Noninvasive methods for the detection of upper and lower airway inflammation in atopic children

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Background: Exhaled nitric oxide (FE_{NO}) and exhaled breath condensate (EBC) are noninvasive methods to assess inflammation.
Objective: To investigate the role of the FE_{NO} and of the EBC pH and IL-5 levels in atopic children.
Methods: We evaluated oral and nasal FE_{NO} and the pH and IL-5 of oral and nasal EBC in children with atopic dermatitis (AD; n = 18), allergic rhinitis (AR; n = 18), intermittent asthma (n = 21), moderate persistent asthma (n = 18), and healthy controls (HCs; n = 16).
Results: Oral FE_{NO} was significantly increased in asthma, whereas the nasal values were increased in AR and asthma in comparison with HCs. The pH of oral EBC was lower in AD and asthma than in AR and HCs, whereas the nasal levels were lower in AD, AR, and asthma than in HCs. The oral IL-5 was higher in AD, AR, and asthma in comparison with HCs, whereas the nasal IL-5 concentrations were higher in asthma and AR than in HCs. In AR, the nasal FE_{NO} correlated with the IL-5 values and with the disease duration. In intermittent asthma, oral and nasal pH inversely correlated with the exacerbations, whereas in moderate asthma, the nasal IL-5 positively correlated with exacerbations. In AD, the oral and nasal IL-5 positively correlated with the serum IgE.
Conclusion: These markers of nasal and bronchial inflammation, accessible with noninvasive techniques, might be useful to identify patients with uncontrolled diseases and to verify the usefulness of new therapeutic approaches.
Clinical implications: These markers are useful tools to monitor the upper and lower airway inflammation in atopic children. (J Allergy Clin Immunol 2006;118:1068-74.)

Key words: *Exhaled nitric oxide, exhaled breath condensate, atopy, airway inflammation, children*

Atopic dermatitis (AD), allergic rhinitis (AR), and asthma have been recently defined as systemic allergic disorders with variable end-organ manifestations.[1,2] AD and AR often coexist with or precede the development of asthma. These atopic diseases share several pathophysiologic features, including inflammatory mediators and allergic triggers.[3,4]

The upper and lower airways classically are the targets of inflammatory processes in AR and asthma, respectively.[3,4] Despite this evidence, inflammatory processes of the lower respiratory tract can be observed in subjects without asthma with AR, and upper airway inflammation may be present in subjects with clinical signs of asthma.[3,5] Furthermore, patients with severe AD and mild asthma have more pronounced late asthmatic responses than patients with asthma without AD.[6] This evidence, taken together, strongly supports the concept of a united airway disease in atopic patients.[2]

Exhaled breath condensate (EBC) and exhaled nitric oxide (FE_{NO}) methods have been recently described as possible tools to assess the inflammatory status in the upper and lower airways and to evaluate the clinical assessment of related diseases[7] despite some unresolved questions about their applications.[8] In particular, the measurement of the oral EBC inversely correlates with other indices of lower airway inflammation, and it decreases during acute exacerbations in asthma.[9,10] FE_{NO} levels are increased in children with asthma, correlate with the number of exacerbations, and are related to the acidification in the airways of subjects with asthma.[10,11] Only a few studies have addressed the comparative use of oral and nasal EBC and FE_{NO} in asthma, AD, and AR.

This work was performed to evaluate the use of noninvasive techniques including the evaluation of oral and nasal FE_{NO} and of the pH and IL-5 levels in the oral and in the nasal EBC for the detection of upper and lower

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Abbreviations used
AD: Atopic dermatitis
AR: Allergic rhinitis
EBC: Exhaled breath condensate
FE_{NO}: Exhaled nitric oxide
HC: Healthy control
ICS: Inhaled corticosteroid

airways inflammation in atopic children with AD, perennial AR, and allergic asthma.

METHODS

Subjects

We studied 18 children with AD, 18 with AR, and 39 with asthma, and 16 age-matched healthy controls (HCs) with no acute respiratory symptoms in the last 4 weeks.

Atopic dermatitis diagnosis was performed at the study entry according to diagnostic criteria of Hanifin and Rajka.[12] AD severity was measured through the SCORAD index.[1] Three children had severe, 8 moderate, and 7 mild AD.

Allergic rhinitis diagnosis was performed at the study entry according to Allergic Rhinitis and its Impact on Asthma guidelines.[13] Six children had intermittent mild, 4 had intermittent moderate severe, 4 had persistent mild, and 4 had persistent moderate severe rhinitis. Patients with AD and AR were enrolled after completing antihistamines or systemic or topical steroid treatment.[14] Children with AD and AR concluded the treatment 3 weeks before entering the study.

Asthma diagnosis and assessment of severity were performed according to Global Initiative for Asthma guidelines.[15] Twenty-one children had intermittent asthma (treated with short-acting β_2-agonists on demand during the last 6 months), and 18 children had moderate asthma (treated with fluticasone proprionate 250 μg inhaler device Diskus 1 a day; GlaxoSmithKline, London, United Kingdom).

During the selection of the patients, 5 patients with unstable rhinitis and 9 patients with unstable asthma were excluded from the study. Unstable disease was defined on the basis of the persistence of nasal or bronchial symptoms with frequent use of rescue medications. Asthma exacerbation was defined as increasing symptoms and signs of asthma (coughing, wheezing, shortness of breath) unresponsive to the patient's routine asthma medication and requiring an unscheduled visit.

No patients had nasal polyposis or bronchial or respiratory tract infections or had a severe exacerbation of asthma resulting in hospitalization during the last month. Within 1 day from the breath condensate collection, all subjects performed pulmonary function tests as recommended by the American Thoracic Society.[16] The study was approved by the local Ethics Committee, and written consent was obtained from parents.

Assessment of atopic status

All subjects were investigated for their clinical history and underwent a careful diagnostic evaluation including skin prick test and total serum IgE measurements. Skin prick test was performed as previously described.[17] House dust mite (*Dermatophagoides pteronyssinus* and *Dermotophagoides farinae*) monosensitized patients were included in the study to select a population as homogeneous as possible and to avoid a bias caused by the seasonal exposure. Serum samples were collected, and total serum IgE and specific IgE levels were determined by CAP System (Pharmacia-Upjohn, Uppsala, Sweden).

Nitric oxide measurement

Oral and nasal FE_{NO} were determined by chemiluminescence (NiOX, Aerocrine, Sweden) following American Thoracic Society recommendations.[18]

To evaluate the nasal FE_{NO}, the nasal samples were obtained from 1 nostril. The patients were instructed to blow up the cheeks to elevate the mouth pressure to isolate the nasal cavity from the rest of the respiratory system.[19] Before the measurements of oral and nasal FE_{NO}, the subjects rinsed out their mouths. The units of FE_{NO} measurements were parts per billion.

EBC collection

The oral EBC was collected using a condenser that allowed the noninvasive collection of the nongaseous components of the expired air (EcoScreen; Jaeger, Wurzburg, Germany). Children were asked to breathe at a normal frequency and tidal volume, wearing a nose clip, for a period of 15 minutes. A temperature of −10°C inside the condensing chamber throughout the collection time produced immediate sample freezing. Samples were stored at −70°C until analysis.

The nasal EBC was collected according to Griese et al,[7] with minor modifications. After applying a nasal mask to the EcoScreen, children were asked to breathe through their noses, with their mouths closed.

Before the collection, the subjects rinsed out their mouths. The oral and nasal condensate, at least 1 mL, were stored at −80°C until analysis.

EBC measurement of pH

A stable pH was achieved in all cases after deaeration/decarbonation of breath condensate specimens by bubbling with argon (350 mL/min) for 10 minutes. pH was then measured within 5 minutes of condensate collection by means of a pH meter (Corning 240; Science Products Division, New York, NY) with a 0 to 14.00 pH range.

EBC measurement of IL-5

Determination of the absolute values of IL-5 in EBC was assessed using commercially available specific ELISAs (Amersham Biosciences, UK Limited, Little Chalfont Buckinghamshire, UK). Limits of detection were less than 2 pg/mL.

Reproducibility

Two successive measurements of oral and nasal FE_{NO}, pH, and IL-5 in EBC of the subjects were assessed for reproducibility by the Bland and Altman test. The degree of reproducibility was expressed as limits of agreements (mean difference + 2 SD) and was as follows: nasal pH, −0.136 and 0.116; exhaled pH, −0.087 and +0.085; nasal FE_{NO}, −21.39 and +19.36; oral FE_{NO}, −1.158 and 1.122; exhaled IL-5, −0.786 and 0.286; nasal IL-5, −0.698 and 0.688. Similar to previous reports, we had an acceptable reproducibility.[20-22]

Data analysis

Data are expressed as mean ± SD. The Kruskal-Wallis test and Dunn procedure were used to compare different groups. The correlations between variables were performed by using Spearman test correlation. The significance was defined as $P < .05$.

RESULTS

Demographic, clinical, and atopic characteristics of the subjects

Demographic, clinical, and atopic characteristics of the subjects enrolled in the study are shown in Table I.

TABLE I. Demographic, clinical, and atopic characteristic of the subjects*

Characteristic	Healthy	AD	AR	Asthma	
				Intermittent	Moderate
Subject number	16	18	18	21	18
Male/female	8/16	11/18	11/18	12/21	12/18
Age (y)	10 ± 2.4	10.1 ± 2.3	9.9 ± 2.9	10 .9 ± 2.3	10.4 ± 2.8
FEV_1 (% predicted)	10 4 ± 7.8	93 ± 6.3	100.7 ± 5	92.3 ± 10.3	87.8 ± 13.3
Total IgE (kU/mL)	87.1 ± 17.1	480.6 ± 297.4	489.7 ± 333.5	771.4 ± 333.8	639.5 ± 231.7
Blood eosinophil number (mm^3)	45.7 ± 17.5	644.6 ± 297.4	585.6 ± 305.3	495.1 ± 209.9	548.6 ± 165.6
Disease duration (y)	None	5.3 ± 2.1	3.6 ± 2.0	4.1 ± 1.2	5.6 ± 2.4
Exacerbation number†	None	None	None	2 ± 0.5	3.8 ± 1.6
RAST to mite (kU/L)	None	24.5 ± 23.8	33.9 ± 32.7	40.5 ± 33.1	42.1 ± 33.5

*Results were expressed as mean ± SD.
†Number of exacerbations during 12 months.

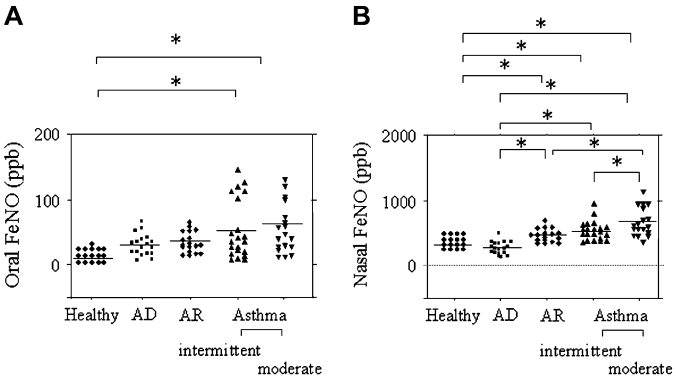

FIG 1. Levels of FE_{NO} of 16 healthy children and 18 with AD, 18 with AR, and 39 with asthma (21 intermittent and 18 moderate). **A,** Levels of oral FE_{NO}. **B,** Levels of nasal FE_{NO}. Individual data are shown. Statistical analysis was by performed Kruskal-Wallis test and Dunn procedure. *$P < .005$.

Exhaled FE_{NO}

The levels of oral FE_{NO} were significantly increased in both (intermittent and moderate) groups with asthma compared with HCs. No significant differences in oral FE_{NO} were found among the AD, AR, and HC subjects. Moreover, no significant differences were found between the 2 groups of subjects with asthma (Fig 1, *A*). The levels of nasal FE_{NO} were significantly increased in AR and in both groups of children with intermittent and moderate asthma with respect to healthy subjects. Significant increase of the nasal FE_{NO} was also found in both groups with asthma compared with AD and AR. Furthermore, statistically significant increase of nasal FE_{NO} was observed in children with moderate asthma with respect to children with intermittent asthma (Fig 1, *B*). The absolute values of nasal and oral FE_{NO} are consistent with previous reports using the same method.[18,19,21]

pH measurements in EBC

The pH levels of oral EBC were significantly lower in children with AD and intermittent and moderate asthma than in HCs. In addition, pH levels were significantly lower in children with intermittent asthma than in children with AD. Furthermore, the oral pH levels were significantly lower in subjects with intermittent and moderate asthma than in subjects with AR. Significantly lower values were also found in children with intermittent than in children with moderate asthma and in AD than in AR (Fig 2, *A*).

The pH levels in nasal EBC were lower in children with AD, AR, intermittent asthma, and moderate asthma than in healthy subjects (Fig 2, *B*).

IL-5 measurements in EBC

The IL-5 levels in oral EBC were significantly higher in subjects with intermittent asthma than in AD, AR, moderate asthma, and HCs (Fig 3, *A*). The concentrations of IL-5 in nasal EBC were significantly higher in children with AR and intermittent and moderate asthma than in HCs. Furthermore, significant differences were found between patients with AD and moderate asthma (Fig 3, *B*).

Correlation among FE_{NO}, pH, IL-5, and biological and clinical outcomes

In AR, the levels of nasal FE_{NO} positively correlated with the levels of IL-5 in nasal EBC (Fig 4, *A*) and with the duration of the disease (Fig 4, *B*).

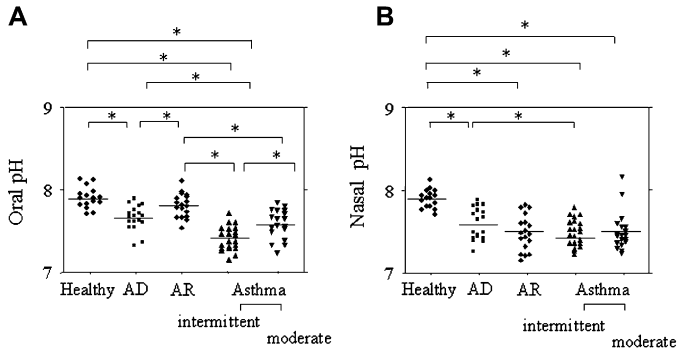

FIG 2. Values of pH oral **(A)** and nasal **(B)** EBC of 16 healthy children, 18 with AD, 18 with AR, and 39 with asthma (21 intermittent and 18 moderate). Individual data are shown. Statistical analysis was performed by Kruskal-Wallis test and Dunn procedure. **P* < .005.

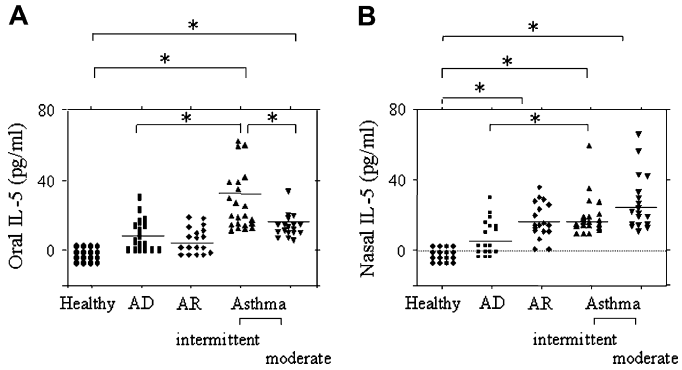

FIG 3. Levels of IL-5 in oral **(A)** and nasal **(B)** EBC of 16 healthy children, 18 with AD, 18 with AR, and 39 with asthma (21 intermittent and 18 moderate). Individual data are shown. Statistical analysis was performed by Kruskal-Wallis test and Dunn procedure. **P* < .005.

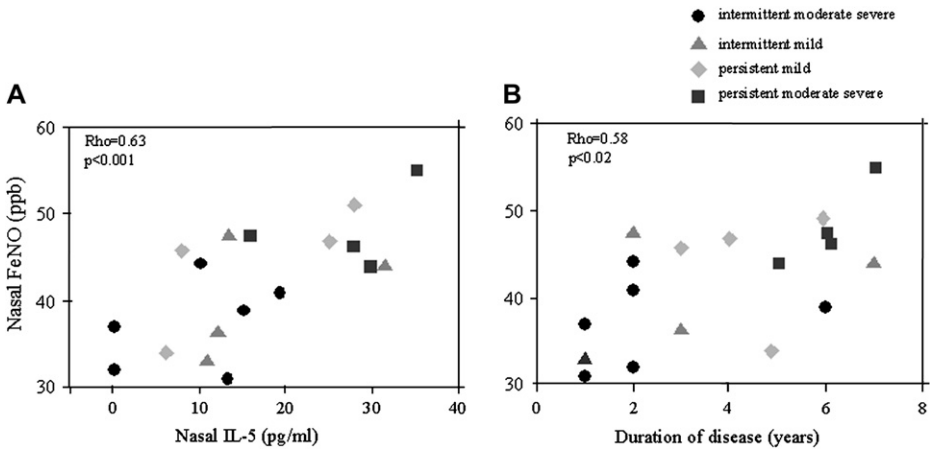

FIG 4. A, Correlation, in 18 children with AR, between nasal FE_{NO} and nasal IL-5 concentrations in EBC. **B,** Correlation between nasal FE_{NO} and the duration of the disease. Individual data are shown. Statistical analysis was performed by using Spearman correlation.

In subjects with intermittent asthma, the presence of exacerbations negatively correlated with the pH values in both oral (Fig 5, *A*) and nasal EBC (Fig 5, *B*). We found a positive correlation between IL-5 concentrations in oral and nasal EBC and the levels of IgE in peripheral blood (Fig 6, *A and B*) in children with AD. Furthermore, in children with moderate asthma, the nasal levels of IL-5 positively correlated with the number of exacerbations (Fig 7). We did not find any correlation between oral or nasal FE_{NO} and IL-5 and the number of asthma exacerbations.

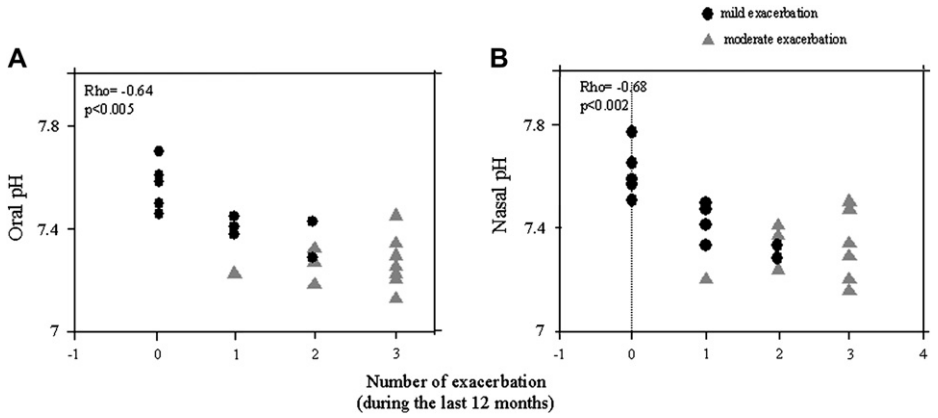

FIG 5. Correlation between the pH values in oral **(A)** and nasal **(B)** EBC with the number of exacerbations in 21 subjects with intermittent asthma. Individual data are shown. *Solid circles,* Children with mild exacerbations. *Solid triangles,* Children with moderate exacerbations. Statistical analysis was performed by using Spearman correlation.

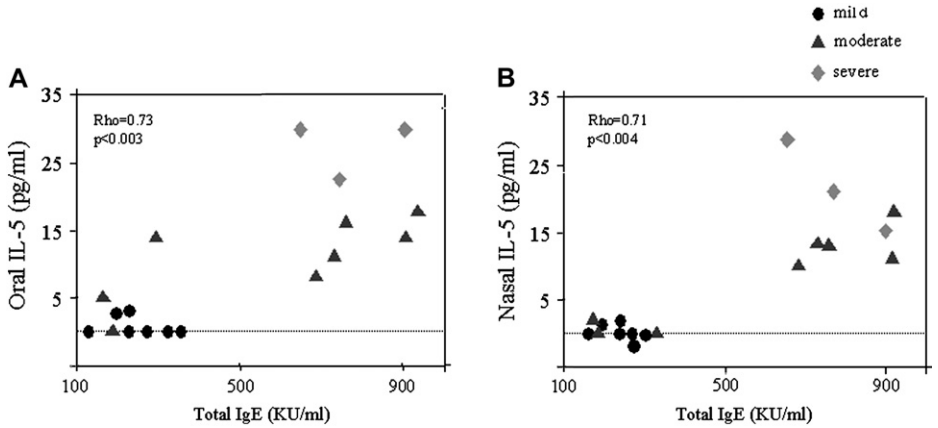

FIG 6. Correlation between the concentration of IL-5 in oral **(A)** and nasal **(B)** EBC with the levels of IgE in peripheral blood in 18 children with AD. Individual data are shown. *Solid circles,* Children with mild AD. *Solid triangles,* Children with moderate AD. *Solid squares,* Children with severe AD. Statistical analysis was performed by using Spearman correlation.

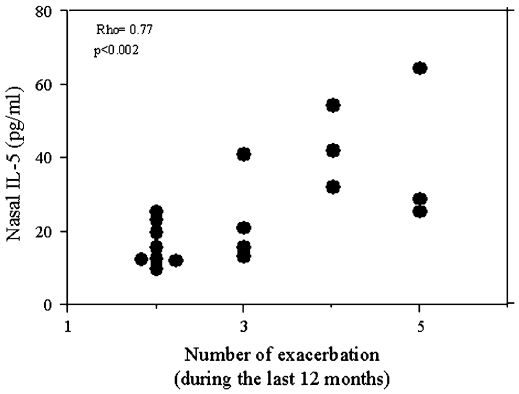

FIG 7. Correlation between the levels of IL-5 in nasal EBC with the number of exacerbations in 18 children with moderate asthma. Statistical analysis was performed by using Spearman correlation.

DISCUSSION

Exhaled nitric oxide and EBC are noninvasive techniques for the assessment of nasal and oral airway inflammation in the atopic subjects with and without asthma.[7,23] The measurement of inflammatory parameters in the nasal and in the bronchial compartments may provide information about the general inflammatory status and about the requirement of a local anti-inflammatory therapy in the absence of clinical evidence.

In this study, we focused our attention on the role played by the FE_{NO} and the levels of pH and IL-5 in the EBC in the upper and lower airways of AD, AR, and asthma. We observed increased levels of oral and nasal FE_{NO} in children with asthma but not in children with AD and AR. Lower pH values were observed in the oral and nasal EBC of children with asthma, AD, and AR. Moreover, the levels of oral IL-5 were increased in children with intermittent asthma, whereas the levels of

nasal IL-5 were increased in AR except in children with asthma.

Exhaled nitric oxide may be used as a sensitive, noninvasive, and reproducible marker of asthma activity[21] that correlates well with eosinophilic airway inflammation. In addition, FE_{NO} levels seem to be associated with a mechanism linking atopy and airway responsiveness in diseases such as AD and AR without symptoms of bronchial obstruction.[24] Recent data showed a relationship among FE_{NO}, respiratory symptoms, lung function, bronchial responsiveness, blood eosinophilia, and high serum levels of total IgE.[25] According to these concepts, our observations of the higher levels of oral and nasal FE_{NO} in patients with asthma are probably a result of the elevated grade of atopy (high levels of serum IgE).[25] Interestingly, the observed absence of a significant difference in oral FE_{NO} between children with intermittent and moderate asthma demonstrates that despite the use of inhaled corticosteroids (ICSs), increased levels of markers of oxidative stress and elevated levels of eosinophils persist in some of these patients. These findings suggest the possibility to use this methodology in children to monitor the efficacy of the therapy on this specific parameter.[26] Moreover, the trend to the increase of oral FE_{NO} in AD and AR with respect to controls supports the hypothesis that this marker might predict and monitor the increased risk to develop bronchial inflammation in atopic children[4] even in the absence of symptoms of asthma.[23]

The concept of an association between the FE_{NO} and the airway inflammation in children is supported by the presence, in AR, of a significant increase of nasal FE_{NO} and of a positive correlation between the nasal FE_{NO} and the levels of nasal IL-5 in EBC. Accordingly, we also found a positive correlation between the nasal FE_{NO} and the duration of rhinitis. These observations further support the concept of the role of the nasal FE_{NO} detection as reliable noninvasive marker useful to monitor the nasal inflammation[5] to predict the risk for developing asthma.[27] The apparent discordance between oral and nasal FE_{NO} and EBC pH in asthma and AD is probably a result of the different causes of modification of the 2 parameters. FE_{NO} is principally a product of activated macrophages, eosinophils, and airway epithelial cells,[28] and the variation of pH is caused by a wide variety of inflammatory pathways.[29] Finally, the increased levels of nasal FE_{NO} observed in patients with moderate asthma are related to a persistent inflammation in the upper airways, suggesting the need to use a nasal anti-inflammatory therapy in these subjects.[3] We did not find any correlations between oral or nasal FE_{NO} and the number of asthma exacerbations, possibly because there are some patients with values overlapping with control subjects. Further studies are necessary to verify the possible use of the measurement of nasal and oral FE_{NO} to predict the risk of exacerbations.[30]

The measurement of the pH values is considered a readily measurable marker of airway disease.[10] Accordingly, we showed lower values of pH in patients with intermittent and moderate asthma than in the other groups of patients and in controls. The relative low increase of the pH values in the oral EBC from patients with moderate asthma with respect to patients with intermittent asthma are probably related to the use of oral ICSs in the conventional treatment of these patients, with a consequent partial reduction of the bronchial inflammatory status.[10] The absence of a statistical difference in the nasal EBC pH values between these 2 groups of subjects, together with the presence of a difference between AD and controls, further supports this hypothesis. It is accepted that the lower values of pH are associated with the presence of an eosinophilic inflammation of the airways in asthma.[9] This concept was expanded by the observed relationship of oral and nasal pH values with the presence of exacerbations. The findings that oral and nasal pH values are lower in AD and AR confirm that in atopics, the presence of the airway inflammation may be considered a marker of progression toward the development of bronchial asthma.[2-5] Moreover, this noninvasive technique can be used to monitor the ongoing nasal and bronchial inflammatory status in children with atopic diseases including rhinitis and asthma. These observations support the concept that subjects with asthma should be well evaluated and treated for rhinitis. This combined nasal and bronchial therapeutic strategy proposed in adults[2,3] should be ideally used for the treatment of upper and lower airway inflammation in children.

IL-5 actively contributes to the local infiltration and activation of eosinophils in asthma and in other allergic diseases.[31] In this study, we observed the relevant contribution of IL-5 in lower and upper airway inflammation associated with asthma. The relatively low concentrations of IL-5 in the oral EBC from patients with moderate asthma with respect to patients with intermittent asthma is probably related to the local treatment with ICSs in patients with moderate asthma. Accordingly, the levels of IL-5 in the nasal EBC of the same patients were further increased with respect to the other groups of subjects. The IL-5 concentrations in both the upper and the lower airways had been demonstrated to be related to the degree of eosinophilic inflammation in asthma.[9] Moreover, previous studies reported high levels of nasal IL-5 in atopic children with asthma and rhinitis,[32] confirming the relevant contribution of upper airway inflammation in asthma.[3] In our children with moderate asthma, the positive correlation between the nasal IL-5 levels and the number of exacerbations of patients with moderate asthma suggests that the persistent uncontrolled inflammation of the upper airways may represent a potential trigger for exacerbations in oral ICS-treated children with asthma. Furthermore, the detection of high concentrations of IL-5 in nasal EBC from children with moderate asthma might be considered a marker of the persistence of airway inflammation, which might lead to the development of exacerbations in this specific group of patients. We did not find any correlations between oral or nasal FE_{NO} or IL-5 and the number of asthma exacerbations. The trend toward increased levels of oral and nasal IL-5 in AD with respect to controls suggests that IL-5 plays a role in eosinophilic inflammation in AD, involving both the upper and lower airways. This

fact was suggested by the positive correlation between oral and nasal IL-5 with the total levels of IgE in peripheral blood of children with AD in association with the degree of the disease. Subjects with allergy were characterized by increased levels of IL-4 and IL-5. The elevated levels of IL-4 may generate the elevated IgE levels found in these subjects.[33]

Taken together, these observations underline the concept that high levels of IL-5 associated with high levels of IgE and a higher degree of eczema in AD may be predictive for a clear risk of inflammation of the upper and lower airways marching to the development of rhinitis and asthma.[34,35]

In conclusion, this study shows a relevant contribution of FE_{NO} and of some mediators present in EBC as markers of upper and lower airway inflammation in children with atopic diseases. The presence of low values of these markers in a subgroup of patients overlapping with the normal subjects suggests the need for further studies to identify a cutoff to define the normal and the pathologic ranges of these parameters. To our knowledge, this is the first report suggesting the potential use of these noninvasive techniques for the identification of children with atopic disorders different from asthma to develop bronchial asthma and to predict, in children with asthma, the development of an exacerbation.

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