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ORIGINAL ARTICLE

Exhaled Nitric Oxide Cutoff Values for Asthma Diagnosis According to Rhinitis and Smoking Status in Japanese Subjects

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ABSTRACT

Background: Measurement of the exhaled nitric oxide fraction (FE_{NO}) has been proposed as a useful diagnostic test for asthma. However, most of the data concerning the FE_{NO} cutoff values for the diagnosis of asthma have not yet examined using standard procedures. Furthermore, there is no detailed study that investigated the cutoff values that takes into account patient factors that influence the FE_{NO} levels.

Methods: FE_{NO} was measured in 142 steroid-naive asthmatics and 224 control subjects using an online electrochemical nitric oxide analyzer in accordance with the current guidelines. Subjects without respiratory symptoms and normal spirometric parameters were included in the control group. Asthma was diagnosed on the basis of the presence of significant airway reversibility and/or airway hyperresponsiveness during clinical follow up 6 months after FE_{NO} measurements.

Results: FE_{NO} was significantly higher in asthmatic patients compared with control subjects ($p < 0.01$). Based on all study subjects, the receiver operating characteristic curves indicated that the cutoff value of FE_{NO} 22 parts per billion (ppb) was associated with the highest combination of sensitivity (90.8%) and specificity (83.9%). Multivariate analysis showed allergic rhinitis, current smoking, and asthma were significant factors influencing the FE_{NO} levels. The cutoff values of FE_{NO} to discriminate asthma from non-asthma ranged from 18 to 28 ppb depending on rhinitis and smoking status.

Conclusions: The cutoff values presented may be useful for the interpretation of FE_{NO} values in the clinical practice.

KEY WORDS

airflow obstruction, airway hyperresponsiveness, airway inflammation, airway reversibility, inhaled corticosteroids

ABBREVIATIONS

AUC, area under the curve; BMI, body mass index; FE_{NO}, exhaled nitric oxide fraction; ppb, parts per billion; ROC, receiver operating curve.

INTRODUCTION

The exhaled nitric oxide fraction (FE_{NO}) is a useful marker to assess airway inflammation.[1] FE_{NO} is elevated in patients with asthma,[1,2] and is associated with airway eosinophilia and airway hyperresponsiveness.[3-5] Furthermore, FE_{NO} has been shown to be able to discriminate among subjects with asthma and those without asthma.[6-16] Therefore, the measurement of FE_{NO} has been proposed as a useful diagnostic test for asthma.[1,6-16]

The FE_{NO} cutoff values for the diagnosis of asthma

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Table 1 Characteristics of the study subjects

	Control	Asthma
Number (male/female)	224 (98/126)	142 (69/73)
Age (years)	39.4 ± 0.9	41.5 ± 1.4
Height (m)	1.64 ± 0.01	1.64 ± 0.01
Body weight (kg)	58.3 ± 0.8	61.2 ± 1.0
Body mass index (kg/m^2)	21.7 ± 0.2	22.7 ± 0.3
Non smoker/Current smoker (n)	172/52	90/52
Non rhinitis/Rhinitis (n)	123/101	48/94
FVC (L)	3.55 ± 0.06	3.61 ± 0.09
%FVC (%)	103.0 ± 0.6	97.7 ± 1.0
FEV_1 (L)	3.08 ± 0.05	2.84 ± 0.08*
FEV_1% (%)	87.0 ± 0.3	78.3 ± 0.9*
%FEV_1 (%)	98.7 ± 0.6	89.1 ± 1.2*
FE_{NO} (ppb)	15.9 ± 0.4	47.3 ± 2.9*

Definition of abbreviations: FVC, forced expiratory capacity; FEV_1, forced expiratory volume in one second; FE_{NO}, exhaled nitric oxide fraction.

All values are Mean ± SE.*$p < 0.01$, for between-group comparisons.

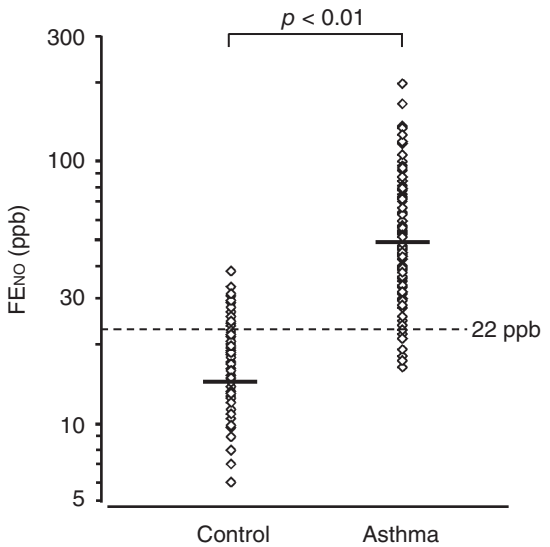

Fig. 1 Scatter plot of the exhaled nitric oxide fraction (FE_{NO}) levels in control subjects and in steroid-naive asthmatic patients. The horizontal solid bars indicated the mean value for each group. The best cutoff value to discriminate between the groups was obtained from the highest combination of sensitivity and specificity and corresponded to 22 ppb.

have been provided in several previous reports.[6-13] However, only a few studies have examined the cutoff values using current standard procedures in a steroid-naive population of adults.[11-13] In addition, it has been recognized that FENO is influenced by patient factors, such as atopy and smoking status.[1,13-26] To date, there is a lack of data on the cutoff values related to patient factors that influence the FENO levels.

In the present study, FENO was measured in steroid-naive asthmatic patients and control subjects in accordance with the current guidelines.[1] The determinants of the FENO levels were evaluated using a multivariate model. Furthermore, we also investigated the cutoff values for the diagnosis of asthma according to patient factors that influence the FENO levels.

METHODS

STUDY SUBJECTS

Adult subjects with and without respiratory symptoms were recruited from the outpatient clinic of Wakayama Medical University. To avoid the influence of the cedar pollen season in Japan, the enrollment was performed from May to December 2009. A detailed interview was performed by physicians. Subjects were excluded if they had a history of lung diseases except for asthma, had a smoking history with more than 20 pack-years, had had an airway infection or were taking any form of corticosteroids, β_2-agonists, leukotriene modifiers, and H_1-antagonists in the 4 weeks preceding the study. Recent quitters were also excluded to stratify the study subjects as either nonsmokers or current smokers (within the past 8 weeks). Afterwards, the subjects underwent measurements for FENO followed by spirometry. Subjects having no respiratory symptoms, no history of asthma and normal spirometric parameters were included in the control group. The diagnosis of asthma was established after the FENO measurements. We performed a clinical follow up of 6 months in the subjects with respiratory symptoms (with or without asthma medication). Chest radiograph and blood examination were performed at the discretion of the physician and patients with a clear alternative diagnosis were excluded. Asthma was diagnosed on the ba-

Table 2 Multivariate model predicting FE_{NO} levels in all subjects

Variables	Coefficient (95% CI)	*p* value
Intercept	0.83 (0.47; 1.19)	<0.0001
Gender (male vs. female)	0.07 (-0.02; 0.17)	0.13
Body mass index (per kg/m^2)	0.01 (-0.00; 0.03)	0.09
Smoking (current smoker vs. non smoker)	-0.27 (-0.37; -0.17)	<0.0001
Rhinitis	0.22 (0.13; 0.31)	<0.0001
Asthma	0.94 (0.84; 1.03)	<0.0001

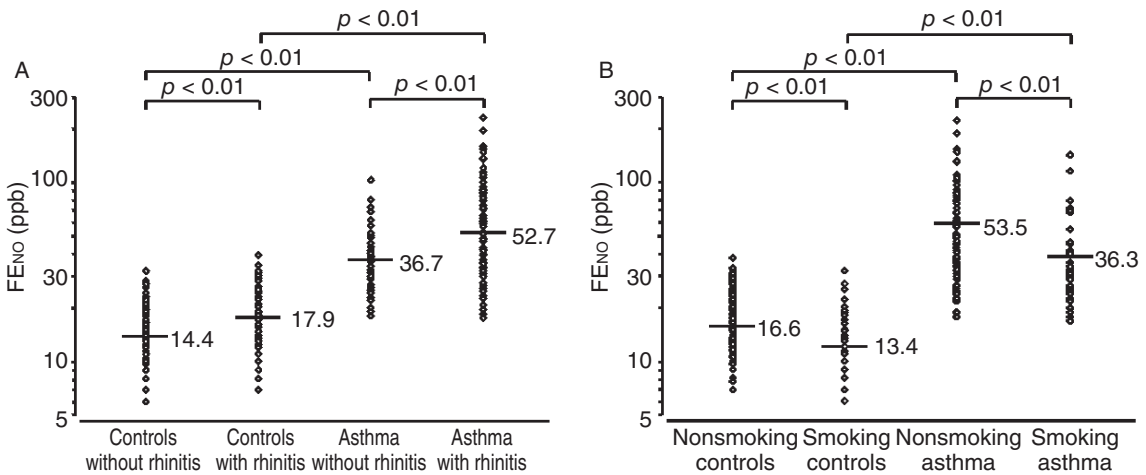

Fig. 2 Scatter plot of the exhaled nitric oxide fraction (FE_{NO}) levels in control subjects and in asthmatic patients according to allergic rhinitis (**A**) and smoking status (**B**). The horizontal solid bars indicated the mean value for each group.

sis of the presence of significant airway reversibility and/or airway hyperresponsiveness during the follow up period. Allergic rhinitis was defined based on compatible symptoms (nasal obstruction, rhinorrhea, sneeze, and/or postnasal drainage) in patients with atopy. Positive specific immunoglobulin E to at least one common allergen (housedust, mite, cedar, cypress, ragweed, cocksfoot, dog, and cat) confirmed the diagnosis of atopy.

The study was approved by the ethics committee of Wakayama Medical University and informed consent was obtained from each subject. Baseline characteristics of the study subjects are presented in Table 1.

STUDY DESIGN

The data obtained from steroid-naive asthmatic patients and control subjects at the initial testing were cross-sectionally analyzed for estimating the cutoff values of FENO.

FENO MEASUREMENTS

FENO was measured by an online electrochemical nitric oxide analyzer (NIOX MINO; Aerocrine AB, Solna, Sweden) as previously described.[26] Measurements of FENO were performed by asking the subjects to empty their lungs and exhale into the device at a constant flow rate of 50 mL/s; the software automatically checks that the breathing manoeuvre is performed according to the current guidelines.[1] Repeated exhalations were performed to obtain two acceptable measurements that agreed within 10% deviation, and the average of these two values was registered. The FENO measurements were performed in the morning. All subjects had fasted and had not smoked for at least two hours before the measurements.

STATISTICAL ANALYSIS

Normality assumptions were met for the logarithm of FENO, the logarithm-transformed value was used in the analysis and then back-transformed values were estimated. Comparisons between groups were made by Mann-Whitney *U* tests. Among the patient factors, age, gender, body mass index, smoking status, allergic rhinitis, and asthma were selected, and the factors influencing the FENO values were analyzed using multiple linear regression analysis. Multiple stepwise forward regression analysis was also used. Receiver operating characteristic (ROC) curves were plotted in order to estimate the cutoff values for diagnosis. An optimal cutoff value was obtained from the highest sum obtained from adding sensitivity and specificity.

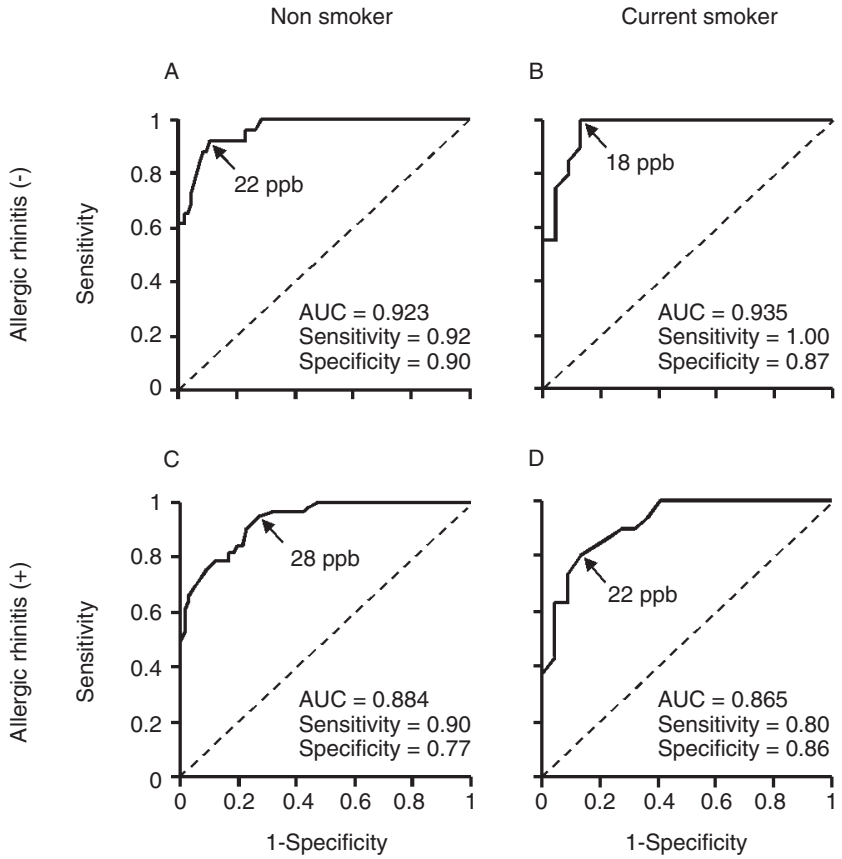

Fig. 3 Receiver operating characteristics (ROC) curves for FE_{NO} in the diagnosis of asthma according to allergic rhinitis and smoking status. (**A**) Nonsmoking subjects without rhinitis (99 control subjects and 27 patients with asthma), (**B**) current smoking subjects without rhinitis (28 control subjects and 21 patients with asthma), (**C**) nonsmoking subjects with rhinitis (73 control subjects and 63 patients with asthma), (**D**) current smoking subjects with rhinitis (24 control subjects and 31 patients with asthma). Data labels represent cutoff values of FE_{NO} (arrow), area under the curve (AUC), sensitivity and specificity in each subgroup.

All data were expressed as mean ± SE and significance was defined as a p value of less than 0.05.

RESULTS

The geometric mean FENO was significantly higher in the asthmatic patients compared with the control subjects (15.9 ppb vs. 47.3 ppb, $p < 0.01$, Fig. 1). ROC curves showed the cutoff value of FENO 22 ppb was associated with the highest combination of sensitivity (90.8%) and specificity (83.9%) for all study subjects (Fig. 1). The area under the curve was 0.896.

In the multivariate analysis, asthma and rhinitis predicted increased FENO levels (coefficient = 0.94, 95% confidence interval [CI]: 0.84-1.03, $p < 0.0001$, and coefficient = 0.22, 95% CI: 0.13-0.31, $p < 0.0001$, respectively). By contrast, current smoking was a significant factor that reduced the FENO levels (coefficient = -0.27, 95% CI: -0.37–0.17, $p < 0.0001$). The effects of age, gender, and body mass index on the FENO levels were not statistically significant (Table 2). Subjects with rhinitis showed significantly higher levels of FENO compared with subjects without rhinitis and the FENO values were significantly lower for current smokers than for nonsmokers in both groups (all $p < 0.01$) (Fig. 2).

Based on the results of the multivariate analysis, the study subjects were divided into four subgroups according to allergic rhinitis and smoking status and the cutoff values for each subgroup were estimated. The optimal cutoff values of FENO to discriminate between the subjects with asthma and those without asthma ranged from 18 to 28 ppb depending on rhinitis and smoking status as shown in Figure 3. The different values of sensitivity and specificity for selected cutoff values of FENO in all subjects and in each subgroup are listed in Table 3.

Table 3 Accuracy of the measurement of FE_{NO} in the diagnosis of asthma

FE_{NO} (ppb)	All		Nonsmoking/non-rhinitis		Smoking/non-rhinitis		Nonsmoking/rhinitis		Smoking/rhinitis	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
14	100.0	57.0	100.0	62.1	100.0	78.3	100.0	42.4	100.0	50.0
16	98.6	67.7	100.0	71.6	100.0	87.0	100.0	53.0	93.3	63.6
18	93.0	75.3	94.1	76.4	85.0	91.3	96.8	57.6	86.7	77.3
20	91.4	78.6	92.3	81.1	80.0	91.3	96.8	68.2	84.4	81.6
22	90.8	83.9	92.3	89.5	70.0	95.7	94.2	69.6	80.0	86.4
24	82.4	88.8	84.6	92.6	60.0	95.7	92.3	72.5	70.0	90.9
26	71.8	91.9	69.2	95.8	55.0	95.7	91.1	73.6	63.3	95.5
28	66.9	93.7	61.5	97.9	50.0	100.0	90.3	77.3	56.7	95.5
30	64.1	95.4	61.5	98.9	50.0	100.0	82.3	81.8	53.3	95.5

DISCUSSION

In the present study, FENO was measured in 142 steroid-naive asthmatic patients and 224 control subjects using the current standard procedures.[1] Based on all study subjects, we have shown that the cutoff value of FENO to discriminate asthma from non-asthma was 22 ppb at a flow rate of 50 mL/second, with a sensitivity and specificity of 90.8% and 83.9%, respectively. Allergic rhinitis and current smoking were significant determinants of the FENO levels. The optimal cutoff values for the diagnosis of asthma ranged from 18 to 28 ppb depending on rhinitis and smoking status. To our knowledge, this is the first study that investigated the cutoff values that took into account patient factors that influence the FENO levels.

Although the current standard procedures for the FENO measurements were recommended in 2005,[1] the cutoff values for the diagnosis of asthma have not yet been established. It is well known that several technical factors influence the measured FENO level, including the expiratory flow rate and collection method. Among these factors, the expiratory flow rate has been demonstrated to have the most critical effect, with FENO declining with an increasing expiratory flow rate.[1] Deykin *et al*. have reported that the diagnostic value of FENO for asthma is not influenced by either the expiratory flow rate or by collection method.[11] They have also shown that the FENO cutoff values were inversely related to the expiratory flow rate.[11] However, most of the studies conducted up to the year 2005 did not include the current standard procedures, and the cutoff values were not determined for the recommended expiratory flow rate of 50 mL/second.[6-8] In addition, the FENO levels are related to inflammation in the asthmatic airway,[3,4,27] and treatment with anti-inflammatory drugs such as corticosteroids results in a marked reduction of these levels.[28] To estimate the FENO cutoff values, we considered that the use of corticosteroids should be prohibited before the measurements. Although many studies have shown that FENO is increased in steroid-naive asthmatics, only a few studies have examined the cutoff values according to the current guidelines.[1] The present study has shown that the cutoff value of FENO for the diagnosis of asthma was 22 ppb at a flow rate of 50 mL/second, which is comparable to the previously published data.[11-13]

The measured FENO level is also influenced by patient factors. Previous studies have shown that there are several determinants of the FENO levels, such as age, gender, height, smoking status, atopy, and rhinitis.[1,13-26] The finding of a higher level of FENO in atopic subjects has been reported,[13-18,22-24] while chronically reduced levels of FENO have been demonstrated in current smokers.[13,15,18,22,25] By contrast, the association between FENO and age, gender, and height is still controversial.[1,17-22] Moreover, it has been reported that the FENO levels in current smokers return to normal levels within 1-8 weeks after smoking cessation.[29,30] We considered these findings as a justification to subsume never-smokers and ex-smokers into the same category. In our study, multivariate analysis showed that significant confounding factors are allergic rhinitis and current smoking, which is consistent with previous reports.[13-18,22-25]

It has been considered that confounding factors that influence the FENO levels are important limiting factors for the implementation of FENO as a diagnostic test for asthma. However, several recent studies have demonstrated that the diagnostic value of FENO for asthma is not influenced by either rhinitis or by smoking.[13-16] In the present study, subjects with rhinitis showed higher levels of FENO compared with subjects without rhinitis and the FENO values were lower for current smokers than for nonsmokers in both controls and asthmatics. However, the FENO levels were significantly higher in the asthma patients compared with the control subjects irrespective to rhinitis and smoking status. Kostikas *et al*. screened a population of steroid-naive young adults including current smokers.[13] They also reported that allergic rhinitis and current smoking were determinants of the FENO levels.[13] However, cutoff values that take

into account these factors were not estimated. Their study comprised 70 controls and 63 asthmatic patients, and each group included a small number of current smokers. In addition, the subjects with concomitant asthma and allergic rhinitis were considered as asthmatics.[13] The present study had a large number of samples that enabled us to estimate the cutoff values according to patient factors that influence the FENO levels. We have shown that the lowest cutoff value was 18 ppb for smoking subjects without allergic rhinitis, and the highest value was 28 ppb for nonsmoking subjects with allergic rhinitis. This analysis also showed that the area under the curves were over 0.85 in all subgroups, indicating that rhinitis and current smoking do not appear to affect the utility of FENO measurements for the diagnosis of asthma. Although further research is needed to establish the cutoff values in patients with other factors that influence the FENO levels, the validity of the cutoff values according to confounding factors may improve the diagnostic value of the FENO measurements for asthma.

In the present study, control subjects were defined on the basis of detailed interviews and spirometry. Asymptomatic patients with asthma may be involved in the control groups. Although the subjects with asthma-like symptoms were carefully excluded according to the guidelines,[31] we made no attempt to validate information by bronchial provocation testing. This test is sensitive for the diagnosis of asthma but has limited specificity,[31,32] and it will not always be practical for physicians to perform bronchial provocation testing before the measurements of FENO. Furthermore, in most of the previous studies, the cutoff value of FENO was assessed by a different measurement system using a chemiluminescene analyzer. However, it has been demonstrated that the FENO values measured by an electrochemical device are reliable, reproducible, and in agreement with those of the chemiluminescene analyzer.[9,33,34]

In summary, this study has provided further data on FENO measurements as a diagnostic test for asthma using the current standard procedures. The cutoff values presented may be useful for the interpretation of FENO values in the clinical practice.

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