

Fractional Exhaled Nitric Oxide: A Potential Biomarker in Allergic Rhinitis?

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Keywords

Allergic rhinitis · Exhaled nitric oxide · Lung function ·
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Abstract

Background: The fractional concentration of exhaled nitric oxide (FeNO) is a surrogate biomarker for Th2-dependent bronchial inflammation. The present study investigated whether FeNO may characterize allergic rhinitis (AR) patients. **Methods:** A total of 553 AR patients (497 males, mean age 28.8 years) were evaluated. Those patients with a high FeNO underwent a 2-year follow-up. **Results:** Increased FeNO was associated with a significantly longer AR duration, impaired lung function, more severe symptoms, and more frequent bronchial hyperresponsiveness (BHR). At follow-up, 22 out of 82 patients (26.8%) with high FeNO levels (>50 ppb) developed asthma. **Conclusions:** AR patients may frequently have high FeNO values, exceeding 50 ppb. This might be associated with an initial impaired lung function, BHR, a perceived worsening of respiratory symptoms, and potential progression to asthma.

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Introduction

Allergic rhinitis (AR) is a relevant risk factor for asthma onset and worsening, as the 2 conditions share some pathophysiological mechanisms [1, 2]. In particular, AR is characterized by a Th2-driven inflammation with prevalent eosinophilic infiltrate [3]. Also, allergic asthma is characterized by mucosal eosinophilic inflammation, bronchial hyperresponsiveness (BHR) to different stimuli, and variable and reversible bronchial obstruction [4]. Hence, airways eosinophilic inflammation represents a common pathogenic pathway in both allergic disorders. Bronchial eosinophilic inflammation may simply be assessed by a surrogate marker, such as the fractional concentration of exhaled nitric oxide (FeNO), during a visit to the clinical practice [5]. NO production is regulated by synthase enzymes, which catalyze the conversion of L-arginine to L-citrulline to generate NO. These enzymes exist in 3 distinct isoforms: endothelial, inducible (iNOS), and neuronal. Recent evidence showed that in allergic asthmatics the upregulation of iNOS in the respiratory epithelium occurs via STAT-6 and the proinflammatory Th2-cytokines interleukin IL-4 and IL-13, with the subsequent production of enhanced NO concentrations in exhaled air [6]. Although FeNO has long been regarded as a surrogate marker of eosinophilic airway inflammation, it is more ac-

The work was conducted at the Navy Medical Service of La Spezia.

curate to consider that FeNO is representative of a Th2-driven local inflammation, specifically of the bronchial mucosa, rather than general eosinophilic inflammation, as measured by blood or induced sputum. FeNO levels correlate better with bronchial eosinophils than with sputum eosinophils [7]. Other data highlight the disconnect between FeNO and eosinophilic inflammation, as suggested by studies with monoclonal antibodies against IL-5 and IL-13. Treatment with mepolizumab, an anti-IL-5 mAb, significantly reduces blood and sputum eosinophils without affecting FeNO levels [8]. In contrast, treatment with lebrikizumab, an anti-IL-13 mAb, significantly reduced FeNO levels without reducing blood eosinophils [9]. Thus, FeNO assessment should be carefully interpreted in the clinical practice [10].

Moreover, AR may precede asthma and thus it should be desirable to have biomarkers potentially able to predict progression to asthma in AR patients. In this regard, it has been suggested that AR patients with BHR may develop successive asthma inception: the so-called asthma march [11]. In addition, AR patients may frequently present impaired lung function, mainly concerning forced expiratory flow at 25–75% of the vital capacity (FEF_{25–75}). It has been proposed that an FEF_{25–75} value <65% predicted may be a marker of early bronchial involvement [12]. It has also been reported that AR children with high FeNO levels develop asthma later [13]. Therefore, we tested the hypothesis that FeNO may be a biomarker in AR patients able to define those with bronchial involvement and the potential of further progression to overt asthma. The aim of this study was to evaluate whether FeNO may characterize patients with AR suggesting a bronchial involvement.

Materials and Methods

Patients

This study included 553 patients (497 males, mean age 28.8 years) suffering from AR. They were Navy soldiers who were referred to the Navy Medical Service for mandatory certification of their physical suitability for specific military training.

The subjects were enrolled in the study on the basis of a diagnosis of AR based on consistency between a positive skin prick test and the occurrence of nasal symptoms after exposure to a sensitizing allergen [1]. The exclusion criteria were: any prior documented history of asthma or referral for asthma symptoms (including cough, wheezing, breathlessness, chest tightness), impaired FEV₁ values (such as <80% of the predicted value) and an FEV₁/FVC ratio <0.7, a positive response to bronchodilation testing, the presence of acute or chronic upper respiratory infections, nasal polyps, clinically relevant septal deviation, previous or current intensive smoking, such as more 10 cigarettes per day, previous or current specific immuno-

therapy, relevant ingestion of nitrate-rich food, and use of nasal or oral corticosteroids, nasal or oral vasoconstrictors, antileukotrienes, and antihistamines during the previous 4 weeks. The subjects with acute upper respiratory airway infection returned after 4 weeks. The Navy Review Board approved the study procedure and written informed consent was obtained from each subject.

Study Design

The baseline visit included: patient history (including AR duration), a clinical examination, visual analogue scale (VAS) assessment of nasal obstruction and breathlessness perception, a skin prick test, nasal rhinoscopy, a lung function assessment (including FVC, FEV₁, FEF_{25–75}, and FEV₁/FVC ratio), FeNO measurement, and methacholine bronchial challenge. The visits were scheduled during the first months of 2013. On the basis of a previous study demonstrating that children with AR and high FeNO values developed asthma after 5 years, we reevaluated the AR patients with high FeNO values after 2 years in order to identify subjects who developed asthma. In this regard, the diagnosis of asthma was based on both asthma symptoms history (cough, dyspnea, wheezing, and chest tightness) and positive bronchodilation testing (increase of FEV₁ values >12% and >200 mL after the inhalation of 400 µg of salbutamol).

VAS Assessment

VAS was used to assess the subjective perception of both nasal and bronchial respiration. The perception of nasal obstruction was measured by rhino-VAS (R-VAS), ranging from 0 (complete nose patency) to 10 cm (complete nose obstruction). Bronchial-VAS (B-VAS) was used to measure the subjective perception of breathlessness, ranging from 0 (completely normal breathing) to 10 cm (severe dyspnea). Patients were asked to position a cross on a line corresponding to their own perception of respiration as previously reported [14].

Skin Prick Test

The skin prick tests were performed according to the European Academy of Allergy and Clinical Immunology guidelines [15]. The panel consisted of: house dust mites (*Dermatophagoides farinae* and *D. pteronyssinus*), cat, dog, a grasses mix (including *Dactylis glomerata*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis*, and *Anthoxanthum odoratum*), a Compositae mix, *Parietaria officinalis*, birch, hazel, olive tree, *Alternaria tenuis*, *Cladosporium*, an *Aspergilli* mix, including positive (histamine) and negative (saline solution) and a control (Stallergenes, Milan, Italy).

Spirometry

Spirometry was performed as outlined by the European Respiratory Society (ERS) [16, 17] using a computer-assisted spirometer (HDPft-2000, nSpire Health, Hertford, UK; predictive value ECCS 1993), with an optoelectronic whirl flow meter. Quality control was conducted daily to achieve a grade A (2 acceptable FEV₁ values within 0.1 L of each other).

Methacholine Bronchial Challenge

Chloride methacholine 1% (Lofarma, Milan, Italy) was used for the bronchial challenge. The test was performed only if basal FEV₁ was equal to or more than 80% of predicted and the basal FEV₁/FVC ratio was equal or more than 0.7, according to internal rules. The dilution schedule was based on American Thoracic Society (ATS) guidelines for methacholine challenge, using a progressive dilution

from 100 mg in 6.25 mL (16 mg/mL) to 0.031 mg/mL [18]. Aerosol was delivered using a dosimetric computerized supply (MEFAR MB3, Marcos, Italy) by a DeVilbiss nebulizer (model 646). The nebulizer output for the MCH test was 0.13 mL/min, $\pm$ 10%.

The testing procedure was performed following the ATS guidelines [18]. The threshold concentration causing a 20% fall of FEV₁ (PC₂₀) was calculated, which indicated the end of testing.

NO Measurement

FeNO was measured with a chemiluminescence analyzer (Eco Medics CLD88 sp, Duernten, Switzerland) before spirometry; the detection limit of the apparatus was 1–5 parts per billion (ppb), as required by ATS guidelines, with a resolution of 1 ppb. The analyzer was calibrated daily using a certified NO mixture. Exhaled NO was recorded with the single-breath method according to published guidelines [5]. 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 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. An FeNO value >25 ppb was considered to be increased according to validated guidelines [5].

Statistical Analysis

Continuous variables are presented as the mean with standard deviation (SD) and categorical variables as the number of subjects and percentage values. The FeNO value was categorized as low (<25 ppb), intermediate ($\geq$ 25 but $\leq$ 50 ppb), and high (>50 ppb). A univariate multinomial logistic regression model was performed to test the effect of the clinical and demographic variables on the FeNO. The odds ratios (OR) associated with FeNO were calculated with their 95% confidence intervals (CI) for each factor from the multinomial logistic model. The likelihood ratio was used as a test of statistical significance and the estimated *p* values were adjusted for multiple comparisons by the Bonferroni correction method. Variables with a *p* value <0.20 were entered into the multivariate analysis, where FeNO was the dependent variable. Multivariate analysis was performed again using the multinomial logistic regression model and the model selection was made using the Akaike information criterion. Differences with a *p* value <0.05 were selected as significant in all of the comparisons. Data were acquired and analyzed in the R v3.3.1 software environment [19].

Results

A total of 553 patients were enrolled in the study. The demographic and clinical characteristics of the participants are summarized in Table 1. Briefly, the mean age was 28.82 years (SD 6.48), the mean AR duration was 5.88 years, the mean R-VAS was 2.6 cm (SD 1.77) and B-VAS was 0.61 cm (SD 1.53). The mean FVC was 105.3% predicted, the mean FEV₁ was 104% predicted, the mean FEV₁/FVC ratio was 0.83, and the mean FEF_{25–75} was 98.3% predicted. Regarding smoking, 80.5% of subjects

Table 1. Demographic and clinical characteristics of the study participants (*n* = 553)

Variable	
FeNO, <i>n</i>	
Low	327 (59.13%)
Intermediate	144 (26.04%)
High	82 (14.83%)
Age, years	28.82 (6.48)
Gender, <i>n</i>	
Male	497 (89.87%)
Female	56 (10.13%)
AR duration, years	5.88 (5.55)
R-VAS, cm	2.60 (1.77)
B-VAS, cm	0.61 (1.53)
FVC, % predicted	105.30 (11.38)
FEV ₁ , % predicted	104.00 (12.24)
FEV ₁ /FVC	83.01 (6.25)
FEF _{25–75} , % predicted	98.28 (27.32)
Smoking, <i>n</i>	
No	445 (80.47%)
Yes	108 (19.53%)
Sensitizations, <i>n</i>	
Only 1	147 (26.58%)
>1	406 (73.42%)
BHR, <i>n</i>	
Negative	405 (73.24%)
Positive	148 (26.76%)
Data are expressed as mean (SD) or <i>n</i> (%).	

were nonsmokers. About 75% of the patients were polysensitized and 73% were negative to methacholine challenge. Categorizing FeNO, it was observed that 327 (59.13%), 144 (26.04%), and 82 (14.83%) patients had low, intermediate, and high FeNO values, respectively.

The descriptive statistics of age, gender, AR duration, R-VAS, B-VAS, FVC, FEV₁, FEV₁/FVC, FEF_{25–75}, smoking habit, sensitization, and BHR, categorized according to FeNO values, are reported in Table 2. In particular, subjects with high FeNO values showed a longer mean AR duration (7.98 years, SD 7.25) than patients with intermediate and low FeNO values (6.35 years, SD 5.90, and 5.14 years, SD 4.69, respectively). The perception of both nasal and bronchial symptoms was higher in patients with a high FeNO. The mean FEF_{25–75} and FEV₁/FVC values were lower in patients with a high FeNO than in patients with intermediate and low FeNO levels [mean (SD): 84.35 (26.16) vs. 92.49 (26.88) and 104.32 (26.01) for FEF_{25–75}; 81.54 (6.36) vs. 81.56 (6.11) and 84.02 (6.1) for FEV₁/FVC, respectively]. In contrast, mean FEV₁ values were similar among low, intermediate, and high

Table 2. Contingency tables and univariate multinomial regression results

Variable	FeNO category, ppb			Univariate analysis			
	low (<25)	intermediate (25 ≤ FeNO ≤ 50)	high (>50)	<i>n</i>	intermediate vs. low FeNO, OR (95% CI)	High vs. low FeNO, OR (95% CI)	<i>p</i> value
Age ^a , years	28.12 (5.9)	29.31 (6.77)	30.77 (7.67)	553	1.03 (1–1.06)	1.06 (1.02–1.1)	0.0352
Gender, <i>n</i>				553			1.0000
Male	295 (59.36%)	131 (26.36%)	71 (14.29%)		1	1	
Female	32 (57.14%)	13 (23.21%)	11 (19.64%)		0.91 (0.47–1.8)	1.43 (0.69–2.97)	
AR duration ^a , years	5.14 (4.69)	6.35 (5.9)	7.98 (7.25)	553	1.04 (1.01–1.08)	1.09 (1.04–1.13)	0.0020
R-VAS ^a , cm	2.34 (1.64)	2.76 (1.89)	3.34 (1.85)	553	1.16 (1.03–1.30)	1.34 (1.18–1.52)	0.0003
B-VAS ^a , cm	0.35 (1.26)	0.67 (1.43)	1.5 (2.21)	553	1.21 (1.04–1.41)	1.49 (1.28–1.72)	<0.0001
FVC, % predicted	105.48 (11.64)	104.57 (11.08)	105.8 (10.93)	553	0.99 (0.98–1.01)	1 (0.98–1.02)	1.0000
FEV ₁ ^a , % predicted	105.79 (11.99)	101.44 (12.08)	101.09 (12.32)	553	0.97 (0.95–0.99)	0.97 (0.95–0.99)	0.0013
FEV ₁ /FVC ^a	84.02 (6.10)	81.56 (6.11)	81.54 (6.36)	553	0.94 (0.91–0.97)	0.94 (0.9–0.97)	0.0003
FEF _{25–75} ^a , % predicted	104.32 (26.01)	92.49 (26.88)	84.35 (26.16)	553	0.98 (0.98–0.99)	0.97 (0.96–0.98)	<0.0001
Smoking ^a , <i>n</i>				553			0.0703
No	250 (56.18%)	121 (27.19%)	74 (16.63%)		1	1	
Yes	77 (71.3%)	23 (21.3%)	8 (7.41%)		0.62 (0.37–1.03)	0.35 (0.16–0.76)	
Sensitizations, <i>n</i>				553			1.0000
Only 1	93 (63.27%)	37 (25.17%)	17 (11.56%)		1	1	
>1	234 (57.64%)	107 (26.35%)	65 (16.01%)		1.15 (0.74–1.79)	1.52 (0.85–2.73)	
BHR ^a , <i>n</i>				553			<0.0001
Negative	309 (76.3%)	88 (21.73%)	8 (1.98%)		1	1	
Positive	18 (12.16%)	56 (37.84%)	74 (50%)		10.92 (6.11–19.54)	158.79 (66.49–379.25)	

Data are expressed as mean (SD) or *n* (%). OR, odds ratio; CI, confidence interval. *p* values were adjusted using the Bonferroni method.

^a Variables included in the multivariate analysis (see the text for abbreviations and further details).

FeNO patients [105.79 (11.99), 101.44 (12.08), and 101.09 (12.32), respectively].

The univariate multinomial logistic regression analysis (Table 2), using the complete set of data, demonstrated a significant association among age, AR duration, R-VAS, B-VAS, FEV₁, FEV₁/FVC, FEF_{25–75}, BHR, and FeNO (*p* = 0.0352, 0.0020, 0.0003, <0.0001, 0.0013, 0.0003, <0.0001, and <0.0001, respectively), as reported in Figure 1. In particular, a 1-unit increase in AR duration, R-VAS or B-VAS, was associated with an increased probability of 4, 16, and 21% (OR 1.04, 1.16, and 1.21, respectively) of having an intermediate FeNO value. The probability of having a high FeNO value was also positively associated with age, AR duration, R-VAS, and B-VAS. Moreover, it was also observed that a 1-unit increase in FEV₁, FEV₁/FVC, and FEF_{25–75} was correlated with a 3, 6, and 2% (OR 0.97, 0.94, and 0.98) and 3, 6, and 3% (OR 0.97, 0.94, and 0.97) decreased chance of having intermediate and high FeNO values, respectively. In addition, the chances of having intermediate and high FeNO values in BHR-positive patients were about 11 and 159 times more likely than in BHR-negative patients (OR 10.92 and 158.25, respectively). Both the statistically significant univariate variables and smoking habit were considered in the multivariate

analysis, which showed a statistically significant effect of both BHR and smoking on the FeNO value (*p* values <0.0001). In particular, BHR-positive patients were about 16 and 435 times more likely to have intermediate and high FeNO values than BHR-negative patients (OR 15.76, 95% CI 7.74–32.1, and OR 433.47, 95% CI 152.25–1234.09, respectively). Regarding smoking, converse findings were obtained, as reported in Table 3. At the follow-up, 22 out of 82 patients (26.8%) with high FeNO values developed asthma, which was diagnosed considering reported asthma symptoms and bronchial reversibility.

Discussion

AR and asthma are closely connected from a pathophysiological point of view. AR may frequently precede asthma onset, and it has in particular been reported that AR duration and sensitization to perennial allergens are relevant risk factors for impaired FEV₁ [20] and for BHR [21] in AR patients. On the other hand, allergic inflammation is typically Th2 dependent. FeNO assessment has been considered a reliable surrogate biomarker of Th2-driven airway inflammation.

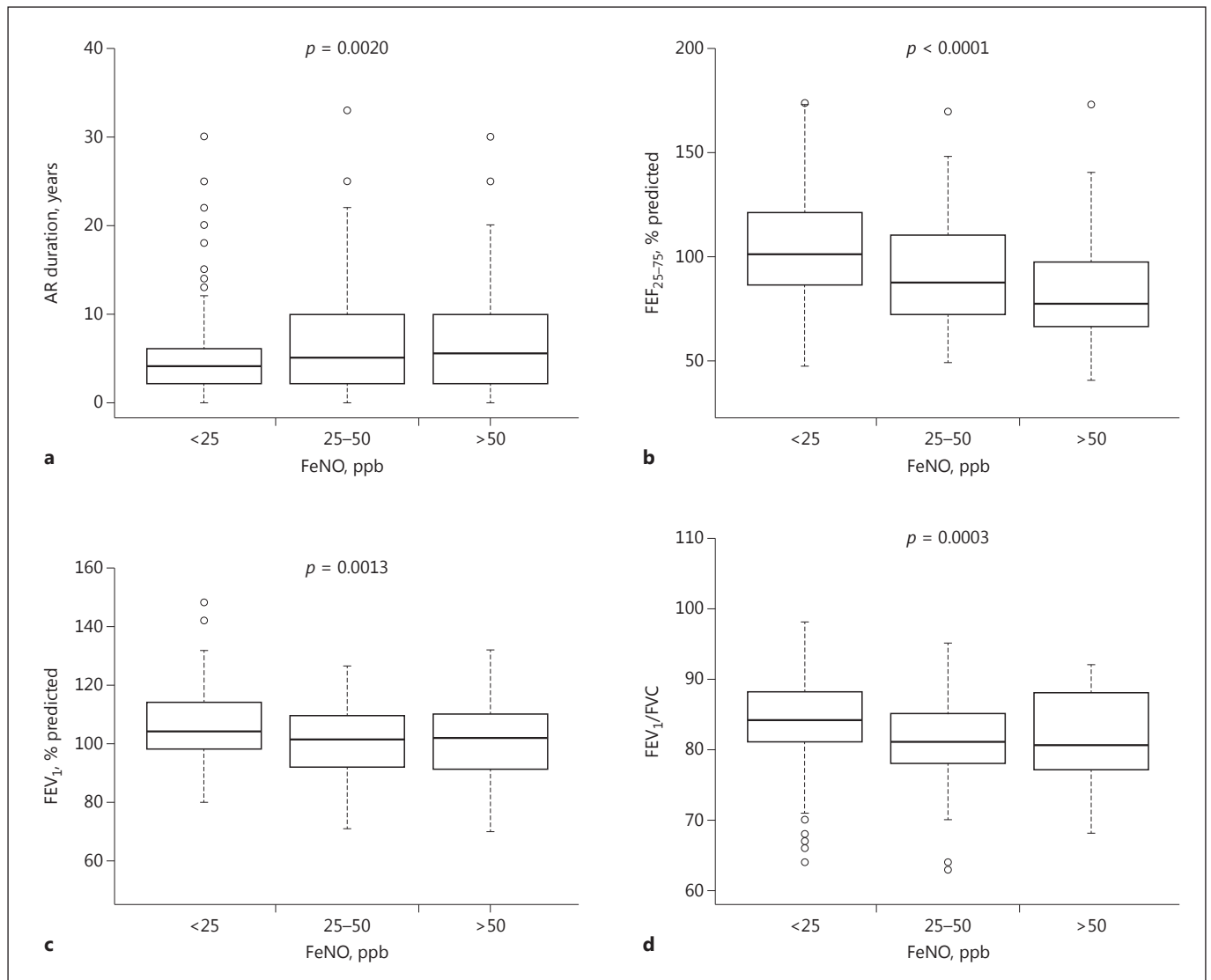


Fig. 1. Disease duration (a), FEF₂₅₋₇₅ values (b), FEV₁ values (c), and FEV₁/FVC ratio (d) in AR patients with low (<25 ppb), intermediate (≥25 but ≤50 ppb), and high (>50 ppb) FeNO values. The *p* values of the univariate multinomial logistic regression models are reported in each plot, comparing the effect of each variable on FeNO.

There are different hypotheses concerning the possible pathogenic role of the nose in affecting the lower airways, including: (i) vagal nasobronchial reflex, (ii) continuum progression of respiratory allergic inflammation, (iii) release of mediators into the blood stream, (iv) postnasal drip, and (v) oral respiration consequent to nasal obstruction. In addition, it has been reported that treating AR may improve comorbid asthma, using intranasal corticosteroids [22, 23], antihistamines [24], and allergen immunotherapy [25].

The primary outcome of the present study was to investigate whether FeNO assessment may characterize patients with AR suggesting a bronchial involvement. About 26% of the global sample had intermediate FeNO values and about 14% had high FeNO values. This highlights the relevant frequency of coexisting bronchial inflammation in AR patients. AR patients with increased FeNO values seem to differ from AR patients with low FeNO values. In fact, AR patients with high FeNO levels have several distinctive characteristics, most notably a longer disease duration. The longer the duration of AR, the more frequent-

Table 3. Multivariate analysis of the effects on FeNO in AR patients

Variable	Intermediate vs. low FeNO	High vs. low FeNO
Intercept	3.6 (0.16–80.67)	0 (0–0.08)
FEV ₁ /FVC	0.97 (0.94–1.01)	1.05 (0.99–1.11)
BHR		
Negative	1	1
Positive	15.76 (7.74–32.1)	433.47 (152.25–1234.09)
Smoking		
No	1	1
Yes	0.25 (0.12–0.5)	0.05 (0.02–0.15)

Results are expressed as OR (95% CI).

ly asthma may develop, which reflects the concept of an “asthma march” [11]. Also of interest is the evaluation of the subjective perception of respiratory symptoms, regarding both nasal obstruction and breathlessness. Patients with high FeNO levels perceive more severe nasal obstruction and dyspnea than normal FeNO subjects. This point may be clinically relevant because it highlights the importance of carefully considering the patient’s perception of symptoms as it may be related to impaired parameters. In addition, patients with high FeNO values have lower readings of lung function parameters, including FEV₁, FEV₁/FVC ratio, and FEF_{25–75}, than those with normal FeNO values. It is of note that the mean values are anyhow within normal references in both subgroups. Finally, AR patients with high FeNO values have BHR more frequently than normal FeNO subjects, and this finding is consistent with the outcomes of previous studies [26–28]. Logistic regression highlighted that the presence of BHR represented a relevant risk for having high FeNO values, whereas smoking was a negative predictive factor.

More interestingly, the follow-up data suggest that some AR patients with high FeNO values may develop asthma over time, confirming a recent study providing evidence that children with AR and high FeNO levels tend to also develop asthma [13]. In fact, about a quarter of AR patients with high FeNO values had asthma after 2 years.

The present findings are consistent with 2 previous studies. The first showed a relevant association between higher FeNO levels and greater asthma burden during the previous year in patients with persistent asthma [29]. The second study reported that AR patients with BHR also had increased FeNO values [30]. Therefore, the present study, which was conducted on a larger group of AR patients and including the follow-up of a selected subgroup, confirmed

the 2 previous reports performed by our group that highlighted the close link between BHR and Th2-driven bronchial inflammation in AR [28, 30]. Thus, these findings underline the concept that measuring FeNO in AR patients might help to identify those with bronchial involvement who are liable to further asthma progression.

The present study has some limitations. These include: (i) the high prevalence of males, (ii) the relatively young age of the patients, (iii) that VAS scores reported only nasal obstruction and breathlessness, (iv) that a FEV₁/FVC ratio <0.7 was considered as an abnormal limit rather than the lower limit of normal, (v) the inclusion of light smokers, and (vi) the lack of a long-term follow-up to confirm these findings. In particular, we limited the follow-up only to the patients with high FeNO values, presuming that high FeNO may predict further asthma onset, as previously reported [13]. Therefore, we considered these subjects as being at risk of asthma and deserving an accurate reevaluation. In fact, for economic reasons, the use of in-depth tests is reserved only in particular conditions. It would have been much more insightful to have compared all AR patients (with low, intermediate, and high FeNO values) after 2 years to determine the relative risk for the development of asthma. Another consideration is that some AR patients, mainly those with high FeNO and BHR, may actually have asthma; indeed, it is well known that asthma symptom perception can be poor, particularly in young male adults, symptoms may vary over time, and as asthma is a contraindication for active military service, some patients may not have been prepared to admit asthma or asthma-like symptoms. However, Italian Navy rules concerning recruitment and periodic visits dictate that allergic subjects, namely those suffering from AR, should be carefully evaluated regarding bronchial involvement through specific tests, including spirometry, bronchodilation testing, and methacholine challenge. However, our conclusions cannot be generalized because of this potential bias and further appropriate studies should be conducted to address these unanswered questions.

On the other hand, the current study is novel compared with our previous studies in that it was conducted in a large AR patient group, performed with a follow-up (even though limited to a subgroup), and it was demonstrated that AR patients with high FeNO values have a more evident bronchial involvement than other AR subjects. In addition, the present outcomes are consistent with the most relevant literature concerning this issue [31–34].

In conclusion, the present study demonstrates that AR patients may frequently have high FeNO values, such as

overt Th2-driven bronchial inflammation. This could be clinically relevant as AR patients with high FeNO values exceeding 50 ppb might have an initial impairment of lung function, BHR, perceived worsening of respiratory symptoms, and potential progression to asthma.

Disclosure Statement

None of the authors have any conflicts of interest to disclose. There was no funding source.

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