

# The Value of Fractional Exhaled Nitric Oxide and Forced Mid-Expiratory Flow as Predictive Markers of Bronchial Hyperresponsiveness in Adults with Chronic Cough



Wuping Bao, MD<sup>a,\*</sup>, Xue Zhang, BMed<sup>a,\*</sup>, Chengjian Lv, BS<sup>a,\*</sup>, Luhong Bao, BS<sup>a</sup>, Junfeng Yin, PhD<sup>b</sup>, Zhixuan Huang, BS<sup>b</sup>, Bing Wang, BMed<sup>a</sup>, Xin Zhou, MD<sup>a</sup>, and Min Zhang, MD<sup>a</sup> *Shanghai, China*

**What is already known about this topic?** Small-airway dysfunction contributes to the pathophysiology of even the mildest forms of classical asthma. It may exist in cough-variant asthma, and its presence could be helpful in predicting bronchial hyperresponsiveness (BHR).

**What does this article add to our knowledge?** Small-airway dysfunction is present in patients with chronic cough and BHR. Forced expiratory flow between 25% and 75% (FEF<sub>25%-75%</sub>) can be used to predict BHR when combined with fractional exhaled nitric oxide (FENO).

**How does this study impact current management guidelines?** FENO > 43 ppb and FEF<sub>25%-75%</sub> < 78.5% strongly predicted positive BHR in Chinese patients. This method is more accessible and convenient than bronchial provocation tests, especially for doctors in primary hospitals.

**BACKGROUND:** Bronchial provocation tests are standard for diagnosing the etiology of chronic cough, but they are time consuming and can induce severe bronchospasm. A safer and faster clinical examination to predict bronchial hyperresponsiveness (BHR) is needed.

**OBJECTIVE:** The objective of this study was to investigate whether small-airway function tests can predict BHR in adult patients with chronic cough.

**METHODS:** A retrospective, cross-sectional study of diagnoses made using spirometry and bronchial provocation test results was performed in 290 patients with chronic nonproductive cough. BHR-predictive values were analyzed via the area under receiver operating characteristic curves (AUCs). Optimal cutoff values were determined by maximizing the sum of sensitivity and specificity.

**RESULTS:** Patients with chronic cough with BHR showed lower forced expiratory flow between 25% and 75% (FEF<sub>25%-75%</sub>), higher fractional exhaled nitric oxide (FENO), and a higher percentage of eosinophils in blood than patients without BHR ( $P < .0001$  for all). The AUCs of FENO and FEF<sub>25%-75%</sub> for a BHR diagnosis were 0.788 (95% CI, 0.725-0.851) and 0.702 (95% CI, 0.641-0.763), respectively. Optimal cutoff values were 43 ppb for FENO and 78.5% for FEF<sub>25%-75%</sub>, with negative predictive values of 85.38% and 81.34%, respectively. The combined use of FENO and FEF<sub>25%-75%</sub> increased the AUC to 0.843 (95% CI, 0.794-0.892), significantly higher than either FENO ( $P = .012$ ) or FEF<sub>25%-75%</sub> ( $P < .0001$ ) alone.

**CONCLUSIONS:** Small-airway dysfunction is present in patients with chronic cough and BHR. FEF<sub>25%-75%</sub> has value as a negative predictive parameter for BHR, especially when combined with FENO. FENO > 43 ppb and FEF<sub>25%-75%</sub> < 78.5% strongly predicted a positive bronchial provocation test. © 2017 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2018;6:1313-20)

**Key words:** Asthma diagnosis; Fractional exhaled nitric oxide; Small-airway function; Bronchial provocation; Cough

Chronic cough (>8 weeks) is one of the most frequent complaints in pulmonary outpatient clinics and severely impairs patients' quality of life. Cough-variant asthma (CVA), first reported by Stanescu and Teculescu in 1970<sup>1</sup> and subsequently named by O'Connell et al in 1991,<sup>2</sup> is one of the most frequent causes of chronic cough<sup>3-6</sup> in adult nonsmokers, accounting for 24% to 29% of chronic cough. CVA is diagnosed on the basis of chronic cough that responds to bronchodilators and the presence of bronchial hyperresponsiveness (BHR),<sup>3-7</sup> which is traditionally demonstrated by performing bronchial provocation tests such as

<sup>a</sup>Department of Respiratory Medicine, Shanghai General Hospital, Shanghai Jiao Tong University, Shanghai, China

<sup>b</sup>School of Mathematical sciences, Tongji University, Shanghai, China

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Corresponding author: Min Zhang, MD, and Xin Zhou, MD, Department of Respiratory Medicine, Shanghai General Hospital, Shanghai Jiao Tong University, No. 100, Haining Road, Shanghai 200080, China. E-mail: [maggie\\_zhangmin@163.com](mailto:maggie_zhangmin@163.com).

\*These authors contributed equally to this work.  
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**Abbreviations used**

|                          |                                                                        |
|--------------------------|------------------------------------------------------------------------|
| ATS-                     | American Thoracic Society                                              |
| AUC-                     | Area under the curves                                                  |
| BHR-                     | Bronchial hyperresponsiveness                                          |
| CalvNO-                  | The alveolar component of fractional exhaled nitric oxide              |
| CVA-                     | Cough-variant asthma                                                   |
| ERS-                     | European Respiratory Society                                           |
| EOS%-                    | Eosinophils percentages in blood                                       |
| EF <sub>50%</sub> -      | Forced expiratory flow at 50% of forced vital capacity                 |
| FEF <sub>75%</sub> -     | Forced expiratory flow at 75% of forced vital capacity                 |
| FEF <sub>25%</sub> -     | Forced expiratory flow at 25% of forced vital capacity                 |
| FEF <sub>25%-75%</sub> - | Forced expiratory flow between 25% and 75%                             |
| FENO-                    | Fractional exhaled nitric oxide                                        |
| FEV <sub>1</sub> -       | Forced expiratory volume in 1 second                                   |
| FVC-                     | Forced vital capacity                                                  |
| MCH-                     | Methacholine                                                           |
| NLR-                     | Negative likelihood ratios                                             |
| NPV-                     | Negative predictive values                                             |
| PD <sub>20</sub> -       | Provocative dose of a substance causing a 20% fall in FEV <sub>1</sub> |
| PLR-                     | Positive likelihood ratios                                             |
| PPV-                     | Positive predictive values                                             |
| ROC-                     | Receiver operating characteristic                                      |

methacholine (MCH) and histamine challenges. Although bronchial provocation tests are considered of great value in confirming or excluding asthma, they are time consuming, expensive, and associated with a small risk of inducing severe bronchospasm. Not all hospitals, especially primary hospitals, perform the bronchoprovocation test. Therefore, clinical examinations that can predict the BHR safely and quickly are needed both for general practitioners and pulmonologists.

Measurement of fractional exhaled nitric oxide (FENO) is widely accepted as a noninvasive biomarker for defining airway eosinophilic inflammation and predicting corticosteroid sensitivity in asthma and other allergic diseases, such as allergic rhinitis. Eosinophilic inflammation is also a key element in the diagnosis and pathogenesis of CVA. FENO values are significantly higher in patients with CVA than in those with chronic cough not attributable to asthma or in healthy volunteers,<sup>8,9</sup> suggesting a potentially useful role for FENO measurement in the etiological diagnosis of chronic cough. Using 30 ppb as the FENO cutoff point, the sensitivity and specificity of FENO for detecting CVA were 75% and 87%, respectively.<sup>8</sup> FENO higher than 33.9 ppb cutoff can positively predict inhaled corticosteroid efficacy for patients with chronic cough.<sup>10</sup> Compared with the bronchoprovocation test, measurement of FENO is extremely easy, quick, and safe to perform.

Asthma is considered a disease predominantly of large airway dysfunction. However, increasing evidence shows that small airways (<2 mm diameter) and surrounding alveolar tissue, traditionally labeled the “quiet zone,” are important sites of inflammation and independently contribute to the clinical expression of asthma regardless of asthma severity.<sup>8</sup> Small-airway function is associated with clinical control, asthmatic exacerbation, nocturnal asthmatic attacks, and exercise-induced asthmatic attacks.<sup>11</sup> Importantly, the small airways are involved in BHR. Changes in the small airways measured with oscillometry correlate with symptoms during provocation tests, whereas proximal airways (forced expiratory volume in 1 second [FEV<sub>1</sub>],

spirometry) do not.<sup>12,13</sup> In addition, more severe BHR was associated with lower forced expiratory flow at 25% to 75% (FEF<sub>25%-75%</sub>) of forced vital capacity (FVC), FEF<sub>50%</sub>, FEF<sub>75%</sub>, and small-airway inflammation in patients who were matched for FEV<sub>1</sub> percent predicted.<sup>14-18</sup> This evidence suggests that small-airway function might predict BHR when used as a single marker or combined with other measurements.

Spirometry, body plethysmography, single/multiple breath nitrogen washout, forced oscillation technique, prolonged sputum induction, and high-resolution computed tomography are used to assess small-airway function noninvasively. Spirometry is the most easily available and widely used of these methods. FEF<sub>25%-75%</sub> obtained by spirometry is significantly lower in asthmatic patients with near-normal FEV<sub>1</sub> and FVC values than in normal volunteers.<sup>19,20</sup> It may therefore be a possible marker of early bronchial impairment and a more sensitive indicator of small-airway function, particularly in allergic subjects with normal FEV<sub>1</sub>, such as those with mild asthma, allergic rhinitis, or CVA.

The aim of this study was to determine the diagnostic value of several noninvasive measures, including FENO and FEF<sub>25%-75%</sub>, alone or in combination, in predicting the presence of BHR in patients with chronic cough.

## METHODS

### Study design and subject selection

We performed a cross-sectional study of diagnostic data from patients with chronic nonproductive cough of at least 2 months' duration who were referred to the Pulmonary Outpatient Clinic of Shanghai General Hospital, Shanghai Jiao Tong University (China) and accepted spirometry and MCH bronchoprovocation tests from June 2016 to December 2016. Patient charts were retrospectively and consecutively evaluated to obtain the detailed clinical history and results of clinical examinations.

Only patients who met all of the following criteria were eligible for study inclusion: history of chronic nonproductive cough, normal chest x-ray or computed tomography results, predicted FEV<sub>1</sub> of 70% or greater with spirometric measurement, and MCH bronchial provocation tests performed.

Patients were excluded if they met the following criteria: respiratory tract infections within 6 weeks before the evaluation, use of inhaled or oral corticosteroid in the previous 4 weeks, or use of montelukast and long-acting  $\beta_2$ -agonists in the previous week.

Descriptive characteristics, clinical history, results of the bronchial provocation test, spirometry, FENO, and percentages of eosinophils in peripheral blood (EOS%) were reviewed and analyzed.

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.

### Spirometric measurements

Spirometric assessments were performed with a spirometer (Jaeger, Hoechberg, Germany) in accordance with the specifications and performance criteria recommended in the American Thoracic Society (ATS)/European Respiratory Society (ERS) Standardization of Spirometry.<sup>21</sup> The following spirometric assessments were reviewed: FVC, FEV<sub>1</sub>, FEV<sub>1</sub>/FVC, FEF<sub>25%</sub>, FEF<sub>50%</sub>, FEF<sub>75%</sub>, and FEF<sub>25%-75%</sub>. FEV<sub>1</sub>, FVC, FEF<sub>25%</sub>, FEF<sub>50%</sub>, FEF<sub>75%</sub>, and FEF<sub>25%-75%</sub> are expressed as percentages of predicted values. FEV<sub>1</sub>/FVC is expressed as an absolute value.

## MCH bronchial provocation tests

MCH bronchial provocation tests were performed with the Jaeger APS Pro system using a Medic-Aid sidestream nebulizer and doubling doses of MCH (0.0725-0.48 mg), following the recommendations of the ATS/ERS Task force: Standardization of Lung Function Testing.<sup>21</sup> FEV<sub>1</sub> was measured 3 minutes after every provocation step. Provocative dose causing a 20% fall in FEV<sub>1</sub> (PD<sub>20</sub>) was recorded, and BHR was defined as present if PD<sub>20</sub> ≤ 0.48 mg. The association between FENO and PD<sub>20</sub> was analyzed in patients with positive BHR.

## FENO measurements

FENO was performed by using a hand-held portable machine (NIOX MINO, Aerocrine AB, Solna, Sweden) at a standard flow rate of 50 mL/s, in accordance with ATS/ERS recommendations.<sup>22</sup> FENO measurements were performed before spirometric assessments and bronchial provocation tests, because the breathing maneuvers involved could distort FENO results.

## Peripheral blood eosinophils

EOS% in peripheral blood were determined by the clinical laboratory of Shanghai General Hospital, Shanghai Jiao Tong University.

## Statistical analysis

Analysis was performed with SPSS software version 19.0 (SPSS Inc., Chicago, Ill). Baseline data are presented descriptively. Normally distributed data are presented as mean ± standard deviation. Normality of distribution was checked with the Kolmogorov-Smirnov test. Comparisons between patients with and without BHR were performed by using the Mann-Whitney test.

Predictive values of single or combined measurements were calculated by constructing receiver operating characteristic (ROC) curves and measuring the area under the curves (AUC).<sup>23</sup> In the ROC plot, the sensitivity (percentage of true positive results) is plotted against 1-specificity (the percentage false positive results). The nearer the curve approaches the top left of the ROC plot, the greater the sum of sensitivity and specificity. ROC plots provide a pure index of the accuracy of a test by demonstrating the limits of the test's ability to discriminate between health and disease over the complete spectrum of operating conditions. Quantification of the overall diagnostic accuracy of a test can be expressed by calculating the AUC of the ROC plot and provides a value between 0.5 (no separation of test values in healthy and disease groups) and 1.0 (perfect separation). Comparison of AUCs was performed with the  $\chi^2$  test in accordance with the method of Hanley and McNeil.<sup>23</sup>

We used MCH bronchial provocation tests as the gold standard for defining BHR. The optimal value of FENO giving the highest sum of BHR diagnostic sensitivity and specificity was used as a cutoff value. Positive predictive values (PPV), negative predictive values (NPV), positive likelihood ratios (PLR), negative likelihood ratios (NLR), and percentages correctly classified were calculated for each cutoff value. PPV is the proportion of positive results that are true positives. NPV is the proportion of negative results that are true negatives.<sup>24</sup> PLR is the probability of a person who has the disease testing positive divided by the probability of a person who does not have the disease testing positive. NLR is the probability of a person who has the disease testing negative divided by the probability of a person who does not have the disease testing negative.<sup>25</sup> The percentage correctly classified is the probability of true positive and true negative results in the whole cohort.<sup>26</sup>

**TABLE I.** Demographic data, spirometric parameters, and values for FENO and peripheral eosinophils of patients with positive or negative bronchial provocation tests

| Characteristic variables             | Positive bronchial provocation | Negative bronchial provocation | P value          |
|--------------------------------------|--------------------------------|--------------------------------|------------------|
| N                                    | 97                             | 193                            |                  |
| Age, y*                              | 43.44 (14.92)                  | 43.18 (14.70)                  | .9414            |
| Male, n (%)                          | 36 (37.11)                     | 81 (41.97)                     | .4265            |
| BMI, kg/m <sup>2</sup>               | 23.38 (3.15)                   | 23.64 (3.76)                   | .6055            |
| Smoking history, n (%)               | 18 (18.56)                     | 11 (5.70)                      | <b>.0014</b>     |
| FVC, %predicted*                     | 97.66 (10.60)                  | 97.08 (11.92)                  | .3869            |
| FEV <sub>1</sub> , %predicted*       | 93.06 (10.99)                  | 97.97 (13.46)                  | <b>.0029</b>     |
| FEV <sub>1</sub> /FVC, %*            | 80.22 (6.60)                   | 84.77 (5.99)                   | <b>&lt;.0001</b> |
| FEF <sub>25%</sub> , %predicted*     | 64.86 (22.15)                  | 80.40 (29.54)                  | <b>&lt;.0001</b> |
| FEF <sub>50%</sub> , %predicted*     | 69.71 (18.48)                  | 90.12 (28.23)                  | <b>&lt;.0001</b> |
| FEF <sub>75%</sub> , %predicted*     | 85.71 (17.14)                  | 95.72 (20.45)                  | <b>&lt;.0001</b> |
| FEF <sub>25%-75%</sub> , %predicted* | 67.57 (19.29)                  | 85.55 (26.14)                  | <b>&lt;.0001</b> |
| FENO, ppb*†                          | 79.52 (54.71)                  | 32.07 (36.84)                  | <b>&lt;.0001</b> |
| EOS in blood, %*‡                    | 6.577 (3.911)                  | 3.643 (3.881)                  | <b>&lt;.0001</b> |
| PD <sub>20</sub> , mg                | 0.2315 (0.2756)                | —                              | —                |

Bold font indicates statistical significance.

BHR, Bronchial hyperresponsiveness; BMI, body mass index; EOS, eosinophils; FEF<sub>25%</sub>, forced expiratory flow at 25% of forced vital capacity; FEF<sub>50%</sub>, forced expiratory flow at 50% of forced vital capacity; FEF<sub>75%</sub>, forced expiratory flow at 75% of forced vital capacity; FEF<sub>25%-75%</sub>, forced expiratory flow at 25% to 75% of forced vital capacity; FENO, fractional exhaled nitric oxide; FEV<sub>1</sub>, forced expiratory volume in 1 s; FVC, forced vital capacity; PD<sub>20</sub>, provocative dose causing a 20% fall in forced expiratory volume in the first second.

\*Mean (SD) values.

†n = 266; 88 for positive BHR, and 178 for negative BHR.

‡n = 160; 56 for positive BHR, and 104 for negative BHR.

Continuous test variables were converted to dichotomous state variables on the basis of the cutoff values, and multiple logistic regression was performed to get a predictive equation. Subsequently, ROC curves were determined for the combined measurements with the dichotomous state variables.

The threshold for statistical significance for all analysis was set at  $P < .05$ .

## RESULTS

### Baseline characteristics

Clinical data from 290 patients with chronic cough who had undergone spirometry and MCH bronchoprovocation tests were included. FENO tests were not performed in 24 of these patients. Positive bronchial provocation was demonstrated in 97 patients (9 patients missing FENO values) and negative bronchial provocation in 193 patients (15 patients missing FENO values).

Most demographic variables did not differ between the groups at baseline (Table I). However, FEV<sub>1</sub> and FEV<sub>1</sub>/FVC were significantly lower in patients with positive MCH challenge ( $P = .003$  and  $P < .0001$ , respectively), although the average values remained within the normal ranges. FEF<sub>50%</sub>, FEF<sub>25%</sub>, and FEF<sub>25%-75%</sub> were also significantly lower in the positive MCH group ( $P < .0001$ ), indicating that small-airway dysfunction was present in these patients. FENO values and EOS% were also higher in the patients with positive challenge tests ( $P < .0001$ ), even though a significantly higher percentage of patients in the BHR group (18.6%) than in the non-BHR group (5.7%) had a history of smoking ( $P = .0014$ ).

**TABLE II.** Optimal cutoff values for the prediction of bronchial hyperresponsiveness

| Characteristic variables  | AUC    | Cutoff values* | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | Correctly classified (%) | PLR  | NLR  |
|---------------------------|--------|----------------|-----------------|-----------------|---------|---------|--------------------------|------|------|
| FEV <sub>1</sub>          | 0.6070 | 100.7          | 79.38           | 40.42           | 40.10   | 79.59   | 53.44                    | 1.33 | 0.51 |
| FEV <sub>1</sub> /FVC     | 0.6877 | 82.96          | 68.04           | 63.73           | 48.53   | 79.87   | 65.17                    | 1.88 | 0.50 |
| FEF <sub>25%</sub>        | 0.6647 | 69.20          | 65.99           | 58.03           | 44.14   | 79.31   | 60.69                    | 1.57 | 0.59 |
| FEF <sub>50%</sub>        | 0.7179 | 79.4           | 74.23           | 59.07           | 47.68   | 82.01   | 64.13                    | 1.81 | 0.44 |
| FEF <sub>75%</sub>        | 0.6592 | 91.6           | 69.07           | 59.59           | 46.21   | 79.31   | 62.76                    | 1.71 | 0.52 |
| FEF <sub>25%-75%</sub>    | 0.7018 | 78.5           | 74.23           | 56.48           | 46.15   | 81.34   | 62.41                    | 1.71 | 0.46 |
| FENO <sup>†</sup>         | 0.7875 | 43             | 71.59           | 82.02           | 66.32   | 85.38   | 78.57                    | 3.98 | 0.35 |
| EOS in blood <sup>‡</sup> | 0.7627 | 3.5            | 76.79           | 71.15           | 58.90   | 85.06   | 73.13                    | 2.66 | 0.33 |

AUC, Area under the curve; BHR, bronchial hyperresponsiveness; EOS, eosinophils; FEF<sub>25%</sub>, forced expiratory flow at 25% of forced vital capacity; FEF<sub>50%</sub>, forced expiratory flow at 50% of forced vital capacity; FEF<sub>75%</sub>, forced expiratory flow at 75% of forced vital capacity; FEF<sub>25%-75%</sub>, forced expiratory flow at 25% to 75% of forced vital capacity; FENO, fractional exhaled nitric oxide; FEV<sub>1</sub>, forced expiratory volume in 1 s; FVC, forced vital capacity; NLR, negative likelihood ratios; NPV, negative predictive values; PLR, positive likelihood ratios; PPV, positive predictive values.

\*Cutoff points of FEV<sub>1</sub>, FEF<sub>25%</sub>, FEF<sub>50%</sub>, FEF<sub>75%</sub>, and FEF<sub>25%-75%</sub> are in percent predicted; the cutoff point of FENO is in ppb; and cutoff points of FEV<sub>1</sub>/FVC and eosinophils in blood are in percent. The cutoff points were selected by maximizing the sum of sensitivity and specificity.

<sup>†</sup>n = 266; 88 for positive BHR, and 178 for negative BHR.

<sup>‡</sup>n = 160; 56 for positive BHR, and 104 for negative BHR.

PD<sub>20</sub> in patients with positive BHR was  $0.232 \pm 0.276$ . A Spearman analysis of the results from patients with positive bronchial provocation tests confirmed that FENO was negatively related to PD<sub>20</sub> ( $r = -0.307$ ,  $P = .004$ ).

### Diagnostic accuracy of single measurements used for BHR prediction

The difference of FEV<sub>1</sub>, FEV<sub>1</sub>/FVC, FEF<sub>25%</sub>, FEF<sub>50%</sub>, FEF<sub>75%</sub>, FEF<sub>25%-75%</sub>, FENO, and EOS% in blood between patients with and without BHR reached statistical significance, indicating that these measurements might predict BHR status. Therefore, we constructed ROC curves to evaluate the prognostic value of these measurements for positive bronchial provocation, and AUCs were calculated (Table II).

The 2 largest AUCs for a positive BHR diagnosis were for FENO (0.788 [95% CI, 0.725-0.851]) and EOS% (0.763 [95% CI, 0.685-0.840]) (Fig 1, A). The next largest AUC was FEF<sub>25%-75%</sub>: 0.702 (95% CI, 0.641-0.763) (Fig 1, B).

### Diagnostic accuracy of FENO combined with FEF<sub>25%-75%</sub>

To determine whether combining measurements would improve BHR prediction, we first repeated the ROC analysis using the 2 measures with the largest AUCs: FENO and EOS%. The AUC was higher for the combination than for either measure alone, but this did not reach the threshold for statistical significance ( $P = .308$  and  $P = .217$ , respectively, compared with FENO and EOS% alone; Fig 2, A).

In an ROC analysis of FENO combined with FEF<sub>25%-75%</sub>, the AUC of FEF<sub>25%-75%</sub> in patients with FENO results was 0.719 ( $n = 266$ ; 95% CI, 0.656-0.782) (Fig 2, B). There was no significant difference between the AUCs of FENO and FEF<sub>25%-75%</sub> ( $P = .130$ ). However, binary logistic regression of the combination of FENO and FEF<sub>25%-75%</sub> produced an AUC of 0.843 (95% CI, 0.794-0.892), which was significantly higher than that for either FENO or FEF<sub>25%-75%</sub> alone ( $P = .0116$  and  $P < .0001$ , respectively).

### Optimal cutoff values for BHR prediction

We calculated the optimal cutoff values of FEV<sub>1</sub>, FEV<sub>1</sub>/FVC, FEF<sub>25%</sub>, FEF<sub>50%</sub>, FEF<sub>75%</sub>, FEF<sub>25%-75%</sub>, FENO, and EOS% in blood for predicting BHR as the values that gave the highest sum

of sensitivity and specificity. The cutoff values for the measures with the highest AUCs were 43 ppb for FENO, 78.5% for FEF<sub>25%-75%</sub>, and 3.5% for EOS% (Table II).

Using the cutoff values in Table II, we converted the continuous test variables to dichotomous state variables by defining FENO  $\geq 43$  ppb as "1," FENO  $< 43$  ppb as "0," FEF<sub>25%-75%</sub>  $\leq 78.5\%$  as "1," and FEF<sub>25%-75%</sub>  $> 78.5\%$  as "0." We then performed logistic regression to get a predictive equation (model) of the combination measurements, followed by multiple logistic regressions on FENO, FEF<sub>25%-75%</sub>, and FENO combined with FEF<sub>25%-75%</sub>. The AUC of the model (FEF<sub>25%-75%</sub> combined with FENO) was 0.830 (95% CI, 0.782-0.879), the AUC of FENO was 0.768 (95% CI, 0.713-0.823), and the AUC of FEF<sub>25%-75%</sub> was 0.667 (95% CI, 0.608-0.726) (Fig 3).

## DISCUSSION

BHR is the key feature of CVA and the main basis on which it is diagnosed. Its presence is generally confirmed with an MCH/histamine bronchial challenge, responsiveness to bronchodilators, or both.<sup>3,27,28</sup> The main disadvantages of bronchial challenge tests are that they are expensive, time consuming, and carry a risk of bronchial spasm. The ability to use simpler, safer, or less-expensive tests, individually or in combination, is needed, especially in primary hospitals without access to bronchial challenge tests.

We have performed a cross-sectional study to evaluate whether measures of small-airway function and airway inflammation, including FENO and FEF, can be used to predict BHR in patients with chronic cough. Increased likelihood of BHR positivity was associated with FENO  $> 43$  ppb and FEF<sub>25%-75%</sub>  $< 78.5\%$ . This association had high specificity, reinforcing the concept that FENO and small-airway dysfunction are strong risk factors of BHR in chronic cough.

FENO is widely accepted as a potential biomarker of airway inflammation, particularly eosinophilic inflammation.<sup>22,29-33</sup> It is an easy-to-obtain and noninvasive measurement that can confirm allergic or eosinophilic inflammation of central large airways, small airways, and alveoli by choosing different flow rates. It can also potentially be used to diagnose asthma with airway eosinophilia with a high degree of discriminatory power, and the ATS guidelines strongly recommend this application.<sup>22</sup> FENO has an

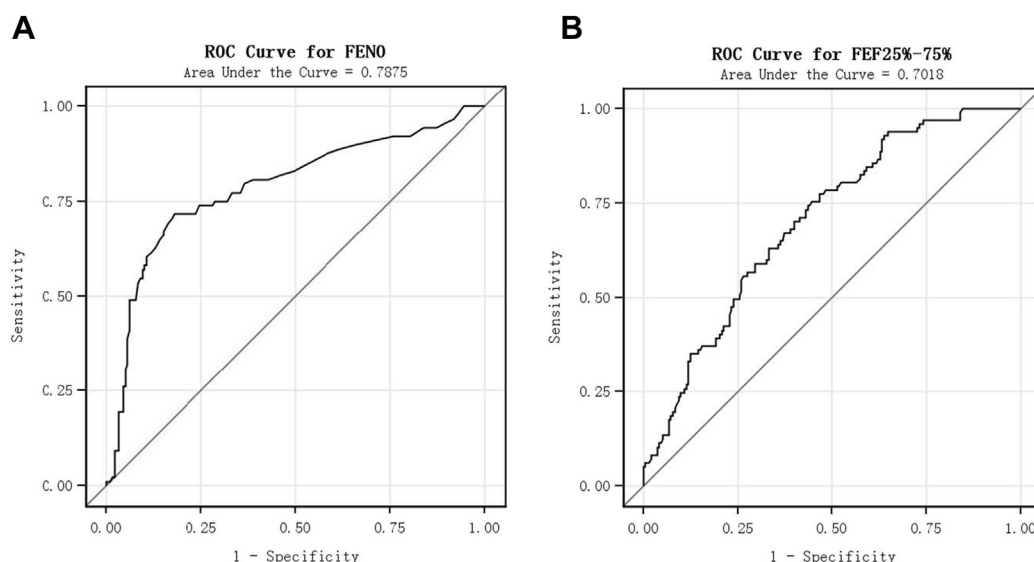


**FIGURE 1.** ROC curves of (A) FENO and (B) FEF<sub>25%-75%</sub> in predicting positive bronchial provocation tests (n = 266 for FENO; n = 290 for FEF<sub>25%-75%</sub>). AUC<sub>FENO</sub> = 0.788 (95% CI, 0.725-0.851). AUC<sub>FEF<sub>25%-75%</sub></sub> = 0.702 (95% CI, 0.641-0.763). AUC, Area under the curve; FEF<sub>25%-75%</sub>, forced expiratory flow at 25% to 75% of forced vital capacity; FENO, fractional exhaled nitric oxide; ROC, receiver operating characteristic.

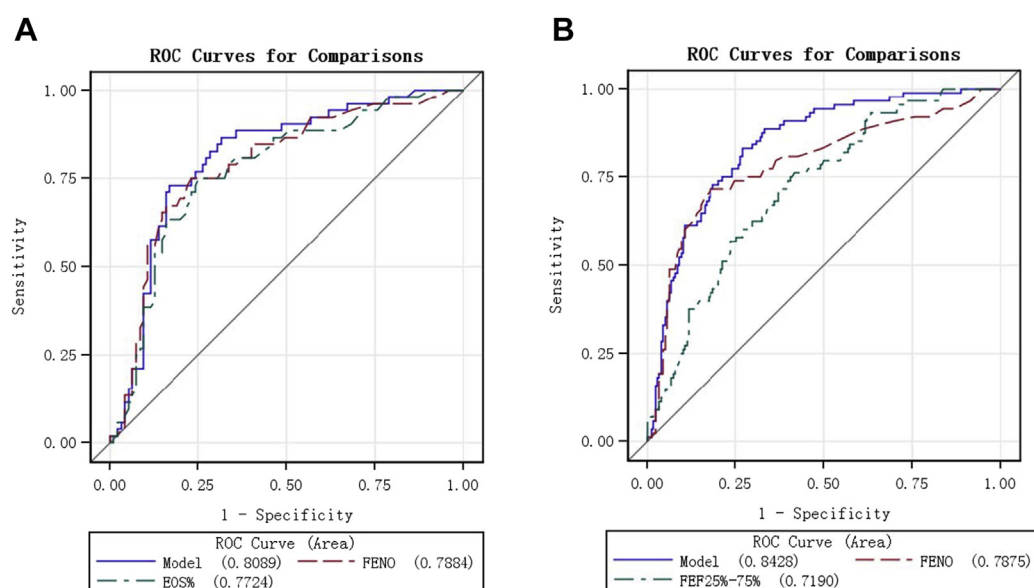


**FIGURE 2.** ROC curves for the models of (A) FENO combined with EOS% and (B) FENO combined with FEF<sub>25%-75%</sub> in predicting positive bronchial provocation. A, n = 147; AUC<sub>Model</sub> = 0.809 (95% CI, 0.735-0.883); AUC<sub>FENO</sub> = 0.788 (95% CI, 0.710-0.867; P = .308, compared with the model); AUC<sub>EOS%</sub> = 0.772 (95% CI, 0.692-0.852; P = .718 and .217, compared with FENO alone and the combined model, respectively). B, n = 266; AUC<sub>Model</sub> = 0.843 (95% CI, 0.794-0.892); AUC<sub>FENO</sub> = 0.788 (95% CI, 0.725-0.851; P = .034, compared with the model); AUC<sub>FEF<sub>25%-75%</sub></sub> = 0.719 (95% CI, 0.656-0.782; P = .130 and P < .0001, compared with FENO alone and the model, respectively). AUC, Area under the curve; FEF<sub>25%-75%</sub>, forced expiratory flow at 25% to 75% of forced vital capacity; FENO, fractional exhaled nitric oxide; ROC, receiver operating characteristic.

important role in determining the causes of chronic cough.<sup>8</sup> In untreated nonsmoking adults with chronic cough, FENO < 30 ppb has high specificity (87%), a high NPV (93%), and a helpful NLR (0.3) for diagnosing the absence of asthma.<sup>8</sup> However, FENO > 30 ppb has relatively low sensitivity (75%) and a weak

PPV (60%) for detecting asthma.<sup>8</sup> In our study, the optimal value of FENO for the clinical prediction of BHR in patients with chronic cough was >43 ppb at a flow rate of 50 mL/s, with a sensitivity and specificity of 71.59% and 82.02%, respectively. PPV and NPV were 0.663 and 0.854, respectively. Accordingly,

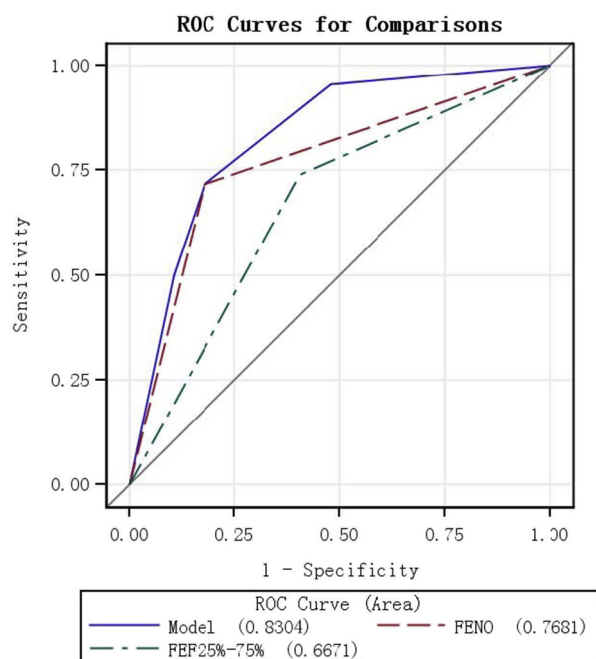


**FIGURE 3.** ROC curve of dichotomous state variables of FEF<sub>25%-75%</sub> combined with FENO (model) in predicting positive bronchial provocation, compared with FENO and FEF<sub>25%-75%</sub> alone ( $n = 267$ ). Dichotomous state variables were separated according to cutoff values of FENO and FEF<sub>25%-75%</sub>.  $AUC_{\text{Model}} = 0.830$  (95% CI, 0.782-0.879);  $AUC_{\text{FENO}} = 0.768$  (95% CI, 0.713-0.823);  $AUC_{\text{FEF25\%-75\%}} = 0.667$  (95% CI, 0.608-0.726). *AUC*, Area under the curve; *FEF<sub>25%-75%</sub>*, forced expiratory flow at 25% to 75% of forced vital capacity; *FENO*, fractional exhaled nitric oxide; *ROC*, receiver operating characteristic.

FENO may have a greater value as a negative predictive parameter in distinguishing patients with BHR from those without. The relatively low sensitivity of FENO limits its use as a sole diagnostic test for BHR and asthma, perhaps because eosinophilic inflammation is not always required as a trigger of BHR development in the very early stage of asthma.<sup>34</sup> However, when added to traditional assessment tools such as spirometry, FENO improved diagnostic accuracy.

Although FENO and BHR reflect different clinical characteristics in asthma, a functional relationship between these 2 measurements cannot be excluded. Some scholars hold that BHR in asthma is partly linked to airway eosinophilic inflammation that can be attenuated by corticosteroids. Moreover, nitric oxide may itself contribute to BHR by increasing airway edema, because it is a latent vasodilator responsible for plasma exudation, and the transformation of nitric oxide into peroxynitrite induces airway hyperresponsiveness in guinea pigs.<sup>35</sup> Our research showed that there is a remarkable difference in FENO values between the patients with chronic cough and positive bronchial provocation results and those with negative results. Moreover, there was a significant association between FENO and PD<sub>20</sub> in patients positive for BHR, indicating that FENO may be a predictive measure for BHR.

Clearly, a cutoff value will be easier for clinicians to use than having to apply equations. Thus, the use of cutoff values is

recommended by the ATS guidelines.<sup>22</sup> In a recently published study, the value of FENO > 34 ppb had a predictive value ( $AUC = 0.62$ ) for provocative concentration of a substance causing a 20% fall in FEV<sub>1</sub> < 16 mg/mL in patients with suspected asthma in whom a bronchodilation test either failed to demonstrate reversibility or was not indicated.<sup>32</sup> Another recent study illustrated that the AUC for FENO was 0.656 (95% CI, 0.600-0.712;  $P < .001$ ) in patients with asthma or chronic obstructive pulmonary disease.<sup>33</sup> Consistent with this, the optimal cutoff point for differentiating BHR from non-BHR in patients with chronic cough with and without BHR was an FENO level of > 43 ppb, within the range of 25 to 50 ppb proposed by recent guidelines<sup>22</sup> for supporting a diagnosis of asthma.

Small airways, traditionally considered to be a silent zone, actually play an important role in asthma similar to that of large airways.<sup>11,36</sup> Small-airway dysfunctions such as airway wall thickening, airway narrowing, and hyperresponsiveness contribute to poor control of asthma, frequent exacerbations, the late-phase allergic response, nocturnal asthma attacks, exercise-induced asthma attacks, and more severe BHR.<sup>11</sup> Small airways may have stronger inflammation than central airways in asthmatic patients.<sup>37</sup> Interestingly, the alveolar component of FENO (CalvNO), estimated by measuring exhaled NO at multiple expiratory flow rates and plotting NO output versus expiratory flow rates, is negatively correlated with FEF<sub>25%-75%</sub>. CalvNO is a specific marker of small-airway dysfunction in stable asthma and is associated with unsatisfactory asthma control in patients with mild untreated asthma.<sup>38,39</sup> Furthermore, many asthmatic patients with normal FEV<sub>1</sub> have air trapping,<sup>40</sup> which is better correlated with CalvNO than with FEV<sub>1</sub>,<sup>41</sup> supporting the notion that abnormalities of peripheral airways are involved in the mildest forms of asthma.

Our finding that the FEF<sub>25%-75%</sub> value of patients with positive bronchial provocation tests is much lower than that of patients with negative bronchial provocation tests confirms that small-airway dysfunction exists in patients with CVA, whose FVC, FEV<sub>1</sub>, and FEV<sub>1</sub>/FVC values are normal. When FEF<sub>25%-75%</sub> alone was used for predicting BHR, the AUC was 0.702 (95% CI, 0.641-0.763), indicating that FEF<sub>25%-75%</sub> alone is insufficient for predicting BHR in patients with chronic cough. Combining FEF<sub>25%-75%</sub> with FENO provided significantly better prediction of bronchial provocation results than either measure alone. Finally, optimal cutoff values of >43 ppb for FENO and <78.5% for FEF<sub>25%-75%</sub> allowed us to discriminate the patients with CVA from the whole cohort of patients in our study, as confirmed in an ROC analysis.

The EOS% in blood better predicted BHR than forced mid-expiratory flow. However, the combination of EOS% and FENO did not improve the predictive accuracy for BHR. Our results show that changes in FENO and EOS% are strongly correlated in patients with chronic cough, which may limit any increase in their power to predict BHR when combined.

There are a number of limitations regarding our study. First, increased FENO levels are positively associated with atopy and with concomitant diseases such as rhinitis and viral infections,<sup>42,43</sup> and the presence of these conditions should be considered when interpreting FENO results. Unfortunately, because of the cross-sectional design of our study and retrospective data collection, we were not able to get more information about concomitant diseases such as rhinitis and atopy.

Second, FENO measurement has been observed to be superior even to bronchoprovocation for asthma diagnosis, in patients with eosinophilic asthma,<sup>44</sup> whereas it has no diagnostic value in noneosinophilic asthma.<sup>44</sup> The diagnostic accuracy of FENO increased when patients with neutrophilic inflammation were omitted from analysis.<sup>33</sup> These differences in results between subsets of patients highlight the importance of study cohort selection, particularly with regard to using sputum eosinophil measurements to exclude patients with noneosinophilic inflammation. A prospective randomized controlled trial including only patients with eosinophilic asthma and with strict limits on concomitant diseases and treatments is needed to confirm the results of our cross-sectional study.

Furthermore, although bronchoprovocation tests are of great value in the diagnosis of CVA, false-positive diagnoses might result if individuals have recently recovered from an infectious disease, or have gastroesophageal reflux disease, allergic rhinitis, or many other conditions.<sup>45</sup> A delayed type of diagnostic study or a prospective study using clinical follow-up to define CVA on the basis of clinical symptoms and signs compatible with the diagnosis together with the presence of BHR is needed to determine the diagnostic value of FENO and FEF<sub>25%-75%</sub> for CVA diagnosis, as compared with bronchial provocation tests.

In conclusion, we found that small-airway dysfunction exists in patients with chronic cough who have a positive MCH challenge. FENO and FEF<sub>25%-75%</sub> may have value as negative predictive parameters in discriminating between chronic cough patients with and without BHR. FENO > 43 ppb and FEF<sub>25%-75%</sub> < 78.5% were highly predictive of a positive bronchial provocation test.

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WB, XZ, and MZ conceived of and designed entire study. CL, BW, and XZ contributed to data collection. WB, XZ, and LB were involved in interpreting spirometric assessments, bronchial provocation tests, and FENO data. JY and ZH performed statistical analysis. WB and XZ wrote the manuscript supervised by MZ and XZ. All authors critically reviewed and approved the final version.

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