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Associated demographics of persistent exhaled nitric oxide elevation in treated asthmatics

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Summary

Background The fraction of exhaled nitric oxide (FENO) is reduced by anti-inflammatory treatment in asthma. However, the FENO level is also regulated by individual demographics and there is considerable variation among clinically stable patients.

Objective We hypothesized that some demographics may be responsible for persistent FENO elevation despite inhaled corticosteroids (ICS) therapy in asthma.

Methods This was a prospective observational study. We initially screened 250 stable asthmatics and determined the FENO cut-off point for identifying poorly controlled asthma defined by one of the following criteria: Asthma control test <20, or forced expiratory volume in one-second % of predicted <80%, or peak expiratory flow variability <80% (Study 1). After 12-weeks, 229 patients who maintained high or low FENO were selected and the independent factors which might contribute to a high FENO were examined (Study 2).

Results A FENO level >39.5 p.p.b. yielded 67% sensitivity and 76% specificity for identifying the patients with poorly controlled asthma. The persistent high FENO group (≥40 p.p.b.) was more likely to be ex-smokers, to show evidence of atopy (positive specific IgE, higher serum IgE and blood eosinophils), and to have allergic comorbidities. Especially, past smoking history, blood eosinophils, and chronic rhinosinusitis were identified to be independent predictors of high FENO. Neither the dose of ICS nor other medication use showed any difference between the groups.

Conclusions and Clinical Relevance These results suggested that past smoking history, blood eosinophilia, and chronic rhinosinusitis are involved in the persistent airway inflammation detected by FENO. Although their relative contributions on FENO values should be further quantified, clarification of the features of the subjects with high FENO might provide clues for adjustment of the treatment approach in asthma.

Keywords airflow obstruction, airway lability, allergic rhinitis, chronic rhinosinusitis, eosinophil, gastro-esophageal reflux disease, immunoglobulin E, inhaled corticosteroids, smoking

Abbreviations ACT, asthma control test; CRS, chronic rhinosinusitis; FENO, exhaled nitric oxide fraction; GERD, gastro-esophageal reflux disease; ICS, inhaled corticosteroids; NOS, nitric oxide synthases.

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Introduction

Airway inflammation is a central features of asthma, and inhaled corticosteroids (ICS) are widely used for the long-term management of the disease [1]. Nitric oxide (NO) is a gaseous signalling molecule that is generated by NO synthases (NOS). The expression of inducible NOS is enhanced by inflammatory stimuli producing large amounts of NO independently of calcium ion flux [2]. Indeed, the exhaled nitric oxide fraction (FENO) is

elevated in asthma [3] and is reduced in a dose-dependent manner by treatment with ICS [4]. However, the FENO is also influenced by individual characteristics such as height, gender, atopy, rhinitis, and smoking status [5–8]. These confounders are considered to be a major limiting factor for the implementation of FE_{NO} measuring as a guide for asthma management [7–10].

Recent studies have shown that sequential changes in FENO are indicative of the loss of asthma control even in smokers and patients with atopy [9, 10], suggesting that sequential FENO measuring may improve asthma management even in the subjects with confounders. However, not all patients with asthma respond to corticosteroids similarly [11] and there is considerable variation in the levels of FENO among clinically stable patients. Indeed, the FENO remains persistently high despite ICS treatment in some individuals. A recent study has shown that the grouping of asthma by FENO provides an independent classification of asthma severity and the subgroups with high FENO are the most reactive and worrisome phenotype [12]. There is also evidence that increased FENO is associated with the loss of asthma control [13, 14] and accelerated decline in pulmonary function [15]. However, it has not been fully elucidated whether or not any patient factors are associated with the persistent FENO elevation in asthma.

In the present study, we hypothesized that some factors may be responsible for high FENO despite ICS treatment. It is well known that the FENO values are modified by asthma control and steroid therapy [16]. All study participants were stable following ICS therapy and the subjects with recent exacerbations of asthma were excluded to control these modifiers. Because the upper limit of the FENO level in patients with stable asthma is still controversial [5, 12, 17, 18], we initially determined the cut-off point for high FENO on the basis of variables for asthma control including asthma control test (ACT), forced expiratory volume in one-second (FEV_1), and peak expiratory flow (PEF) variability (Study 1). After 12-weeks observation, the patients who maintained high or low FENO were selected and the independent factors which might contribute to the persistent FENO elevation were investigated (Study 2).

Methods

Study design

This was a prospective observational study for investigating the relationship between patient factors and the persistent FENO elevation in asthma (Fig. 1). PEF measurement was done at least 8-weeks prior to the study and pulmonary function test, ACT, and the FENO levels were assessed before and 12-weeks after the observation without changes in the treatment. Medical records including smoking history, medication use, and comorbidities such as atopy, allergic rhinitis, chronic rhinosinusitis (CRS), or gastro-esophageal reflux disease (GERD) were obtained.

Study subjects

All subjects were recruited from June 2010 to August 2010 to avoid the influence of the cedar pollen season in Japan. Subjects over 20 years old were eligible if they satisfied the standard criteria for asthma [18]. All patients were stable following the treatment of ICS with or without inhaled long-acting β2-agonist, leukotriene receptor antagonist, or theophylline. Subjects were excluded if they were current smokers, had had an exacerbation of asthma, or had been treated with systemic steroids during and/or 8-weeks prior to the study. Also, patients with poor adherence to the treatment or with other pulmonary diseases were excluded. All ex-smokers and patients with CRS had chest computed tomography to exclude clinically occult emphysema or bronchiectasis. Specific IgE for fungus and anti-neutrophil cytoplasmic antibody was examined to exclude allergic bronchopulmonary aspergillosis or allergic granulomatous angitis at the discretion of the physician.

All comorbidities were identified by specialists on the basis of guidelines [19, 20]. We considered that the presence of nasal polyps by endoscopy and sinus mucosal changes by computed tomography is essential for the diagnosis of CRS because CRS with nasal polyps is characterized by intense eosinophilic inflammation and Th2 polarization [19]. Complete blood cell count, differential count of leucocytes, total serum immunoglobulin E (IgE) levels, and specific IgE for common inhaled allergens (housedust, mite, cedar, cypress, ragweed, cocksfoot, dog, and cat) were examined. Positive specific IgE to at least one allergen was assumed to confirm atopy. This study was approved by the local ethics committee, and informed written consent was obtained from each subject.

FENO measurement

The fraction of exhaled nitric oxide, FENO was measured by an online electrochemical NO analyser (NIOX

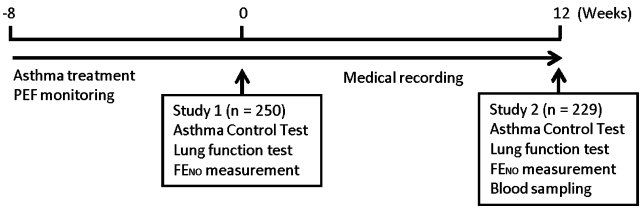

Fig. 1. Study design.

MINO; Aerocrine AB, Solna, Sweden) as previously described [8]. The subjects exhaled at a constant flow rate of 50 mL/s. Exhalations were repeated to obtain two acceptable measurements within 10% deviation, and the average of these two values was registered.

Pulmonary function and ACT measurement

The forced vital capacity (FVC) and FEV_1 were measured as previously described [8]. The morning PEF was monitored and the lowest pre-bronchodilator PEF over a week, expressed as a percentage of the highest PEF (Min%Max), was assumed to represent the PEF variability [21]. The ACT is a questionnaire that assesses the asthma condition according to five items, each of which can be rated on a five point scale [22]. A validated Japanese translation was used.

Determination of cut-off point for FENO levels

The rationale for selecting the cut-off point for high and low FENO was based on previous reports [17, 18] and on a sub-analysis of the data collected during the screening period (Study 1). Using the ROC curve method, we determined the FENO cut-off point for identifying poorly controlled asthma defined by one of the following criteria: ACT score <20, or FEV_1% of predicted <80%, or Min%Max <80%.

Statistical analysis

All data were expressed as mean values ±SD for continuous variables. For categorical variables, the numbers of observations and percentages were given in each category. Comparisons between different subgroups were performed by Fisher's exact test and Kruskal–Wallis test. Multivariate logistic regression analysis was used to assess the association between the binary outcome (FENO $\geq$ 40 p.p.b.) and a set of demographic covariates. The variables with *P*-values <0.20 in the univariate analysis were included in the multivariate model. Spearman's correlation analysis was performed to assess the correlation between the number of eosinophils in the blood and the FENO level. A *P*-value of < 0.05 was considered significant.

Results

During the screening period, the variables for asthma control were obtained from 250 patients, and 51 patients (22.3%) were defined as poorly controlled asthma based on the following criteria: ACT < 20 ($n = 16$), or %FEV_1 <80% ($n = 29$), or Min% Max $< 80\%$ ($n = 30$). A FENO level >39.5 p.p.b. yielded 67% sensitivity and 76% specificity (AUC = 0.749) (Fig. 2). Thus, we selected 40 p.p.b. as the cut-off point for high and low FENO in the subsequent analysis, a value that was within previously published cut-off points ranging from 35 to 50 p.p.b. [17, 23].

After 12-weeks observation, 229 patients who maintained high or low FENO were selected (Table 1). Of the 21 dropouts, nine subjects in the high FENO group and six subjects in the low FENO group were switched over to the other group. Other dropouts were related to either exacerbations of asthma or non-adherence. The selected subjects were divided into the low FENO group ($n = 158$, 69.0%) and the high FENO group ($n = 71$, 31.0%) (Table 2). The baseline FVC and %FVC were similar between the groups, although the FEV_1/FVC ratio ($P < 0.005$) and %FEV_1 ($P < 0.001$) were significantly lower in the high FENO group. The high FE_{NO} group had more exaggerated fluctuations of airway calibre (Min%Max), and more severe symptoms of asthma (ACT) (all $P < 0.001$).

Next, the relationships between the FENO levels and patient demographics were examined (Table 3). Compared to the low FENO group, the subjects with high FENO were more likely to be ex-smokers (low FENO, 21.5%; high FENO 52.1%; $P < 0.001$), although there was no significant difference in pack-years between the groups ($P = 0.39$). Furthermore, the asthmatics with high FENO were more likely to show evidence of atopy such as positive specific IgE (low FENO, 70.2%; high FENO 91.5%; $P < 0.01$), higher serum IgE ($P < 0.001$) and blood eosinophils ($P < 0.001$), and to have allergic comorbidities such as allergic rhinitis (low FENO, 60.8%; high FENO 87.3%; $P < 0.001$), and CRS (low FENO, 7.6%; high FENO 54.9%; $P < 0.0001$). Gender and leukotriene receptor antagonist use tended to be associated with high FENO ($P < 0.1$) but it was not significant.

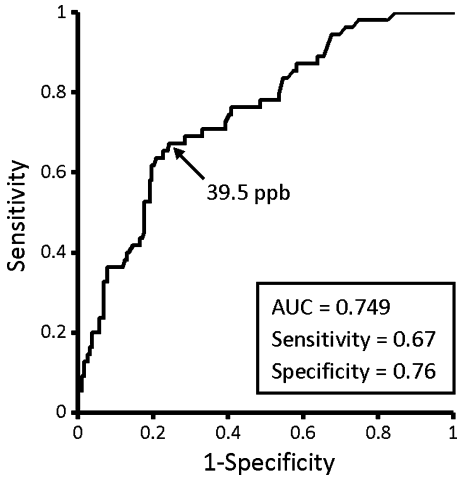

Fig. 2. Receiver operating characteristics (ROC) curve to estimate the FENO cut-off values for identifying poorly controlled asthma. Data labels represent cut-off values of FENO (arrow), area under the curve (AUC), sensitivity and specificity.

Table 1. Characteristics of subjects who maintained high or low FENO levels

Number (female/male)	229 (133/96)
Mean age (years)	46.6 ± 14.7
Body mass index (kg/mm^2)	22.5 ± 3.9
Smoking status (Never : Ex), *n* (%)	158 (69.0) : 71 (31.0)
Atopy, *n* (%)	176 (76.9)
Allergic rhinitis, *n* (%)	158 (69.0)
Chronic rhinosinusitis, *n* (%)	51 (22.3)
GERD, *n* (%)	31 (13.5)
Inhaled corticosteroids, *n* (%)	229 (100.0)
Inhaled long-acting β2 agonist, *n* (%)	99 (43.2)
Leukotriene receptor antagonist, *n* (%)	43 (18.8)
Theophylline, *n* (%)	19 (8.3)
FVC (L)	3.53 ± 0.89
FVC % of predicted (%)	103.7 ± 13.0
FEV_1 (L)	2.71 ± 0.77
FEV_1% of predicted (%)	96.7 ± 15.9
PEF variability (Min%Max) (%)	87.5 ± 6.0
Blood eosinophils (cells/μL)	251 ± 209
Serum IgE levels (IU/mL)	530 ± 1496
FE_{NO} (p.p.b.)	34.6 ± 22.0
Asthma control test (points)	22.9 ± 2.2

GERD, gastro-esophageal reflux disease; FVC, forced vital capacity; FEV1, forced expiratory volume in one-second; PEF, peak expiratory flow; Min%Max, the lowest PEF over a week, expressed as the percentage of the highest PEF; IgE, immunoglobulin E; FENO, exhaled nitric oxide fraction. Mean (SD) values are provided unless otherwise indicated.

Table 2. Pulmonary function test and asthma symptom score by exhaled nitric oxide levels

Characteristics	Low FE_{NO} (<40 p.p.b.) *n* = 158	High FE_{NO} (≥40 p.p.b.) *n* = 71	*P*-value
FVC (L)	3.53 ± 0.91	3.51 ± 0.82	0.76
FVC % of predicted (%)	104.8 ± 13.2	101.1 ± 12.3	0.07
FEV_1 (L)	2.77 ± 0.77	2.59 ± 0.76	0.09
FEV_1/FVC ratio (%)	78.4 ± 9.6	73.4 ± 10.3	<0.005
FEV_1% of predicted (%)	101.1 ± 15.0	87.0 ± 13.4	<0.001
Minimal PEF (L/min)	421 ± 113	398 ± 119	0.06
Maximal PEF (L/min)	468 ± 123	481 ± 129	0.80
PEF variability (Min%Max) (%)	89.9 ± 4.3	82.2 ± 5.7	<0.001
Asthma control test (points)	23.5 ± 2.1	21.6 ± 2.0	<0.001

FENO, exhaled nitric oxide fraction; FVC, forced vital capacity; FEV_1, forced expiratory volume in one-second; PEF, peak expiratory flow; Min%Max, the lowest PEF over a week, expressed as the percentage of the highest PEF. Mean (SD) values are provided.

According to the multivariate logistic regression analysis, blood eosinophilia and CRS were identified to be independent predictors of high FENO (OR 1.78, $P < 0.0001$, and OR 11.71, $P < 0.0001$ respectively) (Table 4). In addition, a past smoking history was also detected as an independent predictor of high FENO (OR 4.14, $P < 0.005$). Indeed, the FE_{NO} was significantly higher for ex-smokers than that for never-smokers, and subjects with CRS showed significantly higher levels of FE_{NO} compared with subjects without CRS (all $P < 0.001$). The number of eosinophils in the blood was significantly associated with the FENO level ($r = 0.57$, $P < 0.0001$) (Fig. 3).

Overall, 65 of 71 subjects (91.5%) with high FENO had at least one of three factors including past smoking history, CRS, and hypereosinophilia defined as a blood eosinophil count >450/μL. Neither the dose of ICS nor other medication use showed any difference between the two groups (Table 3).

Discussion

The present study provides evidence that several factors are responsible for persistent FENO elevation despite ICS treatment in the patients with stable asthma. The subjects with sustained high FENO were characterized by lower %FEV1 values, exaggerated fluctuation of airway calibre, and more severe asthma symptoms. They were more likely to be ex-smokers, to show evidence of atopy, and to have allergic comorbidities. Interestingly, past smoking history, blood eosinophilia, and CRS were identified as independent predictors of persistent FENO elevation in treated asthmatics.

An elevated FENO level is known to represent the presence of airway inflammation [2, 4]. In some individuals, the FENO levels remain high despite adequate conventional therapy [12, 24]. This was also found in our study, and approximately 30% of the subjects had sustained high FENO. This might be explained by the presence of heterogeneous airway inflammation that is relatively resistant to steroids [6, 25–27]. Recently, neutrophilic or persistent eosinophilic inflammation was found in asthmatics with raised FENO despite steroid therapy [25–27]. Furthermore, stepwise increases in ICS or systemic steroids reduce the FENO levels to some extent [28, 29], which might also reinforce this hypothesis. Because FENO measurement identified the subgroup of asthma with persistent airway inflammation, irreversible changes of the airway structure could result in airway obstruction and/or vulnerable airway conditions 12, 25–27]. The persistent high FENO might reflect long-standing airway inflammation and its resultant remodelling [13]. Alternatively, inadequate anti-inflammatory therapy may be a possible explanation for the presence of the persistent FENO elevation. However, in our study, neither the dose of ICS nor other medication use showed any difference between the groups. In addition, neither inhalation instruction nor increasing the ICS dose reduced the sustained high FENO in asthmatic

Table 3. Subject demographics by exhaled nitric oxide levels

Characteristic	Low FENO (<40 p.p.b.)	*n*	High FENO (≥40 p.p.b.)	*n*	*P*-value
Mean age (years)	46.1 ± 15.1	158	48.1 ± 13.9	71	0.38
Gender (male), *n* (%)	64 (40.5)	158	32 (45.1)	71	0.07
Body mass index (kg/mm^2)	22.2 ± 3.8	158	23.4 ± 4.0	71	0.14
Ex-smokers, *n* (%)	34 (21.5)	158	37 (52.1)	71	<0.001
Pack-years (years)	17.7 ± 13.9	35	15.6 ± 9.5	36	0.39
Atopy, *n* (%)	111 (70.2)	158	65 (91.5)	71	<0.01
Allergic rhinitis, *n* (%)	96 (60.8)	158	62 (87.3)	71	<0.001
Chronic rhinosinusitis, *n* (%)	12 (7.6)	158	39 (54.9)	71	<0.0001
GERD, *n* (%)	21 (13.3)	158	10 (14.1)	71	0.87
Blood eosinophils (cells/μL)	173 ± 135	158	420 ± 237	71	<0.001
Serum IgE levels (IU/mL)	390 ± 1281	158	842 ± 1863	71	<0.001
Dose of inhaled corticosteroids (μg/day)*	344 ± 109	158	352 ± 107	71	0.60
Inhaled long-acting β2 agonist use, *n* (%)	65 (41.1)	158	34 (47.9)	71	0.34
Leukotriene receptor antagonist use, *n* (%)	25 (15.8)	158	18 (25.4)	71	0.09
Theophylline use, *n* (%)	13 (8.2)	158	6 (8.5)	71	0.96

*Inhaled corticosteroids, expressed as fluticasone propionate equivalents. FENO, exhaled nitric oxide fraction; GERD, gastro-esophageal reflux disease. Mean (SD) values are provided unless otherwise indicated.

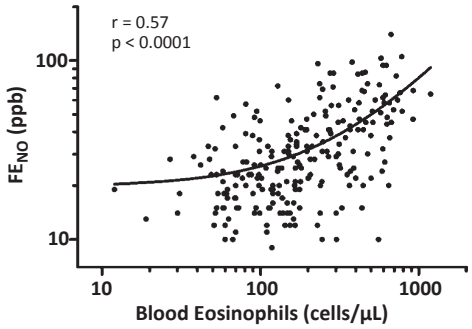

Fig. 3. Relationship between the number of eosinophils in the blood and the FENO levels: The line corresponds to the fitted regression equation. The *r*- value was Spearman's correlation coefficient.

Table 4. Multivariate logistic regression analysis with high exhaled oxide (≥40 p.p.b.) as the outcome

	All asthma (*n* = 229)	
Characteristic	Odds ratio (95% CI)	*P*-value
Female	0.94 (0.49–2.21)	0.89
Body mass index	1.00 (0.90–1.11)	0.98
Ex-smokers*	4.14 (1.67–10.24)	<0.005
Atopy	1.59 (0.33–7.69)	0.56
Allergic rhinitis	3.75 (0.85–16.61)	0.08
Chronic rhinosinusitis*	11.71 (4.00–34.27)	<0.0001
Log number eosinophils in the blood*	1.78 (1.47–2.14)	<0.0001
Log serum immunoglobulin E	1.00 (1.00–1.00)	0.82
Leukotriene receptor antagonist use	2.81 (0.86–9.18)	0.09

*Independent predictors of persistently high exhaled nitric oxide fraction.

children [24]. More than 90% of subjects with high FENO had at least one of three factors including past smoking history, CRS, and hypereosinophilia, which suggests that persistent airway inflammation detected by FENO is probably related to these factors.

Atopy and allergic comorbidities were identified as contributing factors for the persistent high FENO. A significant increase in blood eosinophils was found in treated asthmatics and positive correlations were found between blood eosinophils and FENO levels [30], suggesting that an ongoing recruitment and activation of inflammatory cells may be present in asthmatic airways even when treated by ICS. It is known that eosinophils could be one of the sources of the increased NO production [31] and NO production might be involved in facilitating the migration of eosinophils [32]. Atopy has been known to be associated with airway hyperresponsiveness [21] and asthma patients with atopy show significantly higher levels of FENO than non-atopic patients [5, 7]. In addition, allergic rhinitis raises the FENO significantly [8, 10], and nasal ICS therapy has been reported to reduce the FENO in the patients suffering from asthma and rhinitis [33]. Although CRS was also identified as a predictor for high FENO, it is unclear why only this comorbidity could reach significance in the multivariate analysis. CRS is more often associated with sputum eosinphilia [34] and with damage of the airway mucosa than the other diseases [35]. This additional airway inflammation might enhance the production of exhaled NO. Our definition of CRS, which included both sinus changes and nasal polyps, may have led to the association we observed. Indeed, a recent study showed that increased FENO is more frequent in CRS with nasal polyps compared with CRS without nasal polyps [36]. Moreover, in our study, the mean number of blood eosinophils was significantly higher for subjects with CRS than that for subjects without CRS ($P < 0.0001$), even though 62% of the lat-

ter group had allergic rhinitis (data not shown). The previous studies and ours suggest that the allergic comorbidities function as a potential reservoir of inflammatory mediators, and reciprocally influence the airway inflammation.

There is no doubt that current smoking reduces the FENO levels through reduced production and increased consumption of NO [5, 8, 9]. However, the effects of ex-smoking on the FENO remain to be elucidated. Although steroids have a number of anti-inflammatory actions including the suppression of inducible NOS expression [1, 2], a history of smoking has been reported to impair the effects of steroid therapy in asthma [6, 37]. As shown in Table 3, approximately half of the ex-smokers with asthma showed sustained high FENO. These results suggest that past smoking might have changed the airway condition resulting in a more vulnerable and steroid resistant airway in some ex-smokers with asthma. This phenomenon could be partially explained by the fact that smoking potentially induces nitrative stress [6] and that excessive NO synthesis was found in individuals with refractory asthma [27]. Further study is needed to clarify the long-standing influence of past smoking on airway inflammation.

There would be some limitations in our study. First, the observation period might be too short to select subjects who maintained high or low FENO. Second, a selection bias is possible because the subjects with symptoms suggestive of comorbidities including allergic rhinitis, CRS, and GERD were referred to a specialist. Thus, the data set might not completely represent the population with clinically occult comorbidities [19, 20, 34]. Third, we carefully avoided the cedar pollen season in Japan, but perennial allegen might be a cause of sustained high FENO [23]. Finally, although all subjects had been regularly educated on the correct medication use, we could not verify adherence objectively. Poor adherence could be an explanation for sustained high FENO values in some subjects.

In conclusion, we have shown that past smoking history, blood eosinophilia, and chronic rhinosinusitis are involved in the persistent airway inflammation detected by FENO. Although their relative contributions on FENO values should be furthermore quantified, clarification of the features of the subjects with high FENO might provide clues for adjustment of the treatment approach in asthma.

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