

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

Lin Liu^{1#}, Wen Liu^{1,2#}, Chunhong Liu¹, Dexiang Wang¹, Jiping Zhao¹, Junfei Wang¹,
Jinxiang Wu¹, Tian Liu¹, Yuanyuan Zhang¹, Yahui Liu¹, Liuzhao Cao¹, Liang Dong¹

1. Dept of Pulmonary Medicine, Qilu Hospital of Shandong University, Jinan,
250012, P.R. China

2. Dept of Cadre Health Care, The Second Hospital of Shandong University, Jinan,
250033, P.R. China

#Contributed equally

Running head

Study on small airway function in asthmatics

Correspondence

Liang Dong, Department of Pulmonary Diseases, Qilu Hospital of Shandong University, Jinan, Shandong 250012, China.

Telephone/Fax number :(+86) 053182169331.

E-mail: dl5506@126.com

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.

Conflicts of interest

All the authors have no conflicts of interest and are responsible for the content of the paper.

Authorship and contributorship

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.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/crj.12548

Abstract

Introduction and Objectives: The invasive techniques can be direct and objective to assess small airway function, but they have significant risks and inconveniences for patients and can not 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 [FEF25–75 (%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, $p < 0.001$, 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 - small airway dysfunction - FeNO - IOS

Introduction

Asthma is a very common condition worldwide, which affects estimated 300 million population(1). All age groups can be influenced by this serious global health problem with increasing prevalence in many developing countries, rising treatment costs, and a rising burden for patients and the community(1). Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation and remodeling (2). It is now widely accepted that airway inflammation and remodeling occur not only in the central airways but also in the small airways (3). The forced expiratory volume in one second (FEV1) is deemed a core pulmonary physiology outcome for monitoring asthma control and assessing response to treatment (4). However, FEV1 is considered to mainly reflect the large airways but be not sensitive to small airway obstruction (5). Although small airways account for less than 10% of airflow resistance in normal conditions, evidence shows that their abnormalities significantly contribute to increasing airflow resistance in asthmatic patients (6-8). As a result, the small airway dysfunction may dramatically affect the clinical manifestations and lung function in asthma (9). Dysfunction of small airways leads to poor asthma control even in the mild condition of patients (10). Therefore, it is very important for asthmatic patients to evaluate small airway function early.

The mid-forced expiratory flow (FEF₂₅₋₇₅) is believed to be a marker of small airways (11, 12). However, traditional spirometry requires participators to perform forced expiratory maneuvers, which is difficult for young children and old subjects and hampers reproducibility (13). The invasive techniques such as surgical resection, transbronchial biopsy and optical coherence tomography (OCT)(14) can be direct and objective to assess small airway function, but they have significant risks and inconveniences for patients and cannot be repeated often (15). Some sophisticated techniques such as fractional exhaled nitric oxide (FeNO) and impulse oscillometry (IOS) may surmount such restrictions (16). Therefore, we investigated the relation among FeNO, IOS and small airway function in asthmatic patients, which will provide suggestions to new and more convenient methods to diagnose small airway

dysfunction.

Methods

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) (2). 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.

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 (17). 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 is as follows: FEF25-75 (%pred) < lower limits of normal (LLN) and FEF75 (%pred) < LLN (18).

The Jaeger Master Screen IOS (Jaeger Co, Hoechberg, Germany) was performed in accordance with standard recommendations (19). 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 (F_{res}), respiratory resistance at 5 Hz (R_5), the difference between respiratory resistance at 5 Hz and 20 Hz (R_5-R_{20}) and the reactance area (AX).

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 (20). The performance of FeNO preceded the spirometry.

Biomarkers of peripheral blood

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

Statistical Analysis

SPSS software version 17.0 (SPSS Inc, Chicago, IL, USA) served for statistical analysis of all the recorded data. All variables were presented in the way of mean values $\pm$ 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 association 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 $P < 0.05$.

Results

Baseline

140 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.

The relation between FeNO, IOS and small airway function

The application of Pearson correlations showed negative correlations between FeNO, Fres, AX, EOS and FEF25-75(%pred) ($r=-0.856$, $p<0.01$; $r=-0.851$, $p<0.01$; $r=-0.398$, $p=0.001$; $r=-0.288$, $p=0.014$, respectively) (Figure 1A and Figure 2). AX, Fres and EOS correlated significantly with FeNO ($r=0.331$, $p=0.005$; $r=0.361$, $p=0.002$; $r=0.769$, $p=0.008$, respectively). (Figure 1B, 1C and 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, $p<0.001$, 95% CI: 0.758-0.889; AUC: 0.822, $p<0.001$, 95% CI: 0.749-0.882; AUC: 0.816, $p<0.001$, 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, $p<0.001$, 95% CI: 0.815-0.929). However, the AUC for EOS was below 0.8 (AUC: 0.673, $p<0.001$, 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).

Discussion

Small airways, labeled as “silent zone”, are known as distal airways whose diameters are less than 2mm (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 S 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).

IOS has been demonstrated that it is not complicated to assess small airway function in asthmatic patients (29). 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 (30). The resonant frequency (Fres) and reactance area (AX) all have a relationship with the resistance in small airways(31). 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 MD et al (31) also support this idea. In addition, Takeda T et al (32) demonstrated that AX was correlated with healthy condition, dyspnea, and 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, $p < 0.001$, 95% CI: 0.815-0.929). IOS is a more useful and patient-friendly method in that it is a non-effort dependent method and needs a shorter operator training (33, 34). 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 (34-37). 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 (38). Small particle formulations of ICSs and long-acting β -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 (39-43). 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 (17, 18). 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 (19, 34). During the measurement, buccal air leaks, glottis narrowing or closure and leak around the mouthpiece are common reasons to discard the measurement (19, 34). 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.

Acknowledgements

This study was supported by grants from the National Natural Science Foundation of China (Grant No. 81270072).

References

1. Global Initiative for Asthma: Global Strategy for Asthma Management and Prevention 2015.
Available from: www.ginaasthma.org.
2. Global Initiative for Asthma: Global Strategy for Asthma Management and Prevention 2014.
Available from: www.ginaasthma.org.

3. Hamid Q. Pathogenesis of Small Airways in Asthma. *Respiration*. 2012; 84(1): 4–11.
4. Tepper RS, Wise RS, Covar R, et al. Asthma outcomes: pulmonary physiology. *J Allergy Clin Immunol*. 2012; 129(3): S65–87.
5. Annesi I, Oryszczyn MP, Neukirch F, et al. Relationship of upper airways disorders to FEV1 and bronchial hyperresponsiveness in an epidemiological study. *Eur Respir J*. 1992; 5(9): 1104–1110.
6. Wagner EM, Liu MC, Weinmann GG, et al. Peripheral lung resistance in normal and asthmatic subjects. *Am Rev Respir Dis*. 1990; 141(3): 584-588.
7. Wagner EM, Bleecker ER, Permutt S, et al. Direct assessment of small airways reactivity in human subjects. *Am J Respir Crit Care Med*. 1998; 157(2):447-452.
8. van der Velden J, Barker D, Barcham G, et al. Assessment of peripheral airway function following chronic allergen challenge in a sheep model of asthma. *PLoS One*. 2011; 6(12): e28740.
9. van den Berge M, ten Hacken NH, Cohen J, et al. Small airway disease in asthma and COPD: clinical implications. *Chest*. 2011; 139(2): 412-423.
10. Usmani OS. Small Airways Dysfunction in Asthma: Evaluation and Management to Improve Asthma Control. *Allergy Asthma Immunol Res*. 2014; 6(5): 376-388.
11. Marseglia GL, Cirillo I, Vizzaccaro A, et al. Role of forced expiratory flow at 25%–75% as an early marker of small airways impairment in subjects with allergic rhinitis. *Allergy Asthma Proc*. 2007; 28(1): 74–78.
12. Cirillo I, Klersy C, Marseglia GL, et al. Role of FEF25%–75% as a predictor of bronchial hyperreactivity in allergic patients. *Ann Allergy Asthma Immunol*. 2006; 96(5): 692–700.
13. Nielsen KG, Bisgaard H. The Effect of Inhaled Budesonide on Symptoms, Lung Function, and Cold Air and Methacholine Responsiveness in 2-to5-year-old Asthmatic Children. *Am J Respir Crit Care Med*. 2000; 162(4): 1500-1506.
14. Biermer L. The role of small airway disease in asthma. *Curr Opin Pulm Med*. 2014; 20(1): 23-30.
15. Sandrini A, Taylor DR, Thomas PS, et al. Fractional exhaled nitric oxide in asthma: an update. *Respir*. 2010; 15(1): 57-70.

16. Contoli M, Santus P, Papi A. Small airway disease in asthma: pathophysiological and diagnostic considerations. *Curr Opin Pulm Med*. 2015; 21(1): 68-73.
17. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J*. 2005; 26(2): 319-338.
18. Pellegrino R, Viegi G, Brusasco V, et al. Interpretative strategies for lung function tests. *Eur Respir J*. 2005; 26(5): 948-968.
19. Oostveen E, MacLeod D, Lorino H et al. The forced oscillation technique in clinical practice: methodology, recommendations and future developments. *Eur Respir J*. 2003; 22(6): 1026-1041.
20. Dweik RA, Boggs PB, Erzurum SC, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FeNO) for clinical application. *Am J Respir Crit Care Med*. 2011; 184(5): 602-615.
21. Bjermer L. History and future perspectives of treating asthma as a systemic and small airways disease. *Respir Med*. 2001; 95(9): 703-719.
22. van der Wiel E, Postma DS, van der Molen T, et al. Effects of small airway dysfunction on the clinical expression of asthma: a focus on asthma symptoms and bronchial hyper-responsiveness. *Allergy*. 2014; 69(12): 1681-1688.
23. Shi Y, Aledia AS, Tatavosian AV, et al. Relating small airways to asthma control by using impulse oscillometry in children. *J Allergy Clin Immunol*. 2012; 129(3): 671-678.
24. Lie MK, Christodoulopoulos P, Hamid Q. Small airway inflammation in asthma. *Respir Res J*. 2001; 2(6): 333 -339.
25. van der Wiel E, ten Hacken NH, et al: Small-airways dysfunction associates with respiratory symptoms and clinical features of asthma: a systematic review. *J Allergy Clin Immunol*. 2013; 131(3): 646-657.
26. Balzar S, Wenzel SE, Chu HW. Transbronchial biopsy as a tool to evaluate small airways in asthma. *Eur Respir J*. 2002; 20(2): 254-259.
27. Sunyer J, Jarvis D, Pekkanen J, et al. Geographic variations in the effect of atopy on asthma in the European Community Respiratory Health Study. *J Allergy Clin Immunol*. 2004; 114(5): 1033-1039.

28. Patelis A, Gunnbjörnsdóttir M, Malinovschi A, et al. Population-based study of multiplexed IgE sensitization in relation to asthma, exhaled nitric oxide, and bronchial responsiveness. *J Allergy Clin Immunol*. 2012; 130(2): 397-402.e2.
29. Klug B, Bisgaard H. Measurement of lung function in awake 2-4 year-old asthmatic children during methacholine challenge and acute asthma: a comparison of the Impulse Oscillation Technique, the Interrupter Technique, and Transcutaneous Measurement of Oxygen versus Whole-Body Plethysmography. *Pediatric Pulmonology*. 1996; 21(5):290-300.
30. Lipworth B. Targeting the small airways asthma phenotype: if we can reach it, should we treat it? *Ann Allergy Asthma Immunol*. 2013; 110(4): 233-239.
31. Goldman MD, Saadeh C, Ross D. Clinical applications of forced oscillation to assess peripheral airway function. *Respir Physiol Neurobiol*. 2005; 148(1-2):179-194.
32. Takeda T, Oga T, Niimi A, Matsumoto H, et al. Relationship between small airway function and health status, dyspnea and disease control in asthma. *Respiration*. 2010; 80(2): 120-126.
33. Bikov A, Pride NB, Goldman MD, et al. Glottal aperture and buccal airflow leaks critically affect forced oscillometry measurements. *Chest*. 2015 ; 148(3): 731-738.
34. Price OJ, Ansley L, Bikov A, et al. The role of impulse oscillometry in detecting airway dysfunction in athletes. *J Asthma*. 2016; 53(1): 62-68.
35. Evans TM, Rundell KW, Beck KC, et al. Airway narrowing measured by spirometry and impulse oscillometry following room temperature and cold temperature exercise. *Chest*, 2005; 128(4): 2412-2419.
36. Evans TM, Rundell KW, Beck KC, et al. Impulse oscillometry is sensitive to bronchoconstriction after eucapnic voluntary hyperventilation or exercise. *J Asthma* 2006; 43(1): 49-55.
37. Nakagawa M, Hattori N, Haruta Y, et al. Effect of increasing respiratory rate on airway resistance and reactance in COPD patients. *Respirology*. 2015; 20(1): 87-94.
38. Usmani OS. Treating the small airways. *Respiration*. 2012; 84(6): 441-453.
39. De Backer W, Devolder A, Poli G, et al. Lung deposition of BDP/formoterol HFA pMDI in healthy volunteers, asthmatic, and COPD patients. *J Aerosol Med Pulm Drug Deliv* 2010; 23(3): 137-148.

40. Huchon G, Magnussen H, Chuchalin A, et al. Lung function and asthma control with beclomethasone and formoterol in a single inhaler. *Respir Med.* 2009; 103(1): 41-49.
41. Reddel HK, Gibson PG, Peters MJ, et al. Down-titration from high-dose combination therapy in asthma: removal of long-acting beta(2)-agonist. *Respir Med.* 2010; 104(8): 1110-1120.
42. Barnes N, Price D, Colice G, et al. Asthma control with extrafine-particle hydrofluoroalkane-beclomethasone vs. large-particle chlorofluorocarbon-beclomethasone: a real-world observational study. *Clin Exp Allergy.* 2011; 41(11): 1521-1532.
43. Colice G, Martin RJ, Israel E, et al. Asthma outcomes and costs of therapy with extrafine beclomethasone and fluticasone. *J Allergy Clin Immunol.* 2013; 132(1): 45-54.

Tables

Table 1 Characteristics of patients

	Small airway normal function	Small airway dysfunction	P-value
Number of patients	69	71	-
Age(y)	35.62±13.67	35.13±16.41	0.846
Sex(M/F)	32/37	35/36	0.878
FEV1%pred	84.41±3.44	84.13±3.76	0.634

FEV1/FVC (%)	81.75±5.16	81.05±5.54	0.433
FEF50%pred	88.82±3.36	56.31±12.74	<0.001
FEF75%pred	90.45±5.38	57.91±12.35	<0.001
FEF25-75%pred	87.51±7.51	52.64±13.08	<0.001
FeNO (ppb)	34.46±19.60	54.87±13.21	<0.001
IgE(IU/ml)	86.61±46.85	246.89±96.73	<0.001
EOS (10 ⁹ /L)	0.59±0.15	0.70±0.21	0.002
R5[kPa/(1/S)]	0.34±0.08	0.61±0.20	<0.001
R5-R20[kPa/(1/S)]	0.04±0.02	0.30±0.11	<0.001
AX(kPa /l)	1.17±0.74	2.08±0.62	<0.001
Fres(1/S)	12.93±5.59	19.82±5.40	<0.001

FEV1(%pred), forced expiratory volume in one second; FEV1/FVC, the ratio of forced expiratory volume in one second and forced vital capacity ; 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; FEF25-75(%pred), mid forced expiratory flow of percentages of predicted values; FeNO, fractional exhaled nitric oxide; IgE, immunoglobulin E; EOS, eosinophil; R5, respiratory resistance at 5 Hz; R5-R20, the difference between respiratory resistance at 5 Hz and 20 Hz; AX, reactance area ; Fres, the resonant frequency.

Table 2 Multiple regression statistics for FEF25-75(%pred)

	Unstandardized	SE	Standardized	T -	P-	Co-linearity	Statistics
	coefficient		coefficient	value	value	Tolerance	VIF
Age(y)	0.003	0.045	0.004	0.072	0.943	0.853	1.172
Sex(M/F)	0.482	1.460	0.180	0.330	0.743	0.879	1.137
FEV1%pred	-0.091	0.211	-0.026	-0.433	0.667	0.746	1.340
FEV1/FVC (%)	0.081	0.155	0.034	0.523	0.603	0.637	1.570
FEF50%pred	-0.001	0.067	-0.001	-0.009	0.993	0.643	1.554
FEF75%pred	0.019	0.073	0.018	0.265	0.792	0.572	1.748
FeNO (ppb)	-0.403	0.103	-0.407	-3.906	0.000	0.255	3.929
IgE(IU/ml)	-0.009	0.008	-0.069	-1.142	0.258	0.756	1.323
EOS (10 ⁹ /L)	-5.314	4.644	-0.087	-1.144	0.257	0.475	2.105

R5[kPa/(1/s)]	-2.706	5.824	-0.049	-0.465	0.644	0.250	3.993
R5-R20[kPa/(1/s)]	9.106	7.893	0.093	1.154	0.253	0.425	2.355
AX(kPa /l)	-7.620	2.486	-0.363	-3.065	0.003	0.196	5.091
Fres(1/s)	-0.391	0.205	-0.161	-2.442	0.017	0.385	2.599

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

Table 3 The area under ROC curve on FeNO, AX, Fres and EOS

	AUC	SE	P	95%IC
FeNO (ppb)	0.830	0.035	<0.001	0.758-0.889
AX (kPa /l)	0.822	0.036	<0.001	0.749-0.882
Fres (1/s)	0.816	0.036	<0.001	0.741-0.876
EOS (10 ⁹ /L)	0.673	0.045	<0.001	0.589-0.750
FeNO, AX and Fres	0.881	0.029	<0.001	0.815-0.929

AUC, area under the curve; SE, standard error; 95%IC, confidence interval of 95%

FeNO, fractional exhaled nitric oxide; Fres, the resonant frequency; AX, reactance area EOS, eosinophil.

Table 4 Criterion, sensitivity and specificity of different biomarkers to diagnose small airway dysfunction in asthma.

	Cut-point	Sensitivity (%)	Specificity (%)	likelihood criterion
FeNO (ppb)	38	90.14	72.46	7.35
AX (kPa /l)	1.07	95.77	60.87	2.45
Fres (1/s)	12.65	94.37	57.97	2.25
EOS (10 ⁹ /L)	0.55	61.97	71.01	2.14

FeNO, fractional exhaled nitric oxide; Fres, the resonant frequency; AX, reactance area EOS, eosinophil.

Figure legends

Figure 1. Correlation between FeNO and other indicators in small airway dysfunction group.

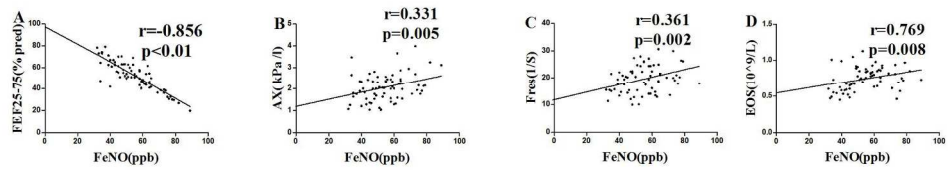
FeNO negatively correlated with FEF25-75(%pred) ($r=-0.856$, $p<0.01$) (A), while had positive correlations with AX, Fres and EOS ($r=0.331$, $p=0.005$; $r=0.361$, $p=0.002$; $r=0.769$, $p=0.008$, respectively). (B, C, D).

Figure 2. Correlation between levels of AX, Fres, EOS and FEF25-75(%pred)

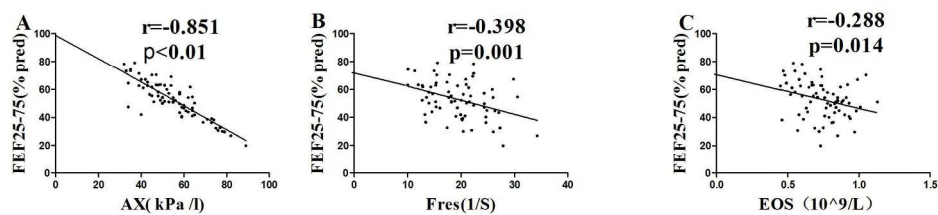
There were negative correlations between AX, Fres, EOS and FEF25-75(%pred) ($r=-0.851$, $p<0.01$; $r=-0.398$, $p=0.001$; $r=-0.288$, $p=0.014$, respectively) .(A, B, C).

Figure 3. Receiver operating characteristic curve analysis of FeNO, AX, Fres and EOS for the diagnosis of small airway dysfunction in asthma.

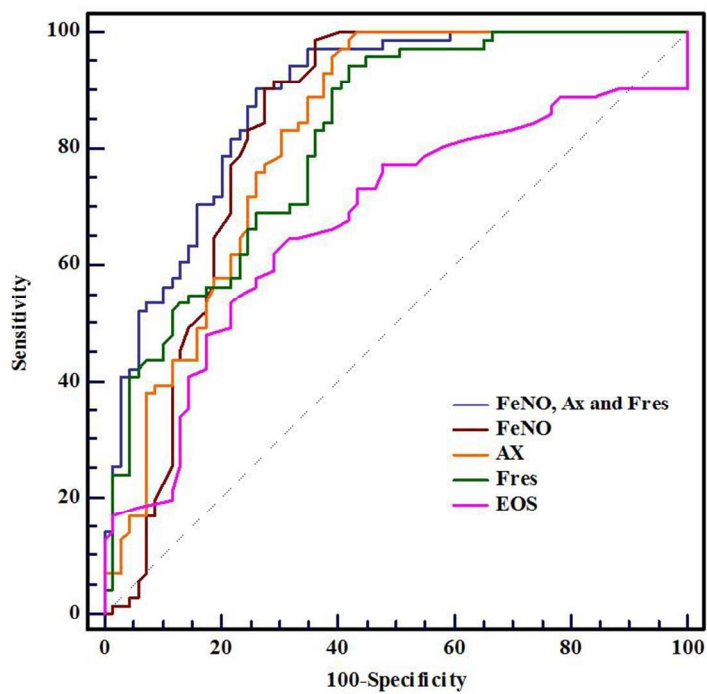
Accepted Article



182x39mm (300 x 300 DPI)



190x50mm (300 x 300 DPI)



282x211mm (96 x 96 DPI)

Accept