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Original Article

Diagnostic utility of fractional exhaled nitric oxide in prolonged and chronic cough according to atopic status

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Abbreviation:
AC, asthmatic cough; AUC, area under the curve; BMI, body mass index; CVA, Cough-variant asthma; CPA, cough-predominant asthma; FEF_{25-75}, forced expiratory flow between 25 and 75%; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; GERD, gastroesophageal reflux disease; NAC, non-asthmatic cough

ABSTRACT

Background: Cough-variant asthma (CVA) and cough-predominant asthma (CPA) are the major causes of persistent cough in Japan. The utility of fractional exhaled nitric oxide (FeNO) measurement in the differential diagnosis of persistent cough has been reported, but the influence of atopic status, which is associated with higher FeNO levels, on the diagnostic utility of FeNO has been unknown.
Methods: We retrospectively analyzed 105 non-smoking patients with prolonged and chronic cough that were not treated with corticosteroids and anti-leukotrienes.
Results: CPA was diagnosed in 37 patients, CVA in 40, and non-asthmatic cough (NAC) in 28. FeNO levels were significantly higher in the CPA [35.8 (7.0–317.9) ppb] and CVA [24.9 (3.1–156.0) ppb] groups than in the NAC group [18.2 (6.9–49.0) ppb] ($p < 0.01$ by Kruskal–Wallis test). The optimal cut-off for distinguishing asthmatic cough (AC; CPA and CVA) from NAC was 29.2 ppb [area under the curve (AUC) 0.74, $p < 0.01$]. Ninety-one percent of subjects with FeNO levels ≥29.2 ppb had AC. Meanwhile, 40% of AC patients had FeNO levels <29.2 ppb. Stratified cut-off levels were 31.1 ppb (AUC 0.83) in atopic subjects vs. 19.9 ppb (AUC 0.65) in non-atopic subjects ($p = 0.03$ for AUC).
Conclusions: Although high FeNO levels suggested the existence of AC, lower FeNO levels had limited diagnostic significance. Atopic status affects the utility of FeNO levels in the differential diagnosis of prolonged and chronic cough.

Introduction

Cough is one of the most common symptoms for which patients seek medical attention.[1] Almost all guidelines have consistently classified cough into three categories according to its duration: acute cough lasting for less than 3 weeks, prolonged or subacute cough lasting for a period of 3–8 weeks, and chronic cough persisting for more than 8 weeks.[2,3] Generally, as cough duration becomes longer, the prevalence of non-infectious causes of cough becomes higher.[2] In Japan, the most prevalent causative diseases of isolated chronic cough have been reported to be cough-variant asthma (CVA), atopic cough, and sinobronchial syndrome, followed by gastroesophageal reflux disease (GERD).[4,5] A recent multicenter study involving isolated cough patients and also those coughed predominantly but complicated by wheeze/dyspnea revealed a high prevalence of CVA and cough-predominant asthma (CPA), accounting for more than 70% of both prolonged and chronic cough.[1]

Fractional exhaled nitric oxide (FeNO) measurement is considered a useful surrogate marker of Th2-driven airway inflammation.[6,7] FeNO levels correlate with sputum eosinophil count,[8] and higher FeNO levels have been reported in asthmatic patients

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compared with non-asthmatic subjects.[9,10] Since FeNO levels are affected by the presence of allergic rhinitis and smoking, Matsunaga *et al.* proposed four cut-off levels for FeNO, stratified by these two factors, to discriminate asthma from non-asthma.[11]

Atopy, which is common in CPA and CVA,[8,12] is also known as one of the factors that affect FeNO levels.[13,14] Although the utility of FeNO measurements in the differential diagnosis of prolonged or chronic cough has been reported,[12,15–18] no study has taken into account the atopic status, which may affect the results. In the present study, we investigated the utility of FeNO measurement in distinguishing asthmatic cough from non-asthmatic cough, in consideration of the atopic status.

Methods

Subjects

We retrospectively analyzed consecutive patients with prolonged and chronic cough lasting for more than 3 weeks. These patients were newly referred to our asthma and chronic cough clinic at Nagoya City University (Nagoya, Japan) and underwent FeNO measurements, from March 2013 to April 2015. Exclusion criteria were 1) prior treatment with inhaled corticosteroids or anti-leukotriene agents; 2) abnormal chest radiograph findings that may explain the cough; 3) fever, blood-stained sputum, or active respiratory infection; and 4) current smokers or former smokers of more than five pack-years or those who quited smoking within for less than 3 months preceding the study. Patients with shortness of breath or wheezing and those with multiple causes of cough were included, as in our previous study.[1] The study was approved by the ethics committee of Nagoya City University Hospital (44-12-0004).

Cough-predominant asthma (CPA) was diagnosed when patients had cough as the predominant symptoms while together with wheeze and/or dyspnea, and either positive airway hyperresponsiveness to methacholine[2] assessed by the continuous methacholine inhalation method (Astograph®; Chest, Tokyo, Japan),[19] or reversible airflow obstruction.[20,21] A diagnosis of CVA was based on an isolated cough, which was relieved by β_2-agonists, and positive airway hyperresponsiveness.[2] Patients with CPA and CVA were combined into the asthmatic cough (AC) group for further analysis.

A diagnosis of non-asthmatic cough (NAC) was made as follows. GERD-related cough was suspected by the presence of 1) classic reflux symptoms of heartburn, indigestion, chest discomfort, throat clearing, dysphonia, dysphagia, and belching and/or 2) typical characteristics of cough that is triggered by phonation, rising, lying, eating, and intake of certain food.[22] A diagnosis was confirmed when cough was relieved by proton pump inhibitors with or without gastrointestinal prokinetic agents.[22,23]

Post-infectious cough was diagnosed when cough was preceded by an acute respiratory tract infection that was not complicated by pneumonia and eventually resolved spontaneously.[2] Sinobronchial syndrome was diagnosed based on findings of chronic sinusitis on sinus imaging and improvement of cough and symptoms related to chronic sinusitis with macrolide antibiotics.[2,24] Atopic cough was diagnosed based on the presence of atopic status and response of coughing to histamine H1 receptor antagonist, but not to inhaled β_2-agonist.[2,25]

Overlapping cases of AC and various causes of non-asthmatic cough (NAC) were categorized as AC subgroups (CPA or CVA).

As previously described, the biological diagnosis of atopy was made by positive specific immunoglobulin E (IgE) against at least one prevalent allergen (>0.70 kU/l), regardless of the level of total IgE.[20] These allergens included house dust mite, mixed Japanese cedar pollen, graminea pollens, mixed weed pollen (ragweed, mugwort, goldenrod, dandelion, and oxeye daisy), Trichophyton, mixed molds (*Candida*, *Penicillium*, *Alternaria*, *Aspergillus*, *Helminthosporium*, and *Cladosporium*), cat dander, and dog dander.[26]

Measurement of study variables

Spirometry was measured with Chestac-8900® (Chest; Tokyo, Japan), according to the American Thoracic Society/European Respiratory Society recommendation.[27]

FeNO measurement

FeNO was measured by Sievers NOA280i chemiluminescence analyzer (GE Analytical Instruments; Boulder, Colorado, USA) at a flow rate of 50 ml/s, according to ERS/ATS recommendations.[6]

Statistical analysis

Statistical analysis was performed with JMP10 Start Statics (SAS Institute Inc., Cary, North Carolina, USA). Normally distributed variables were described as mean (SD) and non-normally distributed variables were described as median (range). Comparison of three groups was made using Chi-square test, ANOVA followed by Tukey—Kramer test, or Kruskal—Wallis analysis followed by Steel—Dwass analysis, as appropriate. Comparison of two groups was made using Mann—Whitney U test. The cut-off value for distinguishing AC from NAC was determined by receiver operating characteristic (ROC) curve analysis. Comparison of area under the curve (AUC) of ROC between atopic subjects and non-atopic subjects was made by DeLong's test.

Univariate analysis was done to evaluate the patient characteristics that were significantly associated with higher FeNO levels; specifically, these factors were age, sex, body mass index (BMI), former smoking status (%), cough duration, diagnosis, atopy, blood eosinophil (/mm^3), total serum IgE (IU/ml), and % predicted forced expiratory volume in 1 s (FEV_1), FEV_1/forced vital capacity (FVC), and forced expiratory flow between 25 and 75% ($FEF_{25–75}$). Variables that had a p value of <0.10 in the univariate analysis were included in the multivariate logistic regression analysis to evaluate which one predict higher FeNO levels (more than the cut-off value calculated by the ROC curve analysis). Correlations between FeNO levels and blood eosinophil count and serum IgE levels were analyzed by Spearman's rank correlation test.

Results

A total of 105 patients were analyzed; final diagnosis was CPA in 37 (35.2%); CVA in 40 (38.1%); and NAC in 28 (26.7%), including 14 patients with GERD (Table 1). Comparison of the patient in the CPA, CVA, and NA groups is shown in Table 2. The CPA group had

Table 1
Etiology of prolonged and chronic cough in our study population (N = 105).

Diagnosis		Number (%)	
Asthmatic cough	Cough-predominant asthma[†]	37 (35%)	77 (73.3%)
	Cough-variant asthma[‡]	40 (38%)	
Non-asthmatic cough	Gastroesophageal reflux disease	14 (13%)	28 (26.7%)
	Sinobronchial syndrome	8 (8%)	
	Post-infectious cough	4 (4%)	
	Atopic cough	2 (2%)	

[†] 32 isolated, 4 with concomitant gastroesophageal reflux disease, 1 with concomitant sinobronchial syndrome.
[‡] 30 isolated, 10 with concomitant gastroesophageal reflux disease.

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Table 2
Comparison of patients with prolonged and chronic cough according to etiology (N = 105).

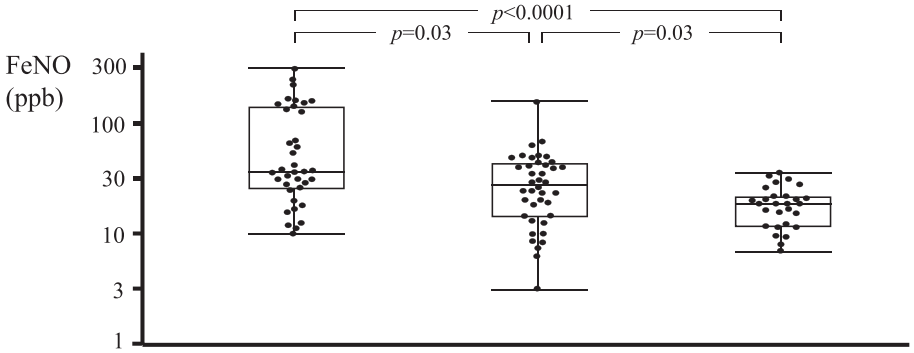
	CPA	CVA	NAC	*p*
Number	37	40	28	
Age (year)	51.7 (17.3)	48.2 (19.4)	43.6 (18.8)	0.23
Male/female (n)	12/25	16/24	10/18	0.79
BMI (kg/m^2)	22.0 (3.5)	22.2 (4.2)	22.0 (4.1)	0.98
Smoking status (former/never)	6/31	3/37	3/25	0.48
Cough duration (month)	17.0 (11.3)	25.0 (9.6)	27.1 (11.5)	0.80
Atopy (n [%])	25 [68]	20 [50]	12 [43]	0.07
Blood eosinophil (/mm^3)	301.7 (246.4)	159.9 (105.3)	140.7 (86.9)	0.004[†]
Total serum IgE (IU/ml)	131.0 (6.4–5010.0)	49.4 (5–1210.0)	58.5 (6.4–758.0)	0.15
FVC (% predicted)	101.5 (17.4)	99.0 (17.6)	103.0 (13.8)	0.61
FEV_1 (% predicted)	93.1 (17.9)	97.6 (16.2)	101.3 (15.9)	0.15
FEV_1/FVC (%)	75.7 (10.8)	81.7 (6.1)	84.9 (7.9)	0.0001[‡]
FEF_{25-75} (% predicted)	63.2 (29.7)	76.1 (26.8)	83.3 (22.4)	0.01[§]

Data are described as mean (SD) or median (range), unless otherwise stated.
CPA, cough-predominant asthma; CVA, cough variant asthma; NAC, non-asthmatic cough; BMI, body mass index; IgE, immunoglobulin E; IU, international unit; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; FEF_{25-75}, forced expiratory flow between 25 and 75%.
[†] CPA vs. CVA: $p = 0.002$; CPA vs. NAC: $p = 0.002$.
[‡] CPA vs. CVA: $p = 0.008$; CPA vs. NAC: $p = 0.0001$.
[§] CPA vs. NAC: $p = 0.01$.

significantly higher blood eosinophil count and lower lung function measures (FEV_1/FVC, FEF_{25-75}) compared with the other two groups. The proportion of atopic subjects was 68% in CPA, 50% in CVA, and 43% in NA ($p = 0.07$). No significant differences were found in the other indices.

FeNO levels were significantly higher in the CPA [35.8 (9.7–317.9) ppb] and CVA [27.6 (3.1–156.0) ppb] groups than in the NAC group [18.3 (6.9–35.4) ppb] (Fig. 1A). Significant differences in FeNO levels were also found between the CPA and CVA groups. The FeNO levels in the NAC group were 18.1 (7.8–35.4) ppb for GERD,

A. Comparison among three groups (CPA, CVA, and NAC)



	CPA (n=37)		CVA (n=40)		NAC (n=28)		*p*
All patients	35.8 (9.7-317.9)		27.6 (3.1-156.0)		18.3 (6.9-35.4)		< 0.0001
・Atopic	41.1 (n=25) (12.4-317.9)	*p*=0.02	34.8 (n=20) (7.3-156.0)	*p*=0.12	20.1 (n=12) (15.9-31.0)	*p*=0.03	0.0006
・Non-atopic	25.5 (n=12) (9.7-164.0)		21.6 (n=20) (3.1-68.5)		15.0 (n=16) (6.9-35.4)		0.13

B. Comparison between AC and NAC

	AC (n=77)		NAC (n=28)	*p*
All patients	33.2 (3.1-317.9)		18.3 (6.9-35.4)	< 0.0001
・Atopic	38.2 (n=55) (7.3-317.9)	*p*=0.002	20.1 (n=12) (15.9-31.0)	*p*=0.03	0.0009
・Non-atopic	23.6 (n=32) (3.1-164.0)		15.0 (n=16) (6.9-35.4)		0.07

Fig. 1. Comparison of FeNO levels in the three groups of patients with prolonged and chronic cough (N = 105). FeNO levels in the CPA and CVA groups were significantly higher compared with those in the NAC group ($p < 0.0001$, by Kruskal–Wallis test). In the CPA and NAC groups, atopic subjects had significantly higher FeNO levels than non-atopic subjects ($p < 0.05$, by Mann–Whitney U test). This figure is presented in logarithmic scales. FeNO, fractional exhaled nitric oxide; ppb, parts per billion; CPA, cough-predominant asthma; CVA, cough-variant asthma; NAC, non-asthmatic cough.

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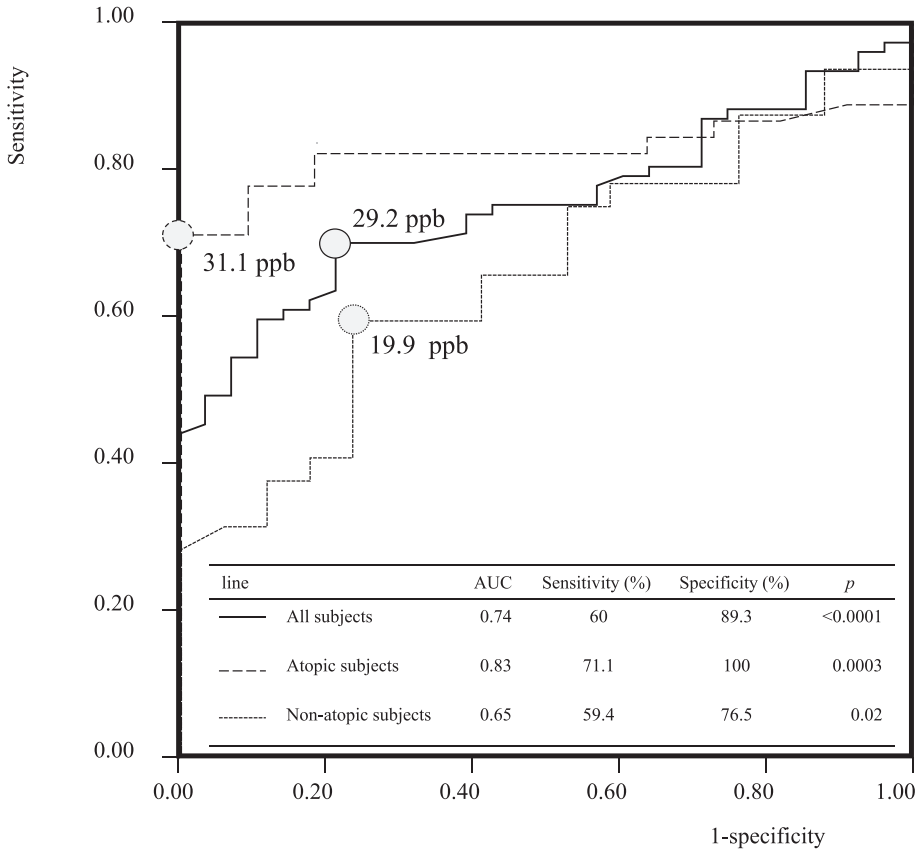

Fig. 2. Receiver operating characteristic curve analysis for distinguishing AC from NAC as a cause of prolonged and chronic cough (N = 105). The overall optimal cut-off value was 29.2 ppb (AUC, 0.74). The cut-off value for atopic subjects was higher (31.1 ppb) than that for non-atopic subjects (19.9 ppb). AC, asthmatic cough; NAC, non-asthmatic cough; AUC, area under the curve; ppb, parts per billion.

17.9 (9.2–35.4) ppb for SBS, 17.1 (6.9–33.4) ppb for PIC, and 19.2 (18.2–20.1) for atopic cough. Similarly, FeNO levels were significantly higher in the combined AC group [33.2 (3.1–317.9) ppb] than in the NAC group ($p < 0.0001$) (Fig. 1B). In the evaluation of FeNO levels based on atopic status, atopic subjects had significantly higher FeNO levels than non-atopic subjects in the CPA, NAC, and AC groups, but not in the CVA group (Fig. 1).

The overall diagnostic utility of FeNO levels to distinguish AC from NAC was examined by ROC curve analysis, which revealed 29.2 ppb (AUC 0.74) as the optimal cut-off value (Fig. 2). In the univariate analysis, which compared patient characteristics based on this cut-off value (high FeNO levels, >29.2 ppb vs. low FeNO levels, ≤29.2 ppb), the factors that were identified to have borderline significant effects on FeNO levels ($p < 0.10$) were etiology of cough (AC/NAC), atopy (%), blood eosinophil (/mm^3), total serum IgE (IU/ml), and FEV_1/FVC (Table 3). Among them, blood eosinophil (/mm^3) and total serum IgE (IU/ml) were excluded from the following multivariate analysis since they were both confounding factors for atopy (%). In the multivariate analysis, atopic status and a diagnosis of AC were identified as independent factors associated with higher FeNO levels (Table 4). The ROC curve analyses based on atopic status revealed different cut-off levels for FeNO levels: 31.1 ppb for atopic subjects and 19.9 ppb for non-atopic subjects (Fig. 2). High AUC (0.83) was obtained in atopic subjects, suggesting a high degree of utility for FeNO measurements. On the contrary, non-atopic subjects had significantly ($p = 0.03$) lower AUC (0.65) than atopic subjects.

Table 3
Univariate analysis of factors affecting patients with prolonged and chronic cough according to FeNO levels (N = 105).

	FeNO ≤ 29.2 ppb	FeNO > 29.2 ppb	*p*
Number	56	49	
Age (year)	46.3 (18.2)	50.4 (19.1)	0.28
Male/female (n)	17/39	21/28	0.18
BMI (kg/m^2)	22.0 ± 3.7	22.2 ± 4.1	0.77
Smoking status (former/never)	6/50	6/43	0.81
Cough duration (month)	5.0 (0.5–480.0)	5.0 (0.2–108.0)	0.41
Diagnosis (AC/NAC)	31/25	46/3	**<0.0001**
Atopy (n [%])	21 [38]	35 [71]	**0.0005**
Blood eosinophil (/mm^3)	145.4 (89.3)	284.9 (227.2)	**0.0001**
Total serum IgE (IU/ml)	49.3 (5.0–758.0)	144.0 (8.4–5010.0)	**0.003**
FVC (% predicted)	102.2 (14.3)	99.4 (18.9)	0.40
FEV_1/FVC	81.8 [7.0]	78.8 [11.0]	**0.09**
FEF_{25-75} (% predicted)	75.8 (24.1)	70.8 (31.5)	0.36

Results with borderline significance ($p < 0.10$) are typed in bold.
FeNO, fractional exhaled nitric oxide; ppb, parts per billion; BMI, body mass index; AC, asthmatic cough; NAC, non-asthmatic cough; IgE, immunoglobulin E; IU, international unit; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity, FEF_{25-75}, forced expiratory flow between 25 and 75%.

Table 4
Multivariate logistic regression models for prediction of FeNO levels in patients with prolonged and chronic cough (N = 105).

Parameter	Odds ratio (95% CI)	*p*
Diagnosis (asthmatic cough)	11.2 (3.3, 52.7)	<0.0001
Atopy (n [%])	3.9 (1.6, 10.0)	**0.003**
FEV_1/FVC (%)	1.0 (0.9, 1.0)	0.99

The results with statistical significance ($p < 0.05$) are typed in bold.
FeNO, fractional exhaled nitric oxide; CI, confidence interval; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity.

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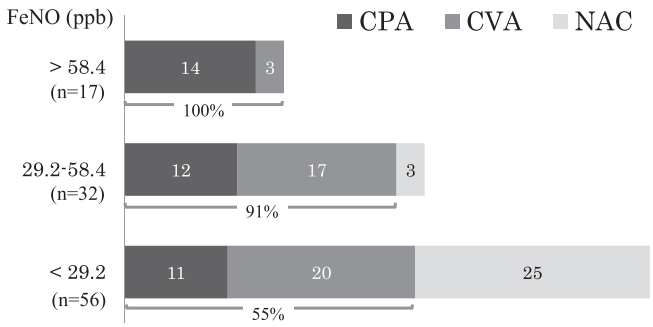

Fig. 3. The stacked bar chart with three categories of FeNO levels in the three groups of patients with prolonged and chronic cough (N = 105). As FeNO levels increase, the proportion of patients with AC becomes higher. The category of FeNO level ≥58.4 ppb was all occupied by patients with AC. However, 55% of subjects who had FeNO level <29.2 ppb were also diagnosed as AC. Low FeNO: <29.2 ppb, Moderate FeNO: 29.2–58.4 ppb, High FeNO: 58.4 ppb. FeNO, fractional exhaled nitric oxide; CPA, cough-predominant asthma; CVA, cough-variant asthma; NAC, non-asthmatic cough; AC, asthmatic cough; ppb, parts per billion.

Figure 3 shows the stacked bar chart of the three groups stratified by FeNO levels; the cut-off value calculated by ROC curve analysis (29.2 ppb) and its two-fold value (58.4 ppb) were used for the stratification. The proportion of AC subjects increased as FeNO levels became higher. The category of high FeNO level (≥58.4 ppb) was occupied by the AC group, which also accounted for 91% of the intermediate FeNO level (29.2–58.4 ppb) category. However, up to 55% of the low FeNO level (<29.2 ppb) category belonged to the AC group. Similar results were obtained when 25 ppb and 50 ppb FeNO levels, which were proposed by the official American Thoracic Society guidelines,[28] were applied in the analysis (data not shown).

FeNO levels significantly correlated with blood eosinophil counts ($\rho = 0.42$, $p < 0.0001$), and serum total IgE titers ($\rho = 0.31$, $p = 0.002$).

Discussion

Our study confirmed that measurement of FeNO levels was a relevant diagnostic procedure for prolonged and chronic cough. We showed that 91% of subjects with intermediate FeNO levels (29.2–58.4 ppb), and all the subjects with high FeNO levels (≥58.4 ppb) belonged to AC groups (Fig. 3). Therefore, the possibility of AC as a cause of chronic cough becomes higher as FeNO levels increase. Previous reports demonstrated that healthy volunteers[12,13,15,29,30] and NAC patients[15–17] rarely had high FeNO levels (≥50 ppb). Therefore, high FeNO levels (≥58.4 ppb) seem to have high specificity for AC.

Nitric oxide has been implicated in the pathophysiology of asthma.[31] Since Th2-driven airway inflammation[32,33] results in the upregulation of inducible nitric oxide synthase (iNOS) in bronchial epithelial cells, high FeNO levels have been observed in asthmatics.[9,10,34] Along with a significant correlation between FeNO and increased sputum eosinophils,[8,35] FeNO is regarded as an indirect marker of eosinophilic airway inflammation that is characteristic of asthma[28] and AC.[36] Although a useful method for evaluating eosinophilic airway inflammation, sputum induction is complex, time-consuming, and carries the potential to induce bronchoconstriction or pharyngeal discomfort. On the other hand, FeNO measurements seem much more suitable for routine clinical practice because it is less invasive and easier to perform.[30]

Previous reports[12,15–18] and our present study of persistent cough patients proposed different FeNO cut-off levels for distinguishing asthmatic subjects (classic asthma predominated by wheezing, CPA, or CVA) from non-asthmatic subjects (healthy control or NAC) (Table 5). Because all these studies consistently used the chemiluminescence method for FeNO measurement, the discrepancies of cut-off levels among these studies may be due to different methods of selection of cases (category of asthma) and control group (non-asthma or healthy control) and the varying patients' characteristics. Although all studies were consistent in the observation that FeNO measurement was a useful diagnostic

Table 5
Previous studies on FeNO measurement in patients with prolonged and/or chronic cough, which consistently used the chemiluminescence method.

Author [reference]	Category of asthma (n)	Control group (n)	Cut-off value (AUC/ Sensitivity/Specificity) case (asthma) vs control (non-asthma)	Cough duration (weeks)	Entry of current s moker	Major differences from our study
Shimoda [12]	CA (92) CVA (90)	HC (90)	20 ppb (NA/72/83) CA vs HC	>8	Included	• Control subjects were all non-atopic. • Higher prevalence of atopy in CA (87.0%)
Chatkin [15]	AC (8) CA (44)	NAC (30) HC (22)	30 ppb (NA/75/87) AC vs NAC	>3	Excluded	• Expiratory flow rate for measuring FeNO was 45 ml/s, not 50 ml/s. • AC were all atopic (100%).
Sato [16]	CA (30) CVA (18)	EB (8) NAC (15)	38.8 ppb (NA/79/91) (CA + CVA) vs (EB + NAC)	>3	Included	• Difference of control groups. • Sputum eosinophilia (≥3%) was included in the criteria of CA and CVA.
Kowal [17]	AC (178)	NAC (362) HC (100)	40 ppb (0.924/88/83) AC vs NAC	>8	Excluded	• All subjects were young adults aged < 45 years old. • Prevalence of atopy in each subgroups was not described. • Median FeNO levels in AC was higher (86 ppb) compared with our study.
Fujimura [18]	CA (10) CVA (11)	Atopic cough (9)	N.A.	>8	N.A.	• No cut-off levels were proposed.
Our study	AC (77)	NAC (28)	(A) 29.2 ppb (0.74/60/89) AC vs NAC (all subjects) (B) 31.1 ppb (0.83/71/100) AC vs NAC (atopic subjects) (C) 19.9 ppb (0.65/59/77) AC vs NAC (non-atopic subjects)	>3	Excluded	–

FeNO, fractional exhaled nitric oxide; ppb, parts per billion; AUC, area under the curve; CA, classical asthma; CVA, cough variant asthma; HC, healthy control; AC, asthmatic cough; NAC, non-asthmatic cough; EB, eosinophilic bronchitis; N.A., not available.

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procedure for prolonged or chronic cough, none took notice of AC patients with low FeNO levels. Our present study revealed that 40% of AC patients accounted for 55% of the patients in the low FeNO level (<29.2 ppb) category. Since FeNO is considered as a surrogate marker of eosinophilic airway inflammation, our results were compatible with the previous report which revealed the prevalence of eosinophilic inflammation (sputum eosinophil >3%) in asthmatics (35–46%).[37,38] These results implied a need to reconsider that about half of patients with AC have low FeNO levels. Namely, a low FeNO level cannot rule out the possibility of AC (Fig. 1 and 3, Table 3).

The difference in FeNO levels between CPA or classic asthma and CVA seems controversial. Although our study and that by Shimoda *et al.*[12] demonstrated these significant differences, other reports reported otherwise.[15,39] Since a degree of mucosal and bronchoalveolar eosinophilia between classic asthma and CVA are similar,[40] there is a possibility that varying influences of confounding factors, such as atopic status[41] (although not significant in the present study), and disease severity may have affected the results.

Several factors may affect the validity of FeNO measurements. In classic asthma, Matsunaga *et al.* proposed that FeNO levels should be evaluated while considering the influence of allergic rhinitis and smoking.[11] In our study, the effects of medications (inhaled corticosteroids[42] and anti-leukotriene agents[43]) and smoking[44] were excluded at study entry. In this study, atopic status and diagnosis were independent determinants of FeNO levels in patients with prolonged and chronic cough. Previous studies also revealed that atopy itself is responsible for elevated FeNO levels even among subjects without asthma or lower airway symptoms,[45–47] which may lead to misdiagnosis of asthma. Therefore, taking atopic status into account seemed to be inevitable when evaluating FeNO levels in prolonged and chronic cough. Although some reports including ours revealed a correlation between FeNO level and non-specific IgE titer,[12,16] more applicable measures are required in clinical practice. Therefore, we proposed cut-off levels based on atopic status. Our results implied the necessity of applying two cut-off levels based on atopic status in the evaluation of prolonged and chronic cough. In addition, FeNO measurement may be less useful in non-atopic subjects than in atopic subjects. To the best of our knowledge, this study was the first to propose different cut-off levels to discriminate AC from NAC based on atopy.

AC is a major cause of prolonged and chronic cough in Japan.[1,48] Although the prevalence of AC in our study (73.3%) seemed higher than those of the US (15–43%), UK (10–26%),[48] and other previous reports in Japan (44–57%),[4,5,49,50] it was similar to a recent multicenter study in Japan (70.6%).[1] Different medical systems, as well as geographical issues and routine diagnostic procedures, might account for this discrepancy. That is, the medical system in Japan, which allows patients to visit specialist clinics on first consult,[48] may have contributed to the high prevalence of treatment-naive AC patients in this study. Additionally, our method of classifying overlapping cases of AC with various causes of NAC to AC subgroups (CPA or CVA) (Table 1) and our inclusion criteria, including patients with shortness of breath or wheezing, might have strengthened this trend.

This study was limited by its retrospective design, which failed to ascertain the presence or absence of allergic rhinitis. Nevertheless, we consecutively recruited patients using a consistent protocol that included spirometry, IgE, and FeNO measurements. Nevertheless, future prospective studies are awaited to reinforce our results.

In summary, FeNO levels were significantly higher in patients with AC than in those with NAC. Although FeNO measurement is useful in the differential diagnosis of prolonged and chronic chough for patients with atopy, its utility for those without atopy seems limited. Intermediate to high FeNO levels might be applicable for the diagnosis of AC, but low FeNO levels do not exclude the diagnosis of AC.

Conflict of interest

MT received research funding from MSD. YK received research funding from GlaxoSmithKline. TO received research funding from Ono Pharmaceutical. ANi received honorarium from Astellas Pharma, AstraZeneca, Boehringer Ingelheim, GlaxoSmithKline, Kyorin Pharmaceutical, Ono Pharmaceutical, MSD, and research funding from Astellas Pharma, Boehlinger Ingelheim, Daiichi-Sankyo, Kyorin Pharmaceutical, Maruho, MSD, Ono Pharmaceutical, Taiho Pharmaceutical, Takeda Pharmaceutical, Teijin. The rest of the authors have no conflict of interest.

Authors' contributions

TA contributed to the performance of pulmonary function tests and FeNO measurements, the acquisition and interpretation of data, and drafting the manuscript. MT contributed to the recruitment of patients, designing and the revision of the manuscript. KF, NT, HI, and HH contributed to the performance of pulmonary function and FeNO measurements. YK, TU, OT, HO, KM, YI, TO, and ANa, contributed to the recruitment of patients. ANi contributed to the recruitment of patients, interpretation of data, and approving the final manuscript.

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