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# Clinical Correlates and Determinants of Airway Inflammation in Pediatric Asthma

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## Abstract

*Background:* Airway inflammation is a key component in the pathophysiology of asthma. However, neither its role in the clinical features of asthma nor the factors affecting the degree of inflammation have been fully defined.
*Methods:* We determined the fractional exhaled nitric oxide concentration ($FE_{NO}$) using a portable device (NIOX-MINO, Aerocrine, Solna, Sweden) in a consecutive sample of 149 asthmatic children aged 6 to 14 years. In order to establish an association with $FE_{NO}$, we analyzed symptoms, spirometric parameters before and after a bronchodilator test, and the impact of asthma on quality of life during the previous 4 weeks. We also investigated how clinical variables that regulate inflammation affected $FE_{NO}$.
*Results:* In patients not treated with inhaled corticosteroids (ICs), $FE_{NO}$ was higher when specific symptoms (wheeze and cough) had been present during the previous 4 weeks; however, we were unable to establish a relationship with symptom frequency, bronchodilator use, asthma crises, hospital admissions, limitation of daily activities, or spirometry results. In patients treated with ICs, $FE_{NO}$ was not related to the clinical expression of asthma, except for a reduced ratio of forced expiratory volume in 1 second to forced vital capacity, both before and after bronchodilation. The main determinant of $FE_{NO}$ level in untreated patients was sensitization to house dust mite. In patients treated with ICs, $FE_{NO}$ was only associated with adherence to therapy.
*Conclusion:* Airway inflammation, as determined by $FE_{NO}$, is only weakly associated with the clinical expression of asthma and spirometry results. Adherence to treatment is the main determinant of the degree of inflammation in patients taking ICs.

**Key words:** Asthma. Nitric oxide. Inflammation. Spirometry. Cross-sectional studies. Signs and symptoms, respiratory. Child. Adolescent.

## Resumen

*Antecedentes:* La inflamación de la vía aérea es un componente fundamental en la fisiopatología del asma, pero aún no se han definido ni su influencia en las manifestaciones clínicas del asma ni los factores que determinan su intensidad.
Métodos: En una muestra consecutiva de 149 niños con asma de 6-14 años, se determinó la fracción exhalada de óxido nítrico ($Fe_{NO}$) mediante un dispositivo portátil (NIOX-MINO®). Se analizó la relación de la $Fe_{NO}$ con los parámetros de la espirometría basal y post-broncodilatación, los síntomas y el impacto del asma sobre las actividades cotidianas referidos a las cuatro semanas previas. Se investigó la influencia sobre la $Fe_{NO}$ de variables que podrían determinar la intensidad de la inflamación de la vía aérea.
Resultados: En los pacientes no tratados con corticoides inhalados (ICS), la $Fe_{NO}$ estaba más elevada cuando algunos síntomas específicos (sibilantes, tos) habían estado presentes en las cuatro semanas previas, pero no había otras asociaciones con la frecuencia de los síntomas, uso de broncodilatador, crisis de asma, hospitalizaciones, limitación de actividades cotidianas o resultados de la espirometría. En los pacientes tratados con ICS, la $Fe_{NO}$ no estaba relacionada con ninguna variable de expresión clínica del asma, excepto con una relación $FEV_1$/FVC disminuida, tanto basal como tras broncodilatación. El principal determinante del nivel de $Fe_{NO}$ en pacientes no tratados fue la sensibilización a ácaros del polvo. En pacientes tratados con ICS, la $Fe_{NO}$ dependía sólo de la adherencia terapéutica.
Conclusión: La inflamación de la vía aérea, determinada mediante $Fe_{NO}$, tiene una relación limitada con la expresión clínica del asma y con la espirometría. El principal determinante de la intensidad de la inflamación en pacientes tratados con ICS es la adherencia terapéutica.

**Palabras clave:** Asma. Óxido nítrico. Inflamación. Espirometría. Estudios transversales. Signos y síntomas respiratorios. Niño. Adolescente.

## Introduction

Asthma is one of the most common diseases in children and adolescents. The current perception of this disease is based on the simultaneous presence of predominantly eosinophilic airway inflammation and reversible airflow obstruction [1]. Asthma therapy targets both these features, mainly through the use of anti-inflammatory drugs such as inhaled corticosteroids (ICs) and bronchodilators ($ß_2$-adrenergic agonists). Whereas airflow obstruction is measured using lung function tests, inflammation could traditionally only be measured in asthmatic children using invasive methods. Nowadays, the fractional exhaled nitric oxide concentration ($FE_{NO}$) is used to assess airway inflammation in children and adults [2,3]. New portable devices [4] make the study of airway inflammation easy.

Use of $FE_{NO}$ as an inflammatory marker makes it possible to assess the clinical correlates of airway inflammation in unselected populations of asthmatic patients. While airway inflammation plays a key role in the pathogenesis of asthma, it is less clear whether symptom intensity or poor lung function is associated with severe inflammation. Some studies have found an association between higher levels of inflammatory markers and more severe clinical expression of asthma [5-8]. However, asthma symptoms not controlled by ICs are better managed by adding long-acting bronchodilators than by increasing the IC dose [9], thus pointing to an important role for noninflammatory airway narrowing in the clinical expression of asthma.

Establishing the main determinants of airway inflammation will allow us to modify therapy and thus better control symptoms.

This study had 2 objectives: to document the clinical features of airway inflammation in asthma by determining the extent to which manifestations of asthma correlate with the underlying inflammation, and to establish the main determinants of airway inflammation in clinical practice. We performed our study in an unselected population of asthmatic children and adolescents.

## Patients and Methods

We invited 151 children aged 6 to 14 years from 7 Spanish primary care centers to participate in the study. Only those with a valid $FE_{NO}$ measurement were included. The final study sample was composed of 149 asthmatic children. Asthma was physician-diagnosed on the basis of the presence of classic asthma symptoms and at least 1 of the following criteria: a positive reversibility test (>12% increase in forced expiratory volume in 1 second [$FEV_1$] after 400 µg of salbutamol), previous hospitalization due to asthma, or 2 or more visits to the emergency room due to asthma. We recruited the study population between September 2007 and March 2008 from children attending the primary care center for any reason, irrespective of whether it was related to asthma. The exclusion criteria were occurrence of a severe asthma attack, inability to complete an acceptable $FE_{NO}$ maneuver, and the presence of other acute or chronic lung disease. The protocol of this study was approved by the Ethics Committee of the Public Health Service of the Principado de Asturias, Spain. Parents and patients gave their written informed consent.

$FE_{NO}$ was measured using a portable nitric oxide analyzer (NIOX MINO, Aerocrine, Solna, Sweden), which provides $FE_{NO}$ measurements at a flow rate of 50 mL/s [4]. The mean of 2 consecutive measurements, in parts per billion (ppb), was accepted as the $FE_{NO}$ value. Patients then underwent a spirometry test [10] and a bronchodilator test by inhaling 400 µg of salbutamol through a spacer chamber. We performed pulmonary function testing both before and after the salbutamol challenge and obtained the following: $FEV_1$, the ratio of $FEV_1$ to forced vital capacity (FVC) and the forced expiratory flow, midexpiratory phase ($FEF_{25-75}$) [11]. Baseline values of $FEV_1$ <80% predicted, $FEV_1$/FVC <75%, and $FEF_{25-75}$ <65% were considered to be low [12]. A positive bronchodilator challenge was defined as an increase >12% in $FEV_1$.

Each patient underwent allergy testing (prick test or specific immunoglobulin [Ig] E in serum) as part of the comprehensive asthma management plan applied in all of the study centers. The centers did not identify which method was used, although the diagnostic criteria were common and previously defined. Allergic sensitization was defined as a specific IgE titer >0.35 $kU_A/L$ or a prick test with a wheal diameter of at least 3 mm (with a positive histamine control and negative saline control). All the patients underwent testing for sensitization to house dust mite, and between 82.6% and 98.7% were tested for sensitization to mould or pollen (grass, tree, weed).

The study pediatrician, who was blind to the $FE_{NO}$ result, conducted a clinical interview, medical chart review, and physical examination. The data collected on clinical status during the previous 4 weeks were as follows: *1)* occurrence of specific asthma symptoms (cough, wheeze, dyspnea, chest tightness); *2)* frequency of daytime symptoms (never, sometimes, ≤2/wk, ≥3/wk, daily) and nighttime symptoms (never, <1/wk, ≥1/wk); *3)* frequency of rescue bronchodilators (never, sometimes, 1/wk, ≥2/wk); *4)* asthma attacks, defined as any worsening of the previous status; *5)* frequency (never, sometimes, frequently) of asthma-related limitations to daily activities (physical activity, school absenteeism, parental absenteeism from work); *6)* use of health care resources (unscheduled visits to the doctor or emergency room due to asthma, hospitalizations due to asthma).

The study pediatrician rated asthma severity and control according to the Global Initiative for Asthma (GINA) guidelines [1] and collected data on the following: present treatment with antileukotrienes or ICs, IC dose (budesonide-equivalent), adherence to therapy (rated as good if over 75% of the prescribed doses were taken, as reported by the patient and parents), physician-diagnosed allergic rhinitis with current activity, nasal or systemic corticosteroids in the previous 4 weeks, and implementation of house dust mite avoidance measures in the patient's bedroom (considering "complete avoidance" the application of daily dust cleaning, washing of sheets and blankets in hot water [≥60º], and use of allergen-impermeable covers for mattresses and pillows). The study pediatrician established the presence of current wheeze or respiratory infection by means of a physical examination.

### *Statistical Analysis*

As $FE_{NO}$ was nonnormally distributed, it was described by means of the median and interquartile range (IQR). The association between $FE_{NO}$ and clinical variables was analyzed using nonparametric tests (Mann-Whitney and Kruskal-Wallis). Separate analyses were performed for patients with or without IC treatment.

The main determinants of $FE_{NO}$ level were investigated through an exploratory multiple linear regression analysis. Natural logarithmic transformation of $FE_{NO}$ ($lnFE_{NO}$) was used as an independent variable, and fixed and random independent variables were defined. Age, gender, and body mass index were included as fixed terms [13]. Random variables were tested using a stepwise approach, with an inclusion probability of 0.05 and exclusion probability of 0.1. Tested variables were selected on a theoretical basis, including those that might cause changes in $FE_{NO}$: sensitization to house dust mite (the main allergen associated with asthma symptoms in winter), complete house dust mite avoidance in the bedroom (see above), exposure to tobacco smoke at home, currently active allergic rhinitis, current respiratory infection, treatment with systemic corticosteroids in the previous 4 weeks, treatment with antileukotrienes for the previous 4 weeks, nasal corticosteroids in the previous 4 weeks, and asthma control status (GINA classification). Separate regression models were constructed for patients with or without IC treatment during the preceding 4 weeks. In the treated patient model, a further 2 random variables were added: daily IC dose (≤200, 201-400, and >400 μg budesonide-equivalent/d) and good adherence to therapy (taking ≥75% of the prescribed doses).

Statistical significance was set at an $\alpha$ level of .05. The results of the analysis carried out with $lnFE_{NO}$ were transformed to $FE_{NO}$ (ppb) to make them easier to interpret.

Table 1. Population Data

| | N=149 |
|---|---|
| Age, y, mean (SD), range | 10.1 (2.1), 6-14 |
| Years with asthma (mean, SD) | 5.5 (3.0) |
| Male, % | 62.4 |
| Weight, kg, mean (SD) | 41.2 (12.7) |
| Height, cm, mean (SD) | 143.7 (13.6) |
| Body mass index, kg/m$^2$, mean (SD) | 19.5 (3.5) |
| Allergic sensitization, % | |
| Any | 94.0 |
| House dust mite | 80.5 |
| Complete house dust mite avoidance measures, %[a] | 33.6 |
| Smoking at home, % | 60.1 |
| Current respiratory infection, % | 11.4 |
| Active allergic rhinitis, % | 35.6 |
| Inhaled corticosteroids (previous 4 weeks), % | 12.2 |
| Current IC treatment, % | 45.0 |
| IC dose (budesonide-equivalent) | |
| ≤200 μg/d, % | 32.8 |
| 201-400 μg/d, % | 56.7 |
| >400 μg/d, % | 10.4 |
| Good adherence to therapy, %[b] | 82.6 % |
| Asthma severity (GINA classification) | |
| Intermittent, % | 47.0 |
| Mild-persistent, % | 35.6 |
| Moderate-persistent, % | 17.4 |

Abbreviations: GINA, Global Initiative on Asthma; IC, inhaled corticosteroids.
[a] Complete house dust mite avoidance measures include daily dust cleaning, washing of sheets and blankets in hot water (≥60°C), and use of allergen-impermeable covers for mattress and pillows.
[b] ≥75% of prescribed doses

## Results

We obtained a valid $FE_{NO}$ measurement from 149 of the 151 patients (98.7%) invited to participate in the study (Table 1). Most of the patients (78%) were studied in the last 4 months of the year (September to December), and 91% were studied at a scheduled visit for asthma. The distribution of the 2 mean $FE_{NO}$ values was very skewed, as many patients had low values: median 33.0 ppb (IQR, 18.0-59.5; range 4.5-217.5). Sixty-seven patients (45%) were taking ICs. Treatment with these agents was related to greater severity of asthma ($\chi^2$=13.1; *P*=.001) and to a higher proportion of patients having uncontrolled or partly controlled asthma ($\chi^2$=9.2; *P*=.010). Children taking ICs had lower $FE_{NO}$ values than untreated children: median 27.5 ppb (IQR, 15.0-51.5) vs 41.8 ppb (IQR, 19.9-62.5; *P*=.044).

For the whole sample, $FE_{NO}$ level was not related to asthma control, use of health care resources, limitation of daily activities, or clinical variables. Only cough in the preceding 4 weeks was associated with a higher $FE_{NO}$ level: median 38.5 ppb (IQR, 19.6-64.0) vs 27.5 ppb (IQR, 11.5-51.3); *P*=.041. $FEV_1$, $FEF_{25-75}$, and $FEV_1$ values after the challenge with salbutamol were not associated with $FE_{NO}$ level, although patients with reduced $FEV_1$/FVC had higher $FE_{NO}$ levels, both before and after inhalation: before, median 64.0 ppb (IQR, 33.1-77.8) vs 32.5 (IQR, 16.9-56.6); *P*=.023; after, median 61.3 ppb (IQR, 48.0-106.5) vs 32.8 (IQR, 17.0-57.1); *P*=.021.

When we performed the analysis separately for treated and untreated patients, clinical expression of asthma was related to level of inflammation only in untreated patients, who had higher $FE_{NO}$ values associated with the occurrence of cough or wheeze in the previous weeks (Table 2). Physical examination, asthma control status, effect on daily activities, and use of health care resources were not associated with $FE_{NO}$ level in treated or untreated patients (Table 3).

Lung function was associated with higher $FE_{NO}$ values only in children treated with ICs (Table 4). Reduced $FEV_1$/FVC (before or after the bronchodilator test) was the spirometric parameter most strongly related to inflammatory measurements. A low $FEV_1$ (baseline or after salbutamol) was independent of $FE_{NO}$ level.

Table 2. Relationships Between $FE_{NO}$ and Clinical Expression of Asthma in Patients With and Without Inhaled Corticosteroid Treatment

| Current Manifestations (Previous 4 Weeks) | | $FE_{NO}$, ppb | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Without IC Treatment (n=82) | | | | With IC Treatment (n=67) | | | |
| | | % | Median | IQR | *P* | % | Median | IQR | *P* |
| *Symptoms* | | | | | | | | | |
| Any symptoms | Yes | 73.8 | 48.0 | 24.5-65.0 | .150[a] | 81.8 | 27.0 | 15.4-61.8 | .265[a] |
| | No | 26.2 | 28.0 | 15.5-58.8 | | 18.2 | 25.5 | 10.6-42.0 | |
| Dyspnea | Yes | 34.1 | 52.5 | 18.8-66.5 | .425[a] | 34.3 | 37.5 | 12.0-72.0 | .164[a] |
| | No | 65.9 | 35.0 | 20.4-61.4 | | 65.7 | 24.0 | 15.6-44.1 | |
| Wheeze | Yes | 36.6 | 54.8 | 29.4-65.0 | .039[a] | 35.8 | 22.5 | 14.3-62.0 | .596[a] |
| | No | 63.4 | 33.0 | 17.3-59.6 | | 64.2 | 27.5 | 15.5-51.0 | |
| Cough | Yes | 65.9 | 48.5 | 24.5-65.5 | .049[a] | 74.6 | 27.0 | 15.9-61.8 | .338 |
| | No | 34.1 | 26.3 | 13.6-56.6 | | 25.4 | 27.5 | 10.8-45.0 | |
| Chest tightness | Yes | 11.6 | 44.5 | 19.6-59.8 | .851[a] | 9.8 | 16.3 | 8.1-41.5 | .175[a] |
| | No | 88.4 | 44.0 | 22.3-65.5 | | 90.2 | 27.5 | 15.0-53.0 | |
| *Frequency of daytime symptoms* | | | | | | .380[b] | | | .083[b] |
| Never | | 37.8 | 33.8 | 17.1-62.8 | | 25.8 | 23.5 | 12.5-39.5 | |
| Sometimes | | 25.6 | 41.8 | 18.9-51.1 | | 28.8 | 49.0 | 18.5-68.5 | |
| ≤ 2/wk | | 17.1 | 57.0 | 31.5-83.0 | | 28.8 | 26.5 | 14.5-61.0 | |
| ≥ 3/wk | | 13.4 | 58.0 | 24.5-65.0 | | 16.7 | 17.0 | 9.5-32.5 | |
| Daily | | 6.1 | 32.5 | 24.5-91.8 | | | 0.0 | | |
| *Frequency of nighttime symptoms* | | | | .211[b] | | | | .606[b] | |
| Never | | 75.0 | 44.0 | 19.6-66.5 | | 62.1 | 27.5 | 15.0-56.3 | |
| <1/wk | | 17.5 | 52.0 | 33.0-63.9 | | 18.2 | 32.0 | 13.5-54.4 | |
| ≥1/wk | | 7.5 | 26.8 | 16.0-38.1 | | 19.7 | 21.0 | 12.0-51.0 | |
| *Frequency of ß$_2$-adrenergic agonists* | | | | | .313[b] | | | | .159[b] |
| Never | | 46.3 | 32.5 | 17.5-61.0 | | 36.4 | 25.5 | 13.8-40.9 | |
| Sometimes | | 26.3 | 51.5 | 28.8-88.8 | | 18.2 | 43.5 | 21.0-64.0 | |
| 1/wk | | 10.0 | 50.8 | 38.6-60.0 | | 10.6 | 49.0 | 23.0-68.5 | |
| ≥2/wk | | 17.5 | 42.3 | 28.9-62.5 | | 34.8 | 20.0 | 9.5-56.0 | |
| *Asthma crisis* | Yes | 25.0 | 53.3 | 23.0-64.3 | .397[a] | 34.3 | 21.0 | 12.0-56.0 | .240[a] |
| | No | 75.0 | 35.5 | 19.6-59.9 | | 65.7 | 32.0 | 16.1-51.4 | |
| *Physical examination* | | | | | | | | | |
| Wheeze | Yes | 11.0 | 51.5 | 22.0-73.3 | .543[a] | 9.0 | 52.5 | 33.1-64.0 | .113[a] |
| | No | 89.0 | 39.5 | 19.5-62.3 | | 81.0 | 24.5 | 14.3-50.3 | |
| *Asthma control (GINA classification)* | | | | | .430[b] | | | | .454[b] |
| Controlled | | 42.7 | 44.0 | 23.0-75.0 | | 19.4 | 27.5 | 13.8-39.5 | |
| Partly controlled | | 37.8 | 33.0 | 18.0-58.0 | | 53.7 | 25.0 | 15.3-50.6 | |
| Uncontrolled | | 19.5 | 42.3 | 25.6-67.0 | | 26.9 | 37.3 | 15.8-65.0 | |

Abbreviations: $FE_{NO}$, fractional exhaled nitric oxide concentration; GINA, Global Initiative on Asthma; IC, inhaled corticosteroid; IQR, interquartile range; ppb, parts per billion.
[a] Mann-Whitney test.
[b] Kruskal-Wallis test.

Table 3. Relationships Between $FE_{NO}$ and Burden of Asthma in Patients With and Without Inhaled Corticosteroid Treatment

| Current Manifestations (Previous 4 Weeks) | $FE_{NO}$, ppb | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Without IC Treatment (n=82) | | | | With IC Treatment (n=67) | | | |
| | % | Median | IQR | *P* | % | Median | IQR | *P* |
| *Limitation of daily activities* | | | | .840[a] | | | | .050[a] |
| Never | 65.9 | 39.8 | 17.8-67.3 | | 59.7 | 23.3 | 15.1-41.9 | |
| Sometimes | 22.0 | 37.5 | 23.3-57.0 | | 25.4 | 51.5 | 23.8-70.3 | |
| Frequently | 12.2 | 54.5 | 19.8-62.5 | | 14.9 | 17.0 | 9.3-69.4 | |
| *Absenteeism, school* | | | | .502[a] | | | | .218[a] |
| Never | 90.2 | 45.8 | 19.4-65.5 | | 82.1 | 27.5 | 15.5-51.0 | |
| Sometimes | 8.5 | 33.0 | 32.5-44.0 | | 14.9 | 21.5 | 14.1-69.0 | |
| Frequently | 1.2 | [b] | [b] | | 3.0 | 10.8 | 9.5- [b] | |
| *Absenteeism, work* | | | | .504[a] | | | | .946[a] |
| Never | 95.1 | 44.0 | 20.4-64.2 | | 93.9 | 27.0 | 15.4-49.9 | |
| Sometimes | 3.7 | 33.0 | 18.5-[b] | | 6.1 | 38.0 | 11.3-67.0 | |
| Frequently | 1.2 | [b] | [b] | | 0.0 | [b] | [b] | |
| *Nonscheduled asthma visits* | | | | | | | | |
| Yes | 7.3 | 61.3 | 30.9-91.5 | .130[c] | 9.0 | 14.0 | 9.3-36.9 | |
| No | 92.7 | 37.5 | 19.1-60.5 | | 91.0 | 27.5 | 16.3-52.3 | |
| *Hospitalization* | | | | | | | | |
| Yes | 0.0 | [b] | [b] | [b] | 3.0 | 10.8 | 9.5- [b] | |
| No | 100.0 | 41.8 | 19.9-62.5 | | 97.0 | 27.5 | 15.8-52.3 | |

Abbreviations: $FE_{NO}$, fractional exhaled nitric oxide concentration; GINA, Global Initiative on Asthma; IC, inhaled corticosteroid; IQR, interquartile range; ppb, parts per billion.
[a] Kruskal-Wallis test.
[b] Insufficient number of patients in the stratum.
[c] Mann-Whitney test.

Table 4. Relationship Between $FE_{NO}$ and Lung Function in Patients With and Without Inhaled Corticosteroid Treatment

| | | $FE_{NO}$, ppb | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Without IC Treatment (n=82) | | | | With IC Treatment (n=67) | | | |
| | | % | Median | IQR | *P* | % | Median | IQR | *P* |
| *Pre-BD lung function* | | | | | | | | | |
| Low $FEV_1$ (<80%) | Yes | 13.4 | 28.0 | 18.0-62.0 | .337 | 9.1 | 28.5 | 19.5-64.0 | .803 |
| | No | 86.6 | 44.0 | 20.5-64.0 | | | 90.9 | 27.5 | 14.6-51.4 |
| Low $FEV_1$/FVC (<75%) | Yes | 7.3 | 49.3 | 26.3-88.0 | .413 | 12.1 | 64.0 | 42.1-74.0 | .019 |
| | No | 92.7 | 41.8 | 19.6-60.5 | | 87.9 | 24.0 | 14.4-46.4 | |
| Low $FEF_{25-75}$ (<65%) | Yes | 15.0 | 44.0 | 21.1-65.4 | .808 | 20.0 | 56.0 | 23.8-70.8 | .027 |
| | No | 85.0 | 41.8 | 19.6-63.5 | | 80.0 | 25.0 | 14.1-47.9 | |
| *Post-BD lung function* | | | | | | | | | |
| Positive BD test[b] | Yes | 15.0 | 53.0 | 29.9-80.4 | .236 | 16.9 | 44.5 | 20.0-64.0 | .217 |
| | No | 85.0 | 37.5 | 19.6-58.4 | | 83.1 | 25.5 | 13.9-49.9 | |
| Low $FEV_1$ (<80%) | Yes | 5.0 | 19.0 | 15.8-44.0 | .160 | 7.7 | 26.5 | 16.0-50.8 | .952 |
| | No | 95.0 | 44.0 | 21.1-63.5 | | | 92.3 | 27.5 | 14.6-51.4 |
| Low $FEV_1$/FVC (<75%) | Yes | 2.5 | 55.0 | 51.5-[c] | .155 | 6.3 | 66.8 | 44.1-180.5 | .020 |
| | No | 97.5 | 37.5 | 19.9-62.5 | | 93.7 | 25.5 | 14.6-49.4 | |
| Low $FEF_{25-75}$ (<65%) | Yes | 2.6 | 55.0 | 51.5-[c] | .450 | 12.7 | 46.8 | 11.0-68.1 | .577 |

Abbreviations: BD, bronchodilator; $FE_{NO}$, fractional exhaled nitric oxide; $FEF_{25-75}$, forced expiratory flow, midexpiratory phase; $FEV_1$, forced expiratory volume in 1 second; FVC, forced vital capacity; IC, inhaled corticosteroid; IQR, interquartile range.
[a] Mann-Whitney test
[b] Change in $FEV_1 \geq 12\%$ after BD
[c] Insufficient number of patients in the stratum

 

Table 5. Determinants of $FE_{NO}$ in Patients With and Without Inhaled Corticosteroid Treatment (Multiple Linear Regression)

| | Without IC Treatment (n=82) | | | With IC Treatment (n=67) | | |
|---|---|---|---|---|---|---|
| | % Change | 95% CI | P | % Change | 95% CI | P |
| *Fixed terms* | | | | | | |
| Age (each year) | +10.8% | 2.1% to 20.4% | .015 | +5.13% | –3.9% to 14.9% | .271 |
| Male sex | +34.2% | –2.7% to 85.2% | .072 | –21.81% | –46.7% to 13.5% | .204 |
| Body mass index, $kg/m^2$ | –3.2% | –7.7% to 1.4% | .166 | +4.92% | –1.3% to 11.4% | .121 |
| *Random terms* | | | | | | |
| Sensitization to house dust mite | +112.8% | 51.3% to 199.2% | .000 | | | |
| Adherence to therapy[a] | | | | –47.2% | –68.6% to –11.1% | .017 |

Abbreviations: CI, confidence interval; $FE_{NO}$, fractional exhaled nitric oxide; IC, inhaled corticosteroid.
[a] ≥75% of prescribed doses
Regression coefficients have been exponentially converted to percentage of change in $FE_{NO}$ in order to aid understanding.

Table 5 shows the results of the multivariate analysis of the determinants of $FE_{NO}$. In untreated patients, the only significant variable was sensitization to house dust mite. In treated patients, the only significant factor related to $FE_{NO}$ level was adherence to therapy. Other variables were excluded in the final model. If the variable "any allergic sensitization" was included in the multivariate model instead of "house dust sensitization", it was excluded in the final models.

## Discussion

Inflammation plays a key role in asthma, and ICs are the most effective option for its management. However, the association between inflammation and clinical expression of asthma seems to be weak. In untreated patients, symptoms such as cough and wheeze are related to the degree of airway inflammation, whereas treatment with ICs apparently overrides any association between symptoms and $FE_{NO}$. There is no consensus on the clinical expression of airway inflammation. Studies with small samples have found a relationship between $FE_{NO}$ and asthma symptoms [5], particularly cough [6,7], in patients with and without IC treatment. In a somewhat larger study, Warke et al [8] also found an association between $FE_{NO}$ and recent symptoms and the degree of asthma control. However, other researchers have not shown differences in $FE_{NO}$ between patients with active asthma and those who have been in remission for 12 months or longer [14,15]. One recent publication even found an inverse relationship between $FE_{NO}$ and asthma symptoms in adults [16]. To some extent, this lack of consensus could be an effect of IC treatment. ICs act on airway inflammation in a fast, powerful, and dose-dependent way [17-21], and this effect can confound any analysis of the relationship between inflammation and the clinical expression of asthma. Patients with more severe or more poorly controlled asthma are treated with ICs more often, or are given higher doses, with the result that they have a lower $FE_{NO}$ than patients with less severe asthma. Similarly, there is no association between the level of airway inflammation and the burden of asthma on the daily activities of children and their families. Although we did not specifically measure asthma-related quality of life, these results are consistent with those of Ehrs et al [22].

Consensus does exist on the lack of association between baseline $FEV_1$ and underlying inflammation, both in children and adults, with or without IC treatment [3,5,8,23,24]. Results for other spirometric parameters, such as $FEF_{25-75}$ have been discordant [3,8], and the relationship between inflammation and postbronchodilator $FEV_1$ has received little attention in the literature [3]. Although widely used in asthma management protocols, $FEV_1$ is generally normal in most asthmatic children and does not help to discriminate between children with more or less severe asthma [25]. We found no association between lung function and inflammation in untreated patients, although children taking ICs had a higher $FE_{NO}$ value in relation to some airflow obstruction indexes, mainly a reduced $FEV_1$/FVC. $FE_{NO}$ is a marker of residual inflammation in children taking ICs [26], and our results show that residual inflammation cannot be recognized on the basis of symptoms, physical examination, $FEV_1$, or bronchodilator response, although a decreased $FEV_1$/FVC ratio may aid diagnosis.

Holt et al [27] used factor analysis to clarify the clinical expression of asthma. Markers of allergic inflammation and airway hyperresponsiveness contribute to one factor ("inflammation/allergy"), while symptoms, baseline pulmonary function, and bronchodilator response each contribute to other clinical features of asthma, which are not inflammation-dependent. We found little association between inflammation and other clinical parameters; therefore, this multifactorial approach seems feasible. We did not measure airway hyperresponsiveness, which has been shown to be strongly associated with airway inflammation [3,24,28].

Exposure to specific allergens leads to airway inflammation in sensitized patients [29,30]. We have shown here that sensitization to a prevalent winter allergen is the main determinant of airway inflammation in asthmatic children who are not treated with ICs. Consistent with the reports of

other authors [31], we were unable to show that house mite avoidance could reduce airway inflammation in mite-sensitized patients. Allergic airway inflammation, however, is highly sensitive to ICs. Exposure to allergens in patients taking ICs is no longer a key determinant of airway inflammation, and the residual inflammation observed in this group depends mainly on adherence to therapy.

Our study has 3 potential limitations. First, we used $FE_{NO}$ as a surrogate marker of airway inflammation. Exhaled nitric oxide is raised in asthmatic patients because of the inflammatory upregulation of inducible NO synthase in airway epithelial cells, and $FE_{NO}$ level correlates with the levels of other markers of airway inflammation [2,32]. The method we used to determine $FE_{NO}$—a portable hand-held device consisting of an electrochemical sensor—complies with the American Thoracic Society/European Respiratory Society standard for $FE_{NO}$ measurement [33]. The results correlate well with those of the gold standard method, chemiluminescence, although they are not fully interchangeable [4,34-36]. Second, our sample size may have been too small to reveal significant differences in $FE_{NO}$ with regard to the clinical expression of asthma. Although our sample was larger than those of other pediatric studies, more extensive studies will be necessary before sound conclusions can be drawn. We investigated whether the lack of association could be explained by the weakness of nonparametric statistical methods; however, parametric analysis using logarithmic transformation of $FE_{NO}$ yielded exactly the same results. Finally, the main determinant of $FE_{NO}$ level in IC-treated patients, adherence to therapy, was determined by interviewing patients and their parents and was not directly measured. Overreporting of adherence is common, both by parents and children [37], and is a potential source of bias.

In conclusion, we established only a weak association between the strength of airway inflammation, as estimated by $FE_{NO}$, and the clinical expression of asthma in schoolchildren and adolescents. In children not treated with ICs, allergen exposure is the main determinant of inflammation, and this cannot be reduced by commonly recommended avoidance measures. Poor adherence to therapy is the chief cause of persistent inflammation in patients treated with ICs.

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## References

1. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2006 report. Available at www.ginasthma.com
2. Warke TJ, Fitch PS, Brown V, Taylor R, Lyons JDM, Ennis M, Shields MD. Exhaled nitric oxide correlates with airway eosinophils in childhood asthma. Thorax. 2005;57:383-7.
3. Zietkowski Z, Bodzenta-Lukaszyk A, Tomasiak MM, Skiepko R, Szmitkowski M. Comparison of exhaled nitric oxide measurement with conventional tests in steroid-naive asthma patients. J Investig Allergol Clin Immunol. 2006;16:239-46.
4. Menzies D, Nair A, Lipworth BJ. Portable exhaled nitric oxide measurement. Comparison with the "gold standard" technique. Chest. 2007;131:410-4.
5. Spergel JM, Fogg MI, Bokszczanin-Knosala A. Correlation of exhaled nitric oxide, spirometry and asthma symptoms. J Asthma. 2005;42:879-83.
6. Li AM, Lex C, Zacharasiewicz A, Wong E, Erin E, Hansel T, Wilson NM, Bush A. Cough frequency in children with stable asthma: correlation with lung function, exhaled nitric oxide, and sputum eosinophil count. Thorax. 2003;58:974-8.
7. Chatkin JM, Ansarin K, Silkoff PE, McClean P, Gutierrez C, Zamel N, Chapman KR. Exhaled nitric oxide as a noninvasive assessment of chronic cough. Am J Respir Crit Care Med. 1999;159:1810-3.
8. Warke TJ, Mairs V, Fitch PS, McGovern V, Ennis M, Shields MD. Exhaled nitric oxide in relation to the clinical features of childhood asthma. J Asthma. 2004;41:745-51.
9. Greenstone IIG, Ni Chroinin M, Lasserson TJ, Ducharme F. Combination of inhaled long-acting beta2-agonists and inhaled steroids versus higher dose of inhaled steroids in children and adults with persistent asthma. Cochrane Database of Systematic Reviews 2005, Issue 4. Art. No.: CD005533. DOI: 10.1002/14651858.CD005533.
10. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CPM, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J. Series "ATS/ERS Task Force: Standardisation of lung function testing". Standardisation of spirometry. Eur Respir J. 2005;26:319-38.
11. Roca J, Sanchís J, Agustí-Vidal A, Segarra F, Navajas D, Rodríguez Roisín R, Casán P, Sans S. Spirometric reference values for a Mediterranean population. Bull Eur Physiopathol Respir. 1986;22:217-24.
12. Oliva C, Gómez D, Sirvent J, Asensio O, and Grupo de Técnicas de la Sociedad Española de Neumología Pediátrica. Respiratory function assessment in cooperative patients. Part I. Spirometry and bronchodilator reversibility testing. An Pediatr (Barc). 2007;66:393-406.
13. de Winter-de Groot KM, van der Ent CK, Prins I, Tersmette JM, Uiterwaal CSPM. Exhaled nitric oxide: the missing link between asthma and obesity? J Allergy Clin Immunol. 2005;115:419-20.
14. Van den Toorn LM, Prins JB, Overbeek SE, Hoogsteden HC, de Jongste JC. Adolescents in clinical remission of atopic asthma have elevated exhaled nitric oxide levels and bronchial hyperresponsiveness. Am J Respir Crit Care Med. 2000;162:953-7.
15. Van den Toorn LM, Overbbek SE, de Jongste JC, Leman K, Hoogsteden HC. Airway inflammation is present during clinical remission of atopic asthma. Am J Respir Crit Care Med. 2001;164:2107-13.
16. Menzies D, Jackson C, Mistry C, Houston R, Lipworth BJ. Symptoms, spirometry, exhaled nitric oxide, and asthma

 

exacerbations in clinical practice. Ann Allergy Asthma Immunol. 2008;101:248-55.
17. Kanniess F, Richter K, Böhme S, Jörres RA, Magnussen H. Montelukast versus fluticasone: effects on lung function, airway responsiveness and inflammation in moderate asthma. Eur Respir J. 2002;20:853-8.
18. Kharitonov SA, Donnelly LE, Montuschi P, Corradi M, Collins JV, Barnes PJ. Dose-dependent onset and cessation of action of inhaled budesonide on exhaled nitric oxide and symptoms in mild asthma. Thorax. 2006;57:889-96.
19. Jones SL, Herbison P, Cowan JO, Flannery EM, Hancox RJ, McLachlan CR, Taylor DR. Exhaled NO and assessment of anti-inflammatory effects of inhaled steroid: dose-response relationship. Eur Respir J. 2006;20:601-8.
20. Beck-Ripp J, Griese M, Arenz S, Köring C, Pasqualoni B, Bufler P. Changes of exhaled nitric oxide during steroid treatment of childhood asthma. Eur Respir J 2002;19:1015-9.
21. Silkoff PE, McClean P, Spino M, Erlich L, Slutsky AS, Zamel N. Dose-response relationship and reproducibility of the fall in exhaled nitric oxide after inhaled beclomethasone dipropionate therapy in asthma patients. Chest. 2001;119:1322-8.
22. Ehrs PO, Sundblad BM, Larsson K. Quality of life and inflammatory markers in mild asthma. Chest. 2006;129:624-31.
23. Senna G, Passalacqua G, Schiappoli M, Lombardi C, Wilcock L. Correlation among FEV1, nitric oxide and asthma control test in newly diagnosed asthma. Allergy. 2007;62:207-8.
24. Steerenberg PA, Janssen NAH, de Meer G, Fischer PH, Nierkens S, van Loveren H, Opperhuizen A, Brunekreef B, van Amsterdam JGC. Relationship between exhaled NO, respiratory symptoms, lung function, bronchial hyperresponsiveness, and blood eosinophilia in school children. Thorax. 2003;58:242-5.
25. Spahn JD, Cherniak R, Paull K, Gelfand EW. Is forced expiratory volume in one second the best measure of severity in childhood asthma? Am J Respir Crit Care Med. 2004;169:784-6.
26. Fitzpatrick AM, Gaston BM, Erzurum SC, Teague WG, National Heart, Lung, and Blood Institute's Severe Asthma Research Program. Features of severe asthma in school-age children: Atopy and increased exhaled nitric oxide. J Allergy Clin Immunol. 2006;118:1218-25.
27. Holt EW, Cook EF, Covar RA, Spahn JD, Fuhlbrigge AL. Identifying the components of asthma health status in children with mild to moderate asthma. J Allergy Clin Immunol. 2008;121:1175-80.
28. de Meer G, van Amsterdam JGC, Janssen NAH, Meijer E, Steerenberg PA, Brunekreef B. Exhaled nitric oxide predicts airway hyper-responsiveness to hypertonic saline in children that wheeze. Allergy. 2005;60:1499-1504.
29. Siddiqui S, Morris J, Avery N, Wyand S, Rood D, Silbart LK. Pulmonary eosinophilia correlates with allergen deposition to the lower respiratory tract in a mouse model of asthma. Clin Exp Allergy. 2008;38:1381-90.
30. Prieto L, Gutiérrez V, Torres V, Uixera S, Marín J. Effect of salmeterol on seasonal changes in airway responsiveness and exhaled nitric oxide in pollen-sensitive asthmatic subjects. Chest. 2002;122:798-805.
31. Gøtzsche PC, Johansen HK. House dust mite control measures for asthma. Cochrane Database of Systematic Reviews 2008, Issue 2. Art. No.: CD001187. DOI: 10.1002/14651858.CD001187.pub3.
32. Cobos N, Pérez-Yarza EG, Sardón O, Reverté C, Gartner S, Korta J. Exhaled nitric oxide in children: a noninvasive marker of airway inflammation. Arch Bronconeumol. 2008;44:41-51.
33. American Thoracic Society, European Respiratory Society. ATS/ERS Recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med. 2005;171:912-30.
34. McGill C, Malik G, Turner SW. Validation of a hand-held exhaled nitric oxide analyzer for use in children. Pediatr Pulmonol. 2006;41:1053-17.
35. Fortuna AM, Feixas T, Casan P. Measurement of fraction of exhaled nitric oxide with the portable NIOX-MINO monitor in healthy adults. Arch Bronconeumol. 2007;43:176-9.
36. Sardón O, Aldasoro A, Korta J, Mintegui J, Emparanza JI, Pérez-Yarza EG. Agreement between two devices for measuring exhaled nitric oxide. An Pediatr (Barc). 2007;67:572-7.
37. Bender BG, Bartlett SJ, Rand CS, Turner C, Wamboldt FS, Zhang L. Impact of interview mode on accuracy of child and parent report of adherence with asthma-controller medication. Pediatrics. 2007;120:e471-7.

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