

## BIOMARKERS

# High Exhaled Nitric Oxide Levels May Predict Bronchial Reversibility in Allergic Children with Asthma or Rhinitis

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**Background.** Allergic asthma and rhinitis may be associated. Airway inflammation is shared by both disorders. The measure of the fractional concentration of exhaled nitric oxide (FeNO) may be considered as a surrogate marker for airway inflammation, mainly in allergic patients. Reversibility to bronchodilation (BD) testing is a functional characteristic of asthma. **Objective.** The aim of this study was to evaluate whether FeNO may predict reversibility to BD in a pediatric cohort of allergic subjects with asthma (180) or rhinitis (150). **Methods.** Lung function (including forced expiratory volume at the first second (FEV<sub>1</sub>), forced volume capacity (FVC), forced expiratory flow at 25–75% of volume capacity (FEF<sub>25–75</sub>)), FeNO measurement, and BD testing were performed in all children. **Results.** Lung function, FeNO, and sensitization type were significantly different in the two groups. A strong correlation was found between FeNO and  $\Delta$ FEV<sub>1</sub> after BD. Two main predictors of reversibility were FeNO values >34 ppb [Odds RatioAdj (ORAdj) = 1.9] and sensitization to perennial allergens (ORAdj = 1.7). **Conclusions.** This study provided evidence that FeNO was strongly related with the response to BD testing and could predict bronchial reversibility in children with allergic rhinitis or asthma. Therefore, a simple FeNO measurement could suggest relevant information about bronchial reversibility.

**Keywords** allergic rhinitis, asthma, bronchodilation, children, nitric oxide, reversibility

## INTRODUCTION

Asthma is the most common chronic disorder in childhood and is characterized by three main pathophysiological characteristics: lower airways inflammation, reversible bronchial obstruction, and bronchial hyperreactivity (BHR) to different stimuli (1). Allergic rhinitis is also characterized by mucosal inflammation and may be frequently associated with asthma (2). Hence, inflammation of airways represents a common pathogenic pathway for both diseases.

The accurate measurement and monitoring of airways inflammation should be a crucial topic in managing allergic patients (3). Unfortunately, a direct assessment of bronchial inflammation needs invasive methods (e.g., bronchial biopsy and/or lavage) or sputum analysis, both are complex and require adequate setting. However, it is actually possible to evaluate bronchial inflammation also by measuring the fractional concentration of exhaled nitric oxide (FeNO), simply during an office visit (4, 5). There is evidence that the FeNO levels well correlate with peripheral or bronchial eosinophilia (6, 7): a marker of asthmatic inflammation. Recently, determinants of exhaled NO in children with atopic asthma have been reported (8). In addition, the role of inducible isoform of nitric oxide synthase and airway inflammation was previously investigated (9).

Moreover, airway inflammation may induce bronchial obstruction: the paramount functional characteristic of asthma. Lung function should be accurately measured in asthmatic patients using the spirometer. Bronchial functional parameters are well standardized and their interpretation is commonly recognized (10, 11). In addition, the reversibility of airflow obstruction is considered as a functional characteristic of asthma. Bronchial reversibility may be spontaneous or induced by bronchodilator agents, mainly  $\beta_2$ -agonists. Therefore, the bronchodilation (BD) testing is usually considered to demonstrate the reversibility of bronchial obstruction. Moreover, BD may also be performed in patients with allergic rhinitis alone: in fact, bronchial reversibility may suggest an initial bronchial impairment in those patients (12). In this regard, it has been recently evidenced that an “asthma march” may exist in allergic rhinitis, such as the progression from initial mild bronchial obstruction to overt reversibility (13).

As FeNO may be considered as a reliable marker of allergic inflammation, this study aimed at evaluating whether its measurement may also predict bronchial reversibility in children with asthma or allergic rhinitis.

## MATERIALS AND METHODS

### Study Design and Setting

This cross-sectional study included 330 consecutive children, median age of 12 years, with allergic rhinitis (150) or asthma (180). All the children were evaluated at the “Ave Gratia Plena” Hospital for rhinitis and/or asthma. The local

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Review Board approved the study and an informed consent was obtained from the parents of each patient.

Detailed clinical history and complete physical examination were performed. The patients were included in the study on the basis of a documented diagnosis of allergic asthma or rhinitis made by primary care pediatricians, according to validated criteria (1, 2). The diagnosis of asthma included consistent symptoms and signs, lung function impairment, and bronchial obstruction reversibility (spontaneous or after adequate pharmacologic intervention). Allergy was documented by medical records, reporting positivity to skin prick test for the most common allergens (including house dust mites, grasses, *Parietaria*, birch, dog, cat, *Alternaria*, *Aspergillus*, ragweed, and olive). Allergy was defined by the concordance between sensitizing allergen and occurrence of symptoms after its inhalation. We excluded all the subjects who met the following criteria: negative skin prick tests, acute or chronic upper respiratory infections, anatomical nasal disorders (i.e., nasal polyps, severe septum deviation, etc.), previous or current specific immunotherapy, and the use of inhaled nasal or oral corticosteroids, nasal or oral vasoconstrictors, long-acting bronchodilators, antileukotrienes, and antihistamines during the previous 4 weeks. In addition, the past history of lower respiratory tract symptoms or treatment with asthma medications were considered as exclusion criteria for the allergic rhinitis group. All patients were previously treated only on demand for drugs (such as antihistamines or short-acting  $\beta_2$ -agonist) alone.

### *Spirometry*

Spirometry was performed using a computer-assisted spirometer (Pulmolab 435-spiro 235, Morgan, UK), with optoelectronic whirl flow meter. It was performed as stated by the American Thoracic Society (ATS) and the European Respiratory Society (ERS) (10, 11).

### *BD Test*

The BD testing was performed according to international guidelines and using a salbutamol metered dose of 400 mcg. Reversibility (bronchodilator responsiveness, BDR) was considered if an increase of at least 12% of FEV<sub>1</sub> was achieved from baseline, according to ERS/ATS guidelines (10, 11).

### *Nitric Oxide Measurement*

FeNO was measured with a chemiluminescence analyzer (Model 280 Nitric Oxide Analyzer; Severs Instrument Inc., Boulder, CO, USA); the detection limit of the apparatus was 1–5 ppb, as required by ATS guidelines (4), 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 (4). Children inhaled to total lung capacity from NO-free air and exhaled a single breath (without nose clip) through a mouthpiece at a mouth pressure of 45 cm water and at an expiratory flow of 50 ml/s. Mouth pressure was displayed on a computer screen 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 and Data Definitions*

Descriptive statistics were first performed and quantitative parameters were reported as medians with quartiles. Qualitative data were reported as frequencies and percentages. Comparison of qualitative data among various groups of patients was made by the  $\chi^2$ -test (or by the Fisher's exact test in case of expected frequencies of less than five). Comparison of quantitative variables between the two groups of patients was made using the nonparametric counterpart U Mann–Whitney test, whenever the normality assumption was not fulfilled.

Linear correlation between pairs of quantitative variables was evaluated by Spearman's correlation coefficient ( $r$ ). For the purpose of the analysis, correlation coefficients of  $>0.8$  were considered as very strong, from 0.6 to 0.79 were considered as strong, from 0.4 to 0.59 were considered as moderate, from 0.2 to 0.39 were considered as weak, and  $<0.2$  were considered as very weak.

Multiple logistic regression analysis was performed for all patients (patients with rhinitis and patients with asthma) to evaluate the role of different independent variables in relationship with the presence of BDR.

Before performing logistic regression, some continuous predictors were dichotomized on the basis of the receiver operating characteristic (ROC) curve analysis, and sensitivity and specificity were calculated. The following predictors were evaluated in the logistic regression model: age of patient ( $>11$  vs.  $\leq 11$  years), duration of disease ( $>4$  years vs.  $\leq 4$  years), FVC ( $\geq 91\%$  vs.  $<91\%$  of predicted), FEF<sub>25–75</sub> ( $\leq 61\%$  vs.  $>61\%$  of predicted), FeNO ( $>34$  ppb vs.  $\leq 34$  ppb), and sensitization to perennial allergens. For the multivariate analysis, the step-down strategy was chosen; the effect was expressed as adjusted odds ratio (OR<sub>Adj</sub>) and 95% confidence interval (CI) and was tested by the likelihood ratio (LR) test.

The area under the ROC curve (AUC) of the model was used as an indicator of the model's ability.

For the analysis, a  $p$ -value of  $<.05$  for two-tailed  $t$ -test was considered as significant. A statistical software program (StatSoft Italia s.r.l. 2005. Statistica, Vigonza, Italy) was used for all the analyses.

### *Results*

Globally, 330 children (184 males and 146 females) were included in this study. Table 1 shows the demographic, clinical, and functional characteristics of the patients divided into two groups: 150 patients with allergic rhinitis and 180 patients with allergic intermittent asthma. Gender did not affect the clinical parameters. The disease duration was longer in asthmatic children than in children with rhinitis. All asthmatic children had controlled asthma. The median values of FVC, FEV<sub>1</sub>, and FEF<sub>25–75</sub> were significantly higher in patients with rhinitis (95%, 88%, and 73% of predicted, respectively) compared with asthmatic children (92%, 81%, and 69% of predicted, respectively). In

TABLE 1.—Demographic and clinical characteristics of patients.

|                                                                     | Patients with rhinitis<br>N = 150 | Patients with asthma<br>N = 180 | p-Value            |
|---------------------------------------------------------------------|-----------------------------------|---------------------------------|--------------------|
| Gender – males, N (%)                                               | 81 (54)                           | 103 (57.2)                      | .2 <sup>a</sup>    |
| Age (years) median                                                  | 10                                | 13                              | .15 <sup>b</sup>   |
| Duration of pathology (year), median [IQR]                          | 3 [2–6]                           | 6 [2–7]                         | .001 <sup>b</sup>  |
| FVC, median [IQR], % of predicted                                   | 95 [89–99]                        | 92 [87–93]                      | .019 <sup>b</sup>  |
| FEV <sub>1</sub> , median [IQR], % of predicted                     | 88 [84–91]                        | 81 [75–86]                      | <.001 <sup>b</sup> |
| FEF <sub>25–75</sub> , median [IQR], % of predicted                 | 73 [64–81]                        | 69 [60–75]                      | <.001 <sup>b</sup> |
| Bronchodilator responsiveness N (%)                                 | 53 (35.3)                         | 171 (95)                        | <.001 <sup>b</sup> |
| FEV <sub>1</sub> percentage of change (post–pre-test), median [IQR] | 8 [5–17]                          | 18 [13–24.5]                    | <.001 <sup>b</sup> |
| FeNO in ppb; median [IQR]                                           | 27 [21–35]                        | 34 [29–38]                      | <.001 <sup>b</sup> |
| Sensitized to perennial allergens pz, N (%)                         | 55 (36.6)                         | 97 (53.8)                       | .001 <sup>a</sup>  |

Notes: All percentages in round brackets are calculated over the total number of subjects reported at the top of column. All numbers in square brackets represent first to third quartiles. IQR, interquartile range.

<sup>a</sup>Chi-squared test.

<sup>b</sup>U Mann–Whitney test.

addition, BDR was significantly more frequent in children with asthma than in children with rhinitis (respectively, 95% and 35.3%,  $p < .001$ ).

The median FeNO value was significantly ( $p < .001$ ) higher in asthmatic children (34) than in children with rhinitis (27), as reported in Table 1. A significantly greater number of children with asthma were sensitized to perennial allergens than those with rhinitis (53.8% vs. 36.6%)

The FeNO values were strongly correlated with  $\Delta$ FEV<sub>1</sub> after BD ( $r = 0.69$ ) in asthmatic children, but they were moderately related in patients with rhinitis ( $r = 0.53$ ), as reported in Figure 1.

To evaluate possible predictive factors for BDR, the entire population of patients was more deeply investigated. A ROC curve analysis was first performed in order to find, for quantitative predictors, the best cutoff value. The results of this analysis are shown in Table 2 and Figure 2. As shown in Table 2 and Figure 2, four variables had an interesting predictive ability (AUC ROC > 0.70): age, duration of disease, FEF<sub>25–75</sub> % of predicted, and FeNO in ppb. Thus, the following predictors were evaluated in the logistic regression model: age (>11 years vs. ≤11 years), duration of disease (>4 years vs. ≤4 years), FEF<sub>25–75</sub> (≤61% vs. >61% of predicted), FeNO levels (FeNO >34 ppb vs. ≤34 ppb), and sensitization

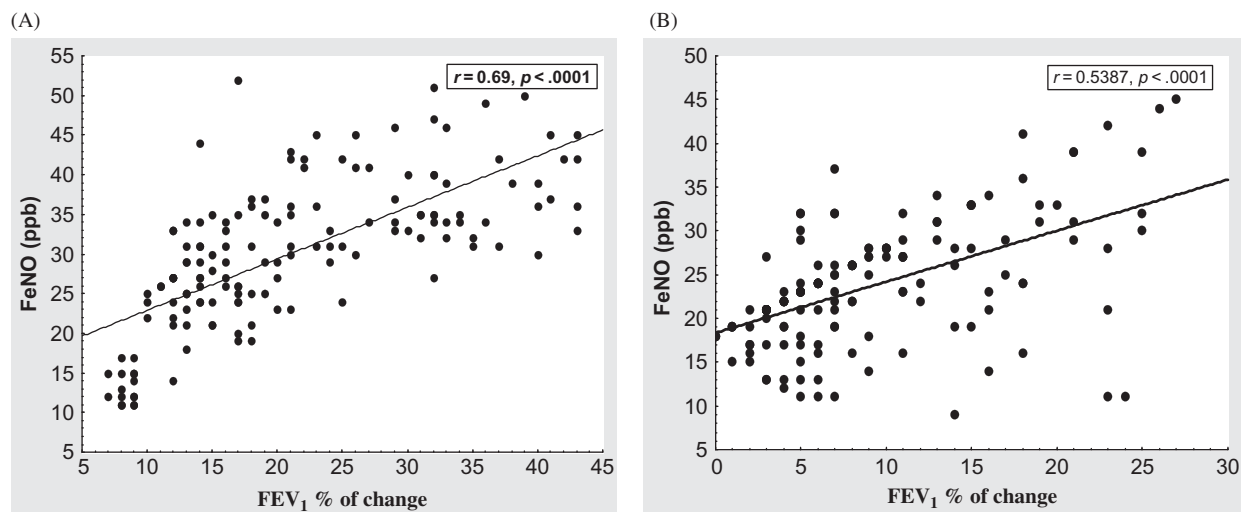


FIGURE 1.—Relationship between FeNO levels and  $\Delta$ FEV<sub>1</sub> after BD testing in asthmatic children (A) and in children suffering from allergic rhinitis (B).

TABLE 2.—Sensitivity, specificity, and best cutoff value for FeNO as predictor of BDR.

| Predictors                          | Sensitivity | Specificity | PPV  | NPV  | AUC (95% CI)     | Best cutoff value |
|-------------------------------------|-------------|-------------|------|------|------------------|-------------------|
| Age (years)                         | 0.71        | 0.72        | 0.24 | 0.87 | 0.71 (0.63–0.77) | >11               |
| Duration of disease (years)         | 0.60        | 0.78        | 0.24 | 0.93 | 0.77 (0.75–0.81) | >4                |
| FVC % of predicted                  | 0.91        | 0.29        | 0.15 | 0.78 | 0.57 (0.51–0.6)  | ≥91               |
| FEF <sub>25–75</sub> % of predicted | 0.51        | 0.89        | 0.24 | 0.91 | 0.73 (0.63–0.79) | ≤61               |
| FeNO in ppb                         | 0.8         | 0.77        | 0.26 | 0.97 | 0.88 (0.77–0.92) | >34               |

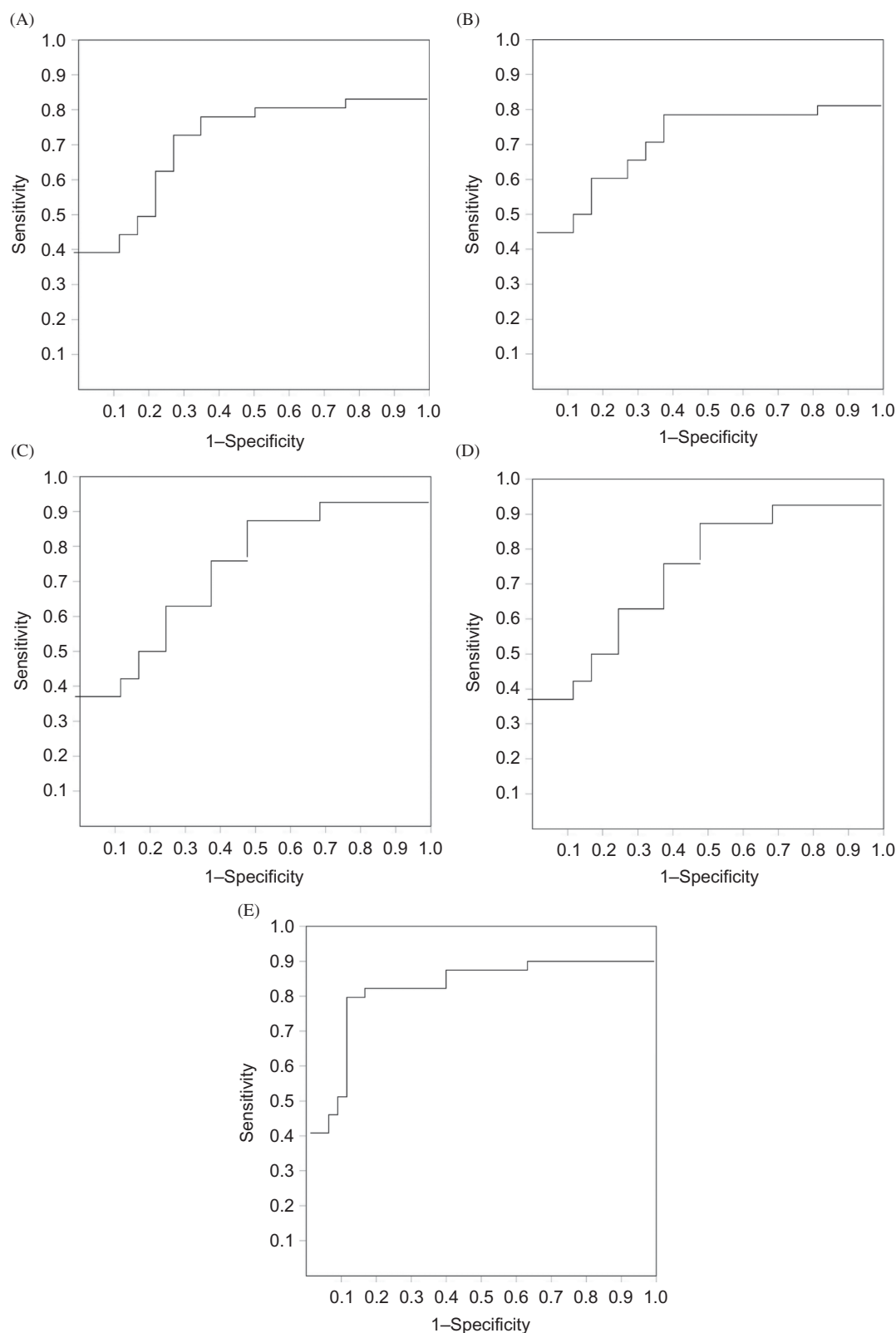


FIGURE 2.— ROC curves obtained for (A) age, (B) duration of disease, (C) FVC % of predicted, (D) FEF<sub>25-75</sub> % of predicted, and (E) FeNO in ppb. AUC was 0.71, 0.77, 0.57, 0.73, and 0.88 respectively.

to perennial allergens. Those variables for patients with BDR and without BDR are presented in Table 3.

Very significant differences were found with respect to age category, duration of disease, FeNO, and spirometric

parameters. In addition, 57.5% of children with BDR were sensitized to perennial allergens, while only 21.6% of children without BDR were sensitized to perennial allergens.

TABLE 3.—Demographic and clinical parameters in patients with BDR (BDR+) and in patients without BDR (BDR—).

| Population                             | BDR+, N = 224 | BDR—, N = 106 | p-Value |
|----------------------------------------|---------------|---------------|---------|
| Age, >11 years                         | 132 (58.9)    | 25 (23.5)     | .004    |
| Duration of disease >4 years           | 128 (57.1)    | 27 (25.4)     | .001    |
| FVC >91% of predicted                  | 49 (21.8)     | 91 (85.8)     | .006    |
| FEF <sub>25–75</sub> ≤61% of predicted | 61 (27.2)     | 11 (10.3)     | <.004   |
| FeNO >34 ppb                           | 148 (66)      | 33 (31.1)     | <.0001  |
| Sensitization to perennial allergens   | 129 (57.5)    | 23 (21.6)     | .0004   |

Notes: Values denote N (%). All percentages in round brackets are calculated over the total number of subjects reported at top of the column.

TABLE 4.—Best fitting logistic regression models for independent predictors of the presence of BDR.

| Variables                            | ORAdj | 95% CI  | p-Value |
|--------------------------------------|-------|---------|---------|
| FeNO >34 ppb                         | 1.9   | 1.7–9.1 | <.005   |
| Sensitization to perennial allergens | 1.7   | 1.1–7.4 | <.005   |

Notes: Outcome variable:  $\Delta$  FEV<sub>1</sub> ≥12. AUC of the model: 0.90.

### Multivariate Logistic Analysis

A logistic regression model was performed to evaluate the role of different independent variables in relationship with BDR (Table 4). Two predictors were significantly associated with BDR: FeNO levels >34 ppb (ORAdj = 1.9) and sensitization to perennial allergens (ORAdj = 1.7). The model's discriminative ability was very satisfactory: the AUC was 0.90.

## DISCUSSION

Allergic inflammation is present both in asthma and allergic rhinitis (14). FeNO assessment has been considered as a surrogate marker of airway inflammation, mainly in allergic patients (4). Moreover, an association between nasal inflammation and bronchial airflow limitation (15) has been reported, as well as it has been demonstrated that nasal eosinophil count was strongly related to eosinophil sputum (16). On the other hand, BDR is the pathognomonic functional characteristic of asthma. Thus, BD testing gains paramount importance to define asthma diagnosis in the presence of overt respiratory symptoms. As FeNO measurement is a simple tool to assess bronchial inflammation, this study aimed at investigating whether FeNO may also predict BDR in children with allergic asthma or allergic rhinitis.

First, this study demonstrated that asthmatic children, as expected, had reduced values of spirometric parameters and higher FeNO levels in comparison with children suffering from allergic rhinitis. In addition, sensitization to perennial allergens was more frequent in asthmatic children. This finding may be clinically relevant as allergic inflammation typically depends on allergen exposure. Thus, chronic allergen exposure induces persistent allergic inflammation (17). The duration of allergic disorder is a relevant risk factor for worsening of respiratory function, both at nasal and bronchial levels (12, 13).

Second, concerning BDR, most of asthmatic children had BDR, whereas only one-third of children with allergic rhinitis had BDR. This fact might suggest an initial bronchial involvement in these children with rhinitis and the clinician should be quite suspicious about the presence of asthma, delving into history for asthma symptoms. Subdividing both groups according to BDR, it appears that children with BDR had more elevated FeNO levels and more frequently sensitized to perennial allergens. To reinforce this finding, a strong relationship between FeNO levels and BDR in asthmatic children and a moderate relationship in children with rhinitis were demonstrated. Thus, a vicious circle among perennial exposure to the causal allergen, chronic inflammation, airflow limitation, and BDR, which is more evident in asthmatic patients seems to exist.

More interestingly, the logistic regression highlighted that the presence of >34 ppb FeNO values represented a relevant risk (ORAdj = 1.9) for having BDR as well as sensitization to perennial allergens (ORAdj = 1.7) in allergic children. These findings were confirmed by the ROC curve with an optimal reliability (AUC = 0.90).

All these data underline the close link between allergic inflammation and BDR in patients with asthma or rhinitis sensitized to perennial allergens. Thus, this study might suggest that a FeNO value of >34 ppb may predict BDR with good reliability.

In addition, a previous study demonstrated a positive correlation between exhaled Nitric Oxide (eNO) and percentage change in FEV<sub>1</sub> (18). A further study reported that FeNO levels significantly correlated with the degree of bronchial hyperresponsiveness, bronchodilator reversibility, allergen skin prick tests, serum Immunoglobulin E (IgE), and total eosinophil count; FeNO levels were also higher in patients with nocturnal symptoms and in patients requiring  $\beta$ -agonist use at least once weekly (19). A more recent study showed that in allergic and nonallergic asthmatics, FeNO was significantly correlated with bronchial hyperresponsiveness to histamine, reversibility of airway obstruction, serum Eosinophil Cationic Protein (ECP) levels, and blood eosinophilia (20). Therefore, all these previous studies were consistent with this article, confirming the significant association between FeNO and BDR.

The relevance of this study is supported by the consistent number of enrolled children and by contemporaneous assessment of two different groups of patients. However, a possible limitation of this study is that it concerns a very

specific group of asthmatic children, such as steroid naïve patients with normal baseline lung function. In fact, the inclusion of steroid-treated patients as well as children with impaired baseline lung function would very probably have changed the findings considerably.

In conclusion, this study provides evidence that FeNO is strongly related with the response to BD testing and may predict BDR in children with allergic rhinitis or asthma. Therefore, a simple FeNO measurement could suggest relevant information about bronchial reversibility.

#### DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article. This study had no sponsorship.

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