Journal of Asthma and Allergy

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Small-Airway Function Variables in Spirometry, Fractional Exhaled Nitric Oxide, and Circulating Eosinophils Predicted Airway Hyperresponsiveness in Patients with Mild Asthma

Wuping Bao[1,*]
Xue Zhang[1,*]
Junfeng Yin[2,*]
Lei Han[1,*]
Zhixuan Huang[2]
Luhong Bao[1]
Chengjian Lv[1]
Huijuan Hao[1]
Yishu Xue[1]
Xin Zhou[1]
Min Zhang[1]

[1]Department of Respiratory and Critical Care Medicine, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, People's Republic of China; [2]School of Mathematical Sciences, Tongji University, Shanghai, People's Republic of China

*These authors contributed equally to this work

Correspondence: Min Zhang
Department of Respiratory and Critical Care Medicine, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, 100 Haining Road, Hongkou District, Shanghai, 200080, People's Republic of China
Tel +86 21 63071428
Email maggie_zhangmin@163.com

Purpose: Patients with variable symptoms suggestive of asthma but with normal forced expiratory volume in 1 second (FEV_1) often fail to be diagnosed without a bronchial provocation test, but the test is expensive, time-consuming, risky, and not readily available in all clinical settings.

Patients and Methods: A cross-sectional study was performed in 692 patients with $FEV_1 \geq 80\%$ predicted; normal neutrophils and chest high-resolution computed tomography; and recurrent dyspnea, cough, wheeze, and chest tightness.

Results: Compared with subjects negative for AHR (n=522), subjects positive for AHR (n=170) showed increased FENO values, peripheral eosinophils (EOS), and R5-R20; decreased FEV_1, FEV_1/Forced vital capacity (FVC), and forced expiratory flow (FEFs) ($P \leq .001$ for all). Small-airway dysfunction was identified in 104 AHR^+ patients (61.17%), and 132 AHR^- patients (25.29%) ($P<0.001$). The areas under the curve (AUCs) of variables used singly for an AHR diagnosis were lower than 0.77. Using joint models of $FEF_{50\%}$, $FEF_{75\%}$, or $FEF_{25\%-75\%}$ with FENO increased the AUCs to 0.845, 0.824, and 0.844, respectively, significantly higher than univariate AUCs (P <0.001 for all). Patients who reported chest tightness (n=75) had lower FEFs than patients who did not ($P<0.001$ for all). In subjects with chest tightness, the combination of $FEF_{50\%}$ or $FEF_{25\%-75\%}$ with EOS also increased the AUCs substantially, to 0.815 and 0.816, respectively (P <0.001 for all versus the univariate AUCs).

Conclusion: FENO combined with $FEF_{50\%}$ and $FEF_{25\%-75\%}$ predict AHR in patients with normal FEV_1. $FEF_{25\%-75\%}$, $FEF_{50\%}$, or $FEF_{25\%-75\%}$ together with EOS also can potentially suggest asthma in patients with chest tightness.

Keywords: asthma diagnosis, small-airway function, fractional exhaled nitric oxide, bronchial provocation, impulse oscillometry

Plain Language Summary

Why was the study done?

Patients with variable symptoms suggestive of asthma but with normal forced expiratory volume in 1 second (FEV_1, reflect large airway function) often fail to be diagnosed without a bronchial provocation test. But the test is expensive, time-consuming, risky, and not readily available in all clinical settings. Thus, we investigated the predictive values of simple and convenient tests, such as spirometry, fractional exhaled nitric oxide (FENO), and circulating eosinophils for predicting airway hyperresponsiveness (AHR) in patients with mild asthma.

What did the researchers do and find?

We performed a retrospective cross-section study, enrolled 692 patients with normal large airway function ($FEV_1 \geq 80\%$ predicted) and recurrent dyspnea, cough, wheeze, or chest tightness. We find that small airway dysfunction ($FEF_{25\%-75\%}$ and $FEF_{50\%}$) do exist in patients with normal FEV_1 values but had typical asthmatic symptoms, and $FEF_{25\%-75\%} < 84.4\%$, or $FEF_{50\%} < 76.8\%$ combine with FENO > 41 ppb predict positive AHR. FEFs combined with circulating eosinophils also can predict AHR effectively in patients with chest tightness.

What do these results mean?

Combining $FEF_{50\%}$ or $FEF_{25\%-75\%}$ with FENO could forego bronchial provocation test to support asthma diagnosis in patients with normal FEV_1 and symptoms suggestive of asthma. $FEF_{25\%-75\%}$, $FEF_{50\%}$, or $FEF_{25\%-75\%}$ together with circulating eosinophils also can potentially suggest asthma in patients with chest tightness.

Introduction

Bronchial asthma (asthma) is a chronic inflammatory airway disease affecting the entire bronchial tree from the large to the small airways (<2 mm diameter).[1] The diagnosis is based on recurrent symptoms of dyspnea, cough, wheeze, and chest tightness, as well as reversible airway limitation or airway hyperresponsiveness (AHR).[2] Mild asthma is the most common type of asthma, often has normal forced expiratory volume in 1 second (FEV_1), representing 50% to 75% of all asthma patients.[3] In nearly 90% of the asthmatic patients with normal FEV_1 in china (Min Zhang, unpublished paper), the bronchial dilation test is negative, so the bronchial provocation test (BPT) is important for confirming or excluding asthma after detailed medical history and physical examination. However, many hospitals do not perform provocation tests because they are expensive, time-consuming, and entail a risk of severe bronchospasm.[2] What's more, pulmonary function testing should be performed in patients with chronic airway disease during COVID-19 pandemic only if it is needed to guide management and limited to the necessary tests, which usually do not include BPT. Therefore, additional ways are needed to predict AHR safely and to detect patients with mild asthma as early as possible in order to relieve their symptoms and prevent the development of chronic inflammation and airway remodeling.

Small-airway dysfunction exists in mild asthma patient, evaluated by a variety of spirometry and IOS measurements. Lower forced expiratory flow between 25% and 75% ($FEF_{25\%-75\%}$) and Forced expiratory flow at 50% of forced vital capacity ($FEF_{50\%}$) are widely used for assessing small-airway function.[4–6] Impulse oscillometry (IOS) may also reliably reflect small-airway function and predict clinical asthma outcomes and AHR.[7–11] FEV in 3 seconds (FEV_3)/FVC is influenced by the airflow velocity in both the central and peripheral airway, normally 95% or greater in adults.[12–14]

In our previous study of patients with chronic cough, patients with AHR had higher fractional exhaled nitric oxide (FENO), a higher percentage of eosinophils in blood (EOS%), and $FEF_{25\%-75\%}$ than patients without AHR.[4] The combination of $FEF_{25\%-75\%}$ and FENO increased the area under the curve (AUC) for AHR diagnosis substantially compared with FENO alone. In that study, $FEF_{25\%-75\%} < 78.5\%$ and FENO > 43 ppb strongly predicted positive AHR in Chinese patients with chronic cough.

In the current study, we used FEFs, peripheral airway resistance as the difference between 5 and 20 Hz (R5-R20), reactance at 5 Hz (X5), resonant frequency (Fres), FEV_3/FVC, FENO, EOS, and EOS% alone or in combination, as potential predictive variables for the presence of AHR, and extended our cross-sectional study to patients with $FEV_1 \geq 80\%$ predicted who had more than 1 typical symptom of asthma, such as variable cough, dyspnea, wheeze, and chest tightness, to confirm the predictive value of small-airway function tests, FENO, and EOS for AHR.

Patients and Methods

Study Design and Subject Selection

A retrospective cross-section of diagnostic data was collected at the initial visit of adult patients with recurrent variable symptoms of dyspnea, cough, wheeze, or chest tightness of at least 8 weeks' duration who were referred to the Pulmonary Outpatient Clinic of Shanghai General Hospital (China). The patients had to undergo a peripheral blood test, spirometry,[15] FENO measurement (NIOX MINO, Aerocrine AB, Solna, Sweden),[11] methacholine challenge testing (MCT), and high-resolution computerized tomography (HRCT, GE Medical System; slice thickness 0.625 mm) from September 2016 to January 2020. The MCT was performed with a Jaeger APS Pro system using a Medic-Aid sidestream nebulizer and doubling doses of methacholine (0.015 to 0.48 mg) following the American Thoracic Society/European Respiratory Society recommendations.[16]

Additional inclusion criteria were age 18–75 years, normal HRCT results, and predicted FEV_1 of 80% or greater with spirometric measurement.

Subjects were excluded if they had had respiratory tract infections in the past 8 weeks; peripheral blood test indicating abnormal hemoglobin, platelets, or neutrophils; use of montelukast, long-acting β_2-agonists, theophylline, anticholinergic agents, or an inhaled or oral corticosteroid in the previous 4 weeks; or having concomitant severe systemic diseases. Patients who had more than a 10 pack-year smoking history, who currently smoked, or who had quit less than 2 years earlier were also excluded.

Descriptive characteristics, clinical history, results of MCT, spirometry, FENO measurement, IOS (Jaeger Co, Hochberg, Germany),[17] EOS, and EOS% were reviewed and analyzed. The FVC, FEV_1, peak expiratory flow (PEF), $FEF_{25\%}$, $FEF_{50\%}$, $FEF_{75\%}$, and $FEF_{25\%-75\%}$ were expressed as percentages of predicted values. FEV_1/FVC and FEV_3/FVC were expressed as the ratios of the absolute values of the variables. Small-airway dysfunction was identified if 2 of $FEF_{50\%}$, $FEF_{75\%}$, and $FEF_{25\%-75\%}$ were lower than 80%. IOS variables R5, R20, R5-R20, X5, and Fres were also collected. For bronchial provocation tests, the provocative dose causing a 20% fall in FEV_1 (PD_{20}) was recorded, and AHR was defined as present if PD_{20} was ≤0.48 mg. Associations of PD_{20} and FENO, EOS, EOS%, FEFs, and IOS were analyzed in patients positive for AHR.

The ethics committee of Shanghai General Hospital, Shanghai Jiao Tong University, approved the protocol. It is a retrospective cross-section study, and the exemption of informed consent will not affect subjects' rights and welfare. Besides, it is very difficult to contact all the patients of our study. Thus, a waiver of informed consent was given for our study (number 2017KY159). We confirm that the patient data was maintained with confidentiality and complied with the Declaration of Helsinki.

Statistical Analysis

Analyses were performed with SPSS software version 19.0 (SPSS Inc, Chicago, Illinois, USA), except for the ROC contrast estimation, ROC contrast test, and 80/20 split-sample cross-validation, which were performed with SAS Proc LOGISTIC version 9.4 (SAS Institute Inc., Cary, NC, USA). Normality of distribution was checked with the Kolmogorov–Smirnov test. Normally distributed data were expressed as mean ± standard deviation (SD) or 95% Confidence interval (CI). Non-normally distributed data were expressed as median and interquartile ranges (IQR). The coefficient of variance (CV) was calculated. Fisher's exact test (discontinuous variables) and the Mann–Whitney test (continuous variables) were performed for the intergroup comparisons. The association between different variables was decided by Spearman correlation.

The prediction performance of each variable was measured as the AUC of the ROC. Furthermore, a multiple logistic model of the 2 variables was fitted, and the resultant AUC of this multiple logistic model was used as a measure of the joint prediction performance. Chi-square test proposed by DeLong et al was used to determine whether the multiple logistic model would significantly improve the prediction performance, defined as the AUC, relative to the marginal models.[18]

MCT was used as the gold standard for defining AHR. The optimal value of the single measurement giving the highest sum of AHR diagnostic sensitivity and specificity was used as a cut-off value.[19,20] Positive predictive values (PPV), negative predictive values (NPV), and percentages correctly classified (PCC) were calculated for each cut-off value.[21,22] The corresponding odds ratios, CI, and *P* values were also calculated.

Continuous variables were converted to dichotomous-state variables on the basis of the cut-off values. Subsequently, ROC curves were determined for the joint models with the dichotomous-state variables.

We constructed and examined all models to predict AHR with repeated five-fold cross-validation (5 repeats). The average AUC of 5 different cross-validation models and the whole-model AUC using the entire data set were calculated. The Error Rate equals abs (Average AUC – Whole Model AUC)/(Whole Model AUC). Accurate classification was also calculated for the test subset.

The threshold for statistical significance for all analyses was set at $P < 0.05$.

Results

Baseline Characteristics

Clinical data from 692 adults were ultimately included. Bronchial provocation tests were positive in 170 patients and negative in 522 patients. Baseline demographics categorized by bronchial provocation test positivity are shown

in Table 1. There were no significant differences in age, sex, smoking history, or body mass index (BMI) between the 2 groups. Subjects with AHR had lower percent predicted of FEV_1, FEV_1/FVC, FEV_3/FVC, and PEF ($P \leq 0.001$ for all). All FEF values, alone or as ratios of their predicted value to that of FEV_1 predicted value, were significantly lower in subjects with AHR than without AHR ($P < 0.001$ for all).

Significantly higher levels of R5-R20 and Fres, lower level of X5 were observed in the AHR group ($P < 0.001$ for all). Furthermore, FENO values, EOS, and EOS% were also dramatically higher in the AHR group ($P < 0.001$ for all) (Table 1).

Mean values of FEV_1, FEV_1/FVC, $FEF_{50\%}$, $FEF_{75\%}$ and $FEF_{25\%-75\%}$ were lower in subjects with chest tightness (n = 75) compared with those without this symptom ($P = 0.009$, 0.008, 0.003, <0.001, and 0.002, respectively). FENO values, EOS, and EOS% values were higher in subjects with than without chest tightness ($P = 0.012$, 0.001, 0.003, and 0.014, respectively).

Correlation Between PD_{20} and FENO, EOS, EOS%, FEFs, and R5-R20

FENO, $FEF_{25\%}$, $FEF_{50\%}$, $FEF_{75\%}$, and $FEF_{25\%-75\%}$ were weakly correlated with PD_{20} by Spearman analysis (Table 2). No significant correlation between PD_{20} and FEV_3/FVC, EOS, EOS%, R5-R20, X5, or Fres was found. There was no difference in PD_{20} between subjects with chest tightness (0.065; IQR, 0.220) and those without this symptom (0.089; IQR, 0.349; $P = 0.764$).

Diagnostic Accuracy of Single Variables Used for Predicting AHR

We created ROC curves to evaluate the ability of many of the variables to predict positive AHR. In the spirometry

Table 1 Demographic Data, Spirometric Variables, IOS Variables, and Values for FENO and Peripheral Eosinophils of Patients with Positive or Negative Bronchial Provocation Tests

Variables	CV for All Subjects (%)	Positive Bronchial Provocation Test	CV for BPT^+ (%)	Negative Bronchial Provocation Test	CV for BPT^- (%)	*P* value
n	-	170	-	522	-	-
Age, years†	33.79	43.90 (14.56)	33.17	43.80 (14.90)	34.02	0.980
Male, n (%)	-	53 (31.18%)	-	203 (38.89%)	-	0.082
BMI, kg/m^2†	14.88	22.94 (2.99)	13.01	23.51 (3.61)	15.37	0.075
Past smoking history (n/%)	-	31 (18.24)	-	97 (18.58)	-	0.204
FVC, % predicted†	11.82	101.10 (10.85)	10.73	101.60 (12.36)	12.17	0.993
FEV_1, % predicted†	**11.95**	**99.33 (11.01)**	**11.08**	**105.50 (12.49)**	**11.84**	**< 0.001**
FEV_1/FVC, %†	**6.72**	**82.55 (5.51)**	**6.67**	**86.87 (5.45)**	**6.28**	**< 0.001**
FEV_3/FVC, %†§	**1.98**	**97.65 (2.26)**	**2.31**	**98.47 (1.76)**	**1.79**	**0.001**
PEF, % predicted†	**16.27**	**90.61 (13.93)**	**15.38**	**96.21 (15.65)**	**16.27**	**< 0.001**
$FEF_{25\%}$, % predicted†	**21.63**	**90.08 (15.87)**	**17.61**	**100.80 (22.10)**	**21.92**	**< 0.001**
$FEF_{50\%}$, % predicted†	**25.55**	**77.26 (17.74)**	**22.96**	**98.20 (23.24)**	**23.66**	**< 0.001**
$FEF_{75\%}$, % predicted†	**31.84**	**74.91 (22.43)**	**29.94**	**101.20 (60.72)**	**29.62**	**< 0.001**
$FEF_{25\%-75\%}$, % predicted†	**25.51**	**75.95 (17.64)**	**23.23**	**97.01 (22.76)**	**23.46**	**< 0.001**
Small-airway dysfunction	-	**104**	-	**132**	-	**< 0.001**
R5-R20, kPa·L^{-1}·s†¶	**94.65**	**0.13 (0.11)**	**88.85**	**0.10 (0.09)**	**95.50**	**< 0.001**
X5, kPa·L^{-1}·s†¶	**97.54**	**−0.12 (0.10)**	**107.93**	**−0.10 (0.08)**	**87.30**	**< 0.001**
Fres, L^{-1}·s†¶	**42.52**	**17.53 (9.02)**	**51.47**	**14.24 (5.03)**	**35.29**	**< 0.001**
FENO, ppb‡	**94.96**	**59 (28–98)**	**75.03**	**24 (15–45)**	**86.65**	**< 0.001**
EOS in blood, % ‡	**95.46**	**5.16 (1.70–7.73)**	**84.47**	**3.37 (1.20–4.40)**	**96.58**	**< 0.001**
EOS in blood, cells/μL‡	**106.10**	**370.6 (120–530)**	**91.90**	**237.2 (80–302.5)**	**108.97**	**< 0.001**
PD_{20}, mg‡	130.68	0.079 (0.026- 0.369)	130.68	–	–	–

Notes: Small-airway dysfunction was identified if 2 of the variables $FEF_{50\%}$, $FEF_{75\%}$ and $FEF_{25\%-75\%}$ were lower than 80%. †Mean (SD) values. ‡Median (IQR) values. § n = 333, 92 for positive bronchial provocation, 241 for negative bronchial provocation. ¶ n = 619, 153 for positive bronchial provocation, 466 for negative bronchial provocation. Bold font indicates statistical significance.

Abbreviations: IOS, impulse oscillometry; FENO, fractional exhaled nitric oxide; CV, Coefficient of variance; BPT^+, positive bronchial provocation test; BPT^-, negative bronchial provocation test; BMI, body mass index; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 second; FEV_3, FEV in 3 seconds; PEF, peak expiratory flow; $FEF_{25\%}$, forced expiratory flow at 25% of FVC; $FEF_{50\%}$, FEF at 50% of FVC; $FEF_{75\%}$, FEF at 75% of FVC; $FEF_{25\%-75\%}$, FEF at 25% to 75% of FVC; EOS, eosinophils; ppb, parts per billion; PD_{20}, provocative dose causing a 20% fall in FEV in the first second; R5, total airway resistance at 5 Hz; R20, central airway resistance at 20 Hz; R5-R20, peripheral airway resistance as the difference between 5 and 20 Hz; X5, reactance at 5 Hz; Fres, resonant frequency.

Table 2 Spearman Correlation Between PD_{20} and Other Variables in BPT^+ Subjects

	N	r	P	95% CI
FENO	**170**	**–0.266**	**0.0005**	**–0.404 ~ –0.116**
EOS	170	–0.054	0.481	–0.208 ~ 0.101
EOS%	170	–0.039	0.609	–0.193 ~ 0.116
$FEF_{25\%}$	**170**	**0.272**	**0.0003**	**0.122 ~ 0.409**
$FEF_{50\%}$	**170**	**0.213**	**0.005**	**0.060 ~ 0.356**
$FEF_{75\%}$	**170**	**0.173**	**0.024**	**0.018 ~ 0.319**
$FEF_{25\%-75\%}$	**170**	**0.229**	**0.003**	**0.077 ~ 0.371**
FEV_3/FVC	92	–0.016	0.881	–0.225~ 0.196
R5-R20	153	–0.026	0.754	–0.188 ~ 0.138
X5	153	0.115	0.158	–0.050 ~ 0.273
Fres	153	–0.064	0.433	–0.226 ~ 0.101

Note: Bold font indicates statistical significance.

Abbreviations: PD_{20}, provocative dose causing a 20% fall in FEV in the first second; BPT^+, positive bronchial provocation test; CI, confidence interval; FENO, fractional exhaled nitric oxide; EOS, eosinophils; $FEF_{25\%}$, forced expiratory flow at 25% of FVC; $FEF_{50\%}$, FEF at 50% of FVC; $FEF_{75\%}$, FEF at 75% of FVC; $FEF_{25\%-75\%}$, FEF at 25% to 75% of FVC; FEV_3, FEV in 3 seconds; FVC, forced vital capacity; R5-R20, peripheral airway resistance as the difference between 5 and 20 Hz; X5, reactance at 5 Hz; Fres, resonant frequency.

measurements, the 2 largest AUCs for a positive AHR diagnosis were 0.763 for $FEF_{25\%-75\%}$ and 0.762 for $FEF_{50\%}$ (Table 3). The FEV_1/FVC, EOS%, EOS, and IOS measurements did not give high AUCs for positive AHR diagnosis.

In patients with chest tightness, the AUCs for positive AHR diagnosis were $FEF_{50\%}$ 0.751 (95% CI, 0.637–0.864), $FEF_{75\%}$ 0.812 (95% CI, 0.708–0.916), $FEF_{25\%-75\%}$ 0.763 (95% CI, 0.651–0.875), FENO 0.731 (95% CI, 0.607–0.855), and EOS 0.706 (95% CI, 0.580–0.832).

Diagnostic Accuracy of Small-Airway Function Variables Combined with FENO and Internal Cross-Validation of the Final Models

To determine whether combining measurements would improve AHR prediction, we repeated the ROC analyses for spirometry measurements combined with FENO. The AUC of $FEF_{50\%}$ combined with FENO was 0.845 (95% CI, 0.812–0.878), which was significantly higher than the AUC of univariate $FEF_{50\%}$ ($P < 0.001$) (Table 4 and Figure 1). Similarly, the other spirometry measurements also had higher AUCs when combined with FENO than they did alone (Table 4) NPV was ≥85.45% for all of the combinations.

We then transformed the continuous variables into binary variables according to the cut-off values shown in the tables and reanalyzed the mentioned above ROC curves. The AUCs of $FEF_{50\%}$ and $FEF_{25\%-75\%}$ combined with FENO remained high (Figure 2).

Table 3 Optimal Cut-Off Values and Other Measures of Usefulness for Predicting Airway Hyperresponsiveness

Characteristic Variables	AUC	Cut-Off Values[†]	Sensitivity %	Specificity %	PPV %	NPV %	PCC %	Odds Ratio	95% CI	P value
FEV_1, % predicted	0.649	95.8	47.65	75.86	39.13	81.65	68.93	0.955	(0.939, 0.971)	< 0.001
FEV_1/FVC, %	**0.713**	**84.67**	**66.47**	**67.62**	**40.07**	**86.10**	**67.34**	**0.867**	**(0.837, 0.898)**	**< 0.001**
FEV_3/FVC, %	0.616	99.06	67.39	51.87	34.83	80.65	56.16	0.817	(0.726, 0.920)	< 0.001
$FEF_{25\%}$, % predicted	0.656	103.8	84.12	41.38	31.85	88.89	51.88	0.973	(0.963, 0.982)	< 0.001
$FEF_{50\%}$, % predicted	**0.762**	**76.8**	**58.82**	**80.46**	**49.50**	**85.71**	**75.14**	**0.950**	**(0.939, 0.960)**	**< 0.001**
$FEF_{75\%}$, % predicted	**0.745**	**81.4**	**66.47**	**69.92**	**41.85**	**86.49**	**69.08**	**0.963**	**(0.955, 0.971)**	**< 0.001**
$FEF_{25\%-75\%}$, % predicted	**0.763**	**84.4**	**70.00**	**68.97**	**42.35**	**87.59**	**69.22**	**0.950**	**(0.940, 0.960)**	**< 0.001**
R5-R20, kPa·L^{-1}·s	0.604	0.88	0.65	100.00	100.00	75.40	75.44	18.961	(3.119, 115.3)	0.001
X5, kPa·L^{-1}·s	0.607	–0.14	43.79	78.76	40.36	81.02	70.11	0.025	(0.003, 0.223)	0.001
Fres, L^{-1}·s	0.634	15.71	56.95	68.32	36.91	82.98	65.53	1.081	(1.046, 1.117)	< 0.001
FENO, ppb	**0.748**	**41**	**65.29**	**78.16**	**49.33**	**87.37**	**75.00**	**1.024**	**(1.019, 1.030)**	**< 0.001**
EOS in blood, %	0.630	3.4	55.88	66.28	35.06	82.19	63.73	1.130	(1.079,1.182)	< 0.001
EOS in blood, cell/μL	0.638	360	41.76	80.65	41.28	80.96	71.10	4.367	(2.413,7.903)	< 0.001
Platelets, ×10^9/L	0.491	269	80.00	24.71	25.71	79.14	38.30	0.995	(0.995,0.996)	< 0.001

Abbreviations: AUC, area under the curve; PPV, positive predictive values; NPV, negative predictive values; PCC: percentages correctly classified; Odds ratio, odds ratio of characteristic variables for predicting AHR; 95% CI, 95% confidence interval of odds ratio; *P* value, the *p* value of the logistic regression test. FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; $FEF_{25\%}$, forced expiratory flow at 25% of FVC; $FEF_{50\%}$, FEF at 50% of FVC; $FEF_{75\%}$, FEF at 75% of FVC; $FEF_{25\%-75\%}$, FEF at 25% to 75% of FVC; R5-R20, peripheral airway resistance as the difference between 5 and 20 Hz; X5, reactance at 5 Hz; Fres, resonant frequency; FENO, fractional exhaled nitric oxide; EOS, eosinophils.

Notes:[†]The cut-off points were selected by maximizing the sum of sensitivity and specificity. Bold font indicates AUC higher than 0.7.

Table 4 Predictive Values of the Combination of Different Variables with FENO in Predicting Airway Hyperresponsiveness

Characteristic Variables	AUC	95% CI of AUC	Sensitivity %	Specificity %	PPV %	NPV %	PCC %	Contrast	95% CI of Contrast	*P* value
FENO + $FEF_{50\%}$	0.845	(0.812,0.878)	83.53	71.65	48.97	93.03	74.57	0.097†	(0.060,0.135)	< 0.001
FENO + $FEF_{75\%}$	0.824	(0.788,0.859)	72.94	77.01	50.82	89.73	76.01	0.076‡	(0.038,0.114)	< 0.001
FENO + $FEF_{25\%-75\%}$	0.844	(0.811,0.876)	80.59	74.14	50.37	92.14	75.72	0.096†	(0.058,0.134)	< 0.001
FENO + FEV_1/FVC	0.807	(0.769,0.844)	85.88	61.69	42.20	93.06	67.63	0.059‡	(0.026,0.092)	0.001

Notes: We used the larger of the 2 univariate AUCs to make the comparison; †Contrast, the difference between AUC of FENO and AUC of bivariate model; ‡Contrast, the difference between AUC of FEFs and AUC of bivariate model; *P* value, contrast's chi-square test for the significance of the contrast.

Abbreviations: FENO, fractional exhaled nitric oxide; AUC, area under the curve; 95% CI, 95% confidence interval of odds ratio; PPV, positive predictive values; NPV, negative predictive values; PCC, percentages correctly classified; $FEF_{25\%}$, forced expiratory flow at 25% of FVC; $FEF_{50\%}$, FEF at 50% of FVC; $FEF_{75\%}$, FEF at 75% of FVC; $FEF_{25\%-75\%}$, FEF at 25% to 75% of FVC; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity.

In patients with chest tightness, the AUCs of $FEF_{50\%}$, $FEF_{75\%}$, and $FEF_{25\%-75\%}$ combined with FENO were 0.880 (95% CI, 0.806–0.954), 0.892 (95% CI, 0.812–0.972), and 0.884 (95% CI, 0.805–0.934), respectively (Table 5).

The error rates between the average AUC of 5 different cross-validation models and the whole-model AUC using the entire data set were lower than 0.05 for all chosen variables, indicating that the data model has stable predictive ability for different data sets (Supplementary Tables S1 and S2).

Diagnostic Accuracy of Small-Airway Function Variables Combined with EOS in Blood

We repeated the ROC analyses for spirometry measurements ($FEF_{50\%}$, $FEF_{25\%-75\%}$, FEV_1/FVC) combined with EOS (Table 6). The AUCs of the combined models were between 0.734 and 0.786, and all had NPV ≥ 85.08%. The AUCs of FEFs combined with EOS were higher in patients with chest tightness than in the population as a whole (0.815, 0.845, and 0.816, for $FEF_{50\%}$, $FEF_{75\%}$, and $FEF_{25\%-75\%}$, respectively; Table 5).

Discussion

Early diagnosis of asthma is very important, not only to relieve the patient's symptoms but also to prevent the development of chronic inflammation and airway remodeling. However, early diagnosis is difficult because patients need to satisfy the 2 criteria of variable symptoms and airflow limitation. This is especially true for mild asthma, which is often undiagnosed because FEV_1 is normal and the bronchodilation test has a high false-negative rate. Our data indicating that small-airway dysfunction is present in asthmatic patients with $FEV_1 \geq 80\%$ predicted may help to support an early diagnosis in patients with mild asthma. Measurements of small-airway function, including $FEF_{50\%}$ and $FEF_{25\%-75\%}$, combined with measures of airway inflammation (FENO or EOS) supported the best prediction of positive AHR diagnosis in subjects who had both typical asthma-like symptoms and $FEV_1 \geq 80\%$ predicted.

Type II airway inflammation increases both EOS and FENO, which are used in asthma diagnosis and the therapeutic response's evaluation of anti-asthma drugs.[23] Our data also showed that the levels of both EOS and FENO were higher in the AHR-positive group. Previous studies in guinea pigs have shown that NO may itself contribute to AHR, by increasing plasma exudation via its vasodilator effect and by its transformation into peroxynitrite, which induces AHR.[24] Furthermore, in our study, a weak correlation between FENO and PD_{20} in patients with positive AHR was observed ($r = -0.266$, $P = 0.0005$), suggesting that FENO is valuable in predicting AHR, which was also noted by Jatakanon et al.[25]

Currently, FENO is particularly helpful in ruling out asthma. Its cut-off value for predicting asthma ranged from 10.5 to 64 ppb in different studies.[26] FENO < 30 ppb has a high specificity (87%) and NPV (93%) for excluding asthma from untreated nonsmoking adults with chronic cough.[27] Schleich et al showed that FENO > 34 ppb had a low predictive value (AUC = 0.62) for predicting AHR in patients with suspected asthma.[28] Schneider et al illustrated that, in their sample as a whole, asthma could be ruled in at FENO > 71 ppb (PPV, 80%) and ruled out at FENO ≤ 9 ppb (NPV, 82%), with an AUC of 0.656.[29] Importantly, when patients with neutrophilic inflammation were omitted, the AUC was 0.745 and asthma could be ruled in at FENO > 31 ppb (PPV, 82%) and ruled out at FENO ≤ 12 ppb (NPV, 81%).[29] Our present study showed that FENO > 41 ppb has a sensitivity of 65.29%,

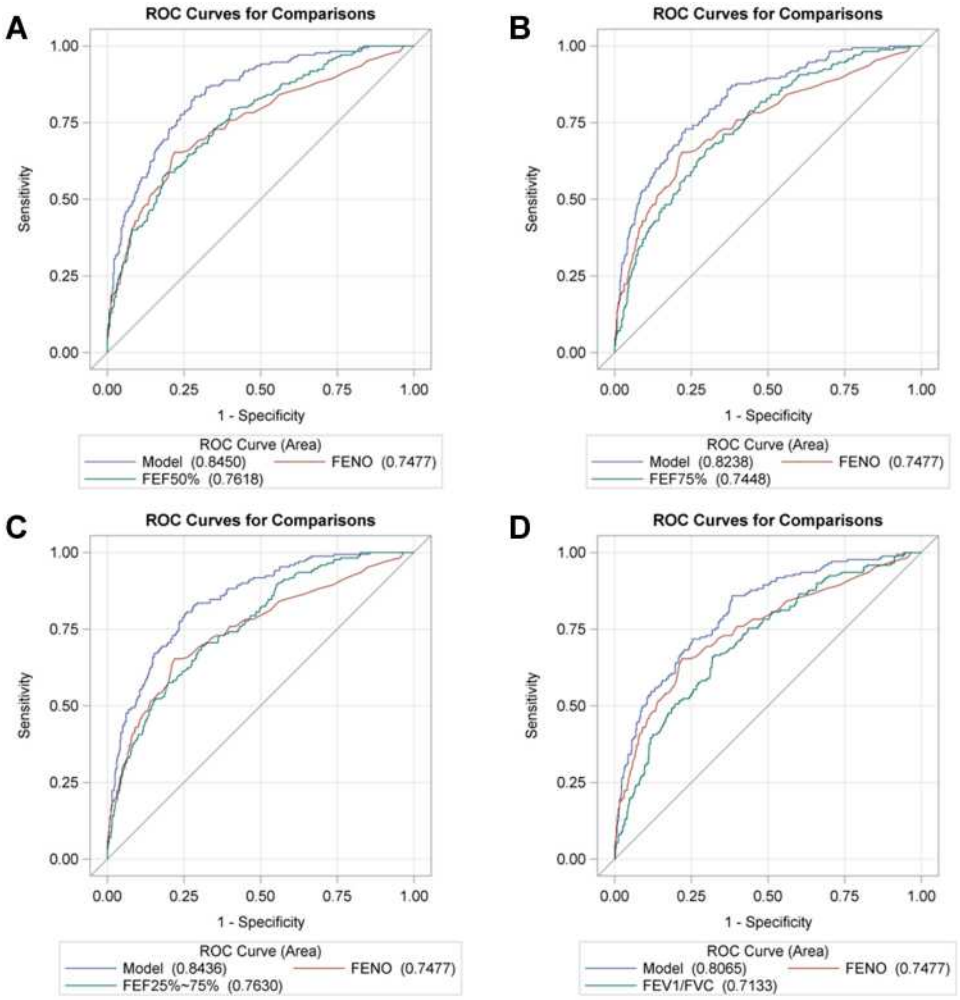

Figure 1 ROC curves for the models of FEFs combined with FENO for predicting positive bronchial provocation tests (n = 692). (**A**) $FEF_{50\%}$ combined with FENO. AUC_{Model} = 0.845 (95% CI, 0.812–0.878); AUC_{FENO} = 0.748 (95% CI, 0.702–0.793; $P < 0.001$, compared with the model); $AUC_{FEF50\%}$ = 0.762 (95% CI, 0.721–0.803; $P < 0.001$, compared with the model). (**B**) $FEF_{75\%}$ combined with FENO. AUC_{Model} = 0.824 (95% CI, 0.788–0.859); AUC_{FENO} =0.748 (95% CI, 0.702–0.793; $P < 0.001$, compared with the model); $AUC_{FEF75\%}$ = 0.745 (95% CI, 0.703–0.786; $P < 0.001$, compared with the model). (**C**) $FEF_{25\%-75\%}$ combined with FENO. AUC_{Model} = 0.844 (95% CI, 0.811–0.876); AUC_{FENO} =0.748 (95% CI, 0.702–0.793; $P < 0.001$, compared with the model); $AUC_{FEF25\%-75\%}$ = 0.763 (95% CI, 0.723–0.802; $P < 0.001$, compared with the model). (**D**) FEV_1/FVC combined with FENO. AUC_{Model} = 0.807 (95% CI, 0.769–0.844); AUC_{FENO} = 0.748 (95% CI, 0.702–0.793; $P = 0.001$, compared with the model); $AUC_{FEV1/FVC}$ = 0.713 (95% CI, 0.669–0.758).

Abbreviations: ROC, receiver operating characteristic; FEF, forced expiratory flow; FENO, fractional exhaled nitric oxide; AUC, area under the curve; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; $FEF_{50\%}$, FEF at 50% of FVC; $FEF_{75\%}$, FEF at 75% of FVC; $FEF_{25\%-75\%}$, FEF at 25% to 75% of FVC.

specificity of 78.16%, PPV of 49.33%, and NPV of 87.37%. The AUC for predicting AHR was 0.748, regardless of the inflammatory type, which is similar to the AUC from Schneider et al when patients with neutrophilic inflammation were omitted.

In our current study, patients positive for AHR, but with FEV_1 in the normal range, had abnormal values of small-airway function variables, obtained with spirometry and IOS. Two-thirds of asthmatic patients with $FEV_1 \geq 80\%$ predicted had small-airway dysfunction, and patients with small-airway dysfunction exhibit a greater likelihood of AHR. $FEF_{50\%}$, $FEF_{75\%}$, and $FEF_{25\%-75\%}$ were weakly correlated with PD_{20}. This might indicate that small-airway dysfunction could be a forerunner of decreased FEV_1 and could be used to detect early disease.

We found the 2 most valuable spirometric variables for predicting AHR were $FEF_{25\%-75\%}$ (AUC = 0.763) and $FEF_{50\%}$ (AUC = 0.762) (Table 3). Those two FEFs were strongly correlated and had equivalent value in predicting AHR. However, because both produced AUC < 0.80, using them singly would be insufficient for predicting AHR in patients with suspected asthma. Thus, we combined the FEFs with FENO or EOS to enhance their predictive value for AHR diagnosis. The AUCs of FEFs combined with FENO were significantly higher than those of the univariate AUCs. This suggests that FEFs combined

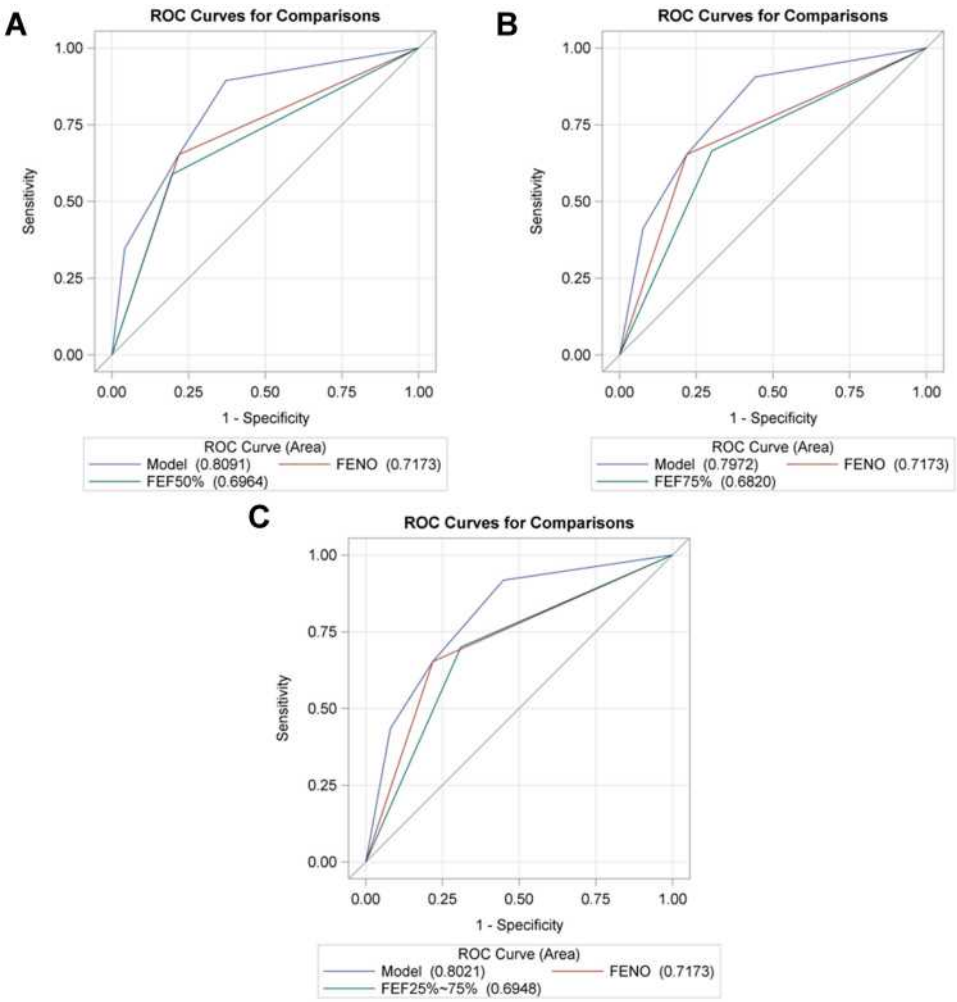

Figure 2 ROC curves of dichotomous state variables of the models of FEFs combined with FENO in predicting positive bronchial provocation tests (n = 692). (**A**) $FEF_{50\%}$ combined with FENO. AUC_{Model} = 0.809 (95% CI, 0.786–0.878); (**B**) $FEF_{75\%}$ combined with FENO. AUC_{Model} = 0.797 (95% CI, 0.768–0.866); (**C**) $FEF_{25\%–75\%}$ combined with FENO. AUC_{Model} = 0.802 (95% CI, 0.780–0.874).

Abbreviations: ROC, receiver operating characteristic; FEF, forced expiratory flow; FENO, fractional exhaled nitric oxide; AUC, area under the curve; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; $FEF_{50\%}$, FEF at 50% of FVC; $FEF_{75\%}$, FEF at 75% of FVC; $FEF_{25\%-75\%}$, FEF at 25% to 75% of FVC.

with FENO (2 noninvasive and convenient measurements) can improve the prediction of AHR diagnosis. The cut-off values had certain difference among different studies possibly because we included patients with mild asthma-like symptoms and normal FEV_1, who had higher FEFs levels than those with more severe symptoms.[5,30–32]

One main limitation of FEF is that it depends on FVC and lung capacity.[33,34] In contrast to FEV_1, $FEF_{25\%-75\%}$ is not normalized to FVC when assessing air-flow obstruction. Therefore, $FEF_{25\%-75\%}$ could be artificially low in individuals with restrictive lung or chest bellows disease (eg, obesity) and could therefore overdiagnose asthma. In our study, mean BMI and FVC were in the normal range (23.37 ± 3.477 kg/m^2 and 101.5% ± 12%, respectively), and neither variable differed between groups.[34,35] Most importantly, all patients in the study had undergone HRCT, therefore guaranteeing that restrictive lung diseases or obesity were excluded and minimizing the possibility of overdiagnosis of asthma in our patients. Furthermore, IOS is a noninvasive and effort-independent alternate test that is done during normal quiet tidal breathing, avoiding the need for any special breathing manoeuvre or any noticeable interference with respiration, and is considered to be more physiological than spirometry[36,37] We evaluated the ability of IOS measurement to assess small-airway function and to predict AHR. In our study, R5-R20 alone exhibited poor predictive value for AHR diagnosis, and the AUC was still lower than 0.80 when we combined R5-R20 and FENO. This finding suggests that when combined with FENO, FEFs provide better value than R5-R20 for

Table 5 Stratified Analysis of Patients with Chest-Tightness: Predictive Values of Combinations of Different Variables with FENO in Predicting Airway Hyperresponsiveness

Characteristic Variables	AUC	95% CI of AUC	Sensitivity %	Specificity %	PPV %	NPV %	PCC %	Contrast	95% CI of Contrast	*P* value
FENO + $FEF_{50\%}$	0.880	(0.806,0.954)	100.00	60.42	58.70	100.00	74.67	0.129†	(0.022, 0.236)	**0.019**
FENO + $FEF_{75\%}$	0.892	(0.812,0.972)	70.37	95.83	90.48	85.19	86.67	0.080‡	(–0.001, 0.161)	0.053
FENO + $FEF_{25\%-75\%}$	0.884	(0.805,0.964)	88.89	75.00	66.67	92.31	80.00	0.121†	(0.018, 0.224)	**0.021**
EOS + $FEF_{50\%}$	0.815	(0.716,0.913)	74.07	79.17	66.67	84.44	77.33	0.064‡	(–0.016, 0.144)	0.115
EOS + $FEF_{75\%}$	0.845	(0.750,0.940)	74.07	89.58	80.00	86.00	84.00	0.033	(–0.017, 0.083)	0.197
EOS + $FEF_{25\%-75\%}$	0.816	(0.715,0.917)	70.37	85.42	73.08	83.67	80.00	0.053‡	(–0.015, 0.120)	0.125

Notes: We used the larger of the 2 univariate AUCs to make the comparison; †Contrast, the difference between AUC of FENO and AUC of bivariate model; ‡Contrast, the difference between AUC of FEFs and AUC of bivariate model; *P* value, significance of the contrast by chi-square test. Bold font indicates statistical significance.
Abbreviations: FENO, fractional exhaled nitric oxide; AUC, area under the curve; 95% CI, 95% confidence interval of odds ratio; PPV, positive predictive values; NPV, negative predictive values; PCC, percentages correctly classified; FEF25%, forced expiratory flow at 25% of FVC; FEF50%, FEF at 50% of FVC; FEF75%, FEF at 75% of FVC; FEF25%-75%, FEF at 25% to 75% of FVC; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; EOS, eosinophils.

Table 6 Predictive Value of the Combination of Different Variables with EOS in Predicting Airway Hyperresponsiveness

Characteristic Variables	AUC	95% CI of AUC	Sensitivity %	Specificity %	PPV %	NPV %	PCC %	Contrast	95% CI of Contrast	*P* value
EOS + $FEF_{50\%}$	0.786	(0.748,0.825)	67.06	76.63	48.31	87.72	74.28	0.148	(0.097,0.200)	< 0.001
EOS + $FEF_{25\%-75\%}$	0.785	(0.747,0.823)	67.65	75.29	47.13	87.72	73.41	0.147	(0.096,0.199)	< 0.001
EOS + FEV_1/FVC	0.734	(0.691,0.777)	56.47	80.84	48.98	85.08	74.86	0.096	(0.045,0.146)	< 0.001

Notes: Contrast, the difference between the AUC of each FEF and the AUC of the bivariate model; we used the larger of the 2 univariate AUCs to make the comparison. *P* value, significance of the contrast by chi-square test.
Abbreviations: EOS, eosinophils; AUC, area under the curve; 95% CI, 95% confidence interval of odds ratio; PPV, positive predictive values; NPV, negative predictive values; PCC, percentages correctly classified; $FEF_{50\%}$, FEF at 50% of FVC; $FEF_{25\%-75\%}$, FEF at 25% to 75% of FVC; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity.

predicting AHR even though they are less physiologically relevant.

Chest tightness is a symptom of asthma that more likely reflects muscle tightness or physical difficulty with moving air that is sensed through proprioception and not through pain pathways.[38–40] In asthmatic patients with normal FEV_1 in our study, the most frequent chief complaints were cough and chest tightness rather than wheeze or dyspnea. Relevant clinical subtypes of asthma, "chest tightness variant asthma"[41] and "chest pain variant asthma"[38,42] have been described in the medical literature. Asthmatic patients who only complain of chest tightness are easily misdiagnosed in clinical practice. We found that decreases of FEV_1, FEV_1/FVC, $FEF_{25\%}$, $FEF_{50\%}$, $FEF_{75\%}$, and $FEF_{25\%-75\%}$ were more serious in subjects with than without chest tightness, indicating that small-airway dysfunction may be involved in the mechanism of chest tightness. The joint model of small-airway function variables ($FEF_{50\%}$, $FEF_{75\%}$, or $FEF_{25\%-75\%}$) and FENO gave particularly high predictive values for AHR in subjects with chest tightness (all of the AUCs ≥ 0.880). In addition, the joint model of EOS with small-airway function variables ($FEF_{50\%}$, $FEF_{75\%}$, or $FEF_{25\%-75\%}$) was highly predictive of AHR in subjects with chest tightness (all of the AUCs ≥ 0.815), which it was not for the population as a whole. Since the cost of peripheral blood cell count is much cheaper than FENO, these tests may provide very economic alternatives for predicting AHR in suspected asthmatics, especially in primary hospitals. The diagnosis of asthma should be strongly considered in patients with lower FEFs, high FENO or high EOS, and the symptom of chest tightness.

The progressive statistical design of this study consisted of several steps. First, the possible influencing variables were found from a Mann–Whitney test. Then, the correlation between the relevant variables and PD_{20} was determined with Spearman analysis. Through the analysis of AUC, the predictive value of those variables was further verified. The repeatability of our data calculation was shown by an 80/20 split-sample cross-validation. The AUCs in the validation sample were close to those in the whole model.

Some limitations also exist in our current study. Firstly, allergic rhinitis and atopy status were not described in our

study, which might influence the diagnostic value of FENO and EOS for BHR. Further comprehensive studies are needed to focus on this issue. Secondly, a larger-scale representative sample of CTVA should be collected to confirm our results. Thirdly, PEF variability measurement needs to be conducted in further prospective study. To overcome the limitations of our current study, larger-scale and multicenter prospective clinical trials should be performed to ensure the integrity of the inspection results.

In conclusion, asthmatic patients suffer from small-airway dysfunction, even though their FEV_1 is within the normal range. Patients with small-airway dysfunction exhibit an increased likelihood of having AHR. In order to improve the diagnosis rate of mild asthma and relieve patients' symptoms as early as possible, we combined 2 simple and noninvasive methods—small-airway function tests and FENO to improve the diagnosis rate of mild asthma. The likelihood of AHR strongly increased with $FEF_{25\%-75\%}$ <84.4%, $FEF_{50\%}$ <76.8%, and FENO >41 ppb. $FEV_{25\%-75\%}$ and $FEF_{50\%}$, derived from spirometry, could be combined with FENO to support asthma diagnosis in patients with normal FEV_1 and symptoms suggestive of asthma, allowing the patient to forego MCT for the diagnosis. $FEF_{25\%-75\%}$ or $FEF_{50\%}$ combined with EOS can also be a very economic method to predict AHR in suspected asthma subjects with chest tightness.

Abbreviations

AUC, Area under the curve; AHR, Airway hyperresponsiveness; BMI, Body mass index; CI, Confidence interval; CV, Coefficient of variance; EOS, Eosinophil count in blood; EOS%, Eosinophil percentage in blood $FEF_{25\%}$, Forced expiratory flow at 25% of forced vital capacity; $FEF_{50\%}$, Forced expiratory flow at 50% of forced vital capacity; $FEF_{75\%}$, Forced expiratory flow at 75% of forced vital capacity; $FEF_{25\%-75\%}$, Forced expiratory flow between 25% and 75% of forced vital capacity; FENO, Fractional exhaled nitric oxide; FVC, Forced vital capacity; FEV_1, Forced expiratory volume in 1 second; FEV_3, Forced expiratory volume in 3 seconds; Fres, Resonant frequency; HRCT, High-resolution computerized tomography; IOS, Impulse oscillometry; IQR, Interquartile ranges; MCT, Methacholine challenge testing; NPV, Negative predictive value; PCC, Percentage correctly classified; PD_{20}, Provocative dose of a substance causing a 20% fall in FEV_1; PPV, Positive predictive value; ROC, Receiver operating characteristic; R5, Total airway resistance at 5 Hz; R20, Central airway resistance at 20 Hz; X5, Reactance at 5 Hz; SD, Standard deviation.

Data Sharing Statement

The data that support the findings of this study are available from Min Zhang upon reasonable request.

Ethics Approval and Consent to Participate

The ethics committee of Shanghai General Hospital, Shanghai Jiao Tong University, approved the protocol, and a waiver of informed consent was given for our study (number 2017KY159). We confirm that the patient data was maintained with confidentiality and complied with the Declaration of Helsinki.

Consent for Publication

Written informed consent for publication of this study was obtained from all participants.

Acknowledgments

The study was supported by National Natural Science Foundation of China (Grant No. 81873402, and 81800020); Appropriate technique application Program of Shanghai Municipal Health System (Grant No.2019SY042); Scientific and Technological Innovation program funded by Science and Technology Commission of Shanghai municipality (Grant No. 20Y11902400); Program of Shanghai Municipal Health System (Grant No.201740039).

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. These authors contributed equally to this work and are considered as co-first authors of the publication: Wuping Bao, Xue Zhang, Junfeng Yin, and Lei Han.

Disclosure

The authors report no conflicts of interest for this work and have reported that no potential conflicts of interest exist

with any companies/organizations whose products or services may be discussed in this article.

References

1. Lipworth B, Manoharan A, Anderson W. Unlocking the quiet zone: the small airway asthma phenotype. *Lancet Respir Med*. 2014;2(6):497–506. doi:10.1016/S2213-2600(14)70103-1
2. Coates AL, Wanger J, Cockcroft DW, et al. ERS technical standard on bronchial challenge testing: general considerations and performance of methacholine challenge tests. *Eur Respir J*. 2017;49(5):1–17.
3. Dusser D, Montani D, Chanez P, et al. Mild asthma: an expert review on epidemiology, clinical characteristics and treatment recommendations. *Allergy*. 2007;62(6):591–604. doi:10.1111/j.1398-9995.2007.01394.x
4. Bao W, Zhang X, Lv C, et al. The value of fractional exhaled nitric oxide and forced mid-expiratory flow as predictive markers of bronchial hyperresponsiveness in adults with chronic cough. *J Allergy Clin Immunol Pract*. 2018;6(4):1313–1320. doi:10.1016/j.jaip.2017.09.026
5. Drewek R, Garber E, Stanclik S, Simpson P, Nugent M, Gershan W. The FEF25-75 and its decline as a predictor of methacholine responsiveness in children. *J Asthma*. 2009;46(4):375–381. doi:10.1080/02770900802492079
6. van der Wiel E, Postma DS, van der Molen T, Schiphof-Godart L, Ten Hacken NH, van den Berge M. 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. doi:10.1111/all.12510
7. Shi Y, Aledia AS, Galant SP, George SC. Peripheral airway impairment measured by oscillometry predicts loss of asthma control in children. *J Allergy Clin Immunol*. 2013;131(3):718–723. doi:10.1016/j.jaci.2012.09.022
8. Manoharan A, von Wilamowitz-moellendorff A, Morrison A, Lipworth BJ. Effects of formoterol or salmeterol on impulse oscillometry in patients with persistent asthma. *J Allergy Clin Immunol*. 2016;137(3):727–33.e1. doi:10.1016/j.jaci.2015.06.012
9. Arıkoglu T, Unlu A, Yıldırım DD, Kuyucu S. The airway hyperresponsiveness to methacholine may be predicted by impulse oscillometry and plethysmography in children with well-controlled asthma. *J Asthma*. 2018;55(11):1166–1173. doi:10.1080/02770903.2017.1407337
10. Ekström S, Hallberg J, Kull I, et al. Body mass index status and peripheral airway obstruction in school-age children: a population-based cohort study. *Thorax*. 2018;73(6):538–545. doi:10.1136/thoraxjnl-2017-210716
11. Dweik RA, Boggs PB, Erzurum SC, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med*. 2011;184(5):602–615. doi:10.1164/rccm.9120-11ST
12. Madan K, Hadda V, Khilnani GC, Guleria R. Isolated reduction of the FEV3/FVC ratio as an indicator of mild airflow obstruction. *Chest*. 2014;145(3):662. doi:10.1378/chest.13-2435
13. Piorunek T, Kostrzewska M, Stelmach-Mardas M, et al. Small airway obstruction in chronic obstructive pulmonary disease: potential parameters for early detection. *Adv Exp Med Biol*. 2017;980:75–82.
14. Morris ZQ, Coz A, Starosta D. An isolated reduction of the FEV3/FVC ratio is an indicator of mild lung injury. *Chest*. 2013;144(4):1117–1123. doi:10.1378/chest.12-2816
15. Miller MR, Hankinson J, Brusasco V, et al. ATS/ERS Task Force. Standardisation of spirometry. *Eur Respir J*. 2005;26:319–338. doi:10.1183/09031936.05.00034805
16. Coates AL, Wanger J, Cockcroft DW, et al. ERS technical standard on bronchial challenge testing: general considerations and performance of methacholine challenge tests. *Eur Respir J*. 2017;49(5):1601526.
17. Wagner EM, Liu MC, Weinmann GG, Permutt S, Bleecker ER. Peripheral lung resistance in normal and asthmatic subjects. *Am Rev Respir Dis*. 1990;141(3):584–588. doi:10.1164/ajrccm/141.3.584
18. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics*. 1988;44(3):837–845. doi:10.2307/2531595
19. Altman DG, Bland JM. Diagnostic tests 2: predictive values. *BMJ*. 1994;309(6947):102. doi:10.1136/bmj.309.6947.102
20. Youden WJ. Index for rating diagnostic tests. *Cancer*. 1950;3(1):32–35. doi:10.1002/1097-0142(1950)3:1<32::AID-CNCR2820030106>3.0.CO;2-3
21. Thornbury JR, Fryback DG, Edwards W. Likelihood ratios as a measure of the diagnostic usefulness of excretory urogram information. *Radiology*. 1975;114(3):561–565. doi:10.1148/114.3.561
22. Hung J, Chaitman BR, Lam J, et al. A logistic regression analysis of multiple noninvasive tests for the prediction of the presence and extent of coronary artery disease in men. *Am Heart J*. 1985;110(2):460–469. doi:10.1016/0002-8703(85)90170-X
23. Castro M, Corren J, Pavord ID, et al. Dupilumab efficacy and safety in moderate-to-severe uncontrolled asthma. *N Engl J Med*. 2018;378(26):2486–2496. doi:10.1056/NEJMoa1804092
24. Sadeghi-Hashjin G, Folkerts G, Henricks PA, et al. Peroxynitrite induces airway hyperresponsiveness in guinea pigs in vitro and in vivo. *Am J Respir Crit Care Med*. 1996;153(5):1697–1701. doi:10.1164/ajrccm.153.5.8630623
25. Jatakanon A, Lim S, Kharitonov SA, Chung KF, Barnes PJ. Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. *Thorax*. 1998;53(2):91–95. doi:10.1136/thx.53.2.91
26. Song WJ, Kim HJ, Shim JS, et al. Diagnostic accuracy of fractional exhaled nitric oxide measurement in predicting cough-variant asthma and eosinophilic bronchitis in adults with chronic cough: a systematic review and meta-analysis. *J Allergy Clin Immunol*. 2017;140(3):701–709. doi:10.1016/j.jaci.2016.11.037
27. Chatkin JM, Ansarin K, Silkoff PE, et al. Exhaled nitric oxide as a noninvasive assessment of chronic cough. *Am J Respir Crit Care Med*. 1999;159(6):1810–1813. doi:10.1164/ajrccm.159.6.9809047
28. Schleich FN, Asandei R, Manise M, Sele J, Seidel L, Is LR. FENO50 useful diagnostic tool in suspected asthma? *Int J Clin Pract*. 2012;66(2):158–165. doi:10.1111/j.1742-1241.2011.02840.x
29. Schneider A, Schwarzbach J, Faderl B, Welker L, Karsch-Völk M, Jörres RA. FENO measurement and sputum analysis for diagnosing asthma in clinical practice. *Respir Med*. 2013;107(2):209–216. doi:10.1016/j.rmed.2012.10.003
30. 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. doi:10.1016/S1081-1206(10)61067-8
31. He L, Wei M, Luo J, et al. Re-evaluation of the diagnostic value of fractional exhaled nitric oxide & its impact in patients with asthma. *Indian J Med Res*. 2018;148(4):441–448. doi:10.4103/ijmr.IJMR_1478_16
32. Feng-Jia C, Xin-Yan H, Geng-Peng L, Yang-Li L, Can-Mao X. Validity of fractional exhaled nitric oxide and small airway function indices in diagnosis of cough-variant asthma. *J Asthma*. 2018;55(7):750–755. doi:10.1080/02770903.2017.1366509
33. Pellegrino R, Viegi G, Brusasco V, et al. Interpretative strategies for lung function tests. *Eur Respir J*. 2005;26(5):948–968. doi:10.1183/09031936.05.00035205
34. Lee H, Chang B, Kim K, et al. Clinical Utility of additional measurement of total lung capacity in diagnosing obstructive lung disease in subjects with restrictive pattern of spirometry. *Respir Care*. 2016;61(4):475–482. doi:10.4187/respcare.04222

35. Forno E, Han YY, Mullen J, Celedón JC. Overweight, obesity, and lung function in children and adults-a meta-analysis. *J Allergy Clin Immunol Pract*. 2018;6(2):570–581.e10. doi:10.1016/j.jaip.2017.07.010
36. Marotta A, Klinnert MD, Price MR, Larsen GL, Liu AH. Impulse oscillometry provides an effective measure of lung dysfunction in 4-year-old children at risk for persistent asthma. *J Allergy Clin Immunol*. 2003;112(2):317–322. doi:10.1067/mai.2003.1627
37. Heijkenskjöld Rentzhog C, Janson C, Berglund L, et al. Overall and peripheral lung function assessment by spirometry and forced oscillation technique in relation to asthma diagnosis and control. *Clin Exp Allergy*. 2017;47(12):1546–1554. doi:10.1111/cea.13035
38. Farr RS, Kopetzky MT, Spector SL, Hurewitz DS. Asthma without wheezing. *Chest*. 1973;63(Suppl):64S–68S. doi:10.1378/chest.63.4_Supplement.64S
39. Global Initiative for Asthma. 2020 GINA Report, Global Strategy for Asthma Management and Prevention. Available at: https://ginasthma.org/gina-reports/. 2020.
40. Taniguchi H, Furuse H, Nakanishi Y, et al. Bronchial biopsy and reactivity in patients with chest tightness relieved with bronchodilator. *J Asthma*. 2017;54(5):479–487. doi:10.1080/02770903.2016.1236940
41. Shen H, Hua W, Wang P, Li W. A new phenotype of asthma: chest tightness as the sole presenting manifestation. *Ann Allergy Asthma Immunol*. 2013;111:226–227. doi:10.1016/j.anai.2013.06.016
42. Taniguchi H, Kanbara K, Hoshino K, Izumi S. Chest pain relieved with a bronchodilator or other asthma drugs. *Allergol Int*. 2009;58(3):421–427. doi:10.2332/allergolint.08-OA-0084