Study on small airway function in asthmatics with fractional exhaled nitric oxide and impulse oscillometry

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Abstract

Objectives: The invasive techniques can be direct and objective to assess small airway function, but they have significant risks and inconveniences for patients and cannot be repeated often. Some sophisticated techniques such as fractional exhaled nitric oxide (FeNO) and impulse oscillometry (IOS) may surmount such restrictions. Therefore, we investigated the relation among FeNO, IOS, and small airway function in asthmatic patients.

Methods: We recruited 140 asthmatic patients including 69 patients with small airway normal function and 71 patients with small airway dysfunction. FeNO, eosinophil (EOS) count and total immunoglobulin E (IgE) in peripheral blood, pulmonary function, as well as IOS were measured.

Results: The levels of FeNO, the reactance area (AX), the resonant frequency Fres and EOS were significantly increased in small airway dysfunction group compared with small airway normal function group ($P < 0.01$ respectively). A multiple regression model showed that FeNO, AX and Fres were correlative factors of mid forced expiratory flow of percentages of predicted values [$\text{FEF}_{25\text{-}75}\%\text{pred}$] ($P < 0.01$, respectively). A receiver operating characteristic (ROC) analysis showed that the combination of FeNO, AX and Fres had a greater area under the ROC curve (AUC) than each of them (AUC: 0.881, $P < .001$, 95% CI: 0.815-0.929).

Conclusion: FeNO and IOS are helpful in diagnosis of small airway dysfunction with high sensitivity and specificity, and FeNO combined with IOS can better evaluate the small airway function in asthmatic patients.

KEYWORDS
asthma, fractional exhaled nitric oxide, impulse oscillometry, small airway dysfunction

1 | INTRODUCTION

Asthma is a very common condition worldwide, which affects estimated 300 million population. Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation and remodeling. It is now widely accepted that airway inflammation and remodeling occur not only in the central airways but also in the small airways. The forced expiratory volume in one second
**Methods**

**2.1 Study population**

The investigation was performed in Pulmonary Outpatient Clinic of Qilu Hospital, Shandong University (China) from July 2014 to July 2015. Patients enrolled in this study were all diagnosed with mild to moderate asthma according to Global Initiative for Asthma (GINA). Patients with smoking history determinedly diagnosed with chronic obstructive pulmonary disease or other lung diseases were excluded. None of selected subjects suffered from chest infections or had oral corticosteroid in the previous 4 weeks. All of the subjects were first seen at our hospital and they had not received regulatory treatment previously. None of them used antiasthma medication (oral or inhaled corticosteroids, short-acting or long-acting bronchodilators, leukotriene modifiers, or antihistamines) at least 24 hours prior to our study.

All the patients underwent pulmonary function test, IOS, FeNO, and blood test.

**2.2 Pulmonary function test and IOS**

Spirometry (Jaeger Co, Hoechberg, Germany) was executed to estimate pulmonary function, which was performed on the basis of American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines. Several parameters were recorded in the form of percentages of predicted values containing forced expiratory volume in one second (FEV1), mid forced expiratory flow (FEF25-75) and instantaneous forced expiratory flow when X% of the forced vital capacity (FVC) has been expired (FEFX%) and their abbreviations were as follows: FEV1(%pred), FEF25-75(%pred), and FEFX% (%pred). The ratio of FEV1 and FVC (FEV1/FVC) were expressed as absolute values. The criteria of small airway dysfunction are as follows: FEF25-75 (%pred) < lower limits of normal (LLN) and FEF75 (%pred) < LLN.

The Jaeger Master Screen IOS (Jaeger Co, Hoechberg, Germany) was performed in accordance with standard recommendations. During IOS measurements, the patients were asked in the sitting position with their heads in a neutral or slightly extended position. The patients used noseclips, and they were instructed to breathe quietly through a mouthpiece. To decrease the shunt compliance of the cheeks, they supported their cheeks and the chin using their hands. The patients did not have artifacts caused by coughing, swallowing, breath holding or vocalization. All IOS data were carefully reviewed by our expert pulmonologists. IOS parameters as follows were recorded including the resonant frequency (Fres), respiratory resistance at 5 Hz (R5), the difference between respiratory resistance at 5 Hz and 20 Hz (R5-R20) and the reactance area (AX).

**2.3 FeNO**

FeNO was performed using a portable device (NIOX MINO, Aerocrine AB, Solna, Sweden) at a flow rate of 50 ml/s, according to ATS/ERS recommendations. The performance of FeNO preceded the spirometry.

**2.4 Biomarkers of peripheral blood**

EOS count and total IgE in peripheral blood were determined by the clinical laboratory of Qilu Hospital, Shandong University.

**2.5 Statistical analysis**

SPSS software version 17.0 (SPSS Inc, Chicago, Illinois, USA) served for statistical analysis of all the recorded data. All variables were presented in the way of mean values ± standard deviations. Comparisons between different subgroups were conducted by unpaired t-test. Pearson’s correlation coefficients were utilized to assess the relationship between parameters. A multiple regression model was...
demonstrated to evaluate whether FEF25–75(%pred) was associated with other parameters. The receiver operating characteristic (ROC) curve was established to define the biomarker which optimally recognized the small airway dysfunction. The best cut-off point, sensitivity, specificity, likelihood criterion as well as confidence interval of 95% (95%CI) were collected and accuracy was determined by the area under the ROC curve (AUC). Statistical significance was considered to exist when \( P < 0.05 \).

### 3 | RESULTS

#### 3.1 | Baseline

One hundred and forty asthmatic patients took part in our investigation and were divided into two subgroups including 69 patients with small airway normal function and 71 patients with small airway dysfunction.

The characteristics of patients were showed in Table 1. There were no statistical differences in terms of age, sex, FEV1 (%pred) and FEV1/FVC. FeNO, EOS, AX, and Fres were significantly higher in the small airway dysfunction group compared with small airway normal function group, while FEF50 (%pred), FEF75 (%pred), and FEF25-75 (%pred) significantly lower. In addition, IgE, R5, and R5-R20 were all statistically different between the two groups.

#### 3.2 | The relation between FeNO, IOS, and small airway function

The application of Pearson correlations showed negative correlations between FeNO, Fres, EOS and FEF25-75 (%pred) \( (r = -0.856, P < 0.01; r = -0.851, P < 0.01; r = -0.398, P = .001; r = -0.288, P = 0.014, \text{respectively}) \), (Figures 1A and 2). AX, Fres and EOS correlated significantly with FeNO \( (r = 0.331, P = 0.005; r = 0.361, P = 0.002; r = 0.769, P = 0.008, \text{respectively}) \). (Figure 1B-1D). A multiple regression model showed that the strongest independent predictors of small airway dysfunction were FeNO, AX and Fres (Table 2).

We performed ROC analysis to find the best biomarker to distinguish small airway dysfunction from small airway normal function in asthmatic patients. The AUC for FeNO, AX, and Fres were all greater than 0.8 (AUC: 0.830,

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<tr>
<th>TABLE 1</th>
<th>Characteristics of patients</th>
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<tbody>
<tr>
<td></td>
<td>Small airway normal function</td>
</tr>
<tr>
<td>Number of patients</td>
<td>69</td>
</tr>
<tr>
<td>Age, y</td>
<td>35.62 ± 13.67</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>32/37</td>
</tr>
<tr>
<td>FEV1%pred</td>
<td>84.41 ± 3.44</td>
</tr>
<tr>
<td>FEV1/FVC, %</td>
<td>81.75 ± 5.16</td>
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<tr>
<td>FEF50%pred</td>
<td>88.82 ± 3.36</td>
</tr>
<tr>
<td>FEF75%pred</td>
<td>90.45 ± 5.38</td>
</tr>
<tr>
<td>FEF25-75%pred</td>
<td>87.51 ± 7.51</td>
</tr>
<tr>
<td>FeNO, ppb</td>
<td>34.46 ± 19.60</td>
</tr>
<tr>
<td>IgE, IU/ml</td>
<td>86.61 ± 46.85</td>
</tr>
<tr>
<td>EOS,10⁹/L</td>
<td>0.59 ± 0.15</td>
</tr>
<tr>
<td>R5, kPa/(1/S)</td>
<td>0.34 ± 0.08</td>
</tr>
<tr>
<td>R5-R20, kPa/(1/S)</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>AX, kPa/L</td>
<td>1.17 ± 0.74</td>
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<tr>
<td>Fres, 1/S</td>
<td>12.93 ± 5.59</td>
</tr>
</tbody>
</table>

Abbreviations: AX, reactance area; Fres, the resonant frequency; EOS, eosinophil; FEF25-75(%pred), mid forced expiratory flow of percentages of predicted values; FEF50(%pred), instantaneous forced expiratory flow when 50% of the forced vital capacity (FVC) has been expired in the form of percentages of predicted values; FEF75(%pred), instantaneous forced expiratory flow when 75% of the forced vital capacity (FVC) has been expired in the form of percentages of predicted values; FeNO, fractional exhaled nitric oxide; FEV1(%pred), forced expiratory volume in one second; FEV1/FVC, the ratio of forced expiratory volume in one second and forced vital capacity; IgE, immunoglobulin E; R5, respiratory resistance at 5 Hz; R5-R20, the difference between respiratory resistance at 5 Hz and 20 Hz.
P < .001, 95% CI: 0.758-0.889; AUC: 0.822, P < .001, 95% CI: 0.749-0.882; AUC: 0.816, P < .001, 95% CI: 0.741-0.876, respectively), and FeNO was slightly better than the other two. Furthermore, FeNO combined with AX and Fres had a greater AUC than each of them (AUC: 0.881, P < .001, 95% CI: 0.815-0.929). However, the AUC for EOS was below 0.8 (AUC: 0.673, P < .001, 95% CI: 0.589-0.750). (Table 3 and Figure 3).

The ROC was performed to determine the best cut-points. The cut-points were picked out by means of the peak sum of sensitivity and specificity. At a cut-point of 38 ppb, FeNO was a best biomarker in diagnosis of small airway dysfunction, whose sensitivity was 90.14% and specificity was 72.46%, with the likelihood ratio of 7.35.

The second best indices were AX and Fres. However, EOS was not good for predicting small airway dysfunction (Table 4).

4 | DISCUSSION

Small airways, labeled as “silent zone,” are known as distal airways whose diameters are less than 2 mm.21 However, recent studies have revealed that small airways are not “silent.”22,23 There is growing consensus that small airways play an important part in asthma. Similar to the inflammation of the large airway, small airway inflammation can also lead to the airway wall thickening, airway narrowing, and hyperresponsiveness,24 which will contribute to poor asthma control and frequent exacerbation.25

FeNO is a latent biomarker of the airway inflammation, and can be used to evaluate inflammation stemming from the central large airways to the small airways.10 Our study established that FeNO was an accurate biomarker in distinguishing small airway dysfunction from small airway normal function at the cut-point of 38 ppb, with high sensitivity and specificity. A recent study revealed that small airways may have a stronger inflammatory condition compared to central airways in asthmatic patients.26 It is well established that FeNO is conducive to indicate airway inflammation, especially for eosinophilic inflammation.19 Balzar et al.26 have demonstrated eosinophil of small airways is increased through trans-bronchial biopsy. Our research showed that FeNO correlated significantly with FEF25-75 (%pred), suggesting that the small airway dysfunction in asthma may be caused by airway inflammation, especially by eosinophilic inflammation.

The European Community Respiratory Health Study (ECRHS) showed allergic reaction accounted for 30% among the risk factors inducing asthma symptoms.27 Secondary to allergic reaction, chronic airway inflammation of asthma can increase FeNO. It is coincidental with our data which showed that levels of IgE and EOS were increased and EOS correlated significantly with FeNO compared with small airway normal function group.28

FIGURE 1 Correlation between fractional exhaled nitric oxide (FeNO) and other indicators in small airway dysfunction group. FeNO negatively correlated with mid forced expiratory flow of percentages of predicted values ($r = -0.856, P < 0.01$) (A), while had positive correlations with AX, Fres, and EOS ($r = 0.331, P = 0.005$; $r = 0.361, P = 0.002$; $r = 0.769, P = 0.008$, respectively), (B-D)

FIGURE 2 Correlation between levels of reactance area (AX), the resonant frequency (Fres), eosinophil (EOS), and mid forced expiratory flow of percentages of predicted values [FEF25-75(%pred)]. There were negative correlations between AX, Fres, EOS, and FEF25-75(%pred) ($r = -0.851, P < 0.01$; $r = -0.398, P = .001$; $r = -0.288, P = .014$, respectively), (A, B, C)
IOS has been demonstrated that it is not complicated to assess small airway function in asthmatic patients. Some parameters of IOS are available. The respiratory resistance at 5 Hz (R5) and 20 Hz (R20) are thought to be on behalf of the total and central airway resistance, respectively, and the difference between them (R5-R20) is considered to represent peripheral airway resistance. The resonant frequency (Fres) and reactance area (AX) all have a relationship with the resistance in small airways. Our study also found that the indices of IOS were elevated and Fres and AX correlated with FEF25-75(%pred) in small airway dysfunction group. It indicates that Both Fres and AX can reflect degree of obstruction in the small airways. Goldman et al. also support this idea. In addition, Takeda et al. demonstrated that AX was correlated with healthy condition, dyspnea, and

<table>
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<th>TABLE 2</th>
<th>Multiple regression statistics for FEF25-75(%pred)</th>
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<tr>
<td>Unstandardized coefficient</td>
<td>SE</td>
</tr>
<tr>
<td>Age, y</td>
<td>0.003</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>0.482</td>
</tr>
<tr>
<td>FEV1%pred</td>
<td>−0.091</td>
</tr>
<tr>
<td>FEV1/FVC (%)</td>
<td>0.081</td>
</tr>
<tr>
<td>FEF50%pred</td>
<td>−0.001</td>
</tr>
<tr>
<td>FEF75%pred</td>
<td>0.019</td>
</tr>
<tr>
<td>FeNO, ppb</td>
<td>−0.403</td>
</tr>
<tr>
<td>IgE, IU/ml</td>
<td>−0.009</td>
</tr>
<tr>
<td>EOS, 10^9/L</td>
<td>−5.314</td>
</tr>
<tr>
<td>R5, kPa/(1/s)</td>
<td>−2.706</td>
</tr>
<tr>
<td>R5-R20 [kPa/(1/s)]</td>
<td>9.106</td>
</tr>
<tr>
<td>AX, kPa/L</td>
<td>−7.620</td>
</tr>
<tr>
<td>Fres, 1/s</td>
<td>−0.391</td>
</tr>
</tbody>
</table>

Abbreviations: AX, reactance area; EOS, eosinophil; FeNO, fractional exhaled nitric oxide; Fres, the resonant frequency; IgE, immunoglobulin E; R5, respiratory resistance at 5 Hz; R5-R20, the difference between respiratory resistance at 5 Hz and 20 Hz; SE, standard error; VIF, variance inflation factors.

**FIGURE 3** Receiver operating characteristic curve analysis of FeNO, reactance area, the resonant frequency, and eosinophil for the diagnosis of small airway dysfunction in asthma

<table>
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<tr>
<th>TABLE 3</th>
<th>The area under receiver operating characteristic curve on fractional exhaled nitric oxide, reactance area, the resonant frequency, and eosinophil</th>
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<tbody>
<tr>
<td>AUC</td>
<td>SE</td>
</tr>
<tr>
<td>FeNO, ppb</td>
<td>0.830</td>
</tr>
<tr>
<td>AX, kPa/L</td>
<td>0.822</td>
</tr>
<tr>
<td>Fres, 1/s</td>
<td>0.816</td>
</tr>
<tr>
<td>EOS, 10^9/L</td>
<td>0.673</td>
</tr>
<tr>
<td>FeNO, AX, and Fres</td>
<td>0.881</td>
</tr>
</tbody>
</table>

Abbreviations: 95%IC, confidence interval of 95%; AUC, area under the curve; AX, reactance area; EOS, eosinophil; FeNO, fractional exhaled nitric oxide; Fres, the resonant frequency; SE, standard error.
level of disease control. Furthermore, our results also found that FeNO combined with AX and Fres had a greater AUC than each of them (AUC: 0.881, $P < .001$, 95%CI: 0.815-0.929). IOS is a more useful and patient-friendly method in that it is a noninvasive dependent method and needs a shorter operator training. Therefore, IOS can also be used to detect other manifestations of small airway dysfunction, such as exercise-induced bronchoconstriction, chronic obstructive pulmonary disease and interstitial lung disease. These results manifested that FeNO associated with IOS could better evaluate the function of small airway in asthmatic patients.

It is known that inhaling corticosteroids is the key therapy to asthma. However, the bulk of inhaler devices in clinical practice mainly use large drug particles and they are invalid to deliver and deposit aerosolized medicine for treatment of the small airway disease in asthma. Small particle formulations of ICSs and long-acting $\beta$-agonist inhaler may lead to better asthma control including small airway dysfunction. However, there still is not a consensus on dose and duration of the medication. It is still a concerning problem how to effectively treat the small airway dysfunction.

Nevertheless, our study has some possible limitations. The FEF 25-75 depends on FVC. If patient is in a poor coordination during lung function measurements, it may lead to excessive diagnosis. Other components of the airway tree may influence IOS measures, especially the upper airways. During the measurement, buccal air leaks, glottis narrowing or closure and leak around the mouthpiece are common reasons to discard the measurement. We had relatively small subjects in this study. Further studies with a larger sample size are required. In addition, our subjects only included mild to moderate asthma and it is not clear whether we can draw a similar conclusion in severe asthmatic patients.

In summary, our research indicates that FeNO and IOS are helpful in diagnosis of small airway dysfunction with high sensitivity and specificity. FeNO combined with IOS can better evaluate the function of small airway in mild to moderate asthmatic patients. Their combination may provide new insights into identification of small airway dysfunction. It may help us identify small airway dysfunction in time, and be available for future studies on therapies. However, further research is needed to find effective treatment for the small airway dysfunction in asthmatic patients.

**ACKNOWLEDGMENT**

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**CONFLICTS OF INTEREST**

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

**AUTHOR CONTRIBUTIONS**

Liang Dong designed this study. Lin Liu, Wen Liu, Chunhong Liu, Dexiang Wang, Jiping Zhao, Junfei Wang, Jinxiang Wu, Tian Liu, Yuanyuan Zhang, Yahui Liu, and Liuzhao Cao collected data. Lin Liu and Wen Liu wrote the paper. All authors contributed to the final version.

**ETHICS**

The study was approved by the Ethics Review Committee for Human Studies in Qilu Hospital of Shandong University. Written informed consent was acquired from all participants.

**REFERENCES**


