Fractional exhaled nitric oxide could identify early spirometry change in clinically suspected asthma patients without airway obstruction

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Abstract
Fractional exhaled nitric oxide (FeNO) has been proposed as a non-invasive biomarker for allergic inflammation seen in asthma. The aim of this study was to assess the ability of FeNO to discriminate spirometry and lung volume measurements between those with and without airway obstruction among subjects with clinically suspected asthma. A retrospective study was conducted. Diagnostic evaluations including spirometry and FeNO testing (NO electrochemical equipment: NIOX VERO; Aerocrine AB, Solna, Sweden) were performed in all subjects. Airway obstruction was defined according to the Standardization of Spirometry of the American Thoracic Society (ATS)/European Respiratory Society (ERS), and 2014 recommendations of the Chinese National Guidelines of Pulmonary Function Test. It was used the Student t test for analysis of continuous variables and the χ2 test for analysis of discrete variables including FeNO levels and lung function metrics. Of the 138 subjects with clinically suspected asthma, airway obstruction was found in 61. There was no significant difference in the mean FeNO levels among subjects with or without airway obstruction (p = 0.241) among un-selected subjects. Likewise, there was no difference in the FeNO levels between aged (≥50 years) and younger subjects (≤50 years) (p = 0.804). A significant proportion of subjects had a normal FeNO level (<25 part per billion, ppb) in spite of having airway obstruction (39/138), 25 had an elevated FeNO level (≥25 ppb) in spite of having no airway obstruction (25/138). Additionally, the airway-obstructed subjects with increased FeNO level had comparable spirometry to those with normal FeNO level (p > 0.05).

However, among subjects without airway obstruction, the forced expiratory volume in 1 s (FEV1)/predicted (pred), maximal expiratory flow at 25% of forced vital capacity (FVC) (MEF25%)/pred, maximal expiratory flow at 50% of FVC (MEF50%)/pred and maximum mid-expiratory flow (MMEF)/pred were significantly lower in the FeNO ≥ 25 ppb group compared to those in the FeNO < 25 ppb group. These analyses indicated that increased FeNO levels could help to determine early spirometry change within clinically suspected asthma subjects without airway obstruction. It is highlighted the importance of FeNO as a phenotype associated with an increased risk of airway obstruction in some subjects in this study.

Keywords
adults, bronchial inflammation, eosinophilic inflammation, FeNO, lung function testing

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Background
In asthma, airway obstruction was associated with a cellular inflammation that involved activated eosinophils, T-lymphocytes, mast cells, macrophages, and dendritic cells. Some of these cells produce an inflammatory factors including histamine, prostaglandins, and leukotrienes, which are associated with cellular bronchial infiltrations in...
FeNO testing has been widely accepted as a marker of eosinophilic airway inflammation both in children and adults with asthma. It has been highlighted the value of FeNO test in characterizing asthma control during treatment with bronchodilators and in predicting response to asthma treatment. It is also associated with the patients’ future risk for exacerbations and developing fixed airway obstruction. Furthermore, many frontline clinicians favor use of FeNO in care of patients with asthma due to the ease of use and non-invasive nature, and this is anticipated to be more prevalent with the advent of newer biologics for patients with eosinophilic asthma.

Unfortunately, to the best of the authors’ knowledge, no data are available on the relationship between lung function fluctuation and FeNO level. To explore the discriminatory ability of FeNO for airway obstruction, an observational study was conducted to investigate the ability of FeNO levels to differentiate between those with and without airway obstruction among subjects with clinically suspected asthma.

Subjects and methods

Subjects

All subjects with chronic cough or chest tightness and no abnormal findings on chest imaging examination referred to our hospital between October 2017 and June 2018 were enrolled in the study. Exclusion criteria were any subjects who had a history of a chronic obstructive pulmonary disease (COPD), asthma, or a previous doctor-diagnosed asthma-COPD overlap (ACO). Subjects who had a confounding pulmonary morbidity such as pulmonary tuberculosis, interstitial lung disease, lung cancer, pulmonary infection and lung edema were also excluded. Additionally, the use of any oral or/and inhaled corticosteroid in the previous 12 weeks was applied as an exclusion criterion. Finally, subjects with negative bronchodilator reversibility testing were also excluded, and the criteria of positive bronchodilator reversibility testing are increase in FEV1 of >12% and >200 mL from baseline, 15–30 min after 400 μg albuterol or equivalent (greater confidence if increase is >15% and >400 mL), otherwise the result is negative. The study flowchart was presented in Figure 1.

FeNO test

FeNO level was measured before Pulmonary Function Test (PFT) according to the guidelines in the user manual training on the NO electrochemical equipment. Subjects were instructed to inhale NO-free air to total lung capacity and immediately exhale fully into the device at a sustained flow rate of 50 mL/s for 10 s, which resulted in display of FeNO value. An increase in FeNO was considered if the value was equal to or higher than 25 ppb.

PFT

All subjects were required to undergo PFT in a reproducible way, and the best values were retained. Percentage predicted values (% pred) were calculated based on reference values for healthy Chinese adults. Airway obstruction was identified using lung function test (Jaeger, Friedberg, German) by an experienced technician according to the Standardization of Spirometry of the ATS/ERS, and 2014 recommendations of Chinese National Guidelines of PFT. The large airway obstruction is defined as FEV1/FVC < lower limits of normal (LLN), while the small airway obstruction is defined as FEV1/FVC ≥ LLN, FVC ≥ LLN, and any two of the three indexes (MEF25%, MEF50%, MMEF) < LLN. According to the recommendations of 2014 Chinese National Guidelines of PFT, the LLN of FVC, FEV1, peak expiratory flow (PEF) and maximal expiratory flow at 75% of FVC (MEF75%) was estimated as 80% of the reference value, the LLN of MMEF, MEF25%, MEF50% was estimated as 65% of the reference value, and the LLN of FEV1/FVC was estimated as 92% of the reference value.

Statistical analysis

The \( t \) and \( \chi^2 \) tests were used to analyze for statistically significant difference among age, FeNO levels, and lung function metrics. Variables were expressed as means ± standard deviations unless
otherwise specified. A \( p \) value of \(<0.05\) was considered statistically significant.

**Results**

*Characteristics of the subjects*

Demographic information of subjects was presented in Table 1. A total of 138 subjects referred to clinic because of persistent respiratory symptoms, who were suggestive of asthma. The average age was 61 years and median age was 50 years with a range between 18 and 85 years.

*FeNO level and PFT data*

The results of FeNO level were reported in Tables 1 and 2. Given the median age among the subjects, the 50 years was opted as threshold. There was no difference in the FeNO level among aged...
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Of all, airway obstruction was found in 61 subjects (61/138), and the FeNO level was comparable between airway obstruction and non-airway obstruction groups ($p = 0.241$, Table 2).

Furthermore, a significant proportion of subjects had a normal FeNO despite of having airway obstruction ($n = 39$, 28.26%), 25 (18.12%) had an elevated FeNO despite of having no airway obstruction. Only 22 (15.94%) who are with FeNO $\geq 25$ ppb simultaneously had airway obstruction. As a consequence, it was found no relationship between occurrence of airway obstruction and elevated FeNO level ($p = 0.719$, Tables 1 and 2).

PFT results were reported in Table 3. Individual spirometric indexes (i.e. FEV1/FVC/pred, FEV1/pred, FVC/pred, MEF75%/pred, MEF25%/pred, MEF50%/pred, PEF/pred, and MMEF/pred) reached statistical significance in subjects with and without airway obstruction.

Based on data above, it was suggested that all subjects have no specific distribution of FeNO value. Then, individual % pred of spirometry were statistically analyzed according to the FeNO level. In subjects with airway obstruction, there was no significant difference in individual all spirometric data ($p > 0.05$, Table 4).

Interestingly, in subjects without airway obstruction, individual some spirometric indexes (i.e., FEV1/pred, MEF25%/pred, MEF50%/pred, and MMEF/pred) reached statistical significance between subgroups with FeNO $\geq 25$ ppb and <25 ppb (Table 4).

**Discussion**

As reported, airway inflammatory phenotype can be measured through FeNO test.$^{11,17}$ On the basis of reported data for type 2 biomarkers in severe

### Table 1. Characteristics of the subjects.

| Variables          | $n = 138$ (%) |
|--------------------|--------------|
| Sex                |              |
| Male               | 61 (44.20)   |
| Years              |              |
| $\leq 50$ years    | 69 (50.00)   |
| FeNO (ppb)         |              |
| $< 25$             | 91 (65.94)   |
| 25–50              | 34 (24.64)   |
| $> 50$             | 13 (9.42)    |
| Airway obstruction |              |
| Present            | 61 (44.20)   |
| FeNO $\geq 25$ ppb| 22* (36.07)  |
| FeNO $< 25$ ppb    | 39 (63.93)   |
| Absent             | 77 (55.80)   |
| FeNO $\geq 25$ ppb| 25 (32.47)   |
| FeNO $< 25$ ppb    | 52 (67.53)   |

The data were number (%).

FeNO: fractional exhaled nitric oxide.

*The $\chi^2$ test was used to analyze the relationship of airway obstruction and FeNO level, $p = 0.719$.

### Table 2. FeNO results for the subjects ($n = 138$).

| Variables | FeNO (ppb) | $p$ Value |
|-----------|------------|-----------|
| Age       |            |           |
| $> 50$ years ($n = 69$) | 27.67 ± 2.83 | 0.804     |
| $\leq 50$ years ($n = 69$) | 26.55 ± 3.49 |           |
| Airway obstruction |            |           |
| Present ($n = 61$) | 30.07 ± 3.62 | 0.241     |
| Absent ($n = 77$) | 24.77 ± 2.80 |           |
| Airway obstruction present |            |           |
| $> 50$ years ($n = 44$) | 27.57 ± 3.10 | 0.270     |
| $\leq 50$ years ($n = 17$) | 36.53 ± 10.28 |           |
| Airway obstruction absent |            |           |
| $> 50$ years ($n = 25$) | 27.84 ± 5.71 | 0.450     |
| $\leq 50$ years ($n = 52$) | 23.29 ± 3.13 |           |

The data were mean ± SD; The t test was used to compare difference in FeNO level between two independent samples.

FeNO: fractional exhaled nitric oxide.

($> 50$ years) and younger subjects ($\leq 50$ years) ($p = 0.804$, Table 2). Of all, airway obstruction was found in 61 subjects (61/138), and the FeNO level was comparable between airway obstruction and non-airway obstruction groups ($p = 0.241$, Table 2).

Increasing FeNO level in subjects with normal spirometry

Based on data above, it was suggested that all subjects have no specific distribution of FeNO value. Then, individual % pred of spirometry were statistically analyzed according to the FeNO level.

In subjects with airway obstruction, there was no significant difference in individual all spirometric data ($p > 0.05$, Table 4).

Interestingly, in subjects without airway obstruction, individual some spirometric indexes (i.e., FEV1/pred, MEF25%/pred, MEF50%/pred, and MMEF/pred) reached statistical significance between subgroups with FeNO $\geq 25$ ppb and <25 ppb (Table 4).

### Table 3. Spirometry results for the subjects ($n = 138$).

| Variable | Airway obstruction ($n = 61$) | Non-airway obstruction ($n = 77$) | $p$ Value |
|----------|-------------------------------|----------------------------------|-----------|
| FEV1/FVC/pred | 85.60 ± 2.19 | 105.80 ± 0.69 | $< 0.0001$ |
| FEV1/pred | 81.19 ± 2.88 | 107.30 ± 1.47 | $< 0.0001$ |
| FVC/pred | 97.79 ± 2.37 | 104.70 ± 1.56 | 0.013 |
| MEF75%/pred | 64.55 ± 3.62 | 106.60 ± 2.14 | $< 0.0001$ |
| MEF25%/pred | 36.47 ± 2.43 | 94.73 ± 2.92 | $< 0.0001$ |
| MEF50%/pred | 47.94 ± 2.86 | 98.55 ± 2.25 | $< 0.0001$ |
| PEF/pred | 78.45 ± 3.03 | 103.70 ± 1.92 | $< 0.0001$ |
| MMEF/pred | 42.14 ± 2.41 | 97.58 ± 2.19 | $< 0.0001$ |

The data were mean ± SD; The t test was used to compare difference in spirometry between subjects with airway obstruction and those without airway obstruction.

FVC: forced vital capacity; FEV1: forced expiratory volume in 1 s; MEF75%: maximal expiratory flow at 75% of FVC; MEF25%: maximal expiratory flow at 25% of FVC; MEF50%: maximal expiratory flow at 50% of FVC; PEF: peak expiratory flow; MMEF: maximum mid-expiratory flow; Pred: Predicted.
asthma, it suggests that biomarkers of inflammation could be considered in identifying and monitoring of asthmatic patients in clinical practice, such as the titration of steroid treatment.\textsuperscript{10,18,19} Then, a hypothesis was made that those with high baseline levels of FeNO would be more likely result in greater decreases in lung function.

In this study, among un-selected subjects, it was found that only 22 (15.94\%) with FeNO \(\geq 25\) ppb simultaneously had airway obstruction, and 25 (18.12\%) with elevated FeNO level had no airway obstruction. It was indicated that there was no relationship between airway obstruction and elevated FeNO level. Previously, a conclusion was shown that no consistent association between accelerated decline in lung function and FeNO in studies of populations with more severe asthma. Whether the normal FeNO levels in the airway obstruction group were associated with airway inflammatory phenotype is unclear; however, it is suspected that they are likely related to other etiologies that mimic asthma.

It was also found no significant difference in FeNO levels in clinically suspected asthma irrespective of whether they had airway obstruction or not. Then, it was further explored whether the increased FeNO levels can be a predicted factor for early decreased spirometry in clinically suspected asthma who did not attach diagnostic criteria of airway obstruction. As supported, in these subjects, it was indicated the FEV1/pred, MEF25%/pred, MEF50%/pred, and MMEF/pred were significantly lower in the FeNO \(\geq 25\) ppb sub-group compared to those in the FeNO < 25 ppb sub-group. This is surprising as, increased FeNO level in clinically suspected asthma with normal spirometry was involved in development of abnormal single lung function index, even it did not attach diagnostic criteria of airway obstruction. This was suggested that FeNO was indicative of association between increased airway inflammation and risk of development of airway obstruction in the study.

It should be noted that the present study had several limitations. This study was a single-center retrospective analysis, and the sample size was low and not calculated. Additionally, methacholine challenge testing was not performed among all subjects, especially in those with normal lung function and elevated FeNO. Besides, the LLN was not calculated in this study. Also, the risk of development of airway obstruction was not established in these subjects. Finally, FeNO level has been known to be affected by a variety of conditions such as smoking, allergic rhinosinusitis, obesity, and obstructive sleep apnea.\textsuperscript{20}

**Conclusion**

Taken together, it was believed that these data provided important and practical information on clinically suspected asthma subjects without airway obstruction, it was also suggested that increased FeNO level in clinically suspected asthma with normal spirometry might be involved in the cumulative risk of development of decreased spirometry.\textsuperscript{21,22}

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**Table 4.** Analysis between spirometry and FeNO level (n = 138).

| Variable | Airway obstruction (n = 61) | p Value | Non-airway obstruction (n = 77) | p Value |
|----------|-----------------------------|---------|-------------------------------|---------|
|          | FeNO < 25ppb (n = 39)       | FeNO \(\geq 25\)ppb (n = 22) | FeNO < 25ppb (n = 52) | FeNO \(\geq 25\)ppb (n = 25) |
| FEV1/FVC/Pred | 85.71 ± 2.69 | 85.40 ± 3.83 | 0.946 | 106.50 ± 0.88 | 104.2 ± 1.005 | 0.115 |
| FEV1/Pred | 82.43 ± 3.66 | 79.00 ± 4.74 | 0.572 | 109.80 ± 1.74 | 102.1 ± 2.45 | 0.013 |
| MEF75%/Pred | 65.23 ± 4.54 | 63.34 ± 6.13 | 0.804 | 108.00 ± 2.62 | 103.5 ± 3.71 | 0.330 |
| FVC/ Pred | 99.29 ± 3.09 | 95.14 ± 3.64 | 0.404 | 106.60 ± 1.89 | 100.9 ± 2.68 | 0.090 |
| MEF25%/Pred | 34.34 ± 2.66 | 40.43 ± 4.86 | 0.356 | 98.77 ± 3.74 | 86.32 ± 4.12 | 0.045 |
| MEF50%/Pred | 48.08 ± 3.61 | 47.69 ± 4.81 | 0.949 | 102.80 ± 2.80 | 89.64 ± 3.16 | 0.005 |
| PEF/Pred | 79.04 ± 3.82 | 77.40 ± 5.10 | 0.797 | 103.60 ± 2.24 | 103.80 ± 3.69 | 0.957 |
| MMEF/Pred | 41.93 ± 2.90 | 42.51 ± 4.34 | 0.980 | 101.20 ± 2.73 | 90.12 ± 3.20 | 0.017 |

The data were mean ± SD; The t test was used to compare difference in spirometry between subjects with FeNO < 25ppb and those with FeNO \(\geq 25\)ppb within airway-obstructed or non-airway-obstructed subgroups.

FVC: forced vital capacity; FEV1: forced expiratory volume in 1 s; MEF75%: maximal expiratory flow at 75% of FVC; MEF25%: maximal expiratory flow at 25% of FVC; MEF50%: maximal expiratory flow at 50% of FVC; PEF: peak expiratory flow; MMEF: maximum mid-expiratory flow; Pred: Predicted; FeNO: Fractional exhaled nitric oxide.
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Authors’ contributions
CC participated in the conception, the design and coordination of the study, collected data, and drafted the manuscript. GQB, WQZ, and LF conceived of the study, and participated in its design and coordination and helped to draft the manuscript. LF participated in collected data.

Declaration of conflicting interests
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Ethical approval
Ethical approval for this study was obtained from the ethics committee of the First Affiliated Hospital of Soochow University. The approval number is (2020) Medical Ethics Committee Grant No. 207.

Informed consent
The informed consent was not sought for the present study, because a retrospective study was conducted. And written informed consent cannot be obtained retrospectively from the subjects included in the study because of the lack of partial information of subjects.

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Availability of data and material
All data generated or analyzed during this study are included in this published article.

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