Exhaled Nitric Oxide in a Population Sample of Adults

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Key Words

Exhaled nitric oxide · Asthma · Rhinitis · Breath analysis · Atopy

Abstract

Background: The relationship between exhaled nitric oxide (FENO) and the diagnosis of asthma in the general adult population is not completely clear. **Objectives:** To investigate the association between FENO and asthma, after controlling for atopy and rhinitis, in a general population sample of adults (mean age 43 years). **Methods:** The cohort of subjects was a sample of subjects who gave their consent to participate in the European Community Respiratory Health Survey II study. **Results:** Atopy, rhinitis and current asthma were positively and current smoking was negatively associated with FENO. Multivariate analysis showed that asthma had a significant predictive effect on FENO ($\beta = 0.53$; 95% CI 0.21–0.84, $p = 0.001$), and the increase in FENO was significantly associated with the risk of current asthma (OR = 1.07, 95% CI 1.00–1.14) by the logistic regression model. Receiver-operator characteristic curve showed that FENO ≥ 18.5 ppb had the best combination of sensitivity (69.2%) and specificity (71%), with a positive predictive value of 24% and a negative

predictive value of 95%, for the diagnosis of asthma. **Conclusions:** Measuring FENO seems to be suitable as an adjunct to questionnaire in the evaluation of asthma in the general population.

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Introduction

Airway inflammation is regarded as a characteristic feature of asthma, even in subjects with newly diagnosed mild disease [1]. Unfortunately, measurement of airway inflammation, particularly in epidemiological studies, is difficult to achieve. In population studies, asthma is defined as the presence of specific respiratory symptoms, alone or as symptoms combined with unspecific airway hyperresponsiveness. However, recent data suggest that airway hyperresponsiveness reflects the presence of remodelling of the airway wall [2], while its relationship with inflammatory cells, that is in induced sputum, is not consistent [3]. Exhaled nitric oxide (FENO) has been proposed as a non-invasive marker of airway inflammation in asthma [4]. FENO levels are increased in subjects with asthma [5–7], and decreased in subjects receiving inhaled

Fig. 1. Sampling patients starting from eligible population in the Turin ECRHS I.

corticosteroids [6, 8]. Measurement of FENO is feasible by a simple, rapid and non-invasive test. In recent years, many epidemiologic studies of asthma have included the measurement of FENO. There is now evidence that FENO levels are higher in atopic subjects [9–11] and even higher in sensitized subjects being exposed to relevant allergens [12, 13]. This may indicate that in atopic subjects the exposure is giving rise to airway inflammation and increased FENO. Measurement of FENO has been found to be a useful diagnostic tool for the screening of patients with a suspected diagnosis of asthma [14], with a high degree of discriminating power [15, 16] and greater diagnostic accuracy than conventional tests [17]. On the other hand, the relationship between FENO and the diagnosis of asthma in the general population is more complex and not fully elucidated. FENO was found to discriminate well subjects with asthma, who have higher levels than normal subjects [10]. In one study population, increased FENO was a very specific finding for allergic asthma only when combined with airway hyperresponsiveness [18]. In a more recent study [19], increased FENO levels were associated with a phenotype characterized by atopy and increased airway responsiveness, regardless of the presence of asthma or asthma-like symptoms.

The aim of this study was to investigate the relationship of FENO with asthma, rhinitis and atopy in a population sample of adults, in the frame of the European Community Respiratory Health Survey II (ECRHS II).

Methods

Sampling

For the purpose of the study the data of the ECRHS II were used. The ECRHS I protocol has been described elsewhere [20]. In each centre, at least 1,500 males and 1,500 females, aged 20–44 years, were randomly selected from the general population. Each participant was sent a brief questionnaire asking about respiratory symptoms (stage 1) and from those who responded, a 20% random sample and a 'symptomatic' sample (those who reported asthma-like symptoms in the short questionnaire) were selected to undergo a more detailed clinical examination (stage 2). The ECRHS II protocol is described in detail elsewhere [21]. In this study (stage 2 of ECRHS II), the cohort of subjects was defined as all subjects who had completed stage 1 of the ECRHS I in Turin and who, having been selected for stage 2, gave their consent to participate in the study and had FENO measured. Starting from a sample of 355 eligible subjects, 177 subjects gave their consent to participate in the present study and 109 had FENO measured (fig. 1). There were no differences in age, sex, and prevalence of atopy, asthma and rhinitis between the 109 subjects who had FENO measured and the 68 subjects who had not.

Questionnaire and Rhinitis and Asthma Definitions

All subjects completed a short, self-completed questionnaire identical to that used for stage 1 in the ECRHS I (see Appendix). They subsequently underwent an interviewer-administered questionnaire that covered medical history and respiratory symptoms, smoking status, occupation, medication and use of health services. Subjects were classified as having allergic rhinitis in the case of a positive response to the question 'Do you have any nasal allergies, including hay fever?' Subjects with 'current asthma' were defined as those reporting asthma in life and at least 1 of these asthma-like symptoms in the last 12 months: wheezing or whistling, tightness in chest, asthma attacks or treatment for medically diagnosed asthma.

Table 1. Demographic and clinical features of study population

	Rhinitis (n = 17)	Current asthma (n = 13)	Atopy ^a (n = 25)	Total (n = 109)
Male	8 (47.1%)	6 (46.2%)	16 (64.0%)	59 (54.1%)
Age, years	40.6 ± 1.5	43.6 ± 0.72	41.0 ± 1.35	43.3 ± 0.66
Current smokers	1 (5.8%)	3 (23.1%)	5 (20.0%)	29 (26.6%)
FEV ₁ , litres	3.16 (2.67–3.66)	2.76 (2.26–3.25)*	3.19 (2.86–3.52)	3.27 (3.11–3.43)
FEV ₁ predicted, %	96.6 (87.3–105.9)	87.4 (73.9–101)	95.6 (89.3–101.9)	102.0 (98.6–105.3)
FENO, ppb	26.6 (17.7–35.6)*	27.7 (18.7–36.7)*	21.9 (15.3–28.4)*	17.5 (15.4–19.6)

Figures in parentheses are 95% CI unless marked otherwise. * $p < 0.001$.

^a Measurements of serum-specific IgE were done in 103 subjects.

Lung Function Testing

Spirometric methods have been reported in detail elsewhere [22]. Briefly, baseline forced vital capacity and forced expiratory volume in 1 s (FEV₁) were measured in all subjects who agreed to these tests. All subjects went through at least 5 trials: all manoeuvres deemed technically satisfactory were recorded and the highest values for forced vital capacity and FEV₁ were compared with predicted values. Subjects with baseline FEV₁ <70% predicted were given 200 µg salbutamol by metered-dose inhaler through a spacer device, and the change in FEV₁ was measured after 20 min as a percentage of the baseline value.

Methacholine Challenge

Bronchial challenge with methacholine was administered to all subjects with baseline FEV₁ >70% predicted, who gave their consent to the test. Methacholine inhalation challenge methods have been described in detail elsewhere [22]. Methacholine was delivered via a Mefar dosimeter (Mefar, Bovezzo, Italy) using 2 methods of challenge (a long and short schedule of doses), depending on whether the subjects had reported respiratory symptoms or not. The test was stopped if the FEV₁ fell by 20% or more, or if the maximum cumulative dose of 2 mg was reached. Salbutamol aerosol was administered to aid recovery when necessary.

Specific Immunoglobulin E

Blood samples were collected for the measurement of serum-specific immunoglobulin E (IgE) to dust mite, cat dander, grass pollen and total IgE using the Pharmacia CAP system (Pharmacia Diagnostics, Uppsala, Sweden). Atopy was defined by specific IgE levels ≥ 0.70 kU/l.

NO Measurement

NO concentration was measured in exhaled air using the offline method. Exhaled air was collected before spirometry according to the American Thoracic Society recommendations [23], by using a pressure-regulated flow-restricted apparatus (Sievers, Boulder, Colo., USA). In brief, subjects without a nose clamp were asked to inhale to total lung capacity from a NO-free air source and, without a breath hold, to exhale to residual volume into a Mylar bag, maintaining a mouth pressure of 10 mm Hg. At this pressure the flow through the valve apparatus was 350 ml/s. The

NO levels were measured within 3 h of collection using a chemiluminescent analyzer (model 280; Sievers).

Statistical Analysis

The distribution of FENO concentrations was log-normally distributed, so that subsequent statistical analysis was carried out on log-transformed data.

Association between current asthma, rhinitis, atopy, FEV₁, bronchial hyperresponsiveness and log-transformed FENO values was determined first by using the Student *t* test and simple linear regression. Independent variables that were univariately associated with log-FENO values (significance level at least $p < 0.05$) were considered for the final linear regression model adjusting for smoking and use of corticosteroids in the last 3 months, 2 factors reported to decrease FENO [8, 24].

To evaluate the effect of FENO on asthma, rhinitis and atopy, logistic regression analysis was performed and associations were expressed as odds ratio (OR) with 95% confidence intervals (CI). Interaction between asthma and rhinitis was tested to evaluate the independent effect of FENO on the 2 conditions.

Finally, in order to determine the best cut-off level of FENO for the diagnosis of asthma, a receiver-operator characteristic (ROC) curve was plotted and the best combination of sensitivity and specificity of FENO for predicting increased probability of asthma in subjects was reported.

Results

FENO concentration was measured in 109 subjects (30 from a symptomatic sample and 79 from a random sample); all subjects also had a spirometry test, 103 of them had a blood sample for specific IgE, 88 a methacholine challenge and 12 had a bronchodilator test. Demographic and clinical characteristics of the subjects are reported in table 1. Twenty-five (24.3%) subjects were atopic, 8 of them (32%) sensitized to dust mite, 8 (32%) to cat dander, 2 (8%) to grass pollen, 4 (16%) both to dust mite and cat

Table 2. Univariate and multivariate linear regression analyses: association (β and 95% CI) between log-FENO level and clinical (atopy, rhinitis, asthma) and functional (FEV₁, bronchial hyperresponsiveness) variables

Log-FENO	Univariate analysis			Multivariate analysis		
	β	95% CI	p	β	95% CI	p
Atopy	0.26	0.09 to 0.51	0.04	0.17	-0.08 to 0.43	0.18
BHR	0.22	-0.07 to 0.50	0.13	-0.11	-0.46 to 0.24	0.52
Rhinitis	0.42	0.14 to 0.71	0.004	0.06	-0.30 to 0.43	0.73
Asthma	0.59	0.29 to 0.90	0.000	0.53	0.21 to 0.84	<0.001
FEV ₁ ^a	0.14	-0.80 to 1.07	0.77	0.32	-0.82 to 1.46	0.58
Current smoking	-0.36	-0.59 to -0.09	0.001	-0.33	-0.56 to -0.10	0.005

BHR = Bronchial hyperresponsiveness. ^a Standardized for age and height.

Table 3. Association (OR and 95% CI) between FENO levels and asthma, rhinitis and atopy: results of univariate and multivariate logistic regression analyses

	Current asthma		Rhinitis		Atopy	
	OR	95% CI	OR	95% CI	OR	95% CI
FENO _{crude}	1.08	1.03–1.14	1.06	1.02–1.11	1.05	1.01–1.09
FENO _{adjusted} ^a	1.07	1.00–1.14	1.01	0.96–1.07	1.04	0.98–1.09

^a OR adjusted for each symptom and smoking habit.

dander, 2 (8%) both to grass pollen and cat dander and 1 (4%) sensitized to all the allergens tested.

According to definitions, 13 subjects (11.9%) had current asthma, 17 (15.6%) had rhinitis and 7 subjects (6.4%) had both asthma and rhinitis. The prevalence of rhinitis in asthmatic patients was 53.8% (7/13). There were no significant differences in age, sex, atopy, race and smoking habits between asthmatic and non-asthmatic subjects. At the time of measurement, 3 asthmatic subjects had been using inhaled corticosteroids (fluticasone 250 μ g twice daily in 1 and budesonide 400 μ g twice daily in 2 subjects) in the last 3 months for asthma. The asthmatic subjects had significantly lower values of FEV₁ than the non-asthmatics (2.76 litres, 95% CI 2.26–3.25 versus 3.34 litres, 95% CI 3.17–3.51; $p < 0.001$).

Of the 109 subjects who had a spirometry test, 97 had an FEV₁ >70% and were asked to have a methacholine challenge; 9 subjects refused the test. Of the 88 subjects who had a bronchial challenge, 14 (15.9%) had a significant bronchial constriction (PD₂₀ FEV₁ <2 mg), 7 of them were asthmatic and 1 had non-allergic rhinitis.

Exhaled NO

FENO values were normally distributed after log transformation. FENO levels were significantly higher in atopic subjects (21.9 ppb, 95% CI 15.3–28.4) than in non-atopic subjects (15.5 ppb, 95% CI 13.6–17.3; $p < 0.05$), in subjects with rhinitis (26.6 ppb, 95% CI 17.7–35.6) than in those without rhinitis (15.8 ppb, 95% CI 13.9–17.6; $p < 0.01$) and in subjects with current asthma (27.7 ppb, 95% CI 18.7–36.7) than in non-asthmatic subjects (16.1 ppb, 95% CI 14.1–18.1; $p < 0.001$).

The results of linear regression analyses, either univariate or multivariate, adjusted for smoking and use of corticosteroids in the last 3 months, are reported in table 2.

Univariate analyses showed that current asthma predicts an increase in FENO levels of about 23% ($\beta = 0.59$ 95%; CI: 0.29–0.90), rhinitis of about 19% ($\beta = 0.42$; 95% CI 0.14–0.71) and atopy of 10% ($\beta = 0.26$; 95% CI 0.09–0.51).

Asthma only had a significant predictive effect on FENO levels ($\beta = 0.53$; 95% CI 0.21–0.84; $p = 0.001$), while atopy and rhinitis had no independent effect on FENO values. No significant association was found between

Fig. 2. ROC curve for the measurement of FENO in the diagnosis of asthma. Data labels feature different cut-off points of FENO levels.

FENO and FEV₁ or bronchial hyperresponsiveness. Current smoking was negatively and independently associated with FENO ($\beta = -0.33$; 95% CI -0.56 to -0.10).

The results of the logistic regression model (univariate and multivariate), which examined the associations between FENO levels and asthma, atopy and rhinitis, are reported in table 3. Univariate analyses showed significant associations between unitary increase in FENO levels and current asthma (OR = 1.08, 95% CI 1.03–1.14), rhinitis (OR = 1.06, 95% CI 1.02–1.11) and atopy (OR = 1.05, 95% CI 1.01–1.09). The multivariate logistic regression analysis showed that asthma was only significantly associated with the increase in FENO (OR = 1.07, 95% CI 1.00–1.14).

ROC Curve

ROC curve (fig. 2) showed that values of FENO ≥ 18.7 ppb had the best combination of sensitivity (69.2%) and specificity (71%), with a positive predictive value of 24% and a negative predictive value of 95% for the diagnosis of asthma.

Discussion

The study shows that FENO levels are increased in subjects (out of a general population random sample) who have a diagnosis of current asthma and are not independently associated with factors known to be risk fac-

tors of asthma, namely atopy and allergic rhinitis. Our results are in agreement with previous studies reporting elevated FENO in allergic asthma [25–27]. Atopy per se has been associated with high levels of FENO. Horvath et al. [9] found elevated levels of FENO among 15 asymptomatic atopic subjects compared with non-atopic controls and Salome et al. [10] found high FENO in atopic subjects compared with non-atopic subjects who were both asymptomatic and without airway hyperresponsiveness. According to a study on a selected population of pulp mill workers [13], it seems that only atopic subjects who had been recently exposed to the relevant allergen had elevated levels of FENO. Atopic subjects who were not exposed to a relevant allergen or had never experienced symptoms of asthma or rhinitis showed normal FENO. We also found increased levels of FENO in atopic subjects compared with non-atopic subjects, but in our population sample the effect of atopy on FENO was not independent. Nearly half our asthmatic subjects had allergic rhinitis, in agreement with the literature, which reports a prevalence of rhinitis in patients with asthma ranging from 50 to 80% [28, 29]. Previous observations have reported increased values of FENO in subjects with allergic rhinitis [30, 31]. We also observed higher values of FENO in subjects with rhinitis, but the effect of rhinitis on FENO was not independent from asthma in our population. The multivariate linear regression analysis showed that asthma only was predictive of increased FENO levels, supporting the view that FENO relates to

airway inflammation in atopic subjects [13]. Our results are different from those of Franklin et al. [19], who found that in adult subjects the elevated FENO values were associated with a phenotype characterized by atopy and increased airway responsiveness, measured as dose-response slope, regardless of the presence of asthma. Possible explanations for the different results may be the largely different prevalence of atopy, which was 67% in the study by Franklin et al. [19], compared to 23% in our study and, possibly, the different way bronchial responsiveness was measured in the 2 studies. In a recently published paper on a large (2,200 subjects) adult general population [32], atopy, in addition to current asthma, was positively and independently associated with FENO. It is possible that our finding that asthma only was predictive of increased FENO levels depends on a selection bias of the sub-sample of population we analysed in the present study. The prevalence of asthma (11.9%) was indeed quite higher than that observed in the larger sample of population analysed in stage 1 of the ECHRS II (3.2%) [33].

Considering the ROC curve, the cut-off point of 18.5 ppb was associated with the highest combination of specificity and sensitivity, which were both nearly 70%, values quite higher than those reported for post-bronchodilator response (increase in FEV₁ >12%) and peak expiratory flow variation (>20%), tests which have been used for detecting asthma, showing a sensitivity of 6 and 10%, respectively [34]. In studies based on population samples enrolled in a clinical setting, the sensitivity of testing FENO has been reported to be much higher in subjects with symptoms suggestive of asthma [14, 17]. In our general population sample, with a 11.9% prevalence of asthma, the high (95%) negative predictive value of the 18.5-ppb cut-off level of FENO appears quite interesting.

In summary, our results from a cross-sectional study of a population sample of adults indicate that current

asthma is associated with higher values of FENO and that asthma is predictive of increased values of FENO.

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Appendix

1. Have you had wheezing or whistling in your chest at any time in the last 12 months?
If 'NO' go to Question 2, if 'YES':
 - 1.1 Have you been at all breathless when that wheezing noise was present?
 - 1.2 Have you had this wheezing or whistling when you did not have a cold?
2. Have you been woken up with a feeling of tightness in your chest at any time in the last 12 months?
3. Have you been woken by an attack of shortness of breaths at any time in the last 12 months?
4. Have you been woken by an attack of coughing at any time in the last 12 months?
5. Have you had an attack of asthma in the last 12 months?
6. Are you currently taking any medicine (including inhalers, aerosols or tablets) for asthma?
7. Do you have any nasal allergies including hay fever?
8. What is your date of birth?
9. Are you male or female?

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