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Clinical usefulness of fractional exhaled nitric oxide for diagnosing prolonged cough

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Summary
Background: Prolonged cough is one of the troublesome symptoms commonly seen in daily practice. Especially, detection of allergic cough such as bronchial asthma (BA), cough variant asthma (CVA) and eosinophilic bronchitis without asthma (EB) is important because the prevalence of these disorders are high. We previously reported fractional exhaled nitric oxide (FeNO) can be a non-invasive marker of allergic airway inflammation. We examined whether FeNO could be applicable for the proper diagnosis of prolonged cough.
Method: About 71 consecutive subjects complaining prolonged cough who gave informed consent for the study were enrolled. FeNO, pulmonary function tests, bronchial hyperresponsiveness (BHR), IgE, and eosinophils in induced sputum and peripheral blood were measured. Final diagnosis of the subjects was 30 with BA, 18 with CVA, 8 with EB, and 15 with other respiratory disorders (Others).
Result: FeNO had significant correlations with non-specific IgE, mite-specific IgE, FEV/FVC, BHR, and eosinophils. The level of cedar-specific IgE was significantly higher in subjects with EB than CVA. FeNO levels in BA and CVA were significantly higher than those in EB and Others. The optimal cutoff level of FeNO was 38.8 ppb with sensitivity of 79.2% and specificity of 91.3% for distinguishing BA and CVA from EB and Others.
Conclusion: FeNO could be used as a diagnostic marker of prolonged cough, especially for the differential diagnosis BA and CVA from EB and others.
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Introduction

Patients with prolonged cough as a chief complaint are commonly seen in outpatient clinics, however proper diagnosis is often difficult.[1] Morice et al. report that the most common diagnoses in patients with chronic cough are cough variant asthma (CVA), sinobronchial syndrome, and gastroesophageal reflux disease (GERD), followed by eosinophilic bronchitis without asthma (EB), chronic bronchitis, post infectious cough, and ACE inhibitor induced cough.[2] In Japan, the prevalence of CVA, atopic cough, and sinobronchial syndrome is high, whereas the prevalence of GERD as a cause of chronic cough tends to be low.[3] Therefore, detection and diagnosis of allergic cough (allergic airway inflammatory disorders) as a cause of prolonged cough is an important clinical concern.

Airway hyperresponsiveness, pulmonary function tests, induced sputum, serum IgE, and skin prick tests are commonly used to diagnose patients with allergic cough. Pulmonary function tests are convenient to evaluate reversibility of airflow limitation, but its sensitivity is low, since patients with mild disease may have normal pulmonary function. Airway hyperresponsiveness and induced sputum tests have higher sensitivity, but are more complex to perform, may trigger bronchoconstriction, and are considered to be relatively invasive procedures. Blood tests such as IgE antibodies are useful to screen the presence or absence of allergies, but they are non-specific for airway pathology.

Fractional exhaled nitric oxide (FeNO) levels are known to be increased in bronchial asthma (BA).[4] Measurement of FeNO is simple, non-invasive, and potentially useful in evaluating allergic airway inflammation.[5,6] Disorders with chronic allergic cough are classified as BA, CVA, and EB. Each disorder has distinct clinical features, and differential diagnosis is essential for treatment.[7] However, accurate diagnosis is often difficult. In this article, we investigate the usefulness of FeNO for the evaluation and differential diagnosis of allergic airway inflammatory disorders in patients with prolonged cough.

Methods

Subjects

Subjects in this study were 71 consecutive patients who visited Department of Pulmonary Medicine, Fukushima Medical University Hospital between January 2004 and January 2007, with a chief complaint of prolonged cough or wheezing lasting more than 3 weeks and gave informed consent for the study. The patients ranged 20—78 years of age, had no abnormalities on chest X-ray or CT scan, had no prior history of treatment for pulmonary disease, and never used oral or inhaled corticosteroids. The study was approved by the Ethics Committee at Fukushima Medical University.

Blood tests

Blood tests included peripheral blood eosinophil count, serum non-specific IgE, and antigen-specific IgE. Radioallergosorbent test for antigen-specific IgE (IgE-RAST) was performed for weeds, mites, house dust, cats, dogs, cedar, cypress, orchard grass, moths, Aspergillus, Candida, and mixed molds. Non-specific IgE was measured by fluorescence enzyme immunoassay (UniCAP; Pharmacia & Upjohn, Uppsala, Sweden). If either the non-specific IgE concentration was $\geq$250 IU/mL, or any antigen-specific IgE was positive ($\geq$0.69 UA/mL), the patient was considered atopic.

Pulmonary function tests

Pulmonary function testing was performed using rolling seal spirometers (Chestac-11 Cyber S-type; Chest MI, Inc., Tokyo, Japan) to measure FVC and FEV_1. Tests were performed by experienced respiratory technicians according to ATS guidelines.[8] The FVC and FEV_1 are expressed as percent predicted values. For airway reversibility testing, a positive result was defined as an improvement in FEV_1 of 200 mL and $\geq$12% from baseline when measured 20 min after inhalation of a short-acting β_2 agonist (salbutamol 200 μg from a pressurized inhaler), or the same improvement after treatment with a long-acting β_2 agonist.

FeNO measurement

FeNO was measured in accordance with ATS/ERS recommendations[9] using a chemiluminescence analyzer (Kimoto, Osaka, Japan) and is expressed as parts per billion. The detailed procedure has been described elsewhere.[5,6] In summary, environmental NO was measured before and after measurement to make sure that levels never exceeded 5 ppb. Measurement was performed with patients in a sitting position and without wearing a nose clip. From total lung capacity without breath holding, the patient exhaled at a constant flow of 50 mL/sec. To eliminate contamination from nasal NO, patients maintained a constant mouth pressure of 16 cm H_2O. Dead space and nasal NO (expiratory peak) and lower airway NO (plateau after peak) were automatically displayed on a monitor using special software. FeNO was measured three times, with differences in measured values within $\leq$10%. The mean value of three measurements was used as data for statistical analysis. FeNO was measured before pulmonary function and airway hyperresponsiveness testing.

Airway hyperresponsiveness

Airway hyperresponsiveness testing using methacholine was performed by the Astrograph method (Jupiter 21; Chest MI, Inc., Tokyo, Japan).[10] Patients inhaled methacholine diluted in physiologic saline (starting with physiologic saline only as a control) at gradually increasing concentrations of 49 μg/mL, 98 μg/mL, 195 μg/mL, 390 μg/mL, 781 μg/mL, 1563 μg/mL, 3125 μg/mL, 6250 μg/mL, and 12500 μg/mL, and airway resistance was continuously measured. A dose-response curve was drawn for methacholine and airway pressure, and the minimum dose of methacholine (Dmin) was calculated as an index of airway responsiveness. Positive airway hyperresponsiveness was defined

as a value <12.5 units.[11] The total cumulative dose of methacholine at the end of inhaling the highest dose was 50 units.

Induced sputum tests

Induced sputum was collected using the method described by Pin et al.[12] with inhalation of 5 mL of 5% hypertonic saline using an ultrasonic nebulizer (Nescojet AZ-11, Azwell, Japan). The sputum samples were stained with Papanicolaou stain and examined by microscopy. Sputum samples were judged to be adequate if alveolar macrophages were present and total percentage of squamous cells was $<10\%$. On each slide, 400 cells other than squamous cells were counted. Observers who counted the cells were blinded to clinical information about the patient. Eosinophilia in the induced sputum was defined as an eosinophil count $\geq 3\%$ of the total cell count.

Differentiation of allergic chronic cough

Patients with allergic airway inflammation associated with prolonged cough[13] are classified as follows: bronchial asthma (BA); cough and wheezing for 3 weeks or longer, sputum eosinophilia, and positive airway hyperresponsiveness or presence of reversible airflow limitation, cough variant asthma (CVA); cough without wheezing for 3 weeks or longer, sputum eosinophilia, and positive airway hyperresponsiveness or presence of reversible airflow limitation, eosinophilic bronchitis without asthma (EB); cough without wheezing for 3 weeks or longer, sputum eosinophilia, but negative airway hyperresponsiveness and no reversible airflow limitation.[14] Within this classification, patients with BA and CVA are defined as the asthmatic group. In patients not meeting these criteria for allergic airway inflammatory disease, a specific diagnosis was made, if possible, based on clinical examination, pulmonary function tests, and imaging studies. These are classified as ''Others'' disorders.

Statistical analysis

Statistical analysis was performed with SPSS for Windows (version 8.0; SPSS, Chicago, IL). The data for FeNO, non-specific IgE, and antigen-specific IgE were not normally distributed, so these were log transformed before analysis. Correlations were examined between FeNO and non-specific IgE, antigen-specific IgE, peripheral blood eosinophil count, sputum eosinophil count, pulmonary function ($FEV_1\%$), and Dmin by using Spearman rank analysis. For BA, CVA and EB, individual parameters were compared. Each parameter was compared using the ANOVA test. Patients were classified as having BA, CVA, EB, or other disorders based on clinical test results, and mean FeNO was compared between four groups. Finally, receiver operating characteristics (ROC) curve was constructed to evaluate the best cutoff level for differentiating asthmatics from other groups. Mean data were expressed as geometric means and 95% confidence intervals, and a two-tailed p value of less than 0.05 were considered significant.

Results

Subject characteristics

Table 1 shows the breakdown of patients in each group. Among 71 patients, 30 (42.3%) had BA, 18 (25.4%) had CVA, 8 (11.3%) had EB, and 15 (21.0%) had other disorders. The other disorders included post infectious cough in 5 patients, postnasal drip in 3, chronic obstructive pulmonary disease in 3, chronic bronchitis in 2, GERD in 1, and sinobronchial syndrome in 1. There were no subjects with bronchiectasis. Table 2 summarizes the characteristics in each group. No significant differences were present among four groups in terms of age, sex, height, weight, or smoking status. Non-smokers were defined as any patient, including ex-smokers, who did not smoke within 1 month before initial evaluation. There were 53 patients (74.6%) in the non-smoking group.

Correlation between FeNO with serum IgE and pulmonary function

Fig. 1 shows the correlation between FeNO levels with non-specific IgE and mite-specific IgE. FeNO showed significantly positive correlations with non-specific IgE ($r = 0.497$, $p < 0.001$) and mite-specific IgE ($r = 0.342$, $p = 0.004$). There was no significant correlation between FeNO and specific IgE other than for mites (data not shown). FeNO showed significantly negative correlations with Dmin and FEV_1/FVC ($r = -0.567$, $p < 0.001$; $r = -0.447$, $p < 0.001$; respectively) (Fig. 2). In addition, FeNO showed significantly positive correlations with eosinophils in both induced sputum and peripheral blood ($r = 0.244$, $p = 0.042$; $r = 0.376$, $p = 0.001$; respectively) (Fig. 3).

Comparison of FeNO levels among the four groups

Fig. 4 shows the FeNO levels in each group. The mean FeNO in each group was: BA 93.5 ppb (95% CI, 72.5–120.7), CVA 46.7 ppb (95% CI, 33.6–64.8), EB 16.4 ppb (95% CI, 10.9–24.8), and Others 21.2 ppb (95% CI, 15.1–29.7). FeNO in the BA group was significantly higher than in the CVA ($p = 0.001$), EB ($p < 0.001$), and Others ($p < 0.001$) groups.

Table 1 Final diagnosis of the patients with chronic cough

Diagnosis	n	(%)
Bronchial asthma	30	42.3
Cough variant asthma	18	25.4
Eosinophilic bronchitis without asthma	8	11.3
Post infectious cough	5	7.0
Post nasal drip	3	4.2
COPD	3	4.2
Chronic bronchitis	2	2.8
Cough with GERD	1	1.4
Sinobronchial syndrome	1	1.4
Total	71	100

Table 2 Comparison of individual parameters

	BA	CVA	EB	Others
Age (yr)	55.5 (48.9—62.5)	48.2 (39.4—57.0)	45.3 (33.3—57.2)	55.5 (47.5—63.5)
Gender (male/female)	20/10	7/11	4/4	8/7
Height (cm)	162.2 (158.2—166.1)	159.3 (154.6—164.0)	161.6 (155.4—167.9)	159.4 (155.0—163.9)
Body weight (kg)	66.6 (58.5—74.7)	58.0 (51.6—64.4)	67.7 (59.1—76.3)	65.9 (58.7—73.1)
Smoker/non-smoker	8/22	3/15	1/7	5/10

Data are expressed as geometric means and 95% CIs. 95% CIs: 95% confidence intervals.

FeNO in the CVA group was also significantly higher than in the EB ($p < 0.001$) and Others ($p < 0.001$) groups. FeNO did not significantly differ between the EB group and Others group ($p = 0.316$). When analysis was limited to non-smokers ($n = 53$), the mean FeNO was BA 85.6 ppb (95% CI, 63.5—115), CVA 40.4 ppb (95% CI, 29.4—55.7), EB 17.6 ppb (95% CI, 11.2—27.7), and Others 18.3 ppb (95% CI, 11.4—29.5). As in the entire study cohort, there were similar significant differences among the groups.

Comparison of individual parameters in BA, CVA and EB

Table 3 compares each parameter between the BA, CVA, and EB group. FeNO was significantly higher in BA and CVA group ($p < 0.001$) than in EB group. The percentage of sputum eosinophils in BA group was significantly higher than that in EB group ($p = 0.037$). But, it did not significantly differ between CVA group and EB group. Mite-specific IgE level was significantly higher in BA group than in EB group. But, in CVA group the difference was not statistically significant. On the other hand, cedar-specific IgE was significantly higher in EB group ($p = 0.02$) than in CVA group.

Cutoff levels to discriminate BA from EB and Others

An ROC curve was constructed to establish a cutoff point to discriminate between asthmatic (BA + CVA) and non-asthmatic (EB + Others) disorders (Fig. 5). The optimal cutoff level of FeNO to discriminate between asthmatics and

non-asthmatic disorders was 38.8 ppb (sensitivity of 79.2% and specificity of 91.3%).

Discussion

In this study, we measured FeNO in patients with chronic cough but who had no abnormalities on imaging studies. FeNO showed statistically significant negative correlations with airway hyperresponsiveness and FEV_1/FVC, and positive correlations with induced sputum and peripheral blood eosinophil counts, non-specific IgE, and mite-specific IgE. On analysis of FeNO in each group, we found significantly higher FeNO levels in BA and CVA group, as compared to EB group. FeNO did not significantly differ between EB group and Others group, thus showing absence of a rise in FeNO in EB patients.

In studies in children,[5,15] as well as in adults, there is a significant positive correlation between FeNO with non-specific IgE and mite-specific IgE. However, the correlation in adults is weaker than in children. This probably can be attributed to a lower prevalence of atopic asthma in adults than in children. On the other hand, FeNO showed significantly positive correlation with peripheral blood and induced sputum eosinophil counts, and significantly negative correlation, as in a previous study,[16] with airway hyperresponsiveness and FEV_1/FVC. Therefore, FeNO may be useful not only as an index of antigen sensitization, but also to evaluate the presence or absence of allergic airway pathology.

Our findings showed a rise in FeNO levels in BA group and CVA group, but not in EB group, which differs from other
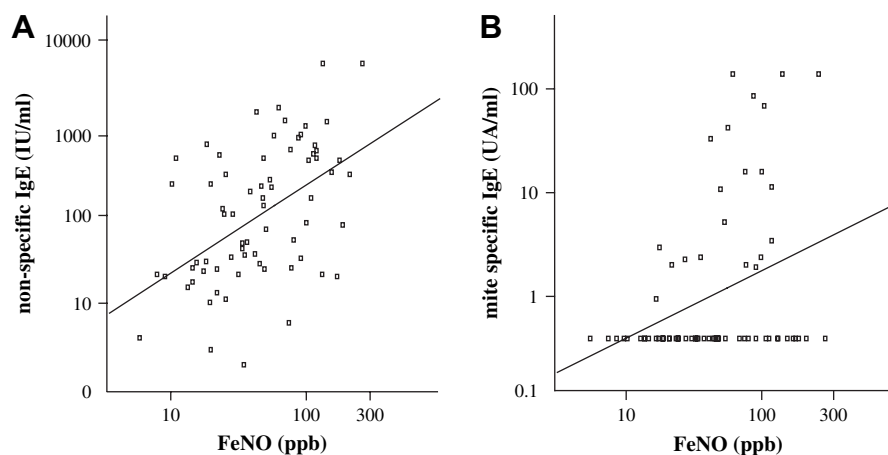

Figure 1 Relationships between FeNO and non-specific IgE levels (A), and FeNO and mite-specific IgE levels (B).
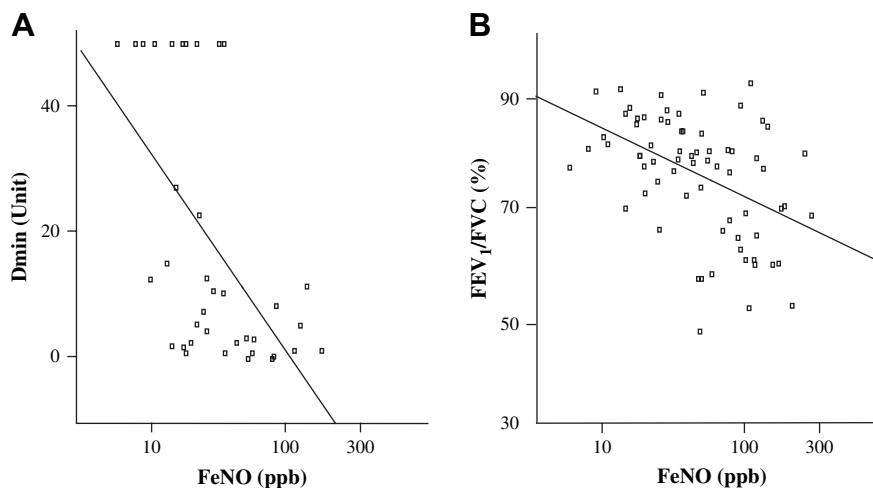
Figure 2   Relationships between FeNO and Dmin (A), and FeNO and FEV$_1$/FVC (B).

reports to date. Only two previous studies have investigated FeNO levels in EB. In one study, Berlyne et al. measured FeNO in EB patients, irrespective of history of use or non-use of corticosteroid therapy, and reported significantly higher levels than in BA patients.[17] In another study, Brightling et al. reported significantly higher FeNO levels in EB (12 ppb) than in asthma (8.5 ppb).[18] EB is a relatively new concept first described by Gibson et al. in 1989. They defined EB as patients with chronic cough and sputum eosinophilia, but without bronchial hyperresponsiveness or reversible airflow limitation.[19] However, in diagnosing EB, Berlyne et al. did not evaluate reversible airflow limitation; they only evaluated airway hyperresponsiveness to distinguish EB from BA. Therefore, patients they diagnosed with EB may have also included those with CVA. In the study by Brightling et al., FeNO was higher in EB than in BA patients. But in their patients with EB, eosinophil counts were elevated both in bronchoalveolar and bronchial lavage fluid, and FeNO levels varied widely between 5 ppb and 30 ppb. This may suggest varying degrees of inflammation in the upper to lower airways. In other words, localization and severity of airway inflammation may vary in EB, thus suggesting different clinical subtypes.

In our study, we evaluated airway hyperresponsiveness and presence or absence of reversible airflow limitation using a β_2 agonist to distinguish EB from CVA. Patients with EB had no increase in FeNO. In addition, FeNO levels were higher in BA than in CVA. When we compared individual parameters between the CVA group and EB group, cedar-specific IgE was significantly higher in the EB group (Table 3). Cedar pollen is the most common type of allergic rhinitis seen in Japan. Japanese cedar pollen granules have a diameter of 30—40 μm, and most of the inhaled pollen is deposited in the nasal cavity and upper airway, without reaching the lower airways.[20] Consequently, our EB patients may have been sensitized to cedar allergen, with inhalation of the allergen causing relatively localized eosinophilic inflammation in the upper airways. In other words, in our patients with EB, eosinophilic airway inflammation was localized, and because of less airway surface area involvement in FeNO production than in CVA and BA, the FeNO levels did not increase. As another possibility, different factors affecting the expression and activation of the nitric oxide synthase (NOS) in BA and EB could be responsible for the FeNO production. Further investigation addressing this issue should be done, for example, using
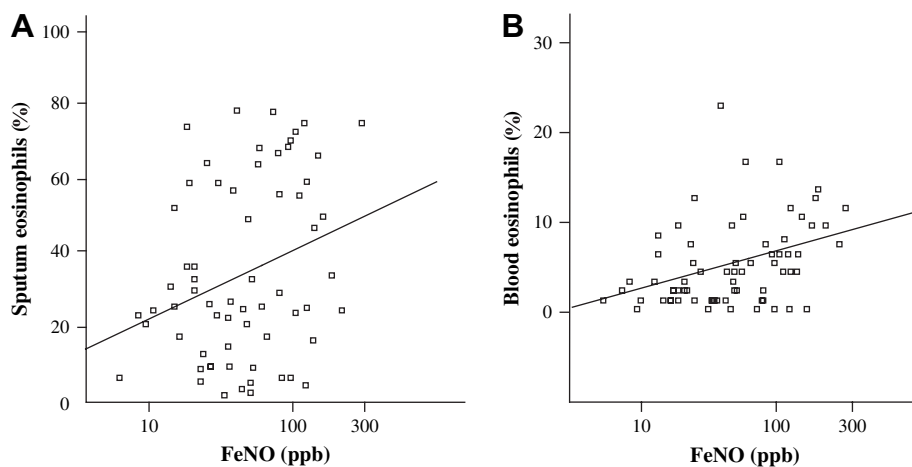

Figure 3   Relationships between FeNO and eosinophils in induced sputum (A) or blood (B).
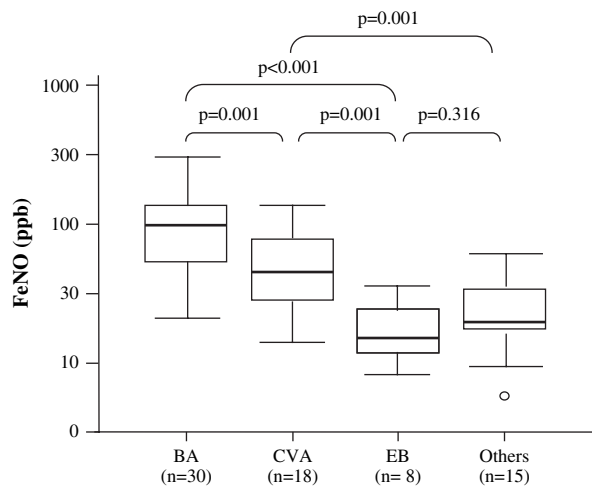
Figure 4 Comparison of FeNO levels among four groups. Box plots for FeNO levels are shown. The thick bar is the median values, and the shaded box represents the interquartile range. Outlying points beyond the inner fences are shown individually.

the method described by Brindicci et al.[21] to differentially measure exhaled NO in the central and peripheral airways, and the immunohistochemistry of airway biopsy specimen for the different types of NOS. Furthermore, sputum neutrophilia might be another reason of lower FeNO levels in EB patients. However, in this study, no significant differences were observed in sputum neutrophils between asthmatics and EB (data not shown) suggesting that the lower FeNO levels in EB group in this study may not be due to the difference in neutrophilic airway inflammation. For the severity of asthma, only 6 patients were classified as

severe asthma, the remaining 24 patients were classified as mild-to-moderate asthma in our patients. Sputum neutrophil and FeNO did not differ between severe and mild-to-moderate asthma.

The effect of smoking on FeNO levels in this study should be discussed. It has been shown that FeNO of steroid naïve smoker asthmatics is lower than non-smoker asthmatics[22] and that generally smoking reduces the levels of FeNO.[9] We also observed same results in our previous study.[6] However, in athmatics of our study (including both BA and CVA), the average levels of FeNO were higher in smoker asthmatics ($n = 11$) than in non-smoker asthmatics ($n = 37$) (112.2 ppb and 63.1 ppb, respectively). Additional analyses revealed no difference in the percentage of eosinophils between smoker and non-smoker asthmatics. However, there was a clear difference in atopic status between two groups. Nine of 11 smoker asthmatics (81.8%) were atopic, however 22 of 37 non-smoker asthmatics (59.5%) were atopic. Furthermore, the average level of non-specific IgE was significantly higher in smoker asthmatics than non-smoker asthmatics (404 IU/mL, 105 IU/mL, respectively, $p < 0.05$). These results suggest that the difference in atopic status might explain the higher FeNO levels in smoker asthmatics in this study.

In studies to date, about 30–40% of patients with CVA go on to develop BA,[23] and airway remodeling begins at an early stage.[24] In EB, the presence of airway remodeling has not been investigated, but in a follow-up study by Berry et al., patients usually had a stable course without progression to bronchial asthma.[25] Therefore, differential diagnosis between EB and asthma is clinically important in terms of treatment and prognosis. But Berry et al. have also reported that some EB patients developed bronchial asthma. This further raise the possible existence of EB clinical

Table 3 Comparison of BA, CVA and EB parameters

	BA ($n = 30$)	CVA ($n = 18$)	EB ($n = 8$)
Age (yr)	55.5 (48.6–62.5)	48.2 (39.4–57.0)	45.3 (33.3–57.2)
Gender (male/female)	20/10	7/11	4/4
Height (cm)	162 (158–166)	159 (155–164)	162 (155–168)
Body weight (kg)	66.6 (58.5–74.7)	58.0 (51.6–64.4)	67.7 (59.1–76.3)
Smoking/non-smoking	8/22	3/15	1/7
FeNO (ppb)	93.5 (72.4–121)**a	46.7 (33.6–64.8)**	16.4 (10.8–24.8)
Blood eosinophils (/μl)	187 (81.4–431)	182 (71–465)	74.6 (15.1–369)
Sputum eosinophil (%)	42.5 (32.8–52.1)*	31.4 (20.8–42.1)	22.9 (11.4–34.3)
Non-specific IgE (IU/ml)	239 (137–414)†	59.3 (20.5–171)	64.7 (21.1–199)
Mite-specific IgE (U$_A$/ml)	1.78 (0.78–4.03)	0.82 (0.34–1.95)	0.41 (0.25–0.66)
House dust specific IgE (U$_A$/ml)	1.78 (1.25–3.97)*	0.78 (0.33–1.81)	0.39 (0.26–0.59)
Cat specific IgE (U$_A$/ml)	0.61 (0.35–0.92)	0.34 (0.32–0.37)	0.41 (0.24–0.70)
Dog specific IgE (U$_A$/ml)	0.53 (0.31–0.92)	0.33	0.41 (0.25–0.67)
Cedar-specific IgE (U$_A$/ml)	1.74 (0.80–3.77)	0.86 (0.38–1.92)*	6.26 (0.90–43.5)
FVC % predicted (%)	94.9 (87.2–103)*	102 (95.7–109)	112 (104–121)
FEV$_1$/FVC (%)	68.4 (64.3–72.4)*a	79.7 (76.7–82.7)	84.3 (80.8–87.9)

Data are expressed as geometric means and 95% CIs.
*$p < 0.05$ and **$p < 0.001$ in comparison with EB.
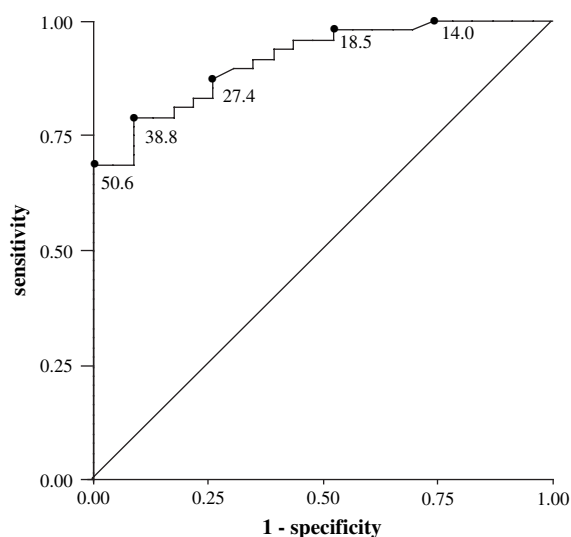a$p < 0.01$ in comparison with CVA.

Figure 5 ROC curve for FeNO to distinguish asthmatic disorders from non-asthmatic disorders; data labels show the selected cutoff points of FeNO levels. The optimal cutoff level of FeNO was 38.8 ppb, sensitivity was 79.2% and specificity was 91.3%.

subtypes as mentioned previously. It is possible that patients with EB who have higher FeNO levels, in other words, more upper to lower airway inflammation, may go on to develop bronchial asthma. Further research concerning this issue is also needed.

Finally, the optimal cutoff level of FeNO for distinguishing asthmatics from non-asthmatics was 38.8 ppb (sensitivity 79.2%, specificity 91.3%). Several cutoff levels of FeNO, in conjunction with other tests, have been recommended in the diagnosis of BA.[26] Using measurement of FeNO alone, a cutoff point of 36 ppb (sensitivity 78%, specificity 60%) has also been reported.[27] In addition, in patients with chronic cough, Chatkin et al. used FeNO cutoff level of 30 ppb (sensitivity 75%, specificity 87%) to define their asthmatic group (including both BA and CVA).[28] Thus, our results support other published findings.

In conclusion, FeNO could be used as a diagnostic marker of prolonged cough. In addition, it could also be useful to distinguish CVA from EB which have both the same eosinophilic airway inflammation.

Conflict of interest

None of the authors have a conflict of interest to declare in relation to this work.

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