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Original Article

# Exhaled Nitric Oxide in Children Under 4 Years of Age With Recurrent Bronchitis

Inés de Mir Messa,* Antonio Moreno Galdó, Nicolás Cobos Barroso, Silvia Gartner, Carlos Martín De Vicente, and Santos Liñán Cortés

*Unidad de Neumología Pediátrica y Fibrosis Quística, Hospital Universitari Vall d'Hebron, Barcelona, Spain*

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ABSTRACT

*Background:* The objective of the study was to assess bronchial inflammation in preschool children with recurrent bronchitis by measuring exhaled nitric oxide.
*Patients and Methods:* The study included patients under 4 years of age with at least 3 episodes of wheezing in the past year (n=63) and a control group (n=30). Exhaled nitric oxide was measured in samples collected offline during spontaneous tidal breathing with a face mask and stored in Mylar balloons.
*Results:* The fractional exhaled nitric oxide concentration ($FE_{NO}$) was higher in the group with bronchitis (mean [SD], 5.3 [1.3] parts per billion [ppb]) than in the control group (4.6 [1.1] ppb) (*P*=.02). There was a significant difference between the control group and children in the bronchitis group not treated with inhaled corticosteroids (*P*<.05), but not between controls and corticosteroid-treated patients. A relationship with eosinophil count was observed in that those with higher counts (>400 mL) had higher $FE_{NO}$ levels (*P*<.01). No relationship was observed between $FE_{NO}$ and a positive methacholine challenge test. Follow-up lasted at least 20 months. The initial $FE_{NO}$ level did not differ significantly according to whether patients were subsequently transient, infrequent, or frequent wheezers (5.2 [0.98] ppb, 5.6 [1.5] ppb, and 4.8 [1.34] ppb, respectively; *P*=.36).
*Conclusions:* In children under 4 years of age with recurrent wheezing bronchitis who were asymptomatic at study entry, a small increase in $FE_{NO}$ was observed although there was a good deal of overlap with the control group.


## Óxido nítrico exhalado en niños menores de 4 años con bronquitis de repetición

*Palabras clave:*
Marcadores de inflamación bronquial
Óxido nítrico exhalado
Niños
Bronquitis
Sibilantes recurrentes

RESUMEN

*Introducción:* El objetivo del estudio ha sido valorar la inflamación bronquial en niños preescolares con bronquitis de repetición, mediante la determinación del óxido nítrico exhalado.
*Pacientes y métodos:* Se incluyó en el estudio a pacientes menores de 4 años con antecedentes de 3 episodios de sibilantes en el último año (n = 63), así como un grupo control (n = 30). Se determinó el óxido nítrico exhalado mediante la técnica de recogida *off-line*, con respiración espontánea a volumen corriente con mascarilla, recogida en bolsa de Mylar.
*Resultados:* El grupo con bronquitis presentó como media una fracción de óxido nítrico en aire exhalado ($FE_{NO}$) más elevada (media±desviación estándar: 5,3 ± 1,3 ppb) que el grupo control (4,6 ± 1,1) (p = 0,02). Hubo una diferencia significativa (p < 0,05) entre el grupo control y los niños con bronquitis que no recibían corticoides inhalados, pero no con los que sí los recibían. Se observó una relación con el número de eosinófilos en sangre, de forma que los que tenían valores más elevados (> 400/Ìl) presentaban concentraciones de $FE_{NO}$ más altas (p < 0,01). No se apreció relación entre la $FE_{NO}$ y el hecho de tener una prueba de provocación bronquial positiva a la metacolina. Se realizó un seguimiento de los pacientes durante más de 20 meses. No hubo diferencias significativas (p = 0,36) en el valor inicial de la $FE_{NO}$ entre los pacientes que

*Corresponding author.
*E-mail address:* fernandezdemir@gmail.com (I. de Mir Messa).

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posteriormente presentaron sibilancias transitorias (5,2 ± 0,98 ppb), infrecuentes (5,6 ± 1,5 ppb) o frecuentes (4,8 ± 1,34 ppb).

*Conclusiones:* Los niños menores de 4 años afectados de bronquitis sibilantes de repetición en fase asintomática presentan una discreta elevación de la $FE_{NO}$, con una amplia superposición de valores con el grupo control.



## Introduction

In recent years, numerous studies have established fractional exhaled nitric oxide (NO) concentration ($FE_{NO}$) as the first useful noninvasive marker of eosinophilic inflammation in the airways.[1,2] Devices for measuring this variable have now been developed for use in daily clinical practice,[3-5] and its applicability as a diagnostic tool has been validated in older children and adults asthma and as a way of assessing patient adherence and response to treatment.[6-8] Moreover, measurement of $FE_{NO}$ has been standardized through guidelines issued by the American Thoracic Society (ATS) and the European Respiratory Society (ERS).[9] These guidelines include practical recommendations for measuring $FE_{NO}$ in infants and preschool children, although in this age group, the technique has not yet been standardized.

In infants and uncooperative preschool children, collecting exhaled air is hindered by certain technical issues and we have less information available on its usefulness in these children, in whom the prevalence of recurrent bronchitis with wheezing is very high.[10] Indeed, approximately 1 out of every 3 children has at least 1 episode of wheezing bronchitis before the age of 3 years, and the cumulative prevalence of such episodes in children aged 6 is 50%.[11,12]

Asthma is a syndrome with a broad clinical spectrum, characterized by inflammation of the airways according to the definition of the Global Initiative for Asthma (GINA).[13] However, in infants and preschool children inflammation is not well characterized and might not even be present in some cases,[14] making diagnosis of asthma in such patients difficult. As a result, a recent consensus document on the management of infants and preschool children with recurrent wheezing bronchitis recommends a symptoms-based approach, and not using the term "asthma" in these children because there is no clear evidence that the pathophysiology of recurrent wheezing bronchitis at this age is similar to that of asthma in older children and adults.[15]

In view of these difficulties, it would be of great use to have a diagnostic test that would distinguish which children at this age have or will develop eosinophilic asthma and will therefore be more likely to respond to treatment with inhaled corticosteroids.

Baraldi et al[16] were the first to report that infants with recurrent bronchitis had significantly elevated $FE_{NO}$ during an acute exacerbation compared to healthy controls and to infants with a first episode of virus-induced wheezing bronchitis. Subsequently, some studies have reported elevated $FE_{NO}$ levels in infants and preschool children with wheezing bronchitis, particularly in those with a predisposition to atopy, compared to healthy children.[17-20]

In view of this background, we set the following objectives for this study: to determine $FE_{NO}$ by means of offline sampling during tidal breathing in a group of young children (<4 years) affected by recurrent wheezing bronchitis during an exacerbation-free period to determine whether $FE_{NO}$ levels were elevated compared to an age-matched group of healthy children. Such an elevation would indicate the presence of persistent eosinophilic bronchial inflammation. In addition, we provided for the possibility of analyzing the variables that might influence the concentrations of $FE_{NO}$ in these children, and assessing whether the baseline values of $FE_{NO}$ might predict which children will progress to persistent wheezing bronchitis.

## Patients and Methods

### *Patients*

Sixty-three consecutive patients with ages ranging from 8 months to 3 years 11 months were enrolled in the study. In the past year, all had experienced 3 or more episodes of acute wheezing bronchitis diagnosed by a physician (bronchitis group). Acute wheezing bronchitis was defined as the presence of acute cough accompanied by wheezing on auscultation, with or without signs of breathing difficulties if bronchodilator treatment had been administered. The study design allowed for one-third of the children to be on treatment with inhaled corticosteroids. Patients with chronic lung diseases (cystic fibrosis, bronchopulmonary dysplasia, etc) or cardiovascular disease were excluded, as were infants born prematurely.

In addition, a group of healthy children (control group) were also studied. These children had been born at term and had no family or personal history of atopy. The control sample was drawn from healthy children who attended check-ups in a health district on the outskirts of Barcelona, Spain.

The calculation used to determine the required sample size was as follows. According to the medical literature, it was estimated that the mean $FE_{NO}$ levels of the control group should be around 5.6 parts per billion (ppb).[16] A difference in $FE_{NO}$ between the control group and the patient group with wheezing of 2.5 ppb was considered clinically significant. Assuming a standard deviation of 3 ppb, an $\alpha$ risk of .05 and a $\beta$ risk of .10, and using a one-tailed hypothesis, 26 patients would be needed in each group. Assuming a drop-out rate of 15%, this number would increase to 30 children. Given that the bronchitis group included children with and without inhaled corticosteroid treatment, to allow comparison of both subgroups with the control group, at least 60 children were to be included in the bronchitis group.

In the bronchitis group, $FE_{NO}$ was only measured when the patient was stable and not experiencing an exacerbation, and provided he or she had not presented bronchitis or any other respiratory infection in the 3 weeks prior to measurement. Children in the control group were not measured if they had had an upper airway infection in the preceding 3 weeks.

The medical history included family history of atopy and passive smoking, as well as other risk factors for recurrent bronchitis. Children with recurrent bronchitis underwent the following tests as part of the study of their underlying disease: complete blood count with total eosinophil count, measurement of total and specific immunoglobulin (Ig) E for the respiratory allergens *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, cat dander, *Alternaria*, *Parietaria*, and olive tree pollen, and skin-prick tests for common respiratory allergens in the Spanish environment (house dust mites, dog and cat dander, and pollen from olive trees and other trees, *Parietaria*, and grasses). Likewise, all underwent a methacholine challenge test, using a modified tracheal auscultation method.[21]

Patients were subsequently scheduled for prospective follow-up visits to distinguish 2 different groups: those who still presented wheezing bronchitis and those who did not. At the end of follow-up, patients were classified as having transient wheezing bronchitis (no episode of bronchitis in the past year), infrequent wheezing bronchitis (≤3 episodes/year), and frequent wheezing bronchitis (>3 episodes/year).

The study was approved by the local clinical research ethics committee, and children were only included after parents had been informed and their written consent had been obtained.

*Methods*

$FE_{NO}$ was determined using an offline sampling method in which exhaled air was collected in a Mylar bag for later analysis. Samples were collected using the Deadspace Discard Bag Collection and Sampling Kit (BSK 01400; Sievers Instruments, Inc., Boulder, Co, USA). This system is compliant with the recommendations of the ATS/ERS[9] for offline measurement of NO, and includes the following elements (Figure 1): *a)* a Zero Air filter for NO, which ensures that inspired air is free of NO, thereby avoiding outside contamination; *b)* a manometer to control expiratory pressure; *c)* a 1.5 L Mylar collection bag, impermeable to outside gases and inert to NO, equipped with a one-way valve; *d)* a valve which on opening allows air to enter the bag thereby facilitating circulation of air through the system; and *e)* a disposable antibacterial and antiviral filter, to which a dual-compartment face mask (Rudolf Face Masks; Pediatric Small 7970 #2, Hans Rudolf, Inc., Mi, USA) is attached to prevent contamination of exhaled air with nasal NO.

The sampling device offers a fixed resistance that ensures that the pressure varies between 5 and 25 cm $H_2O$ depending on the effort made by child during exhalation. This, given the design of the system, would correspond to an expiratory flow of between 50 and 125 mL/s.[22] As was not possible to train small children to maintain a constant expiratory flow, sample collection was done during tidal breathing, and a variable expiratory flow ranging from 0 mL/s (just prior to the start of exhalation) to 125 mL/s was obtained. Expiratory pressures greater than 5 cm $H_2O$ would help close the soft palate, thereby avoiding contamination with air from the upper airways.

**Figure 1.** Device for collecting exhaled air for offline measurement of nitric oxide.

Before the measurement was started, the collection bags were cleaned by evacuating the gas inside using the vacuum pump of the analyzer, filling them with gas free of NO (medical oxygen), and evacuating the gas once again with the vacuum pump.

For sample collection, the children sat in the arms of their mother or father and, once the face mask was placed over the mouth and nose, were allowed to perform 5 to 10 breaths at tidal volume through the NO filter. Then, while the child maintained a stable breathing pattern, the valve was opened for 15 to 45 seconds to collect the exhaled air in the bag (approximate volumes of 50 to 300 mL).

The NO in the air collected in the bags was measured using a chemoluminescence analyzer (LR2000, Logan Research, Rochester, United Kingdom) after connecting the Mylar bag to the Teflon inlet line of the NO analyzer. NO was sampled during the stable or plateau phase of expiration. The analyzer was calibrated daily with an NO cylinder containing 110 ppb.

Two samples were collected per child and analyzed within 2 hours of collection. For each patient, the NO concentration was taken to be the mean of the values obtained from measurement of the 2 bags.

*Statistical Analysis*

Statistical analysis of the data was carried out using the Medcalc program, version 9.0.1.0. The Kolmogorov-Smirnov test was used to assess whether the data were normally distributed. For comparisons between the 2 groups, if the variable was normally distributed, the unpaired *t* test was applied, otherwise the Mann-Whitney nonparametric U test was used. For comparison of 3 or more groups, univariate analysis was done, followed by the Bonferroni test or the Kruskal-Wallis test if the variables were not normally distributed. Qualitative variables were compared using the $\chi^2$ test. Correlation between quantitative variables was assessed using the Pearson correlation coefficient. Results are expressed as means (SD). Statistical significance was set at $P<.05$.

## Results

$FE_{NO}$ was measured in 25 of the 30 children in the control group and 62 of the 63 patients in the recurrent bronchitis group. For the 6 patients who did not complete the test, lack of cooperation (strong crying) was cited as the reason for failure.

The children in the bronchitis group had a mean (SD) age of 25.3 (11.5) months while the mean age of those in the control group was 22.1 (12.2) months. The groups did not differ in terms of their baseline characteristics: age, sex, weight at birth, and gestational age (Table 1). The proportion of children exposed to tobacco smoke at home was greater in the bronchitis group (63%) than in the control group (36%) ($P=.04$).

$FE_{NO}$ levels were greater in the bronchitis group (5.3 [1.3] ppb) than in healthy children (4.6 [1.1] ppb) ($P=.02$), although there was extensive overlap (Figure 2).

When the $FE_{NO}$ levels in the bronchitis group were analyzed by whether the patients were receiving treatment with inhaled corticosteroids (4.9 [1.4] ppb) or not (5.4 [1.3] ppb), only in the latter

**Table 1**
Comparison of Baseline Characteristics of the Control and Bronchitis Groups

| | Control Group (n=25) | Bronchitis Group (n=62) | *P* |
|---|---|---|---|
| Age, mo | 22.1 (12.2) | 25.3 (11.5) | .24 |
| Sex, male/female | 15/10 | 44/18 | .46 |
| Birth weight, g | 3203.1 (442.1) | 3162.8 (528.6) | .74 |
| Gestational age, wk | 39.3 (1.6) | 39.2 (1.3) | .72 |
| Passive smoking | 9 (36%) | 39 (63%) | .04 |

[a] Data are shown as means (SD) or numbers of patients (percentages).

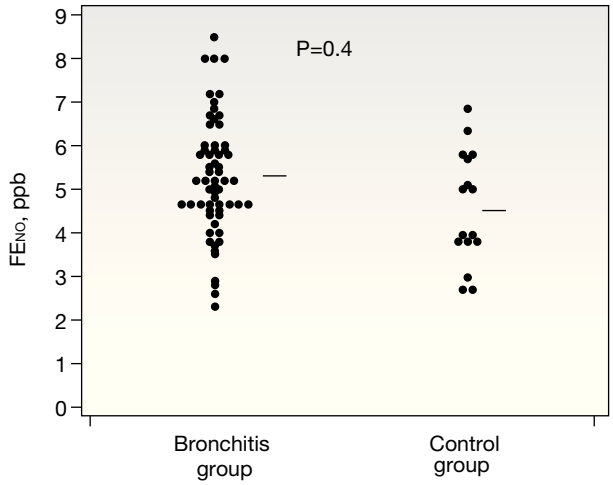


**Figure 2.** Comparison of exhaled nitric oxide levels ($FE_{NO}$) between the group of patients with recurrent bronchitis and the control group (horizontal lines represent mean values). ppb indicates parts per billion.

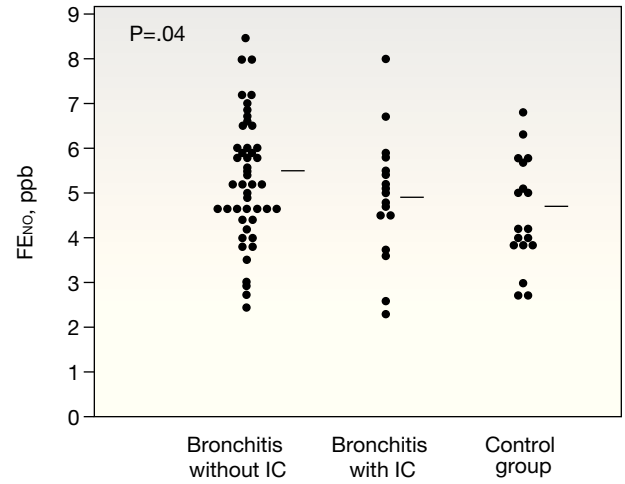


**Figure 3.** Comparison of exhaled nitric oxide levels ($FE_{NO}$) between patients with recurrent wheezing bronchitis who were receiving inhaled corticosteroids (IC) and those who were not at the time of the test (horizontal lines represent mean values).

group were the $FE_{NO}$ levels greater than in the control group (Figure 3).

In the control group of healthy children, $FE_{NO}$ levels did not correlate with age (r=–0.09; *P*=.65), weight at birth (r=–0.28; *P*=.18), gestational age (r=–0.21; *P*=.32), or exposure to tobacco smoke at home (*P*=.2). $FE_{NO}$ levels were, however, higher in healthy girls (5.3 [0.7] ppb) than in healthy boys (4.1 [1.1] ppb) (*P*=.006).

In the group of patients with recurrent bronchitis, $FE_{NO}$ levels did not correlate with age (r=0.24; *P*=.06), gestational age (r=–0.1; *P*=.44), or weight at birth (r=0.02; *P*=.89). Unlike healthy children, there was no correlation between sex and $FE_{NO}$. Likewise, there was no correlation between $FE_{NO}$ and family history of asthma, exposure to tobacco smoke, or the number of acute episodes of bronchitis in the past year (Table 2).

Children in the bronchitis group with an IgE concentration of 100 U/mL or greater had slightly higher $FE_{NO}$ levels (5.99 ppb) than those with a concentration below 100 U/mL (5.13 ppb), a result bordering on statistical significance (*P*=.052). Those with an eosinophil count of 400/μL or more had higher levels of $FE_{NO}$ (6.21 ppb) than those with a lower count (*P*=.01).

In 7 patients, skin-prick tests for respiratory allergens or specific IgE were positive. The median $FE_{NO}$ level was slightly higher (6.7 ppb) in this group than in the group in which these tests were negative (5.2 ppb), but this difference was not statistically significant (*P*=.12).

In children with recurrent bronchitis, bronchial hyperresponsiveness was measured using the PC wheeze (PCw) method. There were no significant differences in $FE_{NO}$ levels between the group with bronchial hyperresponsiveness (PCw<6

**Table 2**
Comparison of Exhaled Nitric Oxide Levels ($FE_{NO}$) Stratified by Different Variables in the Group of Patients With Recurrent Bronchitis[a]

| | $FE_{NO}$ | FENO | *P* |
|---|---|---|---|
| Sex | Boys (n=44) 5.45 (1.4) | Girls (n=18) 4.89 (1.1) | .150 |
| Family history of asthma | No (n=37): 5.25 (1.5) | Yes (n=25): 5.32 (1.17) | .851 |
| Passive smoking | No (n=23): 5.65 (1.3) | Yes (n=39): 5.06 (1.3) | .098 |
| Mother smoked during pregnancy | No (n=47): 5.23 (1.2) | Yes (n=14): 5.26 (1.7) | .937 |
| Eosinophil count | <400/μL (n=51) 5.08 (1.24) | ≥400/μL (n=11): 6.21 (1.4) | .01 |
| Immunoglobulin E | <100 U/mL (n=51): 5.13 (1.3) | ≥100 U/mL (n=11): 5.99 (1.4) | .052 |
| Bronchodilator challenge (PCw) | PCw ≥8 mg/mL (n=20): 5.59 (1.4) | PCw ≤6 mg/mL (n=42): 5.13 (1.3) | .212 |
| No. of episodes of bronchitis in past year | 3-5 (n=27): 5.19 (1.2) | 6-9 (n=28): 5.39 (1.4) ≥10 (n=7): 5.25 (1.7) | .871 |

[a] Data are shown as means (SD) or numbers of patients (percentages).

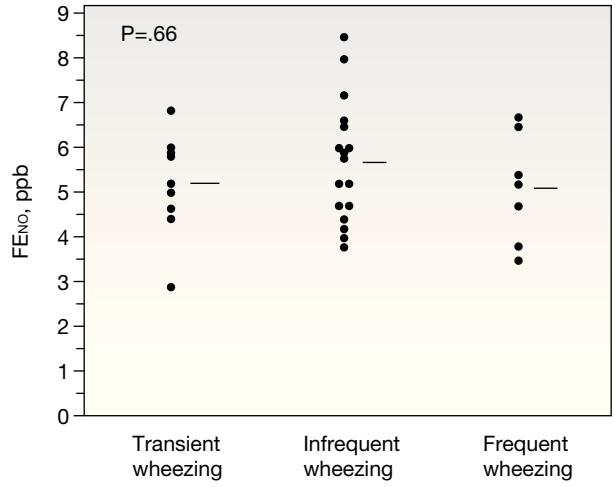


**Figure 4.** Comparison of the initial levels of exhaled nitric oxide ($FE_{NO}$) in patients with recurrent wheezing bronchitis according to whether their episodes in subsequent follow-up were transient, infrequent (1-3 episodes of bronchitis in the past year), or frequent (>3 episodes of bronchitis in the past year). Only patients who were not receiving inhaled corticosteroids when $FE_{NO}$ levels were determined are included. The horizontal lines represent mean values. ppb indicates parts per billion.

mg/mL) and those without bronchial hyperresponsiveness (PCw≥8 mg/mL).

Forty-nine of the 62 patients completed follow-up of 20 months or more. In the remaining patients, follow-up was curtailed because they had changed address or because they did not attend the follow-up visits and could not subsequently be contacted. The mean duration of follow-up was 28.6 months (range, 21.6-41.8 months), and the mean age at the end of follow-up was 53.8 months (range, 35-90 months). At the end of follow-up, 16 patients (32.7%) had not experienced episodes of bronchitis in the past year (transient wheezing), 23 (46.9%) had experienced 1 to 3 episodes (infrequent wheezing), and 10 (20.4%) had experienced 3 or more episodes (frequent wheezing).

The initial $FE_{NO}$ level did not differ significantly according to whether patients were subsequently transient, infrequent, or frequent wheezers (5.2 [0.98] ppb, 5.6 [1.5] ppb, and 4.8 [1.34] ppb, respectively; *P*=.36). Similar results were obtained on considering only patients who were not receiving inhaled corticosteroid therapy when $FE_{NO}$ levels were determined (*P*=.65) (Figure 4). The initial $FE_{NO}$ level was not predictive of the persistence of subsequent episodes of wheezing bronchitis, either in the patients as a whole (area under the diagnostic efficiency curve, 0.48; *P*=.79) or in patients who were not receiving treatment with inhaled corticosteroids (area under the diagnostic efficiency curve, 0.56; *P*=.62)

## Discussion

In this study, we have shown that it is possible to reliably measure $FE_{NO}$ in unsedated children under 4 years of age, and we found that children under 4 with recurrent wheezing bronchitis have slightly increased $FE_{NO}$ levels compared to healthy children, although there was substantial overlap between groups. Moreover, $FE_{NO}$ levels were not predictive of persistence of wheezing bronchitis.

$FE_{NO}$ could be satisfactorily measured in a very high percentage of the children (98.5% of those with wheezing bronchitis and 86.5% of the healthy controls). The children affected by wheezing bronchitis were accustomed to receiving inhaled medication using spacer devices with masks, and the $FE_{NO}$ sample collection device is similar to a spacer device. In contrast, the healthy children had never used a spacer device, and so for some of them, when the sampling device was put in place, rejection was greater, with crying that made it impossible to perform the test.

Normal mean values of $FE_{NO}$ in our series (4.6 ppb) were similar to those found by other authors in previous studies.[16,18,23] In contrast, Franklin et al,[19] who compared single-breath online methods and offline tidal breathing methods in healthy children, found higher mean levels—23.2 ppb and 13.8 ppb, respectively. This highlights the need for specific ranges of normal, given the lack of standardization of the technique.

In the group of children with recurrent bronchitis, $FE_{NO}$ levels were higher than in healthy children, but there was substantial overlap between the 2 groups, indicating that $FE_{NO}$ did not discriminate between children with recurrent bronchitis and healthy children. This finding contrasts with the results of studies of older children and adults which pointed to the good specificity and sensitivity of $FE_{NO}$ for diagnosis of asthma.[6,24] This overlap of values has also been observed in other studies conducted in infants and preschool children.[16,18,20] There may be several reasons for this. $FE_{NO}$ is considered a noninvasive marker of the presence of bronchial eosinophilic inflammation, as it correlates well with the percentage of eosinophils in samples of induced sputum, bronchoalveolar lavage, and bronchial biopsy.[25] In recent years, studies in infants and preschool children using these techniques seem to show that during the first 2 years of life there is no (or very little) eosinophilic inflammation.[14,26] However, such inflammation starts to appear from 3 years onward and is more clearly established from 5 years onwards. In accordance with this, the lack of a clear increase in $FE_{NO}$ in our study might indicate that most of the children with recurrent wheezing bronchitis had another type of bronchial inflammation (probably neutrophilic). In any case, what we did see in our study was a relationship between $FE_{NO}$ levels and markers of eosinophilic inflammation, such as the peripheral eosinophil count and total IgE. Seven patients in whom skin tests or radioallergosorbent tests were positive had higher $FE_{NO}$ levels (6.7 ppb vs 5.2 ppb), although the differences were not significant, given the small number of children with positive tests. Other authors have however found a significant increase in infants with recurrent bronchitis and a history of atopy compared to those with recurrent bronchitis and no history of atopy, although there was also substantial overlap between groups.[20]

Another useful aspect of measuring $FE_{NO}$ is that it can help monitor the progression of asthma and its treatment.[7,8] In our study, the children with recurrent wheezing bronchitis who were not taking inhaled corticosteroids had higher $FE_{NO}$ levels than healthy children, whereas those who were in treatment with inhaled corticosteroids showed no differences with the healthy controls. This fact, along with the comments relating to atopy, indicates that measurement of $FE_{NO}$ could be useful for determining which infants are affected by atopic asthma or are predisposed to developing it. However, the substantial overlap found in our study and in previous studies limits practical applications.

Several factors may limit the validity and utility of measuring $FE_{NO}$ in preschool children and infants:

- Contamination by NO in the environment. The presence of environmental NO contamination at low concentrations of 5 to 10 ppb might affect measurement of $FE_{NO}$ in small children.[27] In our case, to circumvent this problem, the children breathed through an NO filter before the sample of exhaled air was collected.
- Contamination by nasal NO. Although there are authors who consider that nasal NO does not make an important contribution because the paranasal sinuses at this age are not particular pneumatized, thereby limiting contamination, high rates of

elimination of NO from the upper airways have been reported in preterm and term newborn babies.[28] We avoided the possibility of nasal air contamination through the use of a special mask with 2 compartments, placed over the nose and mouth. Furthermore, once the valve of the collection system is closed, the resistance of the system generates a pressure of at least 3 to 5 cm $H_2O$, which helps close the soft palate and prevent paranasal contamination.

– The most important problem is the influence of expiratory flow: $FE_{NO}$ varies with flow; the lower the flow, the higher the $FE_{NO}$ level. A limitation in the technique of collection of exhaled air in infants and preschool children during tidal breathing is that is it impossible to do so at a constant flow (50 mL/s, as recommended by the ATS/ERS[9]). The technique recommended in adults and cooperative children is single-breath exhalation against resistance at a controlled flow of 50 mL/s. Unfortunately, this technique cannot be applied to infants and preschool children. Wildhaber et al[17] have described a similar method using a thoracic compression technique (used to measure forced expiratory flows in infants) with subsequent analysis of the exhaled gas. However, the technique is complicated and requires specialized devices and sedation. Therefore, the most widely-employed technique in infants and preschool children has used tidal breathing. Some authors have modified this technique by using a variable expiratory resistance to maintain a flow of 50 mL/s in children aged over 2 years,[23] or by coupling a pneumotachograph to the circuit of online measurement at tidal volume to select the phase of exhalation when the expiratory flow is 50 mL/s.[29]

In addition to these technical factors, there are physiological variables, such as sex, or environmental ones, such as exposure to tobacco smoke, that may influence concentrations of exhaled NO. There is debate concerning the extent to which sex is an influence. In contrast to the situation in older children, where there are no differences according to sex, in infants somewhat variable results have been reported. In our study, in agreement with Franklin et al,[19] we found significantly higher $FE_{NO}$ levels in girls than in boys in the control group, although such a difference was not found in children with bronchitis. It may be that genetic factors play a part in these differences seen according to sex. Furthermore, in the medical literature, prenatal but not postnatal smoking has been associated with lower $FE_{NO}$ levels in infants.[29,30] We, however, did not find significant differences in the levels of $FE_{NO}$ according to prenatal or postnatal exposure to tobacco, either in the group with wheezing bronchitis or in healthy children.

We have seen in our study that small children with recurrent wheezing bronchitis have greater bronchial hyperresponsiveness than healthy children, as well as higher levels of exhaled NO. In contrast, we have not found a relationship between the presence or severity of bronchial hyperresponsiveness and $FE_{NO}$ levels. This supports the premise that there is no direct relationship between bronchial inflammation and bronchial hyperresponsiveness.[31] The latter may be influenced by other factors such as muscle tone, bronchial wall remodeling, and autonomic dysfunction.

Finally, on the basis of our findings, it does not appear that measurement of $FE_{NO}$ levels in small children can predict the future development of asthma, and there is currently no evidence in the medical literature to support this hypothesis.

Our study and other studies conducted to date in infants and preschool children suggest that, by perfecting and better standardizing the measurement techniques, $FE_{NO}$ could be of clinical use in small children, though less so than in older children, due partly to pathophysiological differences and partly to methodological problems.[32,33]

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