

Exhaled Nitric Oxide May Predict Bronchial Hyperreactivity in Patients with Allergic Rhinitis

Ignazio Cirillo^a Fabio Luigi Massimo Ricciardolo^b Gianmarco Medusei^a
Alessio Signori^c Giorgio Ciprandi^d

^aNavy Medical Service, La Spezia, ^bDivision of Respiratory Disease, Department of Clinical and Biological Sciences, University of Torino, Torino, ^cHealth Science Department, Genoa University, and ^dIRCCS-Azienda Ospedaliera Universitaria San Martino, Genoa, Italy

Key Words

Allergic rhinitis • Bronchial hyperreactivity • FEF_{25–75} • Nitric oxide

Abstract

Background: Nowadays it is possible to assess airway inflammation by measuring the fractional concentration of exhaled nitric oxide (FeNO) during an office visit and there is international consensus on the testing methodology. The aim of this study was to evaluate whether FeNO measurement may be a predictor of bronchial hyperreactivity (BHR) in patients with allergic rhinitis (AR). **Methods:** Two hundred eleven patients (196 males, median age 28.5 years) suffering from persistent AR were evaluated. Values for bronchial function (FVC, FEV₁, and FEF_{25–75}), bronchial provocation tests (methacholine), exhaled nitric oxide (FeNO), a visual analogue scale for nasal and bronchial symptoms, and sensitization were assessed. **Results:** A strong and inverse correlation between FeNO levels and BHR severity was found ($r = -0.58$). FeNO was a predictive factor for BHR, and 37 ppb was found to be the best cutoff (area under the curve 0.90) to define the presence of BHR in patients with AR. **Conclusions:** This study highlights the relevance of FeNO as a possible predictive marker for BHR in AR patients and underlines the

close link between upper and lower airways. Thus, FeNO measurement could be a useful screening tool in identifying subjects with rhinitis at risk of developing asthma.

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Introduction

Allergic rhinitis (AR) is characterized by mucosal inflammation and typical nasal symptoms [1]. AR is frequently associated with asthma, which is in turn characterized by airway inflammation and bronchial hyperreactivity (BHR) [1, 2]. In this regard, many AR patients may have BHR [3]; this condition may mean bronchial involvement and may also suggest a possible evolution in asthma [4]: the so-called ‘asthma march’ [5]. Therefore, the assessment of BHR in a patient with AR may have a relevant prognostic role. BHR is usually investigated via bronchial provocation testing using a variety of stimuli, such as exercise, methacholine (MCH), histamine, or adenosine 5′-monophosphate [6]. MCH challenge has become popular in clinical practice even though it is time-consuming and costly and bears a small risk of inducing severe bronchospasm.

On the other hand, correct assessment of bronchial airway inflammation requires invasive methods (bronchial biopsy and/or lavage) or sputum analysis, both of which are complex and require adequate structures. It is now possible to assess airway inflammation by measuring the fractional concentration of exhaled nitric oxide (FeNO) during an office visit and there is international consensus on the testing methodology [7]. The FeNO measurement is highly reproducible, responsive to changes in the underlying disease state, and predictive of the response to therapeutic intervention with anti-inflammatory medications [8]. Recent studies have highlighted that the FeNO level is highly correlated with the degree of BHR, bronchodilator reversibility, and atopy [9, 10]. Thus, FeNO measurement can be used as a safe, simple, and rapid test and has been demonstrated to be as good as the bronchial provocation test in adult patients with asthma [11–13]. Recently, it was also reported that FeNO levels were strongly and negatively associated with BHR in children with asthma and AR [14]. Therefore, the aim of this study was to evaluate whether FeNO measurement may correlate with BHR also in adult patients with persistent AR.

Materials and Methods

Patients

This cross-sectional study included 211 patients (196 males, mean age 28.5 years), suffering from persistent AR. They were navy soldiers who were referred to the Navy Medical Service for mandatory certification of their health status. The visit included: clinical examination, visual analogue scale (VAS) assessment, a skin prick test, spirometry, and MCH challenge. The visits were performed during the spring immediately before the beginning of the training courses. The Navy Review Board approved the study procedure and written informed consent was obtained from each subject.

Study Design and Setting

A detailed clinical history was taken and a complete physical examination was performed. The patients were enrolled into the study on the basis of a diagnosis of persistent AR made based on concordance between a positive skin prick test and the presence of nasal symptoms after exposure to sensitizing allergen, according to validated criteria [1]. Exclusion criteria were: any prior documented history of asthma or referral for asthma symptoms, including cough, wheezing, dyspnea, and shortness of breath, impaired FEV₁ values (e.g. <80% of the predicted value) and an FEV₁/FVC ratio <0.7, presence of acute (in the last 4 weeks) or chronic upper respiratory infections, anatomical nasal disorders (i.e. nasal polyps, clinically relevant septum deviation, etc.), previous or current intensive smoking such as more than 20 cigarettes a day (screened by expired-CO assessment, e.g. analyzing carboxyhemoglobin and carbon monoxide levels in a single breath using the

Bedfont Micro Smokerlyzer III from Bedfont Scientific Ltd. & De-code, UK), previous or current specific immunotherapy, and use of nasal or oral corticosteroids, nasal or oral vasoconstrictors, antileukotrienes, and antihistamines during the previous 4 weeks. Subjects under drug treatment or with acute upper respiratory airway infection returned after an adequate time.

Age, gender, smoking, duration of rhinitis, rhino-VAS and bronchial VAS, FVC, FEF_{25–75}, FEV₁ at baseline, FeNO, and MCH PC₂₀ were registered for all patients in the analysis.

VAS Assessment

A VAS was used to assess the subjective feeling of both nasal and bronchial respiration. The VAS consisted of an evaluation of symptoms. A rhino-VAS was used to measure the subjective perception of nasal respiration: it ranged from 0 (complete nose obstruction) to 10 cm (complete nose patency). A bronchial VAS was used to measure the subjective perception of lung respiration. Patients indicated their actual perception of breathlessness by marking a VAS. It was hypothesized that the patient's breath perception, determined by their VAS score, could correlate with the degree of bronchial obstruction, as measured by the FEV₁. In this study, the bronchial VAS was a 10-cm vertical line on which 0 implied breathlessness while 10 corresponded to no breathlessness. Initially, patients were instructed to place a mark on the line indicating their ease of breathing at that moment. It was explained that 0 represented breathlessness and 10 no problem breathing. Thus, the lower the numerical score marked by the patient, the greater their perceived breathlessness. With a movable marker, the subject could mark any point on the 10-cm segment which best described his/her perception. No interval marker was visible on the line [15].

Skin Prick Test

It was performed as stated by the European Academy of Allergy and Clinical Immunology [16]. The panel consisted of: house dust mites (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), cat, dog, grasses mix, Compositae mix, *Parietaria officinalis*, birch, hazel, olive tree, *Alternaria tenuis*, *Cladosporium*, and aspergilli mix (Stallergenes, Milan, Italy).

Spirometry

Spirometry was performed using a computer-assisted spirometer (Pulmolab 435-spiro 235; Morgan, UK – predictive values ECCS 1993), with an optoelectronic whirl flow meter. This spirometer fulfills the American Thoracic Society/European Respiration Society (ATS/ERS) standards according to guidelines [17]. It was performed as stated by the ERS [17, 18].

MCH Bronchial Challenge

MCH bronchial challenge was performed in order to evaluate BHR only if basal the FEV₁ was equal to or more than 80% of the predicted value. The aerosol was delivered using a dosimetric computerized supply (MEFAR MB3; Marcos, Italy). The test was performed following the ATS guidelines for MCH challenge [6]. The threshold concentration causing a 20% fall in the FEV₁ (PC₂₀) was calculated.

Degree of BHR Severity

Three categories of BHR were considered on the basis of PC₂₀ as follows: severe, PC₂₀ <1 mg/ml; mild, PC₂₀ between 1 and 4 mg/

Table 1. Demographic and spirometric characteristics of patients

Characteristics	
Age, years	28.5 ± 5.83/27 (19–55)
Gender	
Females	15 (7.1)
Males	196 (92.9)
Smoking	
No	165 (78.2)
Ex-smokers	0
Up to 10 cigarettes/day	19 (9)
Ten or more cigarettes/day	27 (12.8)
Duration of rhinitis, years	4 (0–30)
Rhino-VAS	
1	75 (35.54)
2	52 (24.65)
3	36 (17.06)
4 or more	48 (22.75)
Bronchial VAS	
0	175 (82.94)
1 or more	36 (17.06)
FVC (% predicted)	105.59 ± 10.13/106 (78–131)
FEV ₁ (% predicted)	107.52 ± 11.44/108 (82–132)
FEF _{25–75} (% predicted)	107.20 ± 26.41/107 (49–174)
FeNO	21 (1–256)
BHR	1,383.70 ± 479.73/1,590 (10–1,590)
Categorized BHR	
Negative	168 (79.6)
Borderline	17 (8.1)
Mild	17 (8.1)
Severe	9 (4.3)

Data are expressed as mean ± SD, median (range), and n (%), as appropriate.

ml, and borderline, PC₂₀ ranging from 4 to 16 mg/ml, according to the criteria of the ATS guidelines for MCH challenge [6]. Subjects with no response to the cumulative dose of 16 mg/ml were considered to have normal bronchial responsiveness.

Nitric Oxide Measurement

FeNO was measured with a chemiluminescence analyzer (Quark NObreath; COSMED s.r.l., Italy); the detection limit of the apparatus was 1–5 ppb, as required by ATS guidelines, with a resolution of 1 ppb. The analyzer was calibrated daily using a certified nitric oxide (NO) mixture. Exhaled NO was recorded with the single-breath method according to published guidelines [7]. Subjects inhaled to total lung capacity from NO-free air and exhaled a single breath (without a nose clip) through a mouthpiece at a mouth pressure of 45 cm of water and at an expiratory flow of 50 ml/s. The mouth pressure was displayed on a computer screen in order for the patients to maintain a steady flow. The measurement was rejected if a stable flow was not maintained for at least 6 s of exhalation. NO was measured at the plateau and expressed in ppb.

Statistical Analysis

Values were expressed as means and SD or medians and ranges for continuous variables and numbers and percentages for categorical variables.

Since FeNO had a nonsymmetric distribution, Spearman's correlation coefficient was used to evaluate the association between FeNO and BHR, rhino-VAS and bronchial VAS. These two later characteristics were considered ordinal variables. Pearson's correlation was instead used for all other characteristics. Partial correlation between FeNO and BHR with adjustment for smoke status was also performed. Classical Bonferroni's correction for multiple comparisons was also made.

FEF_{25–75} was further categorized as ≤65% of the predicted value (abnormal values) and >65% (normal values), while BHR was categorized as 'negative' (>the threshold of 15.9 mg/ml) or 'positive' (<15.9 mg/ml). The nonparametric Mann-Whitney test was used to assess differences in gender, smoking, categorical FEF_{25–75}, and categorical BHR on FeNO.

A logistic regression model was used to assess the association between BHR, considered a binary (positive/negative) dependent variable, and FeNO considering also other significant demographic and clinical parameters, such as age, gender, smoking status, and duration of rhinitis. OR for the effect of FeNO on BHR and relative 95% CI are shown.

Moreover, an ROC curve to find the best cutoff of FeNO able to discriminate patients with positive BHR from others was created. The area under the curve (AUC) and its 95% CI were used to evaluate the goodness of the test. $p = 0.05$ was considered statistically significant and SPSS version 18 (IBM Corp., USA) was used for analysis.

Results

In table 1, the characteristics of patients are shown. Most of the patients were males (92.9%) and nonsmokers (only 21.8% were current smokers, the mean was 6 packs per year, there were no ex-smokers, and all smokers stated that they never smoked), with a median duration of rhinitis of 4 years. About a quarter of the patients perceived annoying nasal symptoms, whereas bronchial symptoms were actually almost absent.

Regarding spirometric parameters, all values were within normal reference ranges; the median value of FeNO was 21, while 168 patients (79.6%) were found to be negative for BHR.

There was a negative strong correlation between FeNO and BHR ($r = -0.58$; $p < 0.001$) (table 2; fig. 1). Negative and significant correlations, even if not particularly relevant, were found between FeNO and FEF_{25–75} ($r = -0.23$), FVC ($r = -0.16$), and FEV₁ ($r = -0.22$). Further, controlling for smoking status, the partial correlation between FeNO and BHR was not different from the unadjusted correlation ($r = -0.66$; $p < 0.001$).

Table 2. Coefficients of correlation between demographic, clinical, and spirometric features

		FeNO	FEF _{25–75}	FEV ₁	FVC	Rhino-VAS	Bronchial VAS	BHR	Age	Duration of rhinitis
FeNO	r		–0.23	–0.22	–0.16	0.18	0.38	–0.58	–0.004	0.17
	Sign.		0.001	0.001	0.019	0.007	<0.001	<0.001	0.93	0.011
FEF _{25–75}	r			0.67	0.20	–0.19	–0.33	0.38	–0.14	–0.25
	Sign.			<0.001	0.004	0.007	<0.001	<0.001	0.045	<0.001
FEV ₁	r				0.79	–0.11	–0.28	0.26	–0.17	–0.29
	Sign.				<0.001	0.10	<0.001	<0.001	0.015	<0.001
FVC	r					0.08	–0.09	0.12	0.002	–0.19
	Sign.					0.24	0.20	0.072	0.98	0.006
Rhino-VAS	r						0.35	–0.29	0.10	0.59
	Sign.						<0.001	<0.001	0.15	<0.001
Bronchial VAS	r							–0.32	0.14	0.35
	Sign.							<0.001	0.04	<0.001
BHR	r								–0.022	–0.23
	Sign.								0.75	0.001
Age	r									0.39
	Sign.									<0.001

r = Coefficient of correlation; Sign. = level of significance ($p < 0.05$ was considered statistically significant).

Positive and significant correlations were found between FeNO and both rhino-VAS ($r = 0.18$) and bronchial VAS ($r = 0.38$) and between FeNO and duration of rhinitis ($r = 0.17$). In addition, there was an inverse relationship between bronchial VAS and BHR severity ($r = -0.32$).

No differences regarding FeNO were found between males (median 20; IQR 14–36) and females (median 25; IQR 12–64) ($p = 0.22$), even though there was only a small group of females, or between smokers and nonsmoker patients ($p = 0.20$).

Patients with BHR had a median FeNO value of 57 (range 14–256); this result was significantly different ($p < 0.001$) from that of BHR-negative patients, who showed a lower median value (median 18; range 1–92). Similarly, patients with abnormal FEF_{25–75} values had significantly ($p = 0.02$) higher values of FeNO (median 40; range 14–256) compared with patients with normal FEF_{25–75} values (median 20; range 1–220).

An increase in the probability of being BHR positive was found for an increase of one unit in the FeNO scale [OR (1 unit increase) = 1.11; 95% CI 1.07–1.13; $p < 0.001$]. No confounding effect of age, smoking status, duration of rhinitis, or other clinical characteristics on the relation between FeNO and BHR was highlighted after adjustment for these features in a multivariate model.

From the ROC curve, an AUC of 0.90 (95% CI 0.85–0.96; $p < 0.001$) for FeNO as a test to separate patients negative for BHR from patients positive for BHR (fig. 2) was found. The best cutoff value concerning FeNO was 37; 79.1% (sensitivity) of patients with higher values were positive for BHR, while 90.5% (specificity) of patients with lower values were negative for BHR.

Discussion

AR is a strong risk factor for both onset and worsening of asthma [19], and BHR is a reliable marker of asthma evolution in AR patients [3]. FeNO may be considered a surrogate marker of airway inflammation [10].

The present study provides a series of interesting findings. First, there was a strong inverse relationship between FeNO levels and MCH PC₂₀, e.g. a high intensity of bronchial inflammation, detected by high FeNO values, corresponded to severe BHR as documented by a low PC₂₀. It is important to note that these findings were reported in AR patients.

Second, patients with impaired FEF_{25–75} values, which is considered a reliable marker of early bronchial involvement in AR [20], had higher FeNO levels in comparison

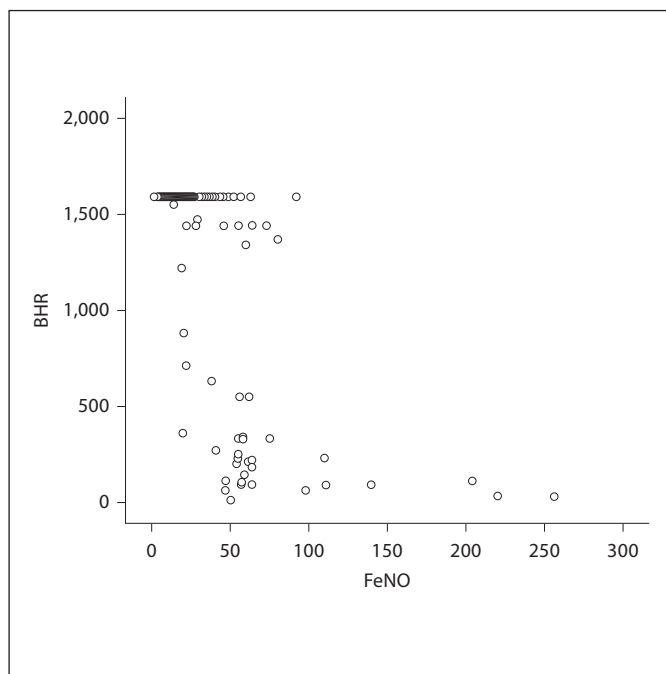


Fig. 1. Scatter plot of FeNO values and BHR degree, expressed as PC₂₀.

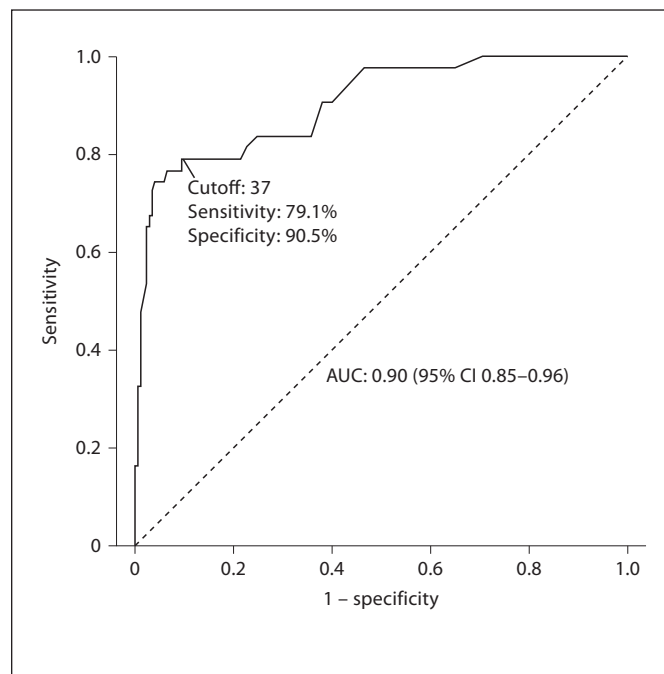


Fig. 2. ROC curve for FeNO levels as a predictor of BHR.

to patients with normal FEF_{25–75} values. This finding underlines the concept that initial bronchial airflow limitation, detected by impaired FEF_{25–75}, is related to bronchial inflammation. In addition, there was an inverse relationship between FEF_{25–75} values and FeNO levels; this phenomenon further confirms the relationship between airflow limitation and inflammation. Furthermore, there was a significant relationship between respiratory symptom severity and more objective parameters, such as FeNO and BHR. However, these correlations were weak, and this might depend on the fact that patients suffered from rhinitis.

The main finding of this study was the definition of a cutoff value for FeNO able to discriminate AR patients with BHR. In fact, an FeNO level ≥ 37 ppb may be considered a reliable marker for BHR presence in AR patients. An AUC of 0.90, a sensitivity of 79.1, and a specificity of 90.5 confirm the validity of the result.

Moreover, the present study confirms a previous one conducted in allergic children with asthma and AR [14]. That study reported that the predictive model established a cutoff value of 32 ppb of FeNO as a predictor of BHR. Both studies confirm previous research and underline the role of measurement of FeNO as a surrogate marker for the BHR test. Furthermore, this study reinforces the

role of FeNO as diagnostic marker for bronchial impairment in allergic patients.

The present study also confirms the results of similar studies. A study was conducted to investigate whether patients with AR and asthma differed from AR patients with or without BHR in terms of the degree of perception of dyspnea and airway inflammation, measured as fractionated exhaled NO [21]. This study concluded that FeNO measurement was useful to assess the inflammatory activity in peripheral airways. Another study aimed to determine the discriminative value of FeNO for asthma and other atopic conditions in the general allergy clinic [22]. Increased FeNO was positively correlated with the presence of respiratory symptoms, airflow reversibility, and total IgE, and it was negatively correlated with PC₂₀ histamine. Thus, these authors concluded that measuring FeNO is a simple and useful test to differentiate AR candidates who will become asthmatics from those with other atopic conditions in a general allergy clinic. Tossa et al. [23] investigated a large group of apprentices. The increase in FeNO was associated with the incidence of BHR (odds ratio 2.00) per unit increase in log parts per billion. Thus, those authors concluded that FeNO measurement is a simple and reproducible test useful in the screening of BHR in workers newly exposed to agents

causing occupational asthma. Keen et al. [24] reported that dysfunction of small conducting airways was associated with airway inflammation assessed by FeNO and BHR in pediatric asthma. Finally, Skiepkio et al. [25] performed a study similar to the present one, but theirs was on a smaller sample (22 patients) and it was restricted to grass allergy. The increase in BHR was negatively correlated with increased FeNO during pollen season. Those authors concluded that FeNO could act as a prognostic marker for identifying patients with asthma risk.

On the other hand, some studies have provided conflicting results. A pediatric study reported that levels of exhaled NO, measured in 32 steroid-naïve atopic children with mild intermittent asthma, were not accurate predictors of BHR degree [26]. Quaedly et al. [27] evaluated a broad spectrum of asthmatics encountered in clinical practice. They reported that BHR and sputum eosinophilia, but not FeNO, were associated with uncontrolled asthma. Another study evaluated the type of infiltrating inflammatory cells [28]. A relationship between BHR and FeNO was not observed in noneosinophilic asthma phenotypes. A further study compared mannitol with MCH challenge [29]. BHR to mannitol was more specific than MCH in the diagnosis of asthma and was more closely associated with ongoing airway inflammation in terms of increased FeNO levels. Finally, a study comparing adenosine with MCH challenge showed that these two stimuli did not agree, and a higher fraction of exhaled NO values

and sputum eosinophil counts were seen in patients with positive adenosine challenge results [30]. Possible reasons for these conflicting findings might be the different phenotypes of asthma (mainly concerning the inflammatory cell types), the characteristic of studied patients in terms of the duration of allergic disorders, exposure to allergens, severity and control of asthma, and intensity of treatment, and the different methodologies used in the studies.

The clinical relevance of the present study may be based on the adequate number of enrolled patients. However, a possible limitation of this study is the fact that there was a selection bias: subjects were navy recruits and mainly male. Therefore, the external validity of the study results is limited.

The main importance of the present study is that it confirms the close relationship between FeNO and BHR also in AR patients, and FeNO measurement could be a useful screening tool to help the allergist to identify AR patients at risk of developing asthma.

In conclusion, this study highlights the relevance of FeNO as a marker for BHR in AR patients and underlines the close link between upper and lower airways.

Disclosure Statement

The authors state that there is no conflict of interest and there was no sponsorship.

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