

Exhaled Nitric Oxide as Screening Tool in Subjects with Suspected Asthma without Reversibility

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Key Words

Asthma diagnosis · Exhaled nitric oxide · Methacholine bronchial challenge · Atopy · Smoking

Abstract

Background: As fractional exhaled nitric oxide (FeNO) has been evaluated only in certain settings for asthma diagnosis, we investigated whether FeNO values could predict positive methacholine challenge testing (expressed as PD₂₀) in subjects with suspected asthma but without spirometric reversibility. **Methods:** Subjects with asthma-like symptoms and negative bronchodilation test were initially evaluated to undergo FeNO measurement and methacholine bronchial challenge. Diagnostic performance of FeNO to predict PD₂₀ to methacholine <800 µg was examined by constructing receiver-operating characteristic curves. **Results:** A total of 112 subjects met the inclusion criteria. In all subjects, FeNO >32 ppb was associated with a sensitivity of 0.47 and a specificity of 0.85 for the identification of the PD₂₀ <800 µg (AUC = 0.691, 95% CI = 0.6–0.775, p = 0.00002). In smokers, FeNO >11 ppb was associated with a sensitivity of 0.85 and a specificity of 0.5 for the identification of PD₂₀ <800 µg (AUC = 0.625, 95% CI = 0.45–0.772, p = 0.18), while in atopics a FeNO level >26 ppb was associated with a sensitivity of 0.55 and a

specificity of 0.85 (AUC = 0.677, 95% CI = 0.53–0.8, p = 0.02).

Conclusions: In subjects with symptoms compatible with asthma but without spirometric reversibility, specific cutoff levels for FeNO levels significantly predict the positive methacholine challenge, with significant confounding factors being atopy and current smoking.

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Introduction

Asthma is a heterogeneous disorder with numerous presentations based on the pattern of symptoms, the triggering factors, the response to treatment and/or the level and type of inflammation [1]. In the diagnosis of asthma in patients without evidence of reversibility and with an intermediate probability of asthma, further investigations need to be made before commencing treatment. These further investigations are mainly concerned with the measurement of bronchial hyperresponsiveness (BHR) and secondly with the assessment of airway inflammation [2].

BHR is a characteristic functional abnormality in asthma. The most widely used method of measuring airway responsiveness relies on measuring response in

terms of change in forced expiratory volume in 1 s (FEV_1), a set time after inhalation of increasing concentrations of histamine or methacholine. In patients with normal or near-normal spirometric values, assessment of airway responsiveness is significantly better than other tests in discriminating patients with asthma from patients with conditions commonly confused with asthma [3]. Bronchial challenge tests present higher sensitivity and specificity compared to other conventional tests but they are time consuming, require experienced technicians and are usually available in specialized centers [4, 5].

The inflammatory component of the disease is considered an important one since it is one of the main features of asthma definition. The fraction of exhaled nitric oxide (FeNO) has been widely evaluated as a marker of eosinophilic airways inflammation, since it is readily measured, provides reproducible results, and is responsive to changes in inhaled corticosteroid doses [6]. Several studies have evaluated FeNO for the discrimination between patients with asthma and nonasthmatics with respiratory symptoms [7, 8], often reporting the superiority of FeNO compared to conventional approaches [9]. Exhaled nitric oxide performed similarly [8] or even better [10] than induced sputum eosinophils in certain settings for the diagnosis of asthma, and in the majority of cases it was clearly superior to simple spirometry and bronchodilator reversibility [9], with a diagnostic performance that was comparable to that of bronchial provocation tests in one study [11]. However, other studies have reported similar specificity but lower sensitivity of FeNO compared to methacholine and mannitol bronchial challenge tests [12]. In a recently published study the value of $FeNO_{50} > 34$ ppb had a high predictive value of $PC_{20} < 16$ mg/ml in patients with suspected asthma in whom a bronchodilating test failed to demonstrate reversibility or was not indicated [13]. Taking all these points together we wanted to determine whether FeNO values could predict positive methacholine challenge testing (expressed as PD_{20}) in subjects with suspected asthma without spirometric reversibility. Possible confounding factors such as smoking habit and atopic status were also assessed.

Methods

Study Subjects

A total of 250 subjects admitted to the outpatient clinics of 424 Army General Hospital and Attikon University Hospital were initially recruited. All subjects gave at least one positive answer for respiratory symptoms related to asthma in a screening

questionnaire based on the European Community Respiratory Health Survey [14]. Briefly, the questionnaire consists of 12 items. These items mainly consider symptoms like wheezing, coughing, tightness, shortness of breath, allergies, use of relief medications and, finally, history of possible asthma attacks. Symptoms were assessed for a 12-month period. The main complaints of our study participants were chronic cough, dyspnea on exertion, and wheezing. Subjects were excluded from the study if they had a previous diagnosis of asthma, were treated with asthma-related medication (inhaled steroids, long-acting β_2 -agonists, leukotriene receptor antagonists), had 12% reversibility after bronchodilation and 200 ml FEV_1 and, finally, had a respiratory infection within the past 8 weeks. Recent smoking quitters were also excluded. The study was approved by the ethics committees of both hospitals and all subjects provided an informed consent.

Measurement of FeNO

FeNO was measured using a portable nitric oxide analyzer (NIOX MINO, airway inflammation monitor; Aerocrine, Solna, Sweden) that provides measurements at 50 ml/s exhalation flow rate, expressed in parts per billion [15, 16]. In all the study subjects FeNO was measured in the morning between 08.00 and 10.00 a.m.

Lung Function

FEV_1 and forced vital capacity (FVC) were measured as components of flow volume using Master Screen Body (Jaeger; Viasys Healthcare, Hoechst, Germany) according to the American Thoracic Society guidelines [17].

Bronchial Hyperresponsiveness

BHR was performed and measured as PD_{20} of methacholine using a commercially available system (APS; Viasys Healthcare) according to the American Thoracic Society guidelines [5]. The test was considered positive with a value of $PD_{20} \leq 4$ μ mol (approximately 800 μ g according to the following equation: μ mol = μ g/MW with methacholine molecular weight of 195.69) [3].

Atopy Status

A positive skin prick test to any of six common aeroallergens (Dermatophagoides mix, cat epithelium, Parietaria, olive, Alternaria, cypress) was used to confirm atopy.

Study Design

On day 1, all initially recruited subjects underwent medical history and physical examination by an experienced pneumonologist, spirometry for measuring FEV_1 /FVC before and after bronchodilation, and BMI measurement. On the same day, subjects underwent FeNO measurement. The following day the eligible study subjects underwent BHR to methacholine. Skin prick tests were also performed within 15 days from the initial recruitment in the eligible study subjects.

Statistical Analysis

Normally distributed data were presented as mean $\pm$ standard deviation (SD), whereas skewed data were presented as median (interquartile ranges). Normality of distribution was checked with the Kolmogorov-Smirnov test. Diagnostic performance of FeNO to predict PD_{20} to methacholine < 800 μ g was examined by con-

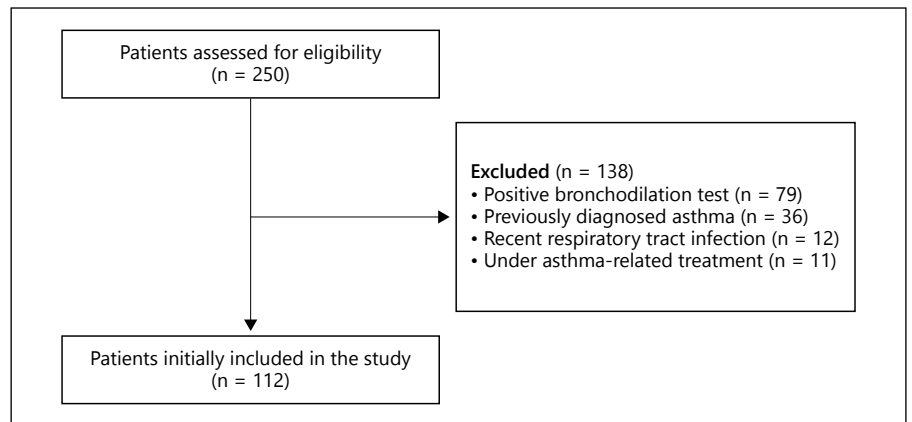


Fig. 1. Flow chart of patients included in the study.

Table 1. Characteristics of study subjects

Study variables	All (n = 112)	Smokers (n = 37)	Atopics (n = 51)
Age, years	25 (22–37)	27 (23–35)	25 (22–34)
Smoking/packs, years	ND	37/10±3	19/9±2
Gender (M/F), n	95/17	30/7	43/8
Body mass index	28±5	27±4	27±4
Atopy, n	51/112	19/37	ND
FEV ₁ (predicted), %	89 (83–99)	91±13	90±12
FEV ₁ /FVC, %	83 (78–87)	83±8	83 (77–86)
Reversibility after bronchodilation, %	5.8±2	6.3±1.5	6.2±3
Fractional exhaled nitric oxide, ppb	20.5 (12–34)	14 (9.5–21)	21 (15–41)
BHR PD ₂₀ , µg	1,320 (520–1,870)	1,230 (470–1,870)	780 (400–1,600)
Considered as positive, n	48/112	17/27	31/51

Normally distributed data are presented as mean ± SD, whereas skewed data are presented as median (interquartile ranges). The PD₂₀ µg was considered positive with a value of PD₂₀ ≤4 µmol (approx. 800 µg according to the following equation: µmol = µg/MW with methacholine molecular weight of 195.69) [3]. PD = Provocative dose; ND = not done.

structing receiver-operating characteristic (ROC) curves. The cut-off value of FeNO was selected according to appropriate statistical methods in order to reflect the optimal sensitivity and specificity of its levels. By considering the data as nonparametric, all simple correlations were performed with Spearman's test. A p value <0.05 was considered significant. Statistical analysis was performed using MedCalc (Mariakerke, Belgium) and GraphPad Prism 3.0 (La Jolla, Calif., USA).

Results

Figure 1 presents the flow chart of patients included in the study. Demographic characteristics of the 112 patients included in the final analysis are presented in table 1.

Associations between FeNO and BHR to Methacholine

There was a significant negative association between FeNO and PD₂₀ to methacholine in all study subjects ($r_s = -0.32$, $p = 0.01$). There was a significant negative association between FeNO and PD₂₀ to methacholine in smoking study subjects ($r_s = -0.27$, $p = 0.03$). Finally, there was a significant negative association between FeNO and PD₂₀ to methacholine in atopic study subjects ($r_s = -0.34$, $p = 0.01$).

ROC Analysis

The diagnostic performance of FeNO in the entire study population as well as in smokers and atopics is presented in table 2. Diagnostic performance was evaluated by using different cutoff points in the range of 10–30 ppb.

Table 2. Diagnostic performance of FeNO in the entire population, smokers and atopics, using different cutoff points

Cutoff points	All		Smokers		Atopics	
	sensitivity	specificity	sensitivity	specificity	sensitivity	specificity
>30 ppb	49 (34–64)	82 (71–90)	12 (2–37)	95 (75–99)	48 (30–67)	85 (62–97)
>25 ppb	51 (36–66)	75 (63–85)	18 (4–43)	90 (68–99)	55 (36–73)	80 (56–94)
>20 ppb	64 (49–78)	60 (47–72)	29 (10–56)	75 (51–92)	68 (49–83)	60 (36–81)
>15 ppb	73 (58–85)	49 (37–62)	53 (28–77)	65 (41–84)	77 (59–90)	45 (23–68)
>10 ppb	81 (64–91)	39 (29–50)	84 (57–97)	55 (32–77)	90 (74–98)	10 (2–32)

Values in parentheses are 95% CI.

Briefly, in the entire population FeNO values >30 ppb are characterized by a specificity of 82% for the identification of $PD_{20} < 800 \mu g$. Similar results were obtained in atopics with a specificity of 85%. In smokers values >30 ppb presented with a specificity of 95% while values >10 ppb showed a sensitivity of 84%.

Group ROC Curve Analysis

All

A FeNO level >32 ppb was associated with a sensitivity of 0.47 and a specificity of 0.85 for the identification of $PD_{20} < 800 \mu g$ (AUC = 0.691, 95% CI = 0.6–0.775, $p = 0.00002$; fig. 2).

Smokers

A FeNO level >11 ppb was associated with a sensitivity of 0.85 and a specificity of 0.5 for the identification of $PD_{20} < 800 \mu g$ (AUC = 0.625, 95% CI = 0.45–0.772, $p = 0.18$; fig. 3).

Atopics

A FeNO level >26 ppb was associated with a sensitivity of 0.55 and a specificity of 0.85 for the identification of $PD_{20} < 800 \mu g$ (AUC = 0.677, 95% CI = 0.53–0.8, $p = 0.02$; fig. 4).

Discussion

In the present study it was shown that FeNO >32 ppb had the higher sensitivity and specificity to identify bronchial hyperresponsiveness to methacholine in patients with asthma-like symptoms but with normal lung function or no evidence of airflow variability according to negative bronchodilation test. This value was lower when atopic status was taken into account and even lower still when patients were current smokers.

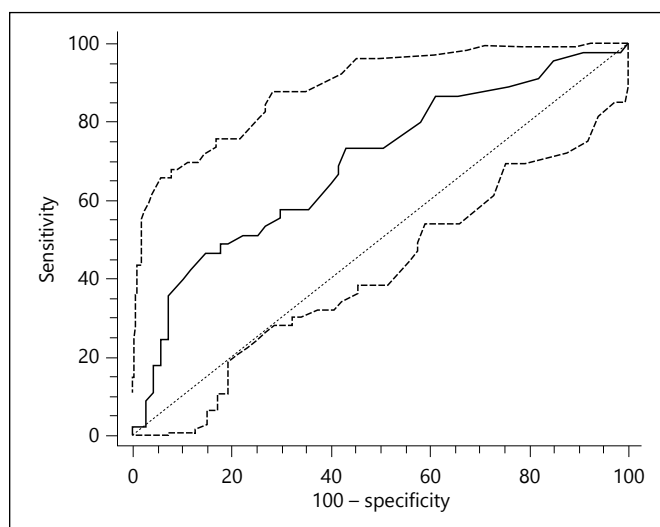


Fig. 2. ROC curve for the evaluation of the prognostic value of FeNO for methacholine $PD_{20} < 800 \mu g$ in all study subjects ($n = 112$). FeNO level >32 ppb was associated with a sensitivity of 0.47 and a specificity of 0.85 for the identification of $PD_{20} < 800 \mu g$ (AUC = 0.691, 95% CI = 0.6–0.775, $p = 0.00002$).

Bronchial hyperresponsiveness and airway inflammation are key, albeit independent, features of asthma [18, 19]. Diagnosis of asthma is based on compatible symptoms along with measurements of lung function, providing an assessment of the severity of airway limitation and its reversibility and variability [2, 3]. In patients with a normal or near-normal spirometry in whom asthma diagnosis remains uncertain, it is more useful to look for evidence of airway hyperresponsiveness and/or airway inflammation [3, 9, 20].

FeNO measurements have been used as a diagnostic tool for asthma in several studies, with promising results in most of them. However, among most of the studies there were major differences regarding the population se-

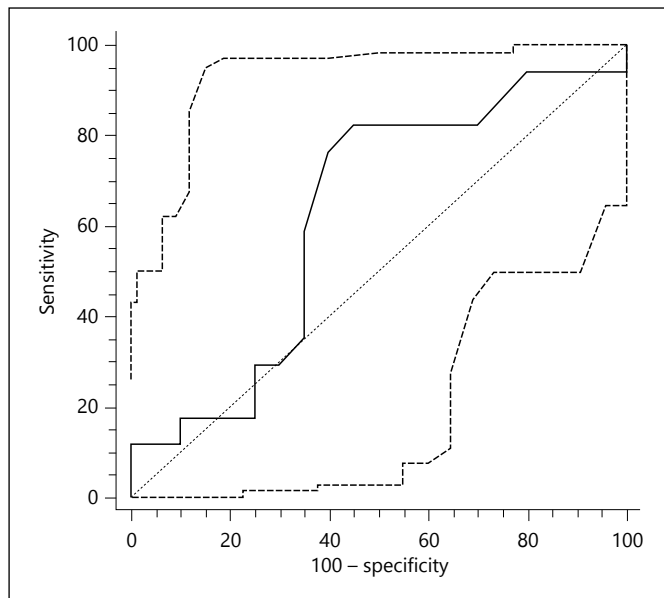


Fig. 3. ROC curve for the evaluation of the prognostic value of FeNO for methacholine $PD_{20} < 800 \mu g$ in smoking subjects ($n = 37$). FeNO level > 11 ppb was associated with a sensitivity of 0.85 and a specificity of 0.5 for the identification of $PD_{20} < 800 \mu g$ (AUC = 0.625, 95% CI = 0.45–0.772, $p = 0.18$).

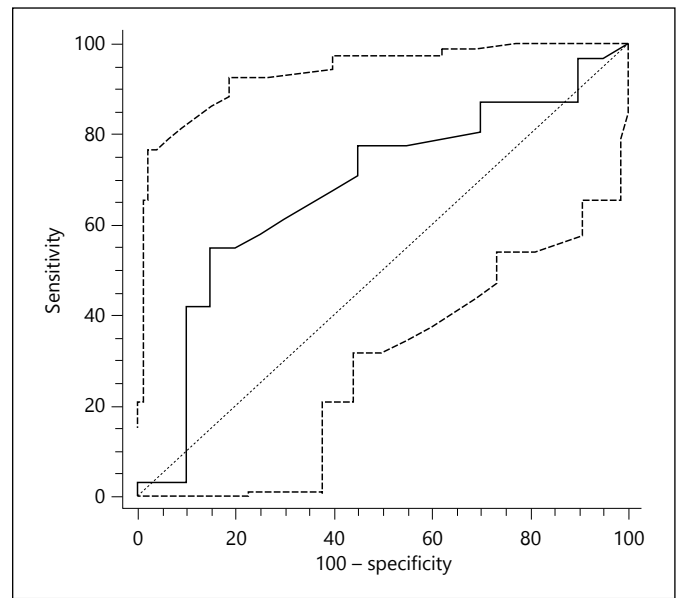


Fig. 4. ROC curve for the evaluation of the prognostic value of FeNO for methacholine $PD_{20} < 800 \mu g$ in atopic subjects ($n = 51$). FeNO level > 26 ppb was associated with a sensitivity of 0.55 and a specificity of 0.85 for the identification of $PD_{20} < 800 \mu g$ (AUC = 0.677, 95% CI = 0.53–0.8, $p = 0.02$).

lected, the percentage of atopic patients and the inclusion or exclusion of smokers. Our study is differentiated from others as the population consisted of subjects with suspected asthma, who were steroid naïve and without evidence of spirometric reversibility, in whom a methacholine challenge was performed in order to confirm or exclude the diagnosis of asthma. Our results are in accordance with a recent study of Schleich et al. [13] in which a similar population was studied. They found that the value of $FeNO_{50} > 34$ ppb had a high predictive value of $PC_{20} < 16$ mg/ml in patients with suspected asthma in whom a bronchodilating test failed to demonstrate reversibility or was not indicated. Their population also included atopic patients and smokers; however no cutoff values of FeNO regarding atopics and smokers were evaluated.

It is well known that FeNO is increased in steroid-naïve patients with asthma [21, 22] and is associated with airway eosinophilia [23]. Moreover, the relevance of bronchial challenge tests to the degree of inflammation is not always consistent, although they represent reliable tools for airway hyperresponsiveness [24]. FeNO has been studied as a screening tool for asthma both in adults [7, 10, 11] and children [25] and as a helpful tool to identify patients with bronchial hyper-

responsiveness to histamine or reversibility to β_2 -agonist among those presenting with chronic respiratory symptoms and normal baseline lung function [13, 23].

Sensitivity and specificity of FeNO measurements in previous studies appear to be acceptable for discriminating asthma from nonasthma [9, 21, 26]. However, it is important to remember that low FeNO values do not exclude the diagnosis of asthma because FeNO levels are normal, especially in nonatopic subjects [27]. On the other hand, atopy influences the levels of FeNO [28] and this was the case in our study as almost 50% of the subjects were atopics and FeNO values > 26 ppb had similar specificity and AUC for positive methacholine challenge to that for the whole population. In the study of Smith et al. [9] a higher percentage of atopic subjects were included, whereas their proportion was not mentioned in the study of Dupont et al. [21].

FeNO levels have been found to be lower in smokers compared to nonsmokers [29] but higher in smokers with asthma than in healthy smokers [30]. However, in previous studies [9, 21] smokers were excluded. In our study, although asthmatic smokers had lower FeNO levels, a value > 11 ppb still had a satisfactory specificity with increased sensitivity and an AUC of 0.625.

We also found a weak although significant negative association between FeNO and PD₂₀ to methacholine in all study subjects, as well as in atopics and smokers. However, the relationship between FeNO and bronchial hyperresponsiveness is controversial and conflicting results have been published [4, 23, 31]. Although FeNO and BHR reflect different parameters in asthma, a functional relationship between these two variables cannot be excluded. It is thought that part of the BHR in asthma is linked to airway eosinophilic inflammation that can be attenuated by corticosteroids [32]. Furthermore, nitric oxide may itself contribute to BHR by increasing airway edema as it is a potent vasodilator responsible for plasma exudation from bronchial vessels, and the transformation of nitric oxide in peroxynitrite was shown to induce airway hyperresponsiveness in guinea pigs [33]. This may be a plausible explanation of the good specificity of FeNO to detect methacholine responsiveness even though increased FeNO may be observed in other pathological conditions too. Finally, the FeNO level >32 ppb that we found to be

associated with positive methacholine challenge is within the range of 25–50 ppb proposed by recent guidelines for supporting a diagnosis of asthma [34].

One possible limitation for the current study might be the argument that it would be more appropriate to study the predictive value of FeNO against the final diagnosis of asthma compared to the methacholine challenge predicting asthma in the same population of patients. We believe that if we used the term ‘final diagnosis of asthma’ without a subjective test already used for asthma diagnosis then we might provide data with significant biases. Following the rules of asthma diagnosis in a specialized center, we decided to use methacholine challenge as the next clinical assessment step after a negative reversibility test.

In conclusion, in subjects with symptoms compatible with asthma but without spirometric reversibility, specific cutoff levels of FeNO significantly predict a positive methacholine challenge, with significant confounding factors being atopy and current smoking.

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