

Exhaled nitric oxide, total serum IgE and allergic sensitization in childhood asthma and allergic rhinitis

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Exhaled nitric oxide (eNO) levels are correlated with several markers of atopy and inflammatory activity in the airways, but the relationship between eNO and total serum IgE has not been fully elucidated in the context of allergic sensitization. The aim of this study was to investigate the relationship between eNO, total serum IgE and allergic sensitization in childhood asthma and allergic rhinitis. eNO levels, lung function, skin prick tests and total serum IgE were determined in 109 children (mean age, 10.4 yr) with mild intermittent asthma and in 41 children (mean age, 10.1 yr) with allergic rhinitis; 25 healthy non-atopic children were recruited as controls. eNO levels (median) were significantly higher in patients with asthma (22.7 p.p.b.) and in those with allergic rhinitis (15.3 p.p.b.) than in healthy controls (5.9 p.p.b.). Children with allergic asthma had higher eNO levels than children with allergic rhinitis. A significant positive correlation was found between eNO and total serum IgE (asthma, $r = 0.42$, $p < 0.0001$; allergic rhinitis, $r = 0.31$, $p < 0.01$), and between eNO and the number of positive skin prick tests (asthma, $r = 0.31$, $p < 0.0001$; allergic rhinitis, $r = 0.39$, $p < 0.01$). eNO levels were better correlated with total IgE than with the number of positive skin prick tests. This correlation was independent of allergic sensitization. High total serum IgE represents a specific and predictive marker of eNO increase in children with asthma or allergic rhinitis. This finding adds further support to the hypothesis that increased serum IgE could be a marker itself of airway inflammation in patients with allergic disease.

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Nitric oxide is a free-radical gas of endogenous origin with multiple biological and pathophysiologic functions, detectable in exhaled air of humans. As exhaled nitric oxide (eNO) is increased in asthmatic patients, attention has been focused on eNO measurement as a simple, non-invasive tool to assess the degree of airway inflammation (1).

In asthmatic patients, a relationship has been shown between eNO levels and other markers of eosinophilic recruitment, detected in blood (2, 3), sputum (4), bronchoalveolar lavage fluid (5) and bronchial biopsies (6). Furthermore, eNO values

have been found to be related to airway hyper-responsiveness (AHR) (2, 3) and total serum IgE, both in adults (7) and in children (2, 3) with asthma.

So far, the relationship between eNO and total IgE has not been fully investigated in the context of allergic sensitization, and, to our knowledge, no study has focused on this relationship in patients with allergic rhinitis. Therefore, the present study aimed to better define the relationship between eNO, total IgE and allergic sensitization, both in asthmatic children and in those with allergic rhinitis.

Materials and methods

Subjects

Enrolled in the study were 109 children with mild intermittent asthma (mean age 10.4 yr, range 7.0–15.7) referred to a University Paediatric Allergy Clinic between November 2002 and September 2003. The diagnosis of asthma was based upon a history of typical respiratory symptoms, physical examination, pulmonary function tests, and response to inhaled β -adrenergic agents according to international guidelines (8). Fifty-one (46.7%) asthmatic children suffered also from allergic rhinitis. All patients had a history of at least one episode of asthma in the past 12 months and were stable at the time of recruitment. Patients were being treated with inhaled β_2 -agonist alone on an as-required basis, and had not used any controller anti-asthmatic therapy during the 3 months prior to the study.

A total of 41 children (mean age 10.1 yr, range 7.2–15.4) with moderate persistent allergic rhinitis were also recruited in the same setting (9). Allergic rhinitis was diagnosed on the basis of clinical history (watery rhinorrhea, blocked nose, sneezing, and nasal itching) and positive skin prick tests to common allergens (9). None of the children had ever presented wheezing or received asthma medication. Antihistamine or inhaled steroid treatment had to have been withdrawn for at least 3 months before the study.

Exclusion criteria for patient recruitment were: (i) history of significant medical illness or parasite infection; (ii) previous or current allergen hyposensitization; (iii) history or signs of respiratory tract infection in the 4 wk prior to the study; and (iv) tobacco smoke exposure in the family.

As a control group, 25 healthy non-atopic children (mean age 10.3 yr, range 7.5–14.9), recruited among secondary school children attending a local church community, were studied. Parents were asked to fill out a health questionnaire regarding the child's clinical history. Healthy controls had no history of airway disease, allergy or significant medical illness, and were not taking any medication. The non-atopic status was defined as the absence of allergic symptoms in the history and negative skin prick tests.

Parents or tutors of the children gave informed consent to the study, which was approved by the local Ethics Committee.

Study design

Subjects attended the Allergy Clinic on two occasions, 7–10 days apart. At the first visit, a

detailed clinical history was collected and skin prick tests (Stallergènes Diagnostics, Antony, France) to 12 common allergens (house dust mites, cat fur, dog dander, *Alternaria A.*, *Aspergillus fumigatus*, olive, mixed-grass pollen, *Parietaria J.*, hen's egg, fresh cow milk, wheat, codfish) were performed. Appropriate positive (histamine dihydrochloride, 10 mg/ml) and negative control solutions were also used. The skin prick test was considered positive if a ≥ 3 mm skin wheal response (mean of maximum and orthogonal midpoint diameters) greater than negative control was recorded within 20 min. At the second visit, a blood sample for total serum IgE assay was obtained. Fractional eNO measurement and pulmonary function tests were sequentially performed. For ethical reasons, controls did not undergo blood sampling for total IgE measurement.

Pulmonary function tests were performed with a Jaeger MasterScope spirometer (Jaeger GmbH, Würzburg, Germany), according to a standardized procedure (10). The best of three maneuvers was recorded as percentage of predicted references values (11). Bronchodilator reversibility was calculated 15 min after the administration of two puffs (200 μ g) of albuterol by a metered-dose inhaler through a spacer (Volumatic®, GSK, Verona, Italy). Children were recruited only if the baseline forced expiratory volume in 1 s (FEV₁) was $> 80\%$ of the mean predicted value for the child's height.

Nitric oxide measurement

Exhaled NO was measured with a fast response chemiluminescence analyzer (NOA Tm280; Sievers Instruments Inc., Boulder, CO, USA) by the on-line single-breath technique, according to the ATS and ERS recommendations for children (12). The analyzer has an NO sampling rate of 200 ml min⁻¹, a detection range of < 0.1 –500,000 parts per billion (p.p.b.) and a response time of 0.02 s for 90% full scale. Briefly, the subjects were asked to perform a single slow exhalation starting from total lung capacity through a mouthpiece and a one-way non-rebreathing valve against a resistance of 16 cm H₂O under a visual biofeedback, to maintain a 50 ml s⁻¹ steady flow. Subjects were at rest, sitting down, having refrained from eating and exercise for at least 1 h, and breathed filtered NO-free air (air filter AFL 01410; Sievers Instruments Inc.) without a nose-clip before the single exhalation maneuver. Ambient air NO was recorded before and after each test. The analyzer was calibrated daily with a zero signal (air filter scrubber, potassium permanganate and charcoal,

ACT 01400; Sievers Instruments Inc.) and certified NO/N₂ calibration gases (Messer, Collegno, Italy) containing 108 p.p.b. and 10.3 parts per million. eNO levels were measured at the plateau (≥ 2 s during an exhalation ≥ 6 s) of the end-exhaled reading and expressed as p.p.b. At least three measurements that varied by $< 10\%$ or two measurements that varied by $< 5\%$ were recorded. The mean value of the respective plateaux was retained according to specific guidelines (12).

Total IgE measurement

Serum was collected and stored at -20°C until assayed. Total IgE were measured using the UniCAP system (Pharmacia Diagnostics AB, Uppsala, Sweden) according to the manufacturer's instructions.

Statistical analysis

All statistical tests were performed using the R statistical language (<http://www.r-project.org>) (13). As data did not necessarily come from multivariate Gaussian distributions, whenever appropriate rank-based methods have been employed. Median values and inter-quartile range (IQR) have been used to describe the distributions of the following variables: eNO (Y), total serum IgE (X), number of positive skin prick tests (Z). Y and X were assessed as natural-log transformed values to obtain comparable measurement scales. Crude and adjusted correlations between these variables were investigated by Kendall's tau coefficient, and the exact conditional (on the data) Mann-Whitney test was used to assess differences between groups. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated using standard methods and the chi-square test was used to compare the rates. The most advantageous cutoff value of total serum IgE in predicting eNO increase in asthmatic patients, with respect to eNO distribution in controls, was

determined by receiver operating characteristic (ROC) curve analysis. All tests were two-sided and a p -value < 0.05 was considered significant.

Results

Characteristics of the patients are reported in Table 1. A total of 91 (83.4%) of the asthmatic, and all patients with rhinitis, were allergic, as demonstrated by positive skin prick tests to one or more allergens.

eNO levels

Patients with asthma as a whole group and patients with allergic rhinitis had significantly higher eNO levels [22.7 (9.1–48.0) p.p.b. and 15.3 (9.4–31.0) p.p.b., respectively] than controls [5.9 (3.4–9.3) p.p.b.] (asthma vs. control group, $p = 0.0001$; allergic rhinitis vs. control group, $p = 0.016$; asthma vs. allergic rhinitis = n.s.) (Table 2). Within the asthma group, higher eNO values were found in allergic [25.6 (11.4–56.2) p.p.b.] than in non-allergic subjects [11.5 (5.4–15.5)] p.p.b. ($p < 0.001$). Patients sensitized to two or more allergens ($n = 67$) had significantly higher eNO levels [34.0 (12.5–65.5) p.p.b.] than mono-sensitized patients ($n = 24$) [14.8 (7.3–23.5) p.p.b.] ($p = 0.001$). In the atopic asthmatics, no difference in eNO levels was found between patients sensitized ($n = 57$) and non-sensitized ($n = 34$) to house dust mites [29.0 (12.2–62.0) p.p.b. vs. 19.0 (8.0–49.0) p.p.b., $p = \text{n.s.}$]. No difference was found in eNO values between non-allergic asthmatic children and controls ($p = \text{n.s.}$). eNO levels in children with allergic rhinitis were lower than in allergic asthma ($p = 0.03$).

Correlation of eNO with total IgE and skin prick tests

A significant positive correlation was found between eNO values and total serum IgE, both in asthmatic patients ($r = 0.42$, $p < 0.0001$) and

Table 1. Characteristics of study population

	Asthma ($n = 109$)	Allergic rhinitis ($n = 41$)	Controls ($n = 25$)
Age, yr (mean $\pm$ s.d.)	10.4 $\pm$ 2.5	10.1 $\pm$ 1.8	10.3 $\pm$ 2.2
M/F ratio	1.3	1.2	1.2
FVC% pred (mean $\pm$ s.d.)	96.7 $\pm$ 12.1	93.8 $\pm$ 8.7	101.1 $\pm$ 8.3
FEV ₁ % pred (mean $\pm$ s.d.)	105.9 $\pm$ 13.7	106.7 $\pm$ 9.9	111.0 $\pm$ 11.2
FEF _{25–75} % pred (mean $\pm$ s.d.)	99.4 $\pm$ 24.7	114.9 $\pm$ 18.3	105.3 $\pm$ 18.3
FEV ₁ % pred (mean $\pm$ s.d.) after bronchodilation	111.3 $\pm$ 11.7	107.9 $\pm$ 10.0	110.8 $\pm$ 10.5
No. subjects sensitized to at least one allergen (%)	91 (83.4)	41 (100.0)	0
No. patients sensitized to house dust mites (%)	57 (52.2)	26 (63.4)	0
No. patients with total serum IgE levels ≥ 175 kU l ⁻¹ (%)	55 (50.4)	21 (51.2)	n.a.

n.a., data not available.

Table 2. Exhaled nitric oxide (eNO), total immunoglobulin E (IgE), and number of positive skin prick tests in asthmatic and allergic rhinitis children

	Asthma			Allergic rhinitis n = 41 (4)	Whole population n = 150	Differences between groups†			
	Atopic n = 91 (1)	Non-atopic n = 18 (2)	All n = 109 (3)			(1) vs. (2)	(1) vs. (4)	(2) vs. (4)	(3) vs. (4)
Variables [median (IQR)]									
eNO* (parts per billion)	25.6 (11.4–56.2)	11.5 (5.4–15.5)	22.7 (9.1–48.0)	15.3 (9.4–31.0)	18.5 (9.2–44.5)	0.0006	0.038	0.053	0.20
IgE (kU l ⁻¹)	196 (89–567)	57 (21–245)	175 (71–456)	183 (80–352)	176 (74–399)	0.004	0.47	0.024	0.96
No. positive skin prick tests	2 (1–3)	–	2 (1–3)	3 (2–3)	2 (1–3)	–	0.53	–	0.032

*The eNO median of the controls [5.9 (IQR 3.4–9.3) p.p.b.] was statistically different from the median of the atopic asthma group ($p < 0.0001$), the all asthma group ($p = 0.0001$), the allergic rhinitis group ($p = 0.016$) and the whole population of patients ($p = 0.0002$). The eNO median of the controls was not statistically different from the median of the non-atopic asthma ($p = \text{n.s.}$).

†p-values for differences between groups: exact Mann–Whitney test for comparison in location of distributions of eNO, total IgE and number of skin prick tests.

in those with allergic rhinitis ($r = 0.31$, $p < 0.01$) (Table 3, Fig. 1). eNO levels were also significantly correlated with the number of positive skin tests in both groups (asthma, $r = 0.31$, $p < 0.0001$; allergic rhinitis, $r = 0.39$, $p < 0.01$). After controlling for skin prick test positivity, eNO values and total serum IgE were still significantly correlated (asthma, $r = 0.42$, $p < 0.0001$; allergic rhinitis, $r = 0.45$, $p < 0.01$). After controlling for total IgE, the relationship between eNO and the number of positive skin prick tests was less evident, but still significant (asthma, $r = 0.23$, $p < 0.05$; allergic rhinitis, $r = 0.30$, $p < 0.05$).

Predicting increased eNO in asthmatic children

The ROC curve analysis showed that the optimal cutoff value of total serum IgE in discriminating asthmatic children with eNO values above the upper quartile of distribution in normal children (9.3 p.p.b.) was 175 kU l⁻¹ (Fig. 2). In predicting eNO levels > 9.3 p.p.b., allergic sensitization

displayed better sensitivity, but lower specificity and lower PPV than the IgE cutoff value (Table 4). Indeed, of the 55 asthmatic children with IgE levels ≥ 175 kU l⁻¹, 51 (92.7%) had increased eNO levels (> 9.3 p.p.b.), whereas, of the 54 asthmatic children with IgE levels < 175 kU l⁻¹, 30 (55.5%) had high eNO values ($\chi^2 = 17.823$, $p < 0.0001$) (Fig. 3). Surprisingly, of the 91 asthmatic children with allergic sensitization (i.e., ≥ 1 allergen positive in the skin prick tests), only 71 (78.0%) had elevated eNO levels (> 9.3 p.p.b.), whereas, of the 18 asthmatic children without any allergic sensitization, 10 (55.5%) had increased eNO values ($\chi^2 = 2.88$, $p = \text{n.s.}$) (Fig. 3).

Discussion

Data emerging from the present study show that eNO values are significantly correlated both with total serum IgE and the number of positive skin prick tests in children with asthma. This association was also detected in patients with allergic rhinitis, thus confirming that allergic rhinitis and asthma may be the expression of a common inflammatory process in different sites of the respiratory tract (14). The correlation of eNO with total serum IgE is stronger than, and independent of, allergic sensitization. Indeed, almost all the asthmatic patients with total IgE levels above the cutoff limit (≥ 175 kU l⁻¹) showed eNO values above the upper quartile of eNO distribution in healthy children, regardless of allergen sensitization. However, both the sensitivity and the negative predictive value of total IgE in discriminating between high and low eNO values in asthmatic children were poor, suggesting that many children may have raised eNO levels while lacking total IgE elevation in serum. Total IgE increase and skin prick test positivity seem, therefore, to represent independent, albeit partially related, risk factors for airway inflammation, being probably the expression

Table 3. Correlations among exhaled nitric oxide (Y), total immunoglobulin E (X), and number of positive skin prick tests (Z) in asthmatic and allergic rhinitis children

	Asthma (n = 109)	Allergic rhinitis (n = 41)	Difference between groups§	Whole population (n = 150)
Correlation				
$R(Y, X)^\dagger$	0.42****	0.31***	0.45	0.40****
$R(Y, Z)^\dagger$	0.31****	0.39***	0.37	0.30****
$R(X, Z)^\dagger$	0.33****	0.30***	0.60	0.31****
$R(Y, X Z)^\ddagger$	0.42****	0.45***	0.82	0.30****
$R(Y, Z X)^\ddagger$	0.23**	0.30**	0.65	0.15***
$R(X, Z Y)^\ddagger$	0.18***	0.06*	0.20	0.15***

* $p > 0.35$; ** $p < 0.05$; *** $p < 0.01$; **** $p < 0.0001$.

†Kendall's tau coefficient for crude correlation between two variables.

‡Kendall's tau coefficient for adjusted correlation between two variables controlling for the third.

§p-values for differences between groups (comparison in the correlation coefficients) by bootstrap resampling.

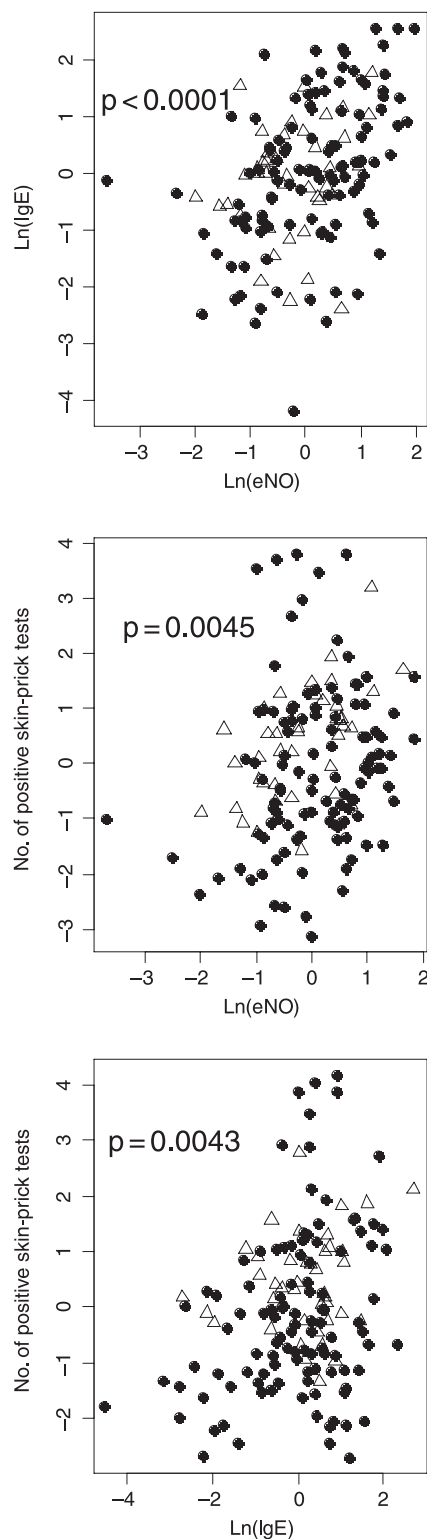


Fig. 1. Scatter plot of the three analyzed variables [eNO (parts per billion; natural log values), total IgE (kU l^{-1} ; natural log values) and number of skin prick tests (observed number of positive skin prick tests)] in study population of patients. Plots refer to adjusted correlation. Asthmatic (dots) and allergic rhinitis (triangles) children are distinguished and p-values come from hypothesis testing for no correlation (see Table 3). Ln, natural log.

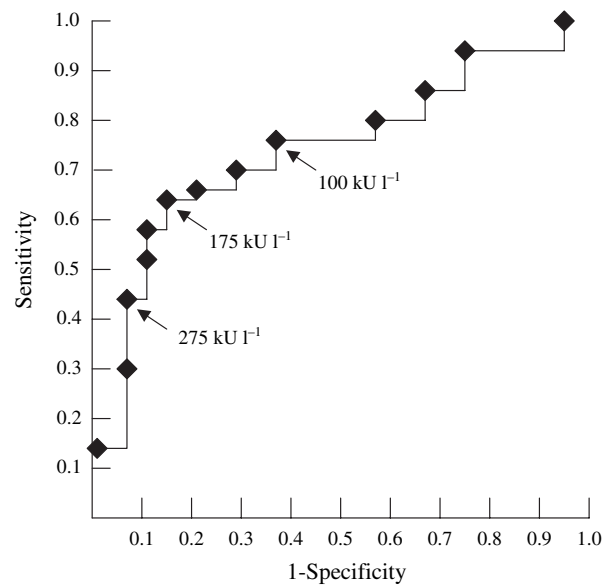


Fig. 2. Receiver operating characteristic (ROC) curve obtained in 109 asthmatic children with different concentrations of total serum IgE by plotting sensitivity and specificity in predicting exhaled nitric oxide (eNO) levels above upper quartile (9.3 p.p.b.) of eNO distribution in controls. Best level to predict an eNO increase is an IgE value of 175 kU l^{-1} .

Table 4. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of total serum IgE and allergic sensitization in discriminating between high (>9.3 p.p.b.) and low (≤ 9.3 p.p.b.) exhaled nitric oxide values in asthmatic children

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Total serum IgE ($\geq 175 \text{ kU l}^{-1}$)	62.9	85.7	92.7	44.4
Allergic sensitization (≥ 1 positive allergen skin prick test)	87.6	28.5	78.0	44.4

of the same phenomenon, i.e., the degree of atopy. It could be also hypothesized that total IgE increase and allergen sensitization may characterize different subtypes of atopic asthma.

The hallmark of atopy is the overproduction of IgE against common environmental allergens. However, evidence exists showing that IgE-mediated mechanisms may operate even in the absence of allergy, and that high total IgE levels represent an independent risk factor for asthma (15, 16). Burrows et al. (17) found the prevalence of asthma was closely related to total serum IgE levels regardless of skin test reactivity to allergens. They speculated that the inflammatory changes in the airway could play a role in the increase of total IgE levels through the milieu of mediators and cytokines generated in the

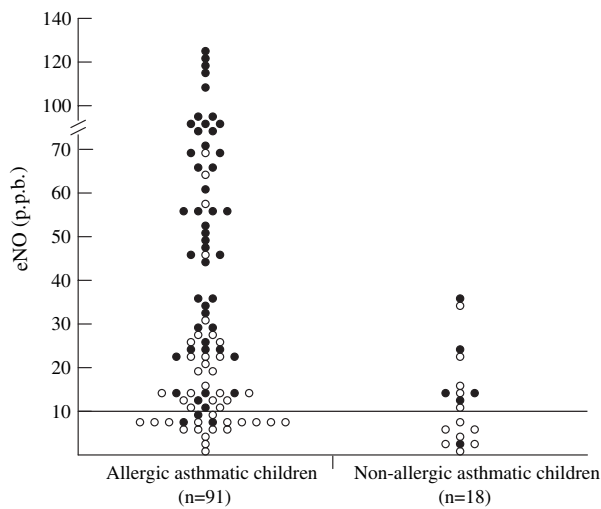


Fig. 3. Exhaled nitric oxide (eNO) in parts per billion (p.p.b.) in children with allergic and non-allergic asthma. Horizontal line represents upper quartile of eNO distribution in controls (9.3 p.p.b.). Closed circles represent children with total IgE ≥ 175 kU l $^{-1}$. Open circles represent children with total IgE < 175 kU l $^{-1}$.

respiratory tract (18). The association of asthma with elevated total IgE has also been demonstrated in children living in a mite-free environment (19), and high levels of total IgE were found to be an independent risk factor for asthma exacerbation (20). One likely explanation is that total serum IgE levels are in some way related to the hallmark of asthma, i.e., AHR, which, in turn, has been attributed to airway inflammation (15, 21).

The significance of increased levels of total serum IgE in individuals without any demonstrable IgE response against common environmental allergens remains speculative. It could simply express an immunological reaction to non-tested allergens or the coexistence of other non-allergic diseases such as parasite infection. It has also been suggested that high total IgE could itself be a marker of airway inflammation in asthmatic patients (15, 18). Humbert et al. (22) showed that the number of Fc ϵ RI-bearing cells in bronchial biopsies both from atopic and non-atopic asthmatic subjects was higher than in normal controls, thus suggesting the possibility of local IgE production in the respiratory mucosa. Further, Kleinjan et al. (23) demonstrated significantly more IgE $^{+}$ B cells and IgE $^{+}$ plasma cells in nasal biopsies from patients with seasonal allergic rhinitis than in non-allergic individuals. These observations support the concept that circulating IgE are not necessarily related to atopic sensitization and that local IgE-mediated mechanisms may occur in airways independently of allergen-specific T-cell/B-cell

interaction. This may explain why eNO levels are more closely correlated with total IgE than with allergic sensitization. In keeping with our data, Strunk et al. (3) recently found that total serum IgE and blood eosinophils, among a variety of biomarkers, better explained eNO variability in children with asthma.

In the present study, eNO levels were significantly higher in asthmatic children and in those with allergic rhinitis than in healthy children. Furthermore, eNO levels were higher in patients with allergic, than with non-allergic, asthma. These findings have been reported by several authors (24–27). When compared to subjects with allergic rhinitis, children with atopic asthma but not those with non-atopic asthma had higher eNO levels.

Previous studies on differences in eNO levels between patients with asthma and allergic rhinitis led to conflicting results (25, 27–29). Various factors may play a role in the discrepancies between different studies, such as the severity of asthma or rhinitis, the rate or type of allergic sensitization and the magnitude of allergen exposure. Prieto et al. (29) found higher eNO levels in asthmatics than in patients with allergic rhinitis, when comparing patients monosensitized to the same allergen. Other authors reported that the difference in baseline eNO between patients with allergic rhinitis and those with asthma, when sensitized to house dust mites, is abolished after specific bronchial challenge (30). These findings stress the similarities in inflammatory changes in subjects with respiratory allergy and suggest that differences in eNO values in allergic individuals, with or without asthmatic symptoms, may be related, primarily, to the level of allergen exposure.

In conclusion, the results of the present study show that increased total IgE levels predict high eNO levels better than allergic sensitization in children with mild intermittent asthma and in those with allergic rhinitis. Moreover, it lends further support to the hypothesis that increased serum IgE could itself be a marker of airway inflammation in patients with allergic respiratory symptoms.

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