indicates better cough-related quality of life.

The following events were recorded for each patient at enrollment: demographic characteristics, history of upper respiratory tract infection (URI), smoking status, disease course, blood white blood cell (WBC) count, blood eosinophil count, blood eosinophil percentage, CSS, and LCQ score. The diagnosis of URI was determined based on a self-report history, such as runny nose, nasal obstruction, sneezing, sore throat, and fever. The LCQ score at enrollment was based on manifestations during the previous 2 weeks.

The pulmonary specialists were blinded to the patients’ FENO values. Empirical therapies (without inhaled or systemic corticosteroids) were prescribed based on clinical experience and cough-related guidelines. The first follow-up was conducted 10 ± 1 days after enrollment to obtain FENO values, CSS, LCQ scores, and drug usages. LCQ scores at the first follow-up were recorded based on manifestations after enrollment. After the total disease course reached 8 weeks, a telephone follow-up was performed to inquire about the patient’s condition.

Of the 223 patients who were initially selected, 20 were found with pulmonary embolism, obsolete pulmonary tuberculosis, rhinitis, or chronic obstructive pulmonary disease; 3 took ACEI; and 2 refused to participate in this trial so that they were excluded (Figure 1). In addition, 9 patients were lost to follow-up. In this case, complete data were obtained for 189 patients with subacute cough, including questionnaires, FENO measurement, and follow-up data.

To investigate factors influencing FENO values in the subjects, patients were divided into in the low-FENO (<25 ppb, n = 136) and high-FENO (≥25 ppb, n = 53) groups, using 25 ppb as the cutoff value of FENO. The demographic and clinical features between the groups were compared.
median and interquartile range (IQR). As the data for FENO had a skewed distribution, values were normally distributed after logarithmic transformation.

Univariate regression analysis was performed with log-transformed values of FENO as dependent variables, and other factors as independent variables. Factors that had a $P$ value $<.05$ and $r^2 > .01$ in univariate regression analyses were then used as independent variables and analyzed by multiple stepwise regression analysis.

Relationships between FENO and possible factors were also analyzed. The statistical power of differences between the low-FENO and high-FENO groups was determined using Student’s $t$ test, the Mann-Whitney U test, or the chi-squared test. Differences to treatment response between the low-FENO and high-FENO groups were analyzed by two-way analysis of variance. A $P$ value $<.05$ was considered statistically significant.

3 | RESULTS

3.1 | Baseline characteristics of patients with subacute cough

General information of the basic characteristics is summarized in Table 1.

3.2 | Correlation between FENO and clinical characteristics

A non-normal distribution was observed from the data of FENO. Univariate linear regression analysis was performed using log-transformed values of FENO as dependent variables and other factors as independent variables (Table 2). FENO levels significantly correlated with gender ($r = .22$, $P = 0$); height ($r = .18$, $P = .02$); weight ($r = .18$, $P = .02$); blood WBC count ($r = .22$, $P = 0$); blood eosinophil count ($r = .37$, $P = 0$); and percentages of blood eosinophils ($r = .33$, $P = 0$). In contrast, there were no correlations between FENO levels and age, disease course at enrollment, CSS, or LCQ scores.

A multiple regression analysis was employed to calculate the beta coefficient ($\beta$) and standard error (SE), respectively, showing that eosinophil count ($\beta = 1.38$, SE = 0.29; $P = 0$) and gender ($\beta = 0.25$, SE = 0.08; $P = 0$) but not height, weight, blood WBC count or percentages of blood eosinophils, were independent factors for FENO levels (Table 3).

3.3 | Comparison of clinical characteristics in patients with different FENO levels

There were no significant differences in gender, height, weight, smoking status, blood WBC count, disease course at enrollment, LCQ total scores, LCQ scores in each domain, total CSS, or daytime CSS between the low-FENO and high-FENO groups (all $P > .05$). However, the percentages of blood eosinophils, blood eosinophil count, age, and nighttime CSS were significantly higher in the high-FENO group than those of the low-FENO group ($P < .05$, Table 1).
3.4 | Comparison of drug therapies in patients with different FENO levels

Medications given to patients included dextromethorphan (10 mL as needed); guaifenesin (10 mL as needed) (both from STADA Pharmaceuticals, Beijing, China); montelukast (10 mg once daily, Hangzhou MSD Pharmaceutical, Hangzhou, China); and roxithromycin (150 mg twice daily when necessary, Yangtze River Pharmaceutical, Taizhou, China). There were no significant differences in drug therapies between the low-FENO and high-FENO groups (P > .05; Table 4).

3.5 | Comparison of clinical outcomes in patients with different FENO levels

CSS and LCQ scores after 10 days of treatment were significantly improved in both the low-FENO and high-FENO groups, as compared with these scores before treatment (P < .05). However, there were no significant differences in CSS and LCQ scores to 10-day treatment response between the groups (P > .05; Figure 2).

Patients with a disease course of more than 8 weeks were observed in both groups. The percentages of patients with a disease course of more than 8 weeks were comparable between the groups, with 12.5% (17/136) in the low-FENO group and 20.8% (11/53) in the high-FENO group (P > .05).

4 | DISCUSSION

In the present study, we aimed to evaluate relationships between FENO and clinical characteristics and outcomes in patients with subacute cough. Our data showed that high blood eosinophil count and gender were independent factors for elevated FENO levels. Nighttime cough was more severe in patients with ≥25 ppb FENO compared with those with FENO <25 ppb. However, with the present study design, no difference was found in treatment response between the two groups.

Data showed that FENO levels significant correlated with blood eosinophil count and gender as well, which are consistent with previous reports. Sato et al. found
that FENO highly correlated with eosinophil counts in both blood and induced sputum in patients with chronic cough. Some studies also showed that FENO values were significantly higher in males than those in females, suggesting that gender may be an important factor affecting FENO.

The United States National Health and Nutrition Examination Survey (NHANES) has reported that the age does not affect FENO levels, in which both women (aged 14-45 years) and men (aged 16-59 years) were involved. In contrast, a national multicenter study has shown that there is a significant positive correlation between FENO levels and age among healthy Chinese people aged 15-80 years. In the present study, the multiple regression analysis did not identify age as an independent factor for FENO levels.

The influence of height and weight on FENO levels also varies from different studies. For example, the height and weight are significantly associated with FENO levels in Caucasians, but not in Arabs or African Americans. In the present study, the multiple regression analysis did not support that the height or weight is as an independent factor influencing FENO levels. A potential explanation is that the conflicting findings among these studies might result from the different ethnicities of the study populations.

Viral infection and bacterial infection of the upper respiratory tract may also be important factors affecting the FENO levels. However, an association between URI and FENO is undecided, based on the data of several previous studies. For example, Huang et al. showed that FENO was lower in children with Mycoplasma pneumoniae pneumonia. Gadish et al. found that FENO levels in infants with acute respiratory syncytial virus (RSV) bronchiolitis were temporarily reduced in the early stage of the disease, and then recovered or increased to higher levels in the recovery phase. However, no significant differences in FENO levels were observed between infants with RSV bronchiolitis and healthy infants. In addition, human rhinovirus infection did not appear to affect FENO levels in patients with asthma. In the present study, we could not determine the etiology of URI at the acute stage. Thus, we could not attribute higher FENO levels in patients to a specific pathogen.

The cutoff value for FENO used to guide clinical practice has differed among studies. For example, the optimal FENO cutoff for diagnosis of asthma has ranged from 20 to 26 ppb. Hsu et al. reported that 33.9 ppb was the optimal

### TABLE 2 Univariate correlation analysis of FENO and potential associated factors

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>.14</td>
<td>.07</td>
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<tr>
<td>Gender</td>
<td>.22</td>
<td>0</td>
</tr>
<tr>
<td>Height</td>
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<td>.02</td>
</tr>
<tr>
<td>Weight</td>
<td>.18</td>
<td>.02</td>
</tr>
<tr>
<td>URI history</td>
<td>−.09</td>
<td>.25</td>
</tr>
<tr>
<td>Blood parameters</td>
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<td></td>
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<tr>
<td>WBC count</td>
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<td>0</td>
</tr>
<tr>
<td>Eosinophil count</td>
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<td>0</td>
</tr>
<tr>
<td>Eosinophils %</td>
<td>.33</td>
<td>0</td>
</tr>
<tr>
<td>LCQ</td>
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<td></td>
</tr>
<tr>
<td>Total scores</td>
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<td>.52</td>
</tr>
<tr>
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<td></td>
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<tr>
<td>Physical</td>
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<td>.48</td>
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<tr>
<td>Psychological</td>
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<td>.65</td>
</tr>
<tr>
<td>Social</td>
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<td>.41</td>
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<tr>
<td>CSS</td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>.10</td>
<td>.18</td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Night</td>
<td>.14</td>
<td>.08</td>
</tr>
<tr>
<td>Day</td>
<td>.03</td>
<td>.74</td>
</tr>
<tr>
<td>Duration of cough</td>
<td>−.05</td>
<td>.50</td>
</tr>
</tbody>
</table>

Abbreviations: CSS, cough symptom scores; LCQ, Leicester Cough Questionnaire; URI, upper respiratory infection; WBC, white blood cell. 

### TABLE 3 Multiple regression analysis of FENO and potential associated factors

<table>
<thead>
<tr>
<th>Variables</th>
<th>β</th>
<th>SE</th>
<th>95% CI</th>
<th>P value</th>
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<tr>
<td>Eosinophil count</td>
<td>1.38</td>
<td>0.29</td>
<td>0.82–1.95</td>
<td>0</td>
</tr>
<tr>
<td>Gender</td>
<td>0.25</td>
<td>0.08</td>
<td>0.09–0.41</td>
<td>0</td>
</tr>
</tbody>
</table>

Factors that had a P value < .05 and r² > .01 in univariate regression analyses were then used as independent variables and analyzed using multiple stepwise regression analysis.

### TABLE 4 Treatments of patients

<table>
<thead>
<tr>
<th></th>
<th>Low-FENO</th>
<th>High-FENO</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, n</td>
<td>136</td>
<td>53</td>
<td>—</td>
</tr>
<tr>
<td>Roxithromycin</td>
<td>Yes/ no</td>
<td>5/131</td>
<td>1/52</td>
</tr>
<tr>
<td>Dextromethorpana</td>
<td>Yes/ no</td>
<td>60/76</td>
<td>25/28</td>
</tr>
<tr>
<td>WBC count</td>
<td>183.67 ± 68.63</td>
<td>181.08 ± 61</td>
<td>.87</td>
</tr>
<tr>
<td>Eosinophil count</td>
<td>.37 0</td>
<td>.33 0</td>
<td></td>
</tr>
<tr>
<td>Guaifenesina</td>
<td>Yes/ no</td>
<td>72/64</td>
<td>29/24</td>
</tr>
<tr>
<td>Dose, mL</td>
<td>187.01 ± 68.32</td>
<td>182.65 ± 60</td>
<td>.77</td>
</tr>
<tr>
<td>Montelukast</td>
<td>Yes/ no</td>
<td>72/64</td>
<td>36/17</td>
</tr>
</tbody>
</table>

*Medications were used as required; dosage is total milliliters over 10 days. Low-FENO, <25 ppb; high-FENO, ≥25 ppb. Continuous data presented as mean ± SD. Student’s t test for continuous variables; chi-square test for numeration data.*
cutoff value of FENO for predicting responses to inhaled corticosteroid therapy for patients with chronic cough. Watanabe et al.26 showed that using 26.5 ppb as the cutoff point of FENO could reduce unnecessary application of inhaled corticosteroid therapy. Based their own data, the American Thoracic Society recommends FENO cutoff on a randomly selected general population in Sweden, with the median FENO value of 16 (IQR 11-22.3) ppb.27 This range was fairly consistent with the distribution of patients with subacute cough in the present study (median 19, IQR 13-26 ppb). Thus, in the present study we used 25 ppb as the low/high cutoff point for FENO levels.

**FIGURE 2** LCQ scores and CSS after treatment. CSS scores after treatment were lower than those at baseline in both groups. LCQ scores after treatment were higher than those at baseline in both groups. There were no significant differences in CSS and LCQ scores to 10-day treatment response between these two groups. *p < .05.
In the present study, we found that 53 patients with high-FENO accounted for 28% of the total 189 patients with subacute cough. Lai et al.\(^9\) recent report suggests that eosinophilic airway inflammation is common in subacute cough (33.6%) by sputum induction for differential cell count. In the same study, the authors found that the common causes of subacute cough were post-infectious cough, eosinophilic bronchitis, cough-variant asthma, and upper airway cough syndrome. Most of the patients enrolled in the present study might have post-infectious cough, based on their history of URI and good clinical outcome. Because sputum induction for differential cell count and spirometry were not conducted, it is too hard to conclude that the cause of cough in patients with protracted nocturnal cough was or was not eosinophilic bronchitis or cough-variant asthma.

Zhang et al.\(^{28}\) showed that levels of FENO in patients with chronic cough positively correlated with total CSS and LCQ scores. However, our data showed that only nighttime cough was more severe in the high-FENO group than in the low-FENO group. These varied observations are possibly because of the variations of populations participated in. In Zhang’s study, 64% of participants were with cough-variant asthma or with eosinophilic bronchitis. However, patients with allergic diseases were excluded in the present study.

In the present study, physicians were blinded to the FENO levels. Patients were treated with antibiotics, antitusive, or montelukast, based on clinical experience and cough-related guidelines. There were no significant differences in drug therapies between the low-FENO and high-FENO groups. After 10 days of treatment, the clinical symptoms and LCQ scores had improved in both groups, with no obvious differences between them. In addition, the percentages of patients with a total disease course of more than 8 weeks were similar between the low-FENO and high-FENO groups.

Montelukast was commonly prescribed for patients with subacute cough, but a randomized controlled trial showed that montelukast was not superior to placebo.\(^{29}\) In our study, 67.9% (36/53) of the patients in the high-FENO group received montelukast, while only 52.9% (72/136) of those in the low-FENO group were given this drug (\(P = .06\)). Patients with high-FENO levels did not appear to benefit additionally from montelukast in the present study. Therefore, prospective randomized controlled studies are needed to validate this finding.

FENO is considered a useful biomarker for predicting a response to corticosteroids in chronic cough and asthma. Some authors in uncontrolled studies have successfully treated cough with a brief course of corticosteroids. This regimen may be tried in those patients whose cough becomes protracted and persistently troublesome.\(^{30}\) However, it remains to be clarified in future whether a short course of corticosteroids is of benefit for subacute cough patients with high-FENO.

In the present prospective study, a few limitations should be noted. First, it was performed in a single-center, and multicenter studies are needed to validate the results. Second, the diagnosis of allergic diseases was solely based on medical history, and the skin prick tests and inhalant screening test for allergens were not applied. Finally, we did not describe the causes for cough lasting more than 8 weeks in 28 patients.

## 5 CONCLUSIONS

Our data show that male gender and a high blood eosinophil count are independent factors for elevation of FENO levels in patients with subacute cough. Patients with higher FENO levels have more severe nocturnal cough. However, FENO levels do not appear to correlate with clinical outcomes or treatment response, at least in the current empirical treatment without corticosteroids. Therefore, the values of FENO in guiding treatment of subacute cough, especially in post-infectious cough, need to be further explored.

## ACKNOWLEDGMENTS

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## CONFLICT OF INTERESTS

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

## AUTHOR CONTRIBUTIONS

All authors read and approved the final manuscript.

- **Participated in the design of the study**: Mingming Jiang, Min Liu, Ying Wang, Lili Xu, Xiaoning Bu, Li An, Hong Zhang, Kewu Huang
- **Carried out the experiments**: Mingming Jiang, Min Liu
- **Performed data analyses**: Mingming Jiang, Min Liu
- **Recruited patients**: Ying Wang, Lili Xu, Xiaoning Bu, Li An, Hong Zhang
- **Drafted the manuscript**: Mingming Jiang, Min Liu
- **Wrote the manuscript**: Kewu Huang

## ETHICS

The study was approved by local ethics review committee and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. All subjects provided written informed consent.
REFERENCES


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