

Utility of nitric oxide for the diagnosis of asthma in an allergy clinic population

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ABSTRACT

Fractionated exhaled nitric oxide (FeNO) expression is increased in airway inflammation and several studies have suggested that FeNO measurement can be useful in patients with asthma. Atopic individuals have increased FeNO levels, indicating that atopy may be a codeterminant in FeNO production. The aim of this study was to determine the discriminative value of FeNO for asthma and other atopic conditions in the general allergy clinic. Patients referred to the outpatient allergy clinic were screened. A standardized questionnaire was taken and atopic status was assessed (skin-prick test or specific plasma IgE). FeNO level and spirometry were measured. If the patient's history was suspect for asthma, a provocative concentration causing a 20% decrease in forced expiratory volume in 1 second (PC₂₀) histamine challenge followed. One hundred fourteen steroid-naïve patients were included. Forty-two subjects were diagnosed as asthmatic patients and 72 were diagnosed as nonasthmatic patients, comprising patients with allergic rhinitis (*n* = 32), nonallergic rhinitis (*n* = 11), urticaria (*n* = 11), eczema (*n* = 7), and other (*n* = 11). Asthmatic patients had a higher FeNO level than nonasthmatic patients (44 ppb versus 17 ppb; *p* < 0.001). Receiver operating characteristic curve analysis revealed the optimal FeNO level to distinguish asthma from nonasthma at 27 ppb, with a sensitivity of 78%, specificity of 92%, a positive predictive value of 86%, and a negative predictive value of 87%. Increased FeNO was positively correlated with the presence of respiratory symptoms (*p* < 0.01), airflow reversibility (*p* < 0.001), total IgE (*p* < 0.001), and negatively correlated with PC₂₀ histamine (*p* = 0.019). Multivariate analysis revealed that atopy was not a significant predictor of FeNO in asthmatic patients. Measuring FeNO is a simple and useful test to differentiate new asthma patients from those with other atopic conditions in a general allergy clinic.

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Fractionated exhaled nitric oxide (FeNO) measurement technique is noninvasive, with minimal discomfort for the patient and good reproducibility.¹ Guidelines for standardized online and offline measurement of FeNO are available.² There is a moderate correlation between FeNO and pulmonary inflammation markers, such as sputum eosinophilia and bronchial biopsies, making it a probable surrogate marker for eosinophilic inflammation in the lungs.^{3,4} Several studies have shown the efficacy of FeNO in diagnosing asthma with higher FeNO values in asthmatic patients.^{5,6} FeNO can also be used in the monitoring of asthma,⁷ allowing a reduction in asthma exacerbation rates and inhaled corticosteroid (ICS) dose.⁸ A recent study concerning difficult-to-treat asthma patients identified FeNO as a predictor of accelerated lung function decline, suggesting that FeNO measurement can be useful in clinical practice.⁹ Several studies

showed that the atopic status of the patient may play a role in the increased FeNO levels, which indicates that atopy may be a codeterminant in NO production.^{10–13} However, Berry *et al.* showed that atopy was not associated with sputum eosinophil count independent of FeNO level.¹⁴

To date, few studies have compared FeNO with reversible bronchoconstriction and bronchial hyperresponsiveness (BHR), current gold standards in diagnosing asthma.^{15,16} In addition, it is unclear whether FeNO can be used to discriminate allergic asthma from other atopic diseases, such as allergic rhinitis, atopic eczema, and urticaria. The aim of this study was to determine whether FeNO can be used as a screening method for the diagnosis of asthma in a population with a high prevalence of atopy.

MATERIALS AND METHODS

Patient Inclusion and Study Design

Eligible patients were all new patients who were referred to a general outpatient allergy clinic from January 2007 until September 2007. Patients using ICSs or oral corticosteroids within 6 weeks before the first visit were excluded from data analysis. All included patients were tested according to a standard diagnostic

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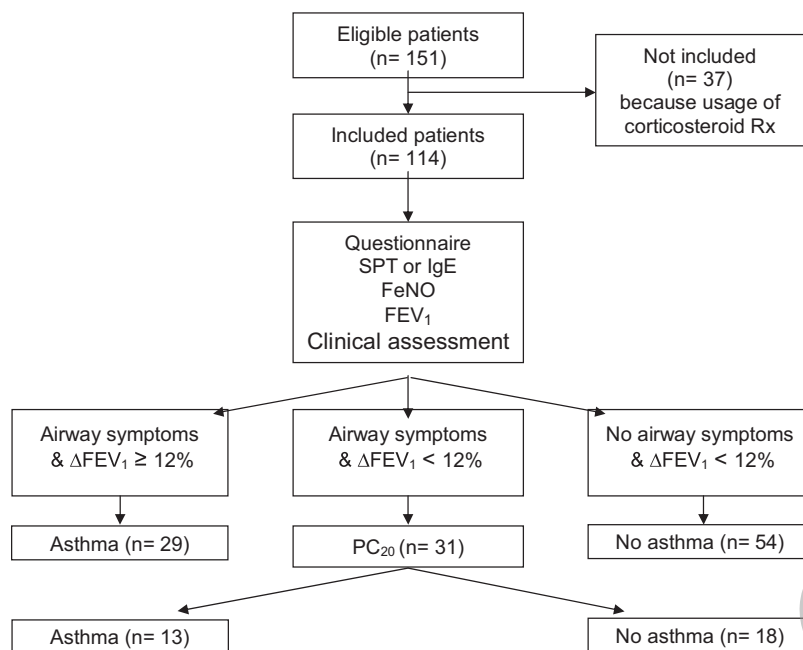


Figure 1. Flowchart asthma screening protocol. Diagnostic protocol for patients referred to the allergy clinic by a general practitioner. SPT, skin-prick test; FEV₁, forced expiratory volume in 1 second; FeNO, fractional exhaled nitric oxide; PC₂₀, provocative concentration of histamine causing a 20% decrease in FEV₁.

protocol (Fig. 1). The current study has an observational and cross-sectional design. It is based on analysis of a routine prospective database that was developed and maintained by the authors with approval of the Institutional Research Board. All patients received written information before the first visit and consented to the diagnostic protocol.

Clinical Parameters

All patients had to complete the standardized questionnaire at first visit and allergic symptoms were scored. Symptoms were divided into nasal and ocular complaints (rhinorrhea, watery eyes, nasal itching, sneezing, headache, facial pain, loss of smell, and nasal blockage), pulmonary complaints (wheezing, coughing, shortness of breath, and exercise intolerance), skin complaints (rash, pruritus, and urticaria), and general complaints (fatigue and nausea).

Atopic status was assessed with skin-prick test (SPT) or determination of specific plasma IgE in patients with eczema or other skin conditions. All subjects were tested for a panel of eight common inhalant allergens: house-dust mite; dog, cat, and horse dander; *Aspergillus fumigatus*; mugwort; and birch and grass pollen (Vivodiagnost; ALK Benelux BV, Groningen, The Netherlands). SPT cutaneous response was compared with a histamine-positive control and a saline solution-negative control. An SPT was considered positive when a wheal diameter of >3 mm was recorded after 15 minutes. IgE and specific plasma IgE were determined with a solid-phase two-step chemiluminescent immunoassay on the Immulite 2000 (Siemens, Los Angeles, CA). Eosinophilic leukocytes were determined

using a hematocytometer with VCS detection on the LH 750 analyzer (Bechman Coulter Co., Miami, FL).

FeNO was measured online at a constant flow rate of 50 mL/s and expressed as parts per billion in accordance with the guidelines of the American Thoracic Society and European Respiratory Society.² FeNO measurements were performed with the Niox-Flex (Aerocrine AB, Solna, Sweden). All FeNO tests were performed before spirometry and the provocative concentration of histamine causing a 20% decrease in forced expiratory volume in 1 second (FEV₁) (PC₂₀) test.

Lower airways obstruction was determined with FEV₁ measurement. FEV₁ was determined by standard spirometry (V_{max} SensorMedics Viasys, type 6200 Encore, 229/6200 Encore, 22 Encore; Cardinal Health, San Diego, CA) before and 15 minutes after inhalation of salbutamol, 400 µg. When the patients' history was suspect for asthma, a PC₂₀ histamine challenge was performed before the second outpatient visit within 6 weeks. In the inhalation challenge test, histamine was administered according to a standardized tidal breathing method (V_{max} SensorMedics, Viasys, as mentioned previously).¹⁷ Treatment with short-acting bronchodilators was allowed up to 8 hours and long-acting bronchodilators and antihistamines up to 48 hours before investigation.

The clinical assessment of the diagnosis of asthma was based on a history of typical respiratory symptoms and an FEV₁ improvement of >12% (of the percent predicted value) and >200 mL or PC₂₀ histamine of ≤8 mg/mL, according to the Global Initiative for Asthma guidelines.¹⁸ Diagnosis of allergic rhinitis and nonallergic rhinitis were based on criteria published in the

Table 1 Patient characteristics of the asthma group and nonasthma group

	Asthma Group (<i>n</i> = 42)	Nonasthma Group (<i>n</i> = 72)	<i>p</i> Value
Age, yr (range)	39 (7–83)	38 (7–87)	>0.05
Gender (% male)	14/42 (33)	29/72 (40)	>0.05
Pulmonary symptoms (%)	38/42 (90)	28/72 (39)	<0.001
Nasal symptoms (%)	39/42 (93)	39/72 (54)	0.02
Skin symptoms (%)	20/42 (48)	34/72 (47)	>0.05
Food symptoms (%)	11/42 (26)	13/72 (18)	>0.05
Atopic sensitization (%)	39/42 (93)	42/72 (58)	<0.001
Current smoking (%)	4/42 (10)	7/72 (10)	>0.05
Family history (%)	25/42 (60)	32/72 (44)	<0.001
Pets (%)	17/42 (40)	6/72 (8)	<0.001
Duration symptoms, yr (range)	6 (1–56)	5 (1–45)	>0.05
Eosinophils blood (%)*	3 (1–10)	3 (0–23)	>0.05
Total IgE, kU/L (range)*	239 (31–2000)	47 (6–2000)	<0.001
FeNO, ppb (range)	44 (6–290)	17 (5–45)	<0.001
Tiffeneau, % (range)	70 (42–95)	77 (69–95)	<0.01
PC ₂₀ , mg/mL (range)*	5 (1–8)	40 (15–40)	<0.001

Data expressed as number/total or median + range.

*Only in subset of patients, see results section and Fig. 1.

FeNO = fractionated exhaled nitric oxide; PC₂₀ = provocative concentration of histamine causing a 20% decrease in forced expiratory volume in 1 second.

Allergic Rhinitis and Its Impact on Asthma guidelines.¹⁹ Diagnosis of atopic eczema and urticaria were confirmed by an independent dermatologist.

Statistical Analysis

Data are presented as medians ± range and mean ± SD as indicated. Results are presented as box plots (with the box showing the median and interquartile range and the vertical bars the 5th and 95th percentiles). Comparisons between groups were tested using nonparametric analysis of variance (Kruskal-Wallis test, three groups or more) or the nonparametric Mann-Whitney *U* test (two groups). Data were correlated by the Spearman rank test. A value of *p* < 0.05 was considered significant.

Multivariate analysis was performed with (natural) log FeNO as dependent variable. Having a pet, pulmonary symptoms, BHR, indoor allergy, and total IgE were the independent variables. These variables had a significance level of at least *p* ≤ 0.1 in a univariate analyses. Variables were excluded using the enter method. All analyses were performed using SPSS Version 17 (SPSS, Chicago, IL). Data are expressed with the independent variable coefficient (β), the standard error (SE), and *p* value. The *R*² is a quantitative measure of how well the independent variables account for the outcome.

Receiver operating characteristic (ROC) analysis was performed to determine a threshold FEV₁ reversibility and FeNO for distinguishing asthma from nonasthma.

The accuracy of the test was quantified as the area under the curve (AUC 95% confidence interval) as well as the diagnostic accuracy (*i.e.*, proportion of correct test results). The level of the cutoff value of FeNO was determined by the maximized sum of sensitivity and specificity.

RESULTS

Subject Characteristics

A total of 151 eligible patients (aged 7–87 years) were referred to our allergy clinic. After excluding patients on ICSs and oral corticosteroids, 114 patients fulfilled the inclusion criteria. Forty-two subjects were diagnosed as asthmatic patients (bronchodilator reversibility [BDR], *n* = 29; and PC₂₀, *n* = 13) and 72 subjects were diagnosed as nonasthmatic patients. The two groups were comparable for sex, age, body mass index (BMI), duration of symptoms, vital capacity (VC), positive family history, and current smoking (Table 1). Atopic sensitization was determined by SPT (*n* = 49), radioallergosorbent test (*n* = 40), or by both (*n* = 25). Eighty-one patients appeared to be atopic, and 33 patients had no sensitizations. Total IgE was determined in 65 of the 114 patients (23 asthmatic and 42 nonasthmatic patients). By definition, asthmatic patients had a significantly decreased PC₂₀ (histamine), a lower FEV₁/VC ratio, and more pulmonary symptoms compared with non-

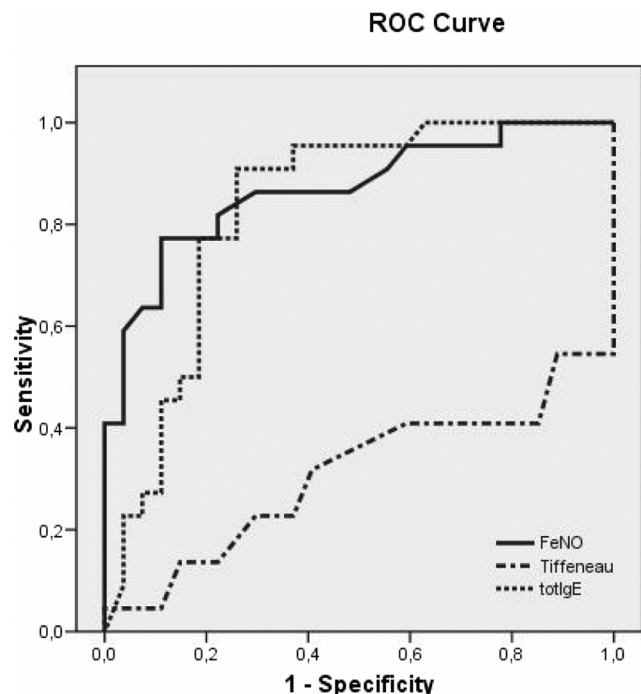


Figure 2. Receiver operating characteristic (ROC) curve for fractionated exhaled nitric oxide (FeNO) level, total IgE, and Tiffeneau (forced expiratory volume in 1 second/forced vital capacity [FEV₁/FVC]) determined for the diagnosis asthma.

asthmatic subjects. Asthmatic patients had significantly higher total plasma IgE (median, 239 kU/L versus 47 kU/L) and were more often atopic (93% versus 58%). They were also more likely to have a pet compared with nonasthmatic patients (40% versus 8%). The analysis also comprised three nonatopic asthma patients. These patients had a median FEV₁/VC ratio of 57% (range, 56–76%) with a nonsmoking history and confirmed reversibility. The average age was higher than the allergic asthmatic patients (63 ± 1 year), as was the IgE level (median, 540 kU/L; range, 31–1049 kU/L). It is not unlikely that they were allergic to other allergens that were not included in our allergy panel. The FeNO concentration was lower than in the allergic asthma patients (median, 18 ppb; range, 17–100 ppb), but this was not significant. The nonasthmatic group comprised the following subgroups: allergic rhinitis ($n = 32$), nonallergic rhinitis ($n = 11$), eczema ($n = 11$), urticaria ($n = 7$), and other ($n = 11$). Because the last subgroup was very heterogeneous and small per number of diagnosis, it was excluded from the subgroup analysis.

Exhaled Nitric Oxide

Asthmatic patients had a higher FeNO level than nonasthmatic subjects (44 ppb versus 17 ppb [mean]; $p < 0.001$). Figure 2 shows the ROC curve for FeNO and the diagnosis of asthma. The AUC was 0.88 (CI,

0.80–0.95). By ROC curve analysis, the optimal threshold FeNO concentration to distinguish asthma from nonasthma (highest sum of sensitivity and specificity) was 27 ppb. This cutoff point for FeNO had a sensitivity of 78% and specificity of 92% for a diagnosis of asthma. Also, FeNO (27 ppb) had a positive predictive value of 86% and a negative predictive value of 87% for a diagnosis of asthma. Diagnostic accuracy was 0.86.

In comparison, an FEV₁ reversibility of 12% had a specificity of 98% but only a sensitivity of 56%. The AUC was 0.77 (CI, 0.67–0.88). A total IgE of >136 kU/L had a sensitivity of 78% and a specificity of 86% with an AUC of 0.85 (CI, 0.75–0.95). On the other hand, a positive history of pulmonary symptoms had a sensitivity of 93% but only a specificity of 65% with an AUC of 0.79 (CI, 0.70–0.88). The Tiffeneau index (FEV₁/FVC) had an even lower AUC of 0.24 (CI, 0.14–0.35). The highest diagnostic accuracy (0.91) was achieved by the combination of FeNO (>27 ppb) and/or presence of BDR. The sensitivity of the combined tests is 87% and the specificity is 90%. Increased FeNO was positively correlated with the presence of respiratory symptoms ($p < 0.001$), airflow reversibility ($p < 0.001$), and total IgE ($p < 0.001$) and was negatively correlated with PC₂₀ histamine ($p = 0.019$). Also, the presence of nasal symptoms was weakly correlated with FeNO ($p = 0.046$). Our results revealed no correlation between FeNO and peripheral blood eosinophils, age, BMI, current smoking, or gender.

Subgroup Analysis

It has been suggested that atopy may be a codeterminant in NO production. A subgroup analysis was performed to see whether FeNO can be used to discriminate allergic asthma from other atopic diseases in our patient population. The asthmatic and allergic rhinitis patients were comparable for age, sex, BMI, current smoking, duration of symptoms, presence of atopy, and family history. Asthmatic patients, however, had a significantly higher total IgE (mean, 239 kU/L versus 46 kU/L) and mean FeNO value (44 ppb versus 21 ppb) when compared with allergic rhinitis patients ($p < 0.001$). The other atopic groups had significantly lower total IgE and FeNO value than asthmatic patients. Nevertheless, allergic rhinitis patients had increased total IgE (mean, 46 kU/L versus 18 kU/L) and FeNO value (21 ppb versus 12 ppb) when compared with nonallergic rhinitis patients ($p < 0.001$; Fig. 3).

Sensitization to indoor allergens resulted in a borderline significant increased mean FeNO compared with sensitization to outdoor allergens only ($p < 0.04$; Fig. 4). Patients with bronchial and nasal symptoms had increased FeNO and total IgE compared with pa-

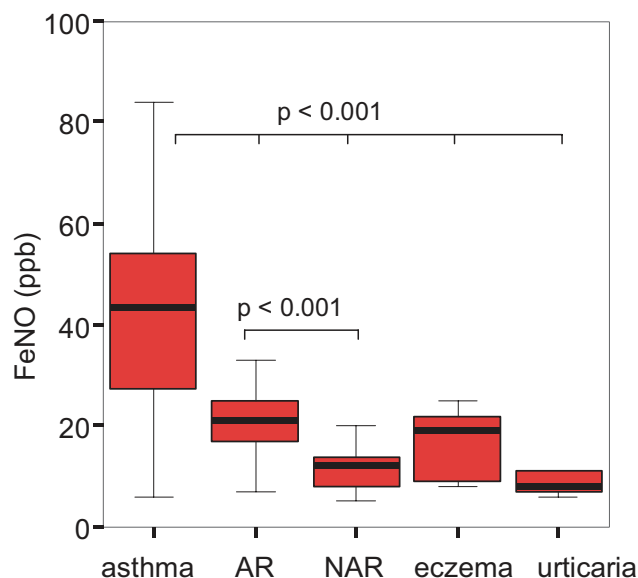


Figure 3. Fractionated exhaled nitric oxide (FeNO) level per diagnosis, (medians and interquartile ranges). Asthma group included patients with rhinitis and eczema. AR, allergic rhinitis; NAR, nonallergic rhinitis.

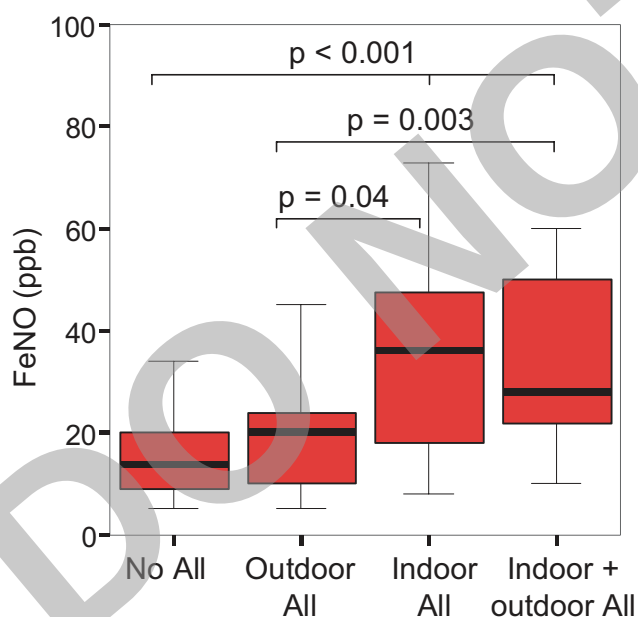


Figure 4. Fractionated exhaled nitric oxide (FeNO) level compared with sensitization to allergens. All, allergy.

tients with only nasal ($p < 0.001$ and $p < 0.001$, respectively) or only bronchial symptoms ($p = 0.02$ and $p = 0.04$, respectively).

Multivariate Analysis

We applied a multiple linear regression model to evaluate if any variables were predictors for an increased FeNO within the study population. The results are summarized in Table 2. In the total study

group ($n = 114$), we evaluated the following multiple linear regression model, with (natural) log FeNO as dependent variable, and pet, current pulmonary symptoms, BHR, indoor allergy, and total IgE as independent variables. The analysis showed that only having a pet adjusted for other covariables was a statistically significant predictor (using the enter method): $\beta = 0.47$ (SE, 0.23); $p = 0.04$. In the asthma group ($n = 42$) and nonasthma group ($n = 72$) we applied the same regression model as mentioned previously and analysis revealed that none of the covariables were statistically significant predictors for FeNO.

DISCUSSION

In a patient population group referred for analysis of symptoms and suspect for allergic disease we confirmed that FeNO measurement is a useful test to distinguish patients with allergic asthma from those with other atopic conditions. Asthmatic patients had a significantly higher FeNO level compared with the nonasthma group (allergic rhinitis, nonallergic rhinitis, atopic dermatitis, and urticaria). The high AUC of the FeNO ROC curve was comparable with that of the histamine challenge test and was even higher than the FEV₁ reversibility. Moreover, used in combination with BDR, the diagnostic accuracy is even higher, suggesting that both tests are partly complementary.

Several studies have shown higher FeNO concentrations in atopic asthma patients than in nonatopic asthmatic patients and nonatopic nonasthmatic subjects.^{10,11,20,21} Atopy is known to enhance FeNO levels even in the absence of allergic symptoms and is therefore considered a relevant confounding factor. In healthy, nonsmoking, adult individuals FeNO levels fall within the 4- to 20-ppb range, indicating that 20 ppb would be the ideal cutoff point between normal and abnormal FeNO. Our results show that FeNO is capable of distinguishing between atopic asthmatic, atopic, and nonatopic patients at a cutoff point of 27 ppb. The analysis also included three nonatopic asthma patients. The results of these three patients did not influence the study results.

According to the literature and also reflected by our data there is a correlation between FeNO levels and total IgE.²² Patients with both bronchial and nasal symptoms had increased FeNO and total IgE compared with patients with only nasal or only bronchial symptoms. In a previous study Braunstahl *et al.* have established that skin test reactivity (atopy score) was significantly related to PC₂₀ methacholine, indicating that allergy is a systemic disease with increased clinical expression (*i.e.*, lower airway involvement) in "severe" atopic subjects.²³ Our findings are in line with those of Gergen *et al.*²⁴ who

Table 2 Multivariate analysis identifying factors that may influence FeNO

	All Patients (n = 114)	Asthma (n = 42)	Nonasthma (n = 72)
Pet at home	$\beta = 0.47$ (SE, 0.23) $p = 0.04$	$\beta = 0.52$ (SE, 0.35) $p = 0.15$	$\beta = 0.40$ (SE, 0.32) $p = 0.29$
Current pulmonary symptoms	$\beta = 0.27$ (SE, 0.29) $p = 0.36$	$\beta = 0.35$ (SE, 0.64) $p = 0.60$	$\beta = 0.16$ (SE, 0.27) $p = 0.59$
BHR	$\beta = 0.31$ (SE, 0.29) $p = 0.28$	$\beta = -0.17$ (SE, 0.55) $p = 0.77$	$\beta = 0.45$ (SE, 0.25) $p = 0.14$
Atopy	$\beta = 0.27$ (SE, 0.23) $p = 0.28$	$\beta = -0.10$ (SE, 0.36) $p = 0.80$	$\beta = 0.28$ (SE, 0.28) $p = 0.38$
Total IgE	$\beta = 0.0004$ (SE, 0.0003) $p = 0.11$ $R^2 = 0.39$	$\beta = 0.0004$ (SE, 0.0003) $p = 0.21$ $R^2 = 0.22$	$\beta = 0.001$ (SE, 0.004) $p = 0.74$ $R^2 = 0.28$

Multivariate analysis was performed with (natural) log FeNO as dependent variable. Having a pet, lung symptoms, BHR, indoor allergy, and total IgE were the independent variables. These variables had a significance level of at least $p \leq 0.1$ in a univariate analyses. Variables were excluded using the enter method. Data are expressed with the independent variable coefficient (β), the standard error (SE) and P value. R^2 is a quantitative measure of how well the independent variables account for the outcome.

BHR = bronchial hyperresponsiveness; FeNO = fractionated exhaled nitric oxide.

showed that the prevalence of asthma and rhinitis is positively correlated with the number of positive SPT reactions to inhalant allergens.

Sensitization to indoor allergens (house-dust mite) resulted in a borderline significant increased FeNO when compared with sensitization to outdoor allergens. In contrast to previous studies, FeNO levels are higher in those sensitized to outdoor allergens (grass pollen).^{25,26} The period of inclusion has not influenced this result. Our patients were included from January to September, a period in which patients are exposed to indoor as well as outdoor allergens. Peripheral blood eosinophils are correlated with FeNO in children and in adult patients with asthma.²⁷ Our results showed no significant correlation between FeNO and blood eosinophils.

The use of ICS is reported to have a strong negative effect on FeNO concentration.^{28,29} Therefore, we excluded all patients who had current ICS therapy from our study. A limitation of the study may be the fact that we did not exclude patients who may have had a viral infection at the time of inclusion. As De Gouw *et al.*³⁰ indicated, FeNO levels are raised during rhinovirus infection and may have influenced our data. However, patients with possible viral infection would have been randomized and equally distributed among the groups. A second limitation concerns the selection bias, because all patients included in the study were referred to an allergy clinic meaning a high *a priori* chance for asthma. Therefore, we are cautious to extrapolate our results to the general pulmonary clinic, although we assume that

FeNO sensitivity will probably be higher. A third limitation is that not all patients underwent a PC₂₀ histamine challenge. Patients with normal lung function and no history of respiratory symptoms were not further evaluated for BHR. We emphasize that this was a real-life study and a positive test in these patients would not have clinical implications because BHR can also be present in normal individuals and those with allergic rhinitis. Crockcroft *et al.* found that the prevalence of BHR to histamine (PC₂₀ $\leq$ to 8 mg/mL) was 10.3% in the entire population. Moreover, there was a progressive increase from 6.1% in the nonatopic group to 33% in the markedly atopic group.³¹

Other factors have to be taken into account as well when interpreting FeNO values. Gender has been described to influence the value of FeNO with 25% lower concentration in female patients.³² In our study there was no difference in FeNO value and gender group. This is in concert with the results established by Olin *et al.*³³ Current smoking has a proven negative association with FeNO concentration as described by Kharitonov *et al.*³⁴ and Robbins *et al.*³⁵ We could not confirm this in our study, probably because of the small amount of smokers in our patient population (10% in the asthma and nonasthma group).

We performed a multivariate analysis to evaluate if any variables, *e.g.*, pulmonary symptoms, gender, age, total IgE, FEV₁, or allergy, were associated with increased FeNO values in our study population. When corrected for the variables there was no sig-

nificant predictor for FeNO in the asthmatic group or the nonasthma group. This suggests that FeNO has a discriminative value for asthmatic and allergic patients with atopy.

Having a pet at home is a significant predictor for increased FeNO when analyzing the entire study population. Almost all of the patients with a pet were asthmatic patients. These patients were probably triggered by animal allergens; hence, the advice for allergen avoidance measures in patients with asthma seems to be justified in this study.

FeNO measurement for asthma diagnosis and monitoring is still not widely implemented in clinical practice. This is probably because of the high costs of the measurement, the relatively low sensitivity of the test, and the conflicting results with regard to the usefulness of this test in guiding asthma therapy.³⁶ However, combining BDR and FeNO can further increase the diagnostic yield. Our study has established that FeNO measurement has a good diagnostic value in differentiating atopic asthma patients from other atopic conditions and can be used in a general allergy clinic.

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