The efficacy of the combination of exhaled nitric oxide and blood eosinophil count in the diagnosis of asthma in China

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Abstract

Background

Tests to identify reversible airflow limitation are important in asthma diagnosis, but they are time-consuming and may be difficult for patients to cooperate. We aim to evaluate the predictive value of fractional exhaled nitric oxide (FeNO) and blood eosinophil (B-Eos) count in asthma diagnosis, and to distinguish patients who could avoid reversibility testing.

Methods

We screened 7463 suspected asthma cases between January 2014 and December 2019 in Chongqing, China, and identified 2349 patients with complete FeNO, B-Eos count, and spirometry data. Of these, 824 were diagnosed with asthma via a positive bronchial-provocation or bronchodilation test.

Results

When FeNO and B-Eos counts were used in combination, the area under the receiver operating characteristic curve (AUC) for diagnosing asthma increased (0.768 vs. 0.745 or 0.728; both \( P < 0.001 \)). The odds ratio for having asthma increased progressively with a gradual increase in FeNO or B-Eos count (both \( P < 0.001 \)). Further analysis of in-series combinations of different threshold values for these biomarkers indicated that moderately elevated biomarker levels (FeNO > 40 ppb and B-Eos > 300 cells/\( \mu l \)) support a diagnosis of asthma because diagnostic specificity was > 95% and the positive likelihood ratio (PLR) was > 10. This conclusion was verified when selecting the data from 2017 to 2019 as the verification cohort.

Conclusion

The combination of FeNO and B-Eos count can improve diagnostic efficacy for asthma. Patients with moderately elevated biomarkers (FeNO > 40 ppb and B-Eos > 300 cells/\( \mu l \)) could be diagnosed with asthma.

Introduction

Asthma is characterized by recurrent respiratory symptoms and a variable expiratory-airflow limitation, affecting approximately 334 million people worldwide.[1, 2] Meanwhile, many asthma patients are still underdiagnosed, which leads to a decrease in work productivity and poor vitality and mental health.[3, 4] The main reason is that the common symptoms of asthma are relatively non-specific,[5] and the objective tests recommended by the Global Initiative for Asthma (GINA), including the bronchial-provocation test (BPT) and the bronchodilation test (BDT), require complex cooperation from patients, over long durations of examination time, and might pose certain risks.[5, 6] Therefore, finding a simple and effective method for diagnosing asthma is an urgent clinical problem.
As asthma is a T-helper 2 (Th2) cell–driven inflammatory disease,[7, 8] even moderate to severe persistent corticosteroid-refractory (defined as Th2-low) asthma has Th2-high features.[9] Fractional exhaled nitric oxide (FeNO) and blood eosinophil (B-Eos) count have been suggested as biomarkers to distinguish airway inflammation in asthma.[10, 11] Unfortunately, FeNO or B-Eos count alone is insufficient to accurately diagnose asthma.[12–14] Although previous studies showed that combining these two biomarkers provides additional predictive information,[15] several limitations have been identified. In some studies, the researchers confirmed that these two biomarkers could be used to identify types of chronic respiratory diseases, but such studies were conducted in the general population.[16, 17] Significant differences in FeNO and B-Eos count between asthmatic and healthy people can lead to overestimating the diagnostic accuracy of these two biomarkers. Meanwhile, some conclusions have been drawn on the basis of selected asthma patients such as adolescents and young adults[18] and might not be applicable to all adult patients. In addition, the diagnosis of asthma in some studies was self-reported (mainly based on non-specific patient symptoms),[17, 19] which led to underdiagnosis of asthma because patients inadequately reported respiratory symptoms to their doctors.[5] Importantly, due to the lack of widely accepted definitions of high FeNO levels and high B-Eos counts, the combination of these two biomarkers for the diagnosis of asthma is worthy of further study.

In the present study, we diagnosed asthma when patients had recurrent respiratory symptoms and a positive result of an objective test (BPT or BDT). We showed the exact profiles of these two biomarkers in asthmatic patients and evaluated the accuracy of FeNO or B-Eos alone or both in combination for the diagnosis of asthma. Finally, we analyzed combinations of different threshold levels of these two biomarkers to identify which patient groups could avoid complex objective tests.

Methods And Materials

Population

We screened all patients who were admitted to the respiratory clinic of Daping Hospital for suspected asthma between January 2014 and December 2019. Patients suspected to have asthma based on their recurrent respiratory symptoms. Inclusion criteria were as follows: (1) age > 12 years; (2) no respiratory infections within the past 7 days; (3) no current treatment with inhaled or oral corticosteroids, leukotriene receptor antagonists, or antihistamines; and (4) no reported history of serious disease in other systems. All examinations were prescribed simultaneously by the same clinician and mostly completed on the same day. It is important to perform the FeNO test before performing spirometry; doctors performing BPT/BDT tests did not know FeNO or B-Eos results. A total of 7463 suspected asthmatic patients were screened (Fig. 1). Due to concerns about the extra costs of the FeNO test or pain caused by venipuncture to obtain blood samples to count B-Eos, as well as the personal diagnosis and treatment habits of clinicians, many data were incomplete. Among them were data from subjects who did not undergo FeNO and B-Eos tests (n = 1492) and participants without FeNO (n = 579) or B-Eos measurements (n = 3043). Ultimately, 2349 patients with complete data were enrolled in the main study, and the conclusions obtained from this cohort were verified with data from incomplete cases.
Fractional exhaled nitric oxide

FeNO was evaluated with an online measurement technique using the Nano Coulomb nitric oxide analyzer (Shangwo Biotechnology Co., Ltd., Jiangsu, China), following the recommendations from the European Respiratory Society (ERS) and the American Thoracic Society (ATS).[20] FeNO results are reported as parts per billion (ppb). See the Supplementary Materials for more measurement details.

Blood eosinophil count

Peripheral venous blood samples were taken, and B-Eos and leukocytes were counted using a Sysmex XN-9000 Hematology Analyzer (Sysmex, Kobe, Japan), a multifunctional automatic hematology analyzer and leukocyte classifier. B-Eos counts were reported along with other leukocyte subpopulations, and the percentage of each subpopulation was calculated.

Spirometry, bronchial provocation test and bronchial dilation test

Baseline spirometry, the BPT, and the BDT were performed using a Jaeger spirometer (Erich Jaeger GmbH, Würzburg, Germany) according to ATS/ERS recommendations.[21] The BPT was performed for patients whose baseline forced expiratory volume in the first second (FEV₁) was > 70% of the predicted value, and it was measured using a Jaeger Aerosol Provocation System.[22] Results were considered positive when the methacholine (Sigma-Aldrich, St. Louis, MO, USA) cumulative dose causing a 20% decrease in FEV₁ (PD₂₀-FEV₁) was < 2.5 mg. The BPT was usually preferred, but the BDT was performed when the patient’s baseline FEV₁ was < 70% of the predicted value. After the patient had inhaled 400 µg albuterol sulfate aerosol (GlaxoSmithKline, Brentford, UK), reversibility test results were considered positive when FEV₁ was increased more than 12% and 200 ml above baseline. Positive BPT results are presented as follows: +/− indicates that the PD₂₀-FEV₁ of methacholine was 1.076–2.500 mg; + indicates that it was 0.294–1.075 mg; ++ indicates that it was 0.035–0.293 mg; +++ indicates that it was < 0.035 mg. BDT results are presented as follows: − indicates that FEV₁ increased less than 12% or 200 ml above baseline after inhalation of 400 µg salbutamol sulfate aerosol; + indicates that FEV₁ increased by 12%–25% and its absolute value increased by 200 ml; ++ indicates that FEV₁ increased by 25%–40% and its absolute value increased by 200 ml; +++ indicates that FEV₁ increased > 40% and its absolute value increased by 200 ml.

Methods of fractional exhaled nitric oxide

During the inspiratory phase, the patient was required to inhale to their total lung capacity through a mouthpiece with a protective filter, which was used to prevent the contamination of ambient nitric oxide. During the exhalation phase, the participant was guided through an animated interface on the device to maintain a correct constant expiratory flow rate (50 ml/s). FeNO measurements were performed prior to spirometry, the methacholine challenge test, and the reversibility test.

Statistical analysis
Statistical analyses were mainly performed using SPSS software version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables are shown as mean ± standard deviation (SD) or as median and interquartile range (IOR), and categorical variables are presented as numbers and percentages. Student’s *t* test was used for normally distributed continuous variables, and the Mann–Whitney *U* test was used for continuous non-normally distributed variables. Categorical variables were analyzed by Pearson’s chi-square test. The relationship between two continuous variables was assessed by determining Spearman’s rank correlation coefficient. Receiver operating characteristic (ROC) curve analysis was performed using MedCalc software version 18.2.1 (MedCalc Software, Ostend, Belgium). The optimal cutoff values of these two biomarkers were obtained based on the highest value of the Youden index. The Hanley–McNeil non-parametric method was employed to compare the area under the ROC curve. The overlap of asthma patients with normal or elevated FeNO and B-Eos count is displayed in a Venn diagram (constructed using the online interactive Venn diagram viewer jvenn [23]). Logistic regression analysis was performed to assess risk factors for asthma or low FEV₁. A forest plot was drawn using GraphPad Prism software version 8 (GraphPad Software, San Diego, CA, USA). The increase in odds ratio was assessed by the Cochran–Armitage trend test. *P*-values < 0.05 were considered to indicate statistical significance unless otherwise specified. To evaluate the impact of bias caused by missing data, we performed sensitivity analyses to verify whether the incomplete data population differed from the main study population.

## Results

### characterization of study population

The main study population included 897 males and 1452 females, of whom 824 patients were diagnosed with asthma. As shown in Table 1, the baseline characteristics of the two groups were consistent (asthma vs. non-asthma; *P* > 0.05), while asthmatic patients had significantly higher white blood cell counts, B-Eos counts, B-Eos percentages, and FeNO levels (7.24 vs. 7.05 × 10⁹/l, 306 vs. 105 cells/µl, 4.5% vs. 1.8%, and 52 vs. 25 ppb, respectively; all *P* < 0.001). Conversely, the percentage of blood neutrophils, forced vital capacity (FVC), forced expiratory volume in the first second (FEV₁), and FEV₁/FVC ratio of asthmatic patients were significantly lower (59.3% vs. 61.9%, 94.4% vs. 96.2%, 79.65% vs. 92.40%, and 68.54% vs. 80.24%, respectively; all *P* < 0.001). Patients with incomplete data (n = 5114) were similar to the main study population with respect to demographic characteristics, proportion of asthma diagnoses, FeNO level, and B-Eos count (*P* > 0.05; Table S1; Figure S1A–B).
Table 1
Characteristics of the study participants (n = 2349).

| Characteristics | Asthma (n = 824) | Non-asthma (n = 1525) | P value |
|-----------------|-----------------|-----------------------|---------|
| Age (years)*    | 46 (36–53)      | 47 (35–55)            | 0.494   |
| Height (cm)*    | 159 (153–165)   | 159 (153–165)         | 0.695   |
| Weight (kg)*    | 59 (52–66)      | 59 (53–67)            | 0.983   |
| BMI (kg/m²)*    | 23.38 (21.23–25.71) | 23.5 (21.21–25.87)  | 0.250   |
| WBC count (× 10⁹/l)* | 7.24 (6.06–8.83) | 7.05 (5.86–8.68)     | < 0.001 |
| %Neu (%)*       | 59.30 (52.50–65.70) | 61.90 (55.20–68.90)  | < 0.001 |
| B-Eos count (cells/µl)* | 306 (148–542)   | 125 (66–238)          | < 0.001 |
| %B-Eos (%)*     | 4.50 (2.10–7.40) | 1.80 (1.00–3.30)      | < 0.001 |
| FeNO (ppb)*     | 52 (25–87)      | 25 (17–35)            | < 0.001 |
| FVC (predicted %)* | 94.40 (83.40–104.38) | 96.20 (86.60–106.90) | 0.001   |
| FEV₁ (predicted %)* | 79.65 (62.95–91.10) | 92.40 (80.55–103.50) | < 0.001 |
| FEV₁/FVC (%)*   | 68.54 (60.4–77.35) | 80.24 (73.06–84.89)  | < 0.001 |
| Gender‡          |                 |                       | 0.679   |
| Female           | 938 (64.60%)    | 514 (35.40%)          |         |
| Male             | 587 (65.40%)    | 310 (34.60%)          |         |
| Objective test‡  |                 |                       | 0.057   |
| BPT             | 1022 (66.30%)   | 520 (33.70%)          |         |
| BDT             | 503 (62.30%)    | 304 (37.70%)          |         |

Notes: Data are presented as median (interquartile range) or number (percentage).

*Data were analyzed using the Mann–Whitney U test.

‡Data were analyzed using Pearson's chi-square test.

Abbreviations: BMI, body mass index; %Neu, percentage of blood neutrophils; WBC, white blood cell; B-Eos, blood eosinophil; %B-Eos, percentage of blood eosinophils; FeNO, fractional exhaled nitric oxide; FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 second.

Correlation between biomarkers and BPT or BDT
We found a weak correlation between FeNO levels and B-Eos counts (Spearman's $\rho$ was 0.460 in asthmatic patients and 0.167 in non-asthmatic patients; both $P<0.001$; Fig. 2A–B). These two biomarkers were significantly related to the cumulative dose of methacholine (Spearman's $\rho = 0.370$ or 0.227; $P<0.001$; Fig. 2C–D). When classified by airway hyperresponsiveness level (according to the cumulative dose of methacholine), FeNO and B-Eos count in the moderate to severe group were higher than in the mild group (73 or 70 vs. 45 ppb, and 402 or 347 vs. 293 cells/µl, respectively; both $P<0.05$; Figure S1C–D). Although FeNO and B-Eos count were related to airway reversibility (Spearman's $\rho = 0.332$ or 0.325; $P<0.001$; Fig. 2E–F), these two biomarkers were not significantly different in any BDT-positive subgroup (grouped by the increase in FEV$_1$ after the BDT; 52 vs. 51 vs. 48.5 ppb, and 290 vs. 312 vs. 328 cells/µl, respectively; all $P>0.05$; Figure S1E–F).

**Diagnostic capabilities of biomarkers**

The area under the receiver operating characteristic curve (AUC) of asthma diagnosis showed no difference between FeNO and B-Eos count ($P=0.212$), but the AUC improved when these two biomarkers were used in combination (0.768 vs. 0.745 or 0.728; both $P<0.001$; Fig. 3A). The AUC of asthma diagnosis presented no difference between B-Eos count and percentage (0.728 vs. 0.727; $P=0.734$; Fig. 3B). When stratified by age (45 years, the median age of all patients), the diagnostic accuracy of these two biomarkers was higher in younger patients (Fig. 3C–D). Whether we included incomplete data or stratified by BPT, BDT, sex (females hardly smoke[24]), or body mass index (BMI), the diagnostic accuracy of these two biomarkers did not considerably change (Figure S2A–F). According to the maximum Youden index, the optimal cutoff values for FeNO and B-Eos count to diagnose asthma were 38 ppb and 203 cells/µl, respectively; the sensitivities and specificities were respectively 62.74% and 81.44%, and 67.23% and 69.9% (Table S2).

**Overlap between asthma patients and normal or elevated biomarkers**

Based on the optimal cutoff value for these two biomarkers (FeNO, 38 ppb; B-Eos, 203 cells/µl), we classified the 2349 patients into four groups: group A (high FeNO, high B-Eos count), group B (high FeNO, low B-Eos count), group C (low FeNO, high B-Eos count), and group D (low FeNO, low B-Eos count). The overlap between asthmatic patients and increased FeNO or B-Eos count is shown in Fig. 4A. The proportion of asthma patients showed no difference between groups B and C (37.8% vs. 29.8%; $P=0.025$, which showed no statistical difference after adjustment by the Bonferroni method; Fig. 4B). The proportion of asthma patients was significantly different between any other two of the four groups (all $P<0.001$). Asthma patients accounted for 75.40% of group A, while in group D, the proportion of asthma was the lowest at 15.5%.

**Diagnostic accuracy for asthma of FeNO and B-Eos combined**
We analyzed the diagnostic accuracies of in-series combinations of different threshold levels of FeNO and B-Eos for asthma (Tables 2 and S3). When linking the different thresholds of these two biomarkers to reach the goals of positive likelihood ratio (PLR) > 10 and a diagnostic specificity > 95% (Fig. 4C–D), which permitted a diagnosis of asthma,[25] the appropriate folding point was FeNO > 40 ppb and B-Eos > 300 cells/μl. Correspondingly, 327 patients were diagnosed, accounting for 39.7% of total asthma patients, and the misdiagnosis rate was as low as 4.5% (Table S4). This conclusion was verified when selecting the data from 2017 to 2019 as the verification cohort (Table S5).
Table 2
Diagnostic accuracy of different combinations of threshold values of these two biomarkers (n = 2349).

| Categories                  | Sensitivity (%) | Specificity (%) | PLR | NLR | PPV (%) | NPV (%) |
|-----------------------------|-----------------|-----------------|-----|-----|---------|---------|
| B-Eos > 100 cells/µl*       |                 |                 |     |     |         |         |
| FeNO > 20 ppb†              | 69.71           | 64.25           | 1.9 | 0.5 | 51.31   | 79.70   |
| FeNO > 30 ppb†              | 58.95           | 80.39           | 3.0 | 0.5 | 61.89   | 78.38   |
| FeNO > 40 ppb†              | 50.98           | 90.38           | 5.3 | 0.5 | 74.12   | 77.33   |
| FeNO > 50 ppb†              | 43.09           | 93.87           | 7.0 | 0.6 | 79.16   | 75.32   |
| FeNO > 60 ppb†              | 35.31           | 96.09           | 9.0 | 0.7 | 83.00   | 73.33   |
| FeNO > 70 ppb†              | 28.76           | 97.62           | 12.1| 0.7 | 86.73   | 71.72   |
| FeNO > 80 ppb†              | 24.26           | 98.35           | 14.7| 0.8 | 88.83   | 70.62   |
| FeNO > 90 ppb†              | 19.75           | 98.85           | 17.2| 0.8 | 90.26   | 69.51   |
| B-Eos > 200 cells/µl*       |                 |                 |     |     |         |         |
| FeNO > 20 ppb†              | 56.07           | 81.10           | 3.0 | 0.5 | 61.58   | 77.36   |
| FeNO > 30 ppb†              | 47.42           | 89.63           | 4.6 | 0.6 | 71.19   | 75.93   |
| FeNO > 40 ppb†              | 41.00           | 94.92           | 8.1 | 0.6 | 81.33   | 74.86   |
| FeNO > 50 ppb†              | 34.66           | 96.76           | 10.7| 0.7 | 85.25   | 73.27   |
| FeNO > 60 ppb†              | 28.40           | 97.93           | 13.7| 0.7 | 88.13   | 71.68   |
| FeNO > 70 ppb†              | 23.13           | 98.74           | 18.4| 0.8 | 90.86   | 70.39   |
| FeNO > 80 ppb†              | 19.51           | 99.13           | 22.4| 0.8 | 92.37   | 69.51   |
| FeNO > 90 ppb†              | 15.89           | 99.39           | 26.1| 0.8 | 93.38   | 68.62   |
| B-Eos > 300 cells/µl*       |                 |                 |     |     |         |         |
| FeNO > 20 ppb†              | 41.83           | 89.93           | 4.2 | 0.6 | 69.18   | 74.10   |

*Baseline screening value of the former biomarker.
†Progressively increasing cutoff values of the combined biomarkers.
‡The optimal diagnostic cutoff value for each biomarker alone.
| Categories               | Sensitivity (%) | Specificity (%) | PLR | NLR | PPV (%) | NPV (%) |
|-------------------------|-----------------|-----------------|-----|-----|---------|---------|
| FeNO > 30 ppb†          | 35.38           | 94.47           | 6.4 | 0.7 | 77.58   | 73.01   |
| FeNO > 40 ppb†          | 30.59           | 97.29           | 11.3| 0.7 | 85.92   | 72.18   |
| FeNO > 50 ppb†          | 25.86           | 98.27           | 15.0| 0.8 | 89.00   | 71.04   |
| FeNO > 60 ppb†          | 21.19           | 98.90           | 19.2| 0.8 | 91.23   | 69.90   |
| FeNO > 70 ppb†          | 17.26           | 99.33           | 25.8| 0.8 | 93.30   | 68.96   |
| FeNO > 80 ppb†          | 14.56           | 99.54           | 31.4| 0.9 | 94.43   | 68.31   |
| FeNO > 90 ppb†          | 11.85           | 99.68           | 36.6| 0.9 | 95.18   | 67.67   |
| B-Eos > 400 cells/µl*   |                 |                 |     |     |         |         |
| FeNO > 20 ppb†          | 31.89           | 93.22           | 4.7 | 0.7 | 71.77   | 71.70   |
| FeNO > 30 ppb†          | 26.97           | 96.28           | 7.3 | 0.8 | 79.67   | 70.93   |
| FeNO > 40 ppb†          | 23.32           | 98.18           | 12.8| 0.8 | 87.36   | 70.32   |
| FeNO > 50 ppb†          | 19.72           | 98.84           | 17.0| 0.8 | 90.16   | 69.50   |
| FeNO > 60 ppb†          | 16.16           | 99.26           | 21.8| 0.8 | 92.17   | 68.66   |
| FeNO > 70 ppb†          | 13.16           | 99.55           | 29.2| 0.9 | 94.04   | 67.96   |
| FeNO > 80 ppb†          | 11.10           | 99.69           | 35.5| 0.9 | 95.05   | 67.48   |
| FeNO > 90 ppb†          | 9.04            | 99.78           | 41.4| 0.9 | 95.72   | 67.00   |
| B-Eos > 500 cells/µl*   |                 |                 |     |     |         |         |
| FeNO > 20 ppb†          | 22.57           | 95.55           | 5.1 | 0.8 | 73.25   | 69.55   |
| FeNO > 30 ppb†          | 19.09           | 97.56           | 7.8 | 0.8 | 80.85   | 69.05   |
| FeNO > 40 ppb†          | 16.51           | 98.80           | 13.8| 0.8 | 88.16   | 68.65   |
| FeNO > 50 ppb†          | 13.95           | 99.24           | 18.3| 0.9 | 90.80   | 68.10   |
| FeNO > 60 ppb†          | 11.43           | 99.51           | 23.5| 0.9 | 92.69   | 67.53   |

*Baseline screening value of the former biomarker.
†Progressively increasing cutoff values of the combined biomarkers.
‡The optimal diagnostic cutoff value for each biomarker alone.
### Comparing the risk of having asthma or reduced lung function between subgroups

The multivariable adjusted odds ratio (aOR) of having asthma was 1.39 (95% confidence interval [CI], 1.13–1.70) for females (Fig. 5A). The aOR of having asthma was significantly higher in group A than in group B or C (17.6 vs. 3.49 or 2.36; both *P* < 0.001; Fig. 5B). The aOR of having asthma increased progressively with a gradual increase in FeNO or B-Eos count (*P* < 0.001; Fig. 5C–D). Correspondingly, the aOR of having reduced lung function (FEV<sub>1</sub> < 80% of the predicted value) was 1.28 (1.07–1.55) for males (Fig. 6A), 2.17 (1.80–2.62) for older patients (age > 45 years) and 2.26 (1.75–2.93) for patients with high leukocyte counts (> 10<sup>10</sup> cells/l). The aOR of having reduced lung function was higher in group A than in group B or C (3.96 vs. 1.88 or 1.86; both *P* > 0.001; Fig. 6B). The aOR of reduced lung function increased progressively with a gradual increase of B-Eos count or FeNO (*P* < 0.001; Fig. 6C–D). These results did not significantly change when we included incomplete data (Figure S3).

### Discussion

The profiles of FeNO level and B-Eos count in asthmatic patients and the accuracy of these two biomarkers in the diagnosis of asthma are unclear. In the present study, we provide a large-cohort analysis of the relationship among FeNO, B-Eos, and asthma in Chinese patients. According to receiver operating characteristic (ROC) curve analysis, combining FeNO and B-Eos counts can improve diagnostic efficacy for asthma. Another interesting finding was that the risk of having asthma increased progressively with a gradual increase in FeNO or B-Eos count. Notably, patients with moderately elevated biomarkers (FeNO > 40 ppb and B-Eos > 300 cells/µl) could be diagnosed with asthma, as diagnostic specificity is > 95% and the PLR > 10. Although diagnostic sensitivity was reduced to 30.59%, these patients benefited from avoiding BPTs, especially
since the simultaneous increase in FeNO and B-Eos count is associated with higher bronchial hyperresponsiveness,[18] which may trigger an acute exacerbation of asthma.

At present, in order to avoid underdiagnosis or overdiagnosis, objective tests must be conducted to confirm the diagnosis of asthma.[5] However, there are several limitations to objective tests.[6] The BPT is time consuming, has a risk of triggering asthma attack, and is generally not available in primary care; the BDT has limited value for distinguishing asthma from chronic airway diseases; and variable peak expiratory flow requires good cooperation and adherence.[26–28] Conspicuously, the UK National Institute for Health and Care Excellence (NICE) recommends that FeNO, a potential indirect predictor of Th2 airway inflammation in asthma, should be measured in all suspected asthma patients.[29] Our data indicated that the optimal cutoff level for FeNO in the diagnosis of asthma was 38 ppb, in line with the recommendation by Japanese Respiratory Society (JRS).[30] who recommend using a FeNO cutoff value of 35 ppb to diagnose asthma. Meanwhile, GINA conservatively points out that measurement of FeNO alone is insufficient to determine or rule out asthma.[1] This is because diagnostic cutoff values for FeNO are mostly concentrated in the intermediate range (25–50 ppb), and these levels can overlap extensively between asthma and other diseases.[31, 32] Therefore, many guidelines recommend that FeNO should be combined with other objective evidence to identify inflammatory respiratory diseases.[1, 26, 29, 33]

B-Eos count, another promising and easy-to-measure biomarker, is more attractive as a means of diagnosing asthma.[6, 34] In this study, we found that the optimal diagnostic cutoff level was 203 cells/µl for B-Eos to identify asthma. Consistent with previous reports,[13, 35] our data indicated that FeNO or B-Eos count alone had only moderate accuracy for diagnosing asthma, so using a single biomarker for this purpose will yield many false negatives and false positives. In all suspected asthma cases, as B-Eos count gradually increased, the risk of having FEV₁ < 80% of the predicted value significantly increased. In addition, previous studies have demonstrated that high B-Eos counts are related to poor asthma control, risk of exacerbations, and response to maintenance of inhaled corticosteroids.[36–38] It is becoming crucial that the measurement of blood eosinophils adds predictive and prognostic information to airway disease.[39]

Although FeNO and B-Eos are part of the Th2 inflammation cascade, these two biomarkers are regulated by different inflammatory pathways.[11] Activation of the Th2 inflammatory cascade leads to secretion of various cytokines, including interleukin-4 and –13 (IL-4, IL-13), which activate nitric oxide synthase to increase FeNO in bronchial epithelial cells. IL-5 acts on IL-5 receptor subunit α (IL5RA), causing eosinophilia.[10] Similar to a previous report by Malinovschi et al.,[16] FeNO was weakly correlated with B-Eos count in this study, but our data also revealed that the correlation between FeNO and B-Eos count was stronger in asthma patients than in non-asthma patients. These results suggested that the combination of FeNO and B-Eos count could help diagnose asthma. Based on ROC curve analysis, the AUC of asthma diagnosis based on both biomarkers combined was higher than that based on single biomarker alone. Since positive and negative predictive values depend on the prevalence of the disease irrespective of the sensitivity and specificity,[25, 40] when we seek threshold levels for a diagnosis of asthma using both biomarkers, the goal is to achieve an ultrahigh PLR (> 10). When linking the different
thresholds of these two biomarkers, the appropriate folding point is found at a FeNO of 40 ppb and a B-Eos count of 300 cells/µl.

Notably, our entire study population included patients with suspected early asthma who underwent the BPT and those with severe symptoms who underwent the BDT, which is more representative of suspected asthma patients of all adult ages. The advantage of this study was the large number of asthmatic patients who were evaluated by spirometry and standardized clinical examination. However, this study also had some limitations. Due to the large proportion of incomplete data, we repeated the main analysis on this incomplete data and examined the risk of selection bias. The results of these secondary analyses were similar to those of our primary analysis. Since the smoking status of the study population was unknown, we calculated the diagnostic accuracy of these two biomarkers in women (who rarely smoke in China[24]), and the results did not change significantly. This indirectly indicated that smoking status had a limited effect on the diagnostic efficiency of these two biomarkers. As this was a retrospective study, although we followed a uniform inclusion procedure, there were still potential selection biases. Moreover, the atopic statuses and comorbidities of patients in this study were not fully known, which might have affected the efficiency of these two biomarkers in diagnosing asthma. These issues are worthy of further evaluation in prospective studies.

In conclusion, there was no difference in diagnostic accuracy for asthma between FeNO and B-Eos count, but the combination of these two biomarkers could improve diagnostic efficacy. Notably, patients with moderately elevated biomarkers (FeNO > 40 ppb and B-Eos > 300 cells/µl) could be diagnosed with asthma, which can be applied as a practical diagnostic tool for asthma in primary care.

**Abbreviations**

FeNO: Fractional exhaled nitric oxide; B-Eos: Blood eosinophil; BPT: Bronchial-provocation test; BDT: Bronchodilation test; GINA: Global Initiative for Asthma; AUC: Area under the receiver operating characteristic curve; PLR: Positive likelihood ratio.

**Declarations**

**ACKNOWLEDGMENTS**

We thank Dr. Youming Zhang from the National Heart and Lung Institute, Imperial College London, UK; and Dr. Neil Barnes from the William Harvey Research Institute, London, UK for critically reading and editing the manuscript. Thanks to Dr. Xin Yao from the First Affiliated Hospital of Nanjing Medical University, China, for guidance on statistical analysis in this study. We thank LetPub (www. letpub.com) for its linguistic assistance during the preparation of this manuscript.

**AUTHORS’ CONTRIBUTIONS**
YH conceived and designed this study. HyC obtained funding. WhL, MC, NnZ, NfN, BD, GqY, SH and CG collected the data. JhL, RH and YbW were involved in data cleaning and verification. LL and JhL analyzed the data. JhL drafted the manuscript. HyC, NB and YH contributed to the critical revision of the manuscript. All authors have read and approved the final manuscript.

FUNDING

This study was supported by a grant from the Training Plan of Clinical Medical Scientific Research Talents of Army Medical University (2019XLC2019).

Availability of data and materials

With the permission of the corresponding author, we can provide patient data without a name or other identifier. Data can be provided after the Article is published. Once the data can be made public, the research team will provide an email address for communication.

ETHICAL APPROVAL

The Ethics Committee of Daping Hospital approved our study protocol and waived written informed consent from patients as it was a retrospective study (Approval No. 2019-79). Patient confidentiality was maintained, and all work was carried out in accordance with the Declaration of Helsinki.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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