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Original Article

Associations of fractional exhaled nitric oxide with airway dimension and mucus plugs on ultra-high-resolution computed tomography in former smokers and nonsmokers with asthma

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Abbreviations:
ACO, asthma-COPD overlap; ALR, airway-to-lung size ratio; BMI, body mass index; COPD, chronic obstructive pulmonary disease; CT, computed tomography; FEF_{25-75}, forced expiratory flow at 25–75% of forced vital capacity; FEV_1, forced expiratory volume in 1 s; FeNO, fractional exhaled nitric oxide; FVC, forced vital capacity; GINA, Global Initiative for Asthma; ICS, inhaled corticosteroid; JRS, Japanese Respiratory Society; LA, lumen area; OCS, oral corticosteroid; PEF, peak expiratory flow; TLV, total lung volume; U-HRCT, ultra-high-resolution computed tomography; WA, wall area; WA%, wall area percent

ABSTRACT

Background: Associations of fractional exhaled nitric oxide (FeNO) with airway wall remodeling and mucus plugs remain to be explored in smokers and nonsmokers with asthma. Ultra-high-resolution computed tomography (U-HRCT), which allows accurate structural quantification of airways >1 mm in diameter, was used in this study to examine whether higher FeNO was associated with thicker walls of the 3rd to 6th generation airways and mucus plugging in patients with asthma.
Methods: The retrospective analyses included consecutive former smokers and nonsmokers with asthma who underwent U-HRCT in a hospital. The ratio of wall area to summed lumen and wall area was calculated as the wall area percent (WA%). Mucus plugging was visually scored.
Results: Ninety-seven patients with asthma (including 59 former smokers) were classified into low (<20 ppb), middle (20–35 ppb), and high (>35 ppb) FeNO groups (n = 24, 26, and 47). In analysis including all patients and subanalysis including nonsmokers or former smokers, WA% in the 6th generation airways was consistently higher in the high FeNO group than in the low FeNO group, whereas WA % in the 3rd to 5th generation airways was not. In multivariable models, WA% in the 6th generation airways and the rate of mucus plugging were higher in the high FeNO group than in the low FeNO group after adjusting for age, sex, body mass index, smoking status, lung volume, and allergic rhinitis presence.
Conclusions: Higher FeNO may reflect the inflammation and remodeling of relatively peripheral airways in asthma in both former smokers and nonsmokers.

Introduction

Asthma is an inflammatory disorder involving proximal and peripheral airways with substantial pathophysiological and clinical

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heterogeneity.[1–5] Asthma is common in both nonsmokers and smokers, and a smoking history modulates airway inflammation.[6] The disease complexity requires a robust assessment of airway inflammation in relation to airway structural changes for more individualized management.

Fractional exhaled nitric oxide (FeNO) is used to evaluate type 2 inflammation characterized by the activation of eosinophils and innate lymphoid cell type 2 as well as the expression of cytokines such as IL-4 and IL-13 that stimulate inducible NO synthase expression in the airways.[7] Clinically, high FeNO is associated with exacerbation risk and lung function decline.[8,9] From a pathophysiological perspective, FeNO may reflect airway wall remodeling and mucus plugging of the airways due to collagen deposition and proliferation of smooth muscle cells and goblet cell hyperplasia.[10] Thus far, increased FeNO is considered to reflect large airway lesions, while peripheral airway/alveolar lesions are reflected in alveolar NO.[11,12] However, whether FeNO is truly confined to proximal airway lesions or may be involved in the relatively peripheral airway lesions in asthma is not fully understood.

Airway morphology can be evaluated using chest computed tomography (CT). On conventional CT images with 512 × 512 matrix and ≥0.5 mm slice thickness, the airway dimensions of the central airways such as the segmental (3rd generation) and subsegmental (4th generation) bronchi are associated with clinical outcomes in patients with asthma.[13] However, the limited image resolution makes it difficult to accurately quantify the dimension of peripheral airways <2 mm in lumen diameter.[14] In contrast, ultra-high-resolution CT (U-HRCT) images with a 1024 × 1024 matrix and 0.25 mm slice thickness enable the evaluation of airways 1–2 mm in lumen diameter, which correspond to the proximal part of the peripheral airway tree.[14,15] On U-HRCT, more than 50% of the 6th generation airways are <2 mm in lumen diameter, and the influence of asthma onset before age 40 years in the airway dimensions can be detected in the 5th generation but not 3rd generation airways in patients with COPD.[14–16] These findings suggest that a more accurate evaluation of the peripheral airways using U-HRCT could increase the understanding of the primary site of FeNO elevation in patients with asthma.

Persistent type 2 inflammation after inhaled corticosteroid (ICS) treatment may be associated with small airway dysfunction and exacerbation frequency in asthma.[17] A conventional CT study showed that the association of increased wall area with higher FeNO is greater in the 6th generation airways than in the 3rd generation airways in never smokers with asthma,[18] although the limited image resolution would cause overestimation of the wall size in relatively peripheral airways such as 6th generation airways.[19] Conventional CT studies have also visually quantified mucus plugging in relation to disease severity and response to treatments, including biologics, in asthma[20–24] and have shown cross-sectional and longitudinal associations between FeNO and mucus plugs.[23,25]

It was hypothesized that high FeNO is associated with wall thickening of the peripheral airways compared with that of the proximal airways and with increased mucus plugging in patients with asthma. In real-world practice, the interpretation of FeNO is important not only in nonsmokers but also in smokers to evaluate asthma control levels after anti-inflammatory treatments.[6,7] Therefore, this study aimed to examine whether FeNO is associated with wall thickening of 3rd to 6th generation airways and with mucus plugging on U-HRCT in both former smokers and nonsmokers with ICS-treated asthma.

Methods

Study design and population

Consecutive patients with asthma who underwent U-HRCT between 2018 and 2020 at Kyoto University Hospital were enrolled into this retrospective cross-sectional study. The diagnosis and severity of asthma were assessed according to the Global Initiative for Asthma (GINA) guideline.[26] Former smokers were defined as those with a life-long smoking history of at least 10 pack-years. Exclusion criteria were as follows: concurrent respiratory disease (except COPD); a history of lung resection; an acute respiratory tract infection or asthma exacerbation in the previous month; smoking history within the past year; insufficient quality of CT such as inappropriate breath-holding during scans, abnormal CT findings such as interstitial lung abnormalities, and unavailability of spirometry and FeNO obtained within an exacerbation-free 3 months of U-HRCT scanning. This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee (No. R1660-5). Written informed consent was waived because of the retrospective nature of this investigation.

Fractional exhaled nitric oxide

FeNO was measured using a NIOX VERO® (Circassia Pharmaceuticals, Oxford, UK) before performing pulmonary function tests according to the recommendations of the American Thoracic Society/European Respiratory Society guidelines (ATS/ERS).[27] In accordance with the JRS Statement, FeNO was classified into three groups: low (<20 ppb), middle (20–35ppb), and high (>35 ppb).[7] Sensitivity analyses were performed using the ATS guidelines, in which FeNO was classified into three groups: low (<25 ppb), middle (25–50 ppb), and high (>50 ppb).[28]

Pulmonary function test

Spirometry was performed after inhalation of a bronchodilator through CHESTAC-8900 (Chest MI Corp, Tokyo, Japan) following the ATS/ERS guidelines.[29] All measurements were repeated 3 times. The predicted forced vital capacity (FVC) and predicted forced expiratory volume in 1 s (FEV_1) were calculated with the Lambda-Mu-Sigma method for the Japanese population.[30] The predicted forced expiratory flow at 25–75% of FVC (FEF_{25-75}) was calculated based on the Global Lung Function Initiative 2012.[31] The predicted peak expiratory flow (PEF) was also calculated as reported.[32]

CT scan acquisition

U-HRCT images at 1024 × 1024 matrix with 0.25 mm slice thickness were obtained using an Aquilion Precision scanner (Cannon Medical, Tokyo, Japan) at full inspiration as previously reported.[14–16] The CT scan conditions were 120 kVp, autoexposure control. Reconstruction was performed using the forward-projected model-based iterative reconstruction solution algorithm.[33]

CT image analyses

Airway structure and total lung volume (TLV) were quantitatively evaluated using SYNAPSE VINCENT software (FUJIFILM Medical, Tokyo, Japan). To measure the wall area percent (WA%), defined as the percentage of wall area (WA) to the sum of WA and lumen area (LA),[13,34] the right apical (RB1), right lateral (RB4), right

posterior basal (RB10), left apicoposterior (LB1+2) and left anteromedial basal (LB10) segmental bronchus (3rd generation) were visually identified. All branches of the 3rd to 6th generation airways in these 5 paths were identified by tracking from the 3rd down to the 6th generation. For each branch, after generating cross-sectional images perpendicular to the longitudinal centerline of the lumen, LA and WA in these cross-sections located in the middle-third portion of the branch were automatically measured, and WA% was calculated and averaged. Finally, these CT measures from the 5 bronchial paths were separately averaged for the 3rd, 4th, 5th, and 6th generations.

Moreover, the airway-to-lung size ratio (ALR), an index for size mismatch between the central airway and lung,[35,36] was obtained by measuring the TLV and lumen internal diameters as 14 branch segments, including the trachea, right and left main bronchus, bronchus intermedius, and the 3rd and 4th airway generations of five paths (RB1, RB4, RB10, LB1, and LB10). ALR was calculated by dividing the geometric mean of the airway lumen diameters for these 14 locations in centimeters by the cube root of TLV in cubic centimeters.[35]

Airway mucus plugs were identified and scored by visual inspection by two CT-experienced pulmonary physicians using a scoring system based on the bronchopulmonary segment anatomy as described previously.[20,37] The scores by the two inspectors were averaged, and the presence of mucus plugs was defined as a score $\geq$1. Furthermore, the presence of mucus plugs was classified into central and noncentral mucus plugs. Patients with central mucus plugs were defined as those with the presence of mucus plugs that were identified in the 3rd or 4th generation airways by at least one inspector, whereas patients with noncentral mucus plugs were defined as those whose mucus plugs were only identified in the 5th or more peripheral generation airways.

Statistics

Continuous variables are expressed herein as the mean (standard deviation, SD) unless indicated otherwise. Interobserver variability for the mucus plug score was assessed using the intraclass correlation coefficient (ICC). Categorical variables are expressed as absolute numbers and percentages. The correlation between mucus plug score and WA% was tested using the Spearman rank correlation test. Continuous and categorical variables were compared among the low, middle, and high FeNO groups using analysis of variance and Fisher's exact test, respectively. Multivariable linear regression and logistic models were constructed to examine whether the high and middle FeNO groups were more closely associated with WA% and the rate of mucus plugging, respectively, than the reference low FeNO group was. Age, sex, and body mass index (BMI) were included as independent variables in the models of nonsmokers and former smokers because the number of nonsmokers with asthma (n = 38) was not enough to include many independent variables. Age, sex, BMI, height, smoking status (nonsmoker/former smoker), and the presence of allergic rhinitis were included in the models of all patients. The normality of the dependent variables in the models was visually confirmed by histograms. Statistical analysis was performed using R software version 4.2.3. A p value less than 0.05 was considered statistically significant.

Results

Patient characteristics

As shown in Figure 1, of 139 patients consecutively undergoing U-HRCT, those with smoking history within the past year, abnormal or poor-quality CT images, and unavailability of spirometry, FeNO or DICOM data were excluded. A total of 97 patients with asthma who had undergone spirometry within 90 exacerbation-free days from U-HRCT were included in this study. All patients received regular ICS treatment, and 59 patients (60.8%) were former smokers. The characteristics of all patients and those stratified by smoking status (nonsmokers/former smokers) are summarized in Table 1. The ICC for the mucus plug score was 0.93 [0.88, 0.95], as shown in Supplementary Figure 1. The prevalence of the central mucus plugs did not differ between nonsmokers and former smokers (38.1% and 37.9%, p = 1.00).

U-HRCT airway measures in patients stratified by FeNO

Based on FeNO levels, patients were divided into a low FeNO group (<20 ppb, n = 24), a middle FeNO group (20–35 ppb, n = 26), and a high FeNO group (>35 ppb, n = 47). Their characteristics are described in Table 2. Age, sex, height, BMI, smoking history, pack years, duration of asthma, medication, FEV_1, FVC, or FEV_1/FVC did not differ between the groups. FEF_{25-75} tended to be lower in the high FeNO group (p = 0.06). As shown in Figure 2, the WA% in the 3rd generation airways tended to differ between the low, middle, and high FeNO groups (p = 0.05). WA% in the 6th generation, but not in the 4th and 5th generations, significantly differed among the 3 groups, and post hoc Tukey's multiple comparisons showed that WA% in the 6th generation was significantly higher in the high FeNO group than in the low FeNO group (p = 0.0002). The presence rate of mucus plugging also differed between the 3 groups. In contrast, ALR and TLV did not differ among the 3 groups. As shown in Supplementary Figure 2, LA and WA did not differ for any generation.

Figure 3 presents representative images showing a thicker wall relative to the lumen size in the 6th generation airway and mucus plugging in a patient with FeNO of 68 ppb.

Separate analyses of the associations of FeNO with U-HRCT findings in former smokers and nonsmokers

Supplementary Table 1 shows the clinical features of the low, middle, and high FeNO groups of former smokers and nonsmokers with asthma. As shown in Figure 4, WA% in the 6th generation airway consistently differed between the low, middle, and high FeNO groups. The presence of mucus plugging significantly differed between the 3 groups in nonsmokers but not in former smokers. Supplementary Figure 3 shows that FeNO values were significantly correlated with WA% and mucus plug score. The prevalence of the central mucus plugs did not significantly differ between the 3 groups irrespective of smoking status (nonsmoker; 75.0%, 0.0%, and 35.7%, p = 0.12; former smoker; 14.3%, 40.0%, and 47.1%, p = 0.32). Supplementary Figure 4 shows that the mucus plug score was significantly correlated with WA% in the 6th generation in both former smokers (rho = 0.49, p < 0.0001) and nonsmokers (rho = 0.32, p = 0.047). In contrast, a significant association between mucus plug scores and WA% in the 3rd to 5th generation airways was found in former smokers but not in nonsmokers.

Multivariable models for WA% and mucus plugging

As shown in Table 3, separate multivariable models in former smokers and nonsmokers were constructed. WA% in the 6th generation airways was higher in the high FeNO group than in the low FeNO group after adjustment for age, sex, and BMI for both models of nonsmokers and former smokers (estimate [95% CI] = 6.38 [2.64, 10.13], p = 0.002, and estimate [95% CI] = 3.55 [0.60, 6.50], p = 0.02, respectively). In contrast, WA% in the 3rd, 4th, and 5th generations

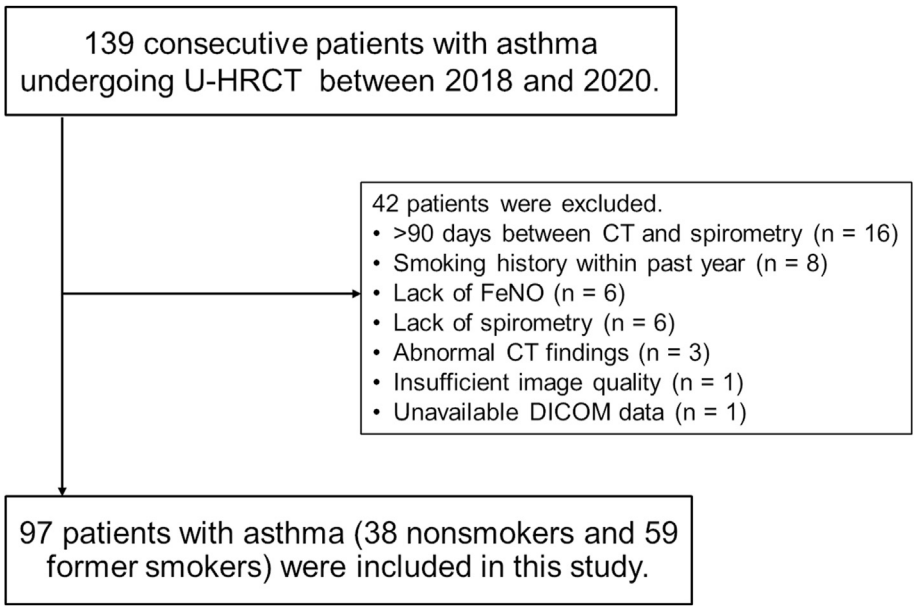

Fig. 1. Patient flow chart for this study. CT, computed tomography; FeNO, fractional exhaled nitric oxide.

was not consistently higher in the high FeNO groups of nonsmokers and former smokers. The high FeNO group tended to be associated with a higher rate of mucus plugging than the low FeNO group in nonsmokers (odds ratio [95% CI] = 5.18 [0.90, 36.0], p = 0.07) but not in smokers (odds ratio [95% CI] = 1.56 [0.42, 5.98], p = 0.51). Moreover, in the models of all patients, WA% in the 6th generation and the rate of mucus plugging were higher in the high FeNO group than in the low FeNO group after adjusting for age, sex, height, BMI,

Table 1
Patient characteristics.

Demographics	All (n = 97)	Nonsmokers (n = 38)	Former Smokers (n = 59)
Age, year	69.7 (9.6)	66.4 (11.8)	71.9 (7.2)
Sex male, n (%)	67 (69.1)	11 (28.9)	56 (95.0)
BMI, kg/m^2	24.4 (3.6)	24.4 (4.1)	24.4 (3.4)
Smokers, n (%)	59 (60.8)	0 (0.0)	59 (100.0)
Pack-years	30.5 (39.4)	0.9 (2.4)	49.6 (40.2)
Duration of asthma, year	16.3 (13.5)	19.5 (12.3)	14.2 (13.9)
%predicted FEV_1, %	77.9 (20.7)	83.2 (22.6)	74.5 (18.8)
%predicted FVC, %	98.8 (17.2)	98.2 (16.6)	99.2 (17.8)
FEV_1/FVC, %	64.5 (9.9)	69.5 (2.4)	61.3 (11.4)
FEV_1/FVC <70%, n (%)	74 (76.2)	19 (50.0)	55 (93.2)
ICS use, n (%)	97 (100.0)	38 (100.0)	59 (100.0)
ICS high dose, n (%)	16 (16.8)	9 (25.0)	7 (11.9)
LABA use, n (%)	84 (86.6)	34 (89.5)	50 (84.7)
LAMA use, n (%)	40 (41.2)	17 (44.7)	23 (39.0)
OCS use, n (%)	7 (7.2)	6 (15.8)	1 (1.7)
Biologic use, n (%)	5 (5.2)	2 (5.3)	3 (5.1)
Severe asthma (GINA), n (%)	76 (78.4)	30 (78.9)	46 (78.0)
Allergic rhinitis, n (%)	41 (42.3)	12 (31.6)	29 (49.2)
Blood eosinophil count, /μl	342.1 (358.7)	365.0 (329.1)	327.3 (378.5)
Serum IgE, IU/mL	549.3 (1058.8)	304.1 (321.5)	704.2 (1309.3)
FeNO, ppb	34.0 [20.0, 60.0]	33.0 [19.3, 64.5]	34.0 [20.3, 57.5]
WA% (3rd), %	57.7 (5.8)	58.5 (6.2)	57.1 (5.2)
WA% (4th), %	60.8 (5.8)	61.5 (6.6)	60.3 (5.2)
WA% (5th), %	63.5 (5.2)	63.4 (6.0)	63.6 (4.6)
WA% (6th), %	62.9 (5.0)	61.54 (5.5)	63.7 (4.5)
ALR	0.03 (0.01)	0.03 (0.01)	0.03 (0.01)
Mucus plug score	1.0 [0.0, 3.5]	1.0 [0.0, 3.4]	0.50 [0.0, 3.3]
Mucus plugging, n (%)	50 (51.5)	21 (55.3)	29 (49.2)
Central mucus plugging, n (%)	19 (38.0)	8 (38.1)	11 (37.9)

Values indicate the mean (standard deviation), median [interquartile range] or number (%). ICS, inhaled corticosteroid; LABA, long-acting beta agonist; LAMA, long-acting muscarinic antagonist; OCS, oral corticosteroid; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; WA%, wall area percent; ALR, airway-to-lung size ratio.

Table 2
Characteristics of patients classified into three groups according to FeNO levels.

Characteristics	Low FeNO	Middle FeNO	High FeNO	*P*
	n = 24	n = 26	n = 47	
Age, year	68.1 (13.7)	70.7 (8.6)	70.0 (7.4)	0.60
Sex male, n (%)	16 (66.7)	18 (69.2)	33 (70.2)	0.95
Height, cm	160.7 (8.9)	162.8 (7.9)	162.4 (9.4)	0.68
BMI, kg/m^2	24.2 (3.6)	24.3 (4.3)	24.5 (3.3)	0.94
Smokers, n (%)	14 (58.3)	16 (61.5)	29 (61.7)	0.96
Pack-years	18.2 (18.9)	35.4 (37.5)	34.2 (46.8)	0.21
Duration of asthma, year	16.9 (15.1)	12.9 (13.9)	17.7 (12.4)	0.34
ICS use, n (%)	24 (100.0)	26 (100.0)	47 (100.0)	NA
ICS high dose, n (%)	7 (29.2)	3 (11.5)	6 (12.8)	0.10
LABA use, n (%)	23 (95.8)	20 (76.9)	41 (87.2)	0.14
LAMA use, n (%)	11 (45.8)	12 (46.2)	17 (36.2)	0.62
OCS use, n (%)	1 (4.2)	1 (3.8)	5 (10.6)	0.45
Biologic use, n (%)	3 (12.5)	0 (0.0)	2 (4.3)	0.13
Severe asthma (GINA), n (%)	22 (91.7)	21 (80.8)	33 (70.2)	0.11
Allergic rhinitis, n (%)	11 (45.8)	7 (26.9)	23 (48.9)	0.18
Blood eosinophil count, /μl	191.9 (196.3)	396.3 (470.5)	388.8 (336.5)	0.06
Serum IgE, IU/mL	687.3 (1834.9)	531.2 (686.1)	493.1 (676.1)	0.78
FEV_1, L	1.99 (1.02)	1.89 (0.56)	1.99 (0.53)	0.80
%predicted FEV_1, %	78.2 (29.5)	76.6 (21.6)	78.5 (14.2)	0.93
FVC, L	3.16 (1.00)	3.30 (0.90)	3.25 (0.91)	0.87
%predicted FVC, %	97.5 (18.0)	100.5 (16.3)	98.6 (17.6)	0.82
FEV_1/FVC, %	65.1 (9.4)	61.7 (13.3)	65.6 (7.5)	0.25
PEF, L/min	6.10 (2.72)	5.80 (1.76)	6.18 (1.61)	0.73
%predicted PEF, %	86.0 (32.1)	82.6 (27.0)	85.8 (15.9)	0.84
FEF_{25-75}, L/min	1.45 (1.62)	1.00 (0.69)	0.91 (0.41)	0.06
%predicted FEF_{25-75}, %	63.8 (55.0)	53.1 (38.2)	45.8 (19.7)	0.15

Values indicate the mean (standard deviation) or number (%). Continuous and categorical variables were compared among the low, middle, and high FeNO groups using analysis of variance and Fisher's exact test, respectively. ICS, inhaled corticosteroid; LABA, long-acting beta agonist; LAMA, long-acting muscarinic antagonist; OCS, oral corticosteroid; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; PEF, peak expiratory flow; FEF_{25-75}, forced expiratory flow between 25 and 75% of FVC.

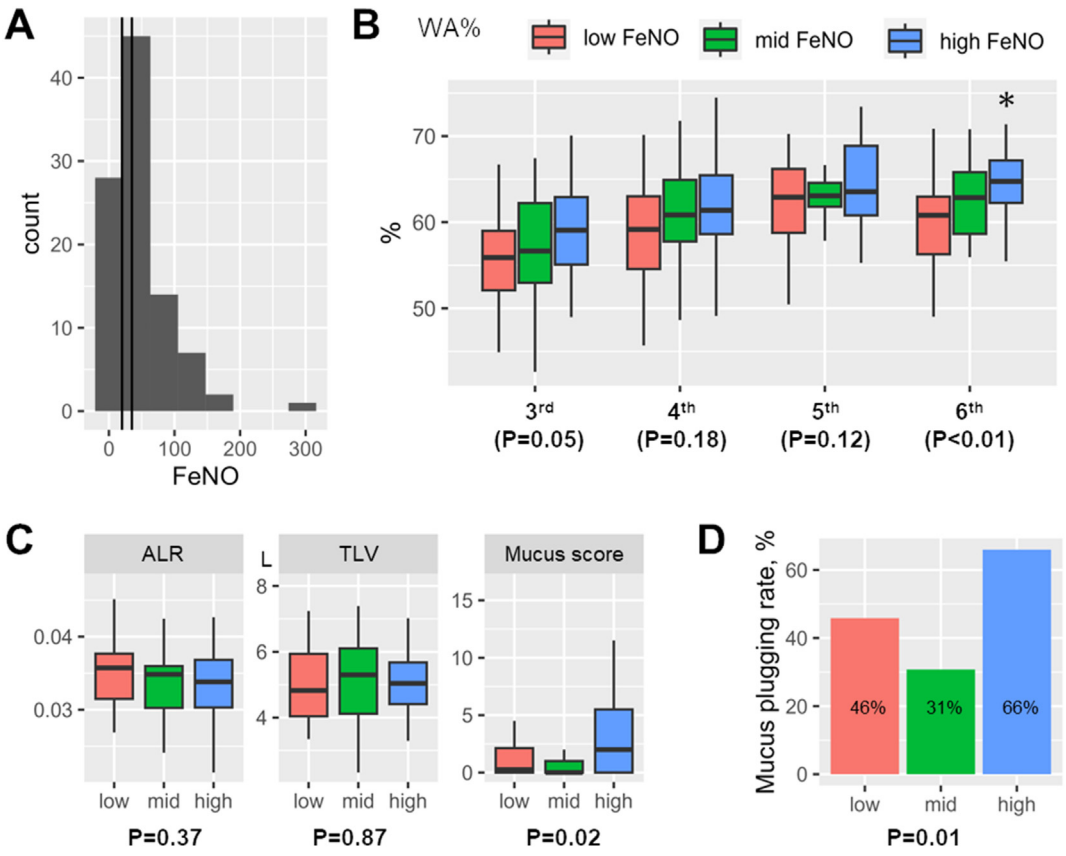

Fig. 2. Comparisons of ultra-high-resolution CT measures in patients with asthma stratified by fractional exhaled nitric oxide. **A.** Distribution of fractional exhaled nitric oxide (FeNO). Patients with asthma were divided into a low FeNO group (<20 ppb, n = 24), a middle FeNO group (20–35 ppb, n = 26), and a high FeNO group (>35 ppb, n = 47). **B.** Wall area percent (WA%) in the 6th generation airway differed between the 3 groups. **C.** The airway-to-lung size ratio (ALR), total lung volume (TLV), and mucus plug score were compared. **D.** The rate of mucus plugging presence was compared. P values for WA% and the rate of mucus plugging were calculated based on analysis of variance and Fisher's exact test, respectively. *p < 0.05 compared to the low FeNO group based on post hoc Tukey comparisons.

smoking status, TLV, and presence of allergic rhinitis (WA%: estimate [95% CI] = 4.57 [2.29, 6.84], p = 0.0001, Mucus plugging: odds ratio [95% CI] = 3.23 [1.07, 10.34], p = 0.04). As shown in Supplementary Table 2, there were significant interactions between the FeNO group and smoking status on WA% in the 3rd and 5th generation airways.

Sensitivity analyses

Sensitivity analyses were performed by classifying patients into low (<25), middle (25–50), and high (>50) FeNO groups based on the ATS statement[28] instead of the Japanese guidelines. Supplementary Figure 5 shows similar associations of high FeNO with WA% in the 6th generation and mucus plugging on U-HRCT. As shown in Supplementary Table 3, separate multivariable models of nonsmokers and former smokers showed that WA% in the 6th generation was consistently higher in the high FeNO group than in the low FeNO group after adjusting for age, sex, and BMI. Moreover, the models of all patients showed that the high FeNO group was significantly associated with a higher WA% in the 6th generation airways and a higher rate of mucus plugging after adjusting for age, sex, height, BMI, smoking status, TLV, and the presence of allergic rhinitis.

Discussion

This study examined airway morphology on U-HRCT in ICS-treated patients with asthma stratified by FeNO and showed that WA% in the 6th generation airways and the rate of mucus plugging were higher in the high FeNO group than in the low FeNO group. Moreover, separate analyses of former smokers and nonsmokers consistently showed an association between high FeNO levels and WA% in the 6th generation airways. Although conventional CT studies have shown the association of WA% in the 6th generation airways and mucus plugging with higher FeNO in nonsmokers with asthma,[18,25] to our knowledge, this is the first study to analyze airway dimension and mucus plugs on U-HRCT with higher measurement accuracy and show the association of higher FeNO with higher WA% and the presence of mucus plugs in nonsmokers and former smokers with asthma.

Our data provide a novel insight into the pathogenetic background of FeNO in former smokers and nonsmokers with asthma. Although approximately half of patients with asthma are ever smokers,[6] many studies have excluded smokers. In this context, the observed associations of higher FeNO with higher WA% in the 6th generation airways, but not the 3rd to 5th generation airways, in both nonsmokers and former smokers are relevant for more efficient use of FeNO in real-world practice. Studies using a combination of CT and histology have shown that WA% on CT is associated with airway epithelial thickness and an increased smooth muscle layer on pathology.[38–40] Therefore, the present findings suggest that higher FeNO may reflect airway inflammation and remodeling in the more peripheral airways than the central segmental airways irrespective of smoking status.

The duration of asthma did not differ between the low, mid, and high FeNO groups. Although a previous study showed that a longer

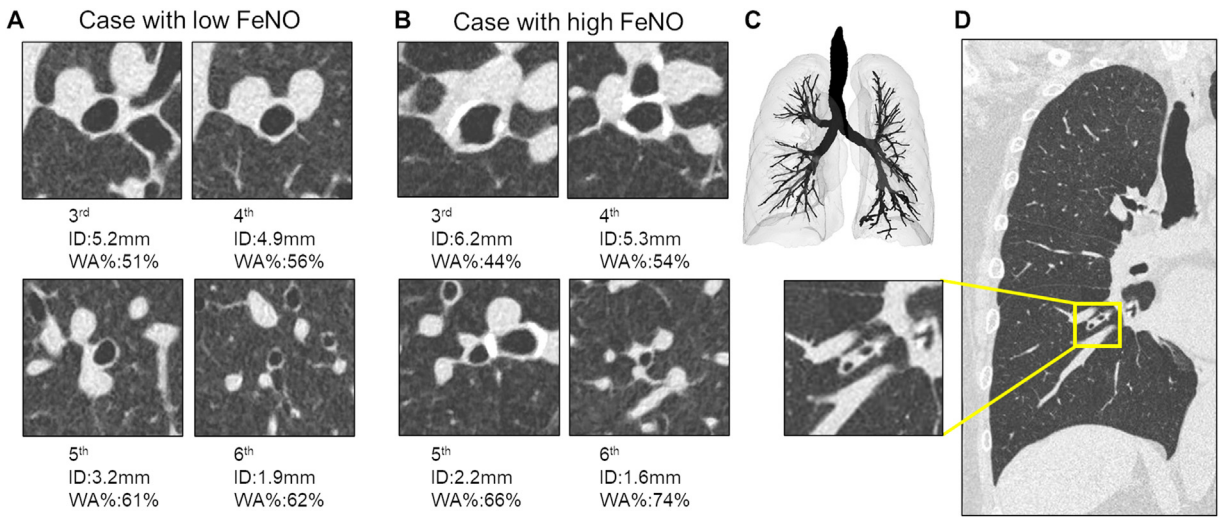

Fig. 3. Representative ultra-high-resolution CT images of nonsmokers with asthma with low and high fractional exhaled nitric oxide. **A and B.** Cross-sectional images of the 3rd, 4th, 5th, and 6th generation airways in a patient with nonsmoking asthma with low fractional exhaled nitric oxide (FeNO) and a patient with nonsmoking asthma with high FeNO. **C and D.** Visualization of the association between airway tree and lung sizes and lumen occlusion by mucus plugging in the same patient with high FeNO.

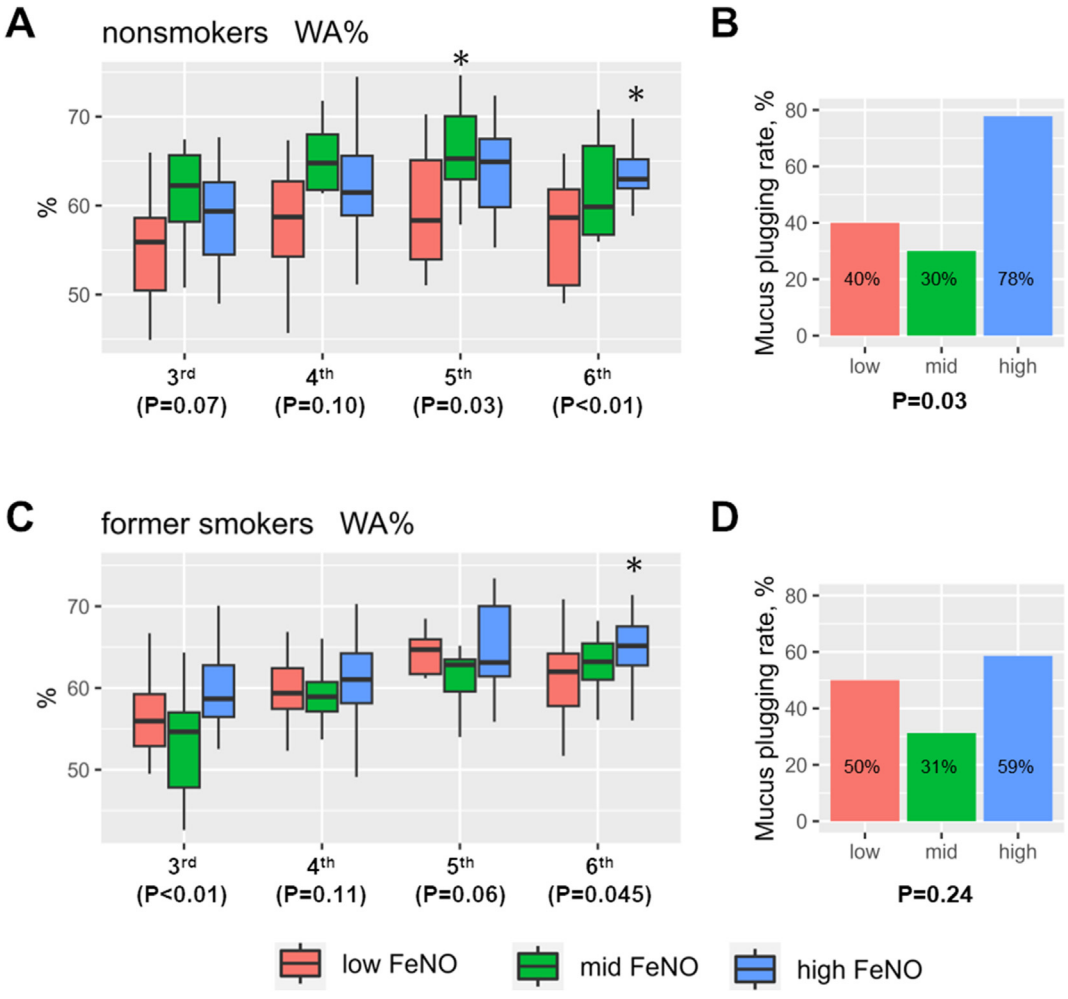

Fig. 4. Separate analyses in nonsmokers and former smokers with asthma: Associations of fractional exhaled nitric oxide groups with ultra-high-resolution CT findings. Nonsmokers (n = 38) and former smokers (n = 59) with asthma were divided into a low FeNO group (<20 ppb), a middle FeNO group (20-35 ppb), and a high FeNO group (>35 ppb). **A and B.** Wall area percent (WA%) and the presence of mucus plugging on ultra-high-resolution CT in nonsmokers. **C and D.** WA% and mucus plugging in smokers. P values for WA% and the rate of mucus plugging were calculated based on analysis of variance and Fisher's exact test, respectively. *p < 0.05 compared to the low FeNO group based on post hoc Tukey comparisons.

Table 3
Multivariable models for associations of FeNO levels with wall area percent and mucus plugging on ultra-high-resolution CT.

Nonsmoker (N = 38)	Low FeNO	Middle FeNO	High FeNO
WA% (3rd)	0 (Ref)	4.63 [-0.28, 955]	2.49 [-1.79, 6.77]
WA% (4th)	0 (Ref)	4.56 [-1.17, 10.29]	3.74 [-1.25, 8.73]
WA% (5th)	0 (Ref)	5.31 [0.24, 10.38]*	3.92 [-0.49, 8.32]
WA% (6th)	0 (Ref)	3.89 [-0.42, 8.19]	6.38 [2.64, 10.13]*
Mucus plugging, yes	1 (Ref)	0.60 [0.08, 4.30]	5.18 [0.90, 36.0]
Former smoker (N = 59)	Low FeNO	Middle FeNO	High FeNO
WA% (3rd)	0 (Ref)	−2.44 [-6.16, 1.28]	2.92 [-0.41, 6.25]
WA% (4th)	0 (Ref)	−1.62 [-5.27, 2.02]	1.24 [-2.02, 4.50]
WA% (5th)	0 (Ref)	−2.09 [-5.45, 1.27]	1.59 [-1.42, 4.60]
WA% (6th)	0 (Ref)	1.24 [-2.06, 4.53]	3.55 [0.60, 6.50]*
Mucus plugging, yes	1 (Ref)	0.44 [0.09, 1.93]	1.56 [0.42, 5.98]
All patients (N = 97)†	Low FeNO	Middle FeNO	High FeNO
WA% (6th)	0 (Ref)	2.35 [-0.27, 4.97]	4.57 [2.29, 6.84]*
Mucus plugging, yes	1 (Ref)	0.45 [0.12, 1.56]	3.23 [1.07, 10.37]*

Each model for nonsmokers and former smokers included the FeNO group (high FeNO, middle FeNO, and reference low FeNO), age, sex, and body mass index as independent variables and wall area percent (WA%) or the presence of mucus plugging as a dependent variable. Multivariable linear regression and logistic models were made for WA% and mucus plugging, respectively. Values indicate the estimate [95% confidence interval, 95% CI] for all the models except for mucus plugging and the odds ratio [95% CI] for the model for mucus plugging.

* Indicates a p value < 0.05.

† Models for all patients were made after confirming the absence of a significant interaction between the FeNO group and smoking. In addition to the variables used for the models of nonsmokers and former smokers, smoking status, height, CT lung volume, and presence of allergic rhinitis were included as independent variables for the models of all patients.

duration of asthma could cause airway wall thickening,[41] the influence of the disease duration on airway morphology might be small in this study.

In nonsmokers, WA% in the 3rd and 4th generations airways tended to differ between the low, mid, and high FeNO groups ($p = 0.07$ and 0.10), whereas WA% in the 5th and 6th generation airways significantly differ between the groups ($p = 0.03$ and $P < 0.01$). Moreover, multivariable models showed that the high FeNO group was associated with higher WA% in the 6th generation, but not in the 3rd to 5th generation airways. In a previous conventional CT study including nonsmokers with asthma,[18] simple spearman correlation tests showed significant correlations between FeNO value and wall area normalized by body surface area for all the 3rd to 6th generations, but the significant association was detected only in the 6th generation when age, lumen area, disease duration, ICS dose, blood eosinophil and IgE were adjusted by partial rank correlation tests. We believe that our findings are important because they confirmed those previous findings by using U-HRCT that allowed a more accurate evaluation of the wall dimension of the airways.[14]

The high FeNO group also had a higher rate of mucus plugging on U-HRCT than the low FeNO group in the main and sensitivity analyses including all patients with asthma. Considering the potential influence of limited image resolution on mucus plug assessment, our findings extend a previous finding of the association between mucus plugging on conventional CT and higher FeNO.[23]

In the separate analyses of nonsmokers and former smokers, a significant difference in the rate of mucus plugging between the low, middle, and high FeNO groups was found in nonsmokers but not in former smokers. A higher mucus plug score was associated with a higher WA% in the 6th generation airways but not in the 3rd to 5th generation airways in nonsmokers, whereas the mucus plug score was associated with WA% in all the 3rd to 6th generation airways in former smokers. These findings are in line with a previous study showed distinct impacts of mucus plugs on lung function and exacerbation frequency in smokers and nonsmokers with asthma.[21]

Moreover, 93.2% of former smokers exhibited FEV_1/FVC <70% and met criteria of asthma-COPD overlap (ACO).[42] The observed different associations between the mucus plugging and WA% in nonsmokers and former smokers might reflect the distinct pathophysiological features in asthma and ACO, respectively. While eosinophilic inflammation is central and induces mucus plugging in nonsmoking asthma,[22] coexisting eosinophilic and neutrophilic inflammation, termed mixed granulocytic inflammation, is common in ACO.[43] These different airway inflammatory states potentially affect microbiota[44] and viscosity of mucus plugging.[45] Furthermore, CT studies have shown that WA% of the central airways was greater in smokers with chronic bronchitis than those without[46] and greater in patients with ACO than in nonsmoking asthma.[47] Taken together, we speculate that mucus plugging may develop in relation to the wall thickening of the relatively peripheral airways in nonsmoking asthma and that a smoking history may change the airway inflammatory status, nature of the mucus plugging, and the process of wall remodeling, which could affect the association between mucus plugging and the locations of the airways with thicker walls.

This study also showed no association between FeNO and ALR. ALR is an index of CT dysanapsis representing the size mismatch between the central airway and lung. CT dysanapsis has been shown to be associated with a wide variation in lung function in healthy subjects, COPD development, and increased susceptibility to inhaled ambient air pollutants, leading to lower lung function.[35,36,48] Our finding of a lack of association between FeNO and ALR in patients with asthma suggests that CT dysanapsis may not affect type 2 airway inflammation in patients with asthma. However, further studies are warranted to test whether CT dysanapsis could affect the host response to inhaled allergens and sensitization/inflammation in patients with asthma.

All patients were treated with ICS, and 47 were categorized into the high FeNO group based on the JRS statement[7] in this study. Considering that higher FeNO and mucus plugging are associated with type 2 inflammation, such as sputum eosinophils and IL-4, and are predictive of response to biologics in patients with severe asthma,[20,23,49] the observed associations of higher FeNO with higher WA% in the 6th generation (but not in the 3rd or 4th generation) and mucus plugging suggest that higher FeNO even after ICS treatment might reflect uncontrolled type 2 inflammation in the relatively peripheral airways and further rationalize FeNO use for the selection of biologics in the management of severe asthma.

There are several limitations in this study. First, the sample size was relatively small. The separate analysis of former smokers and nonsmokers might have been underpowered. However, the significant association of FeNO with WA% in the 6th-generation airways was confirmed in both former smokers and nonsmokers, increasing the validity of the main finding in this study. Second, the cross-sectional nature of the study could not allow establishing a direct causal relationship between FeNO and airway structure. Third, although this study consecutively included patients undergoing U-HRCT, the retrospective nature of the study design might have caused bias. Future research should adopt a prospective longitudinal design to more accurately track changes in FeNO and airway structure over time.

In conclusion, higher FeNO was associated with a higher wall area relative to the lumen area in the 6th generation airways rather than 3rd to 5th generation airways in both nonsmokers and former smokers with asthma treated by ICS. These data further rationalize the use of FeNO to estimate inflammation and remodeling in the relatively peripheral airways in the clinical management of diseases.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.alit.2024.01.013.

Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

YH contributed to the acquisition, analysis, and interpretation of data and drafted the manuscript. NT contributed to the design of the work and the analysis and interpretation of data and drafted the manuscript. HM contributed to the acquisition, analysis, and interpretation of data and edited the manuscript. KS contributed to the interpretation of data and edited the manuscript. RS and TO contributed to the acquisition, analysis, and interpretation of data. HS, AS and SS contributed to the acquisition and interpretation of data. TH contributed to the design of the work and interpretation of data.

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