

Exhaled nitric oxide fractions are well correlated with clinical control in recurrent infantile wheeze treated with inhaled corticosteroids

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Fractional exhaled nitric oxide (FeNO) is a non-invasive marker of bronchial inflammation in asthma. However, the interest of FeNO measurement remained limited in infantile wheeze. The aim of this prospective study was to evaluate the feasibility and reproducibility of FeNO off-line measurement in very young children with recurrent wheeze and to assess whether clinical control of infantile wheeze correlates with FeNO levels. Two exhalation samples were collected in mylar balloon during quite tidal breathing. FeNO measurements were performed off-line by a NO analyzer. The participating patients were aged ≤ 36 months, wheezes had started before the age of 24 months, and they were receiving maintenance treatment with inhaled corticosteroids for at least 3 months duration. The studied population comprised of 40 uncontrolled infants with persistent wheezy respiratory symptoms, median age 14.5 months, and 40 with optimal controlled infantile wheeze, median age 14 months. The reproducibility was excellent ($r = 0.95$; $p < 0.0001$). There was a significant difference in FeNO levels between the groups of persistent wheeze and well-controlled infants: 19.8 (2.5–99.3) ppb vs. 7.7 (0.6–29.5) ppb, $p < 0.0001$. At a FeNO level > 15 ppb, the predictive values for uncontrolled disease were as follows: positive predictive value = 65%, negative predictive value = 90%. FeNO levels were not increased by atopy or passive tobacco. Off-line assessment of FeNO is feasible, reproducible, and well accepted in wheezy very young children. Optimal clinical control of infantile wheeze appeared to be associated with the control of bronchial inflammation when evaluated by FeNO measurements.

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Bronchial inflammation has an important role in the pathophysiology of asthma and recurrent infantile wheeze, and asthma management depends mainly on the inflammation control by inhaled corticoids (ICS). Airway inflammation can be evaluated by the non-invasive measurement of exhaled nitric oxide fractions (FeNO). The online measurement of FeNO is well standardized, reproducible, and easily performed. The off-line method allows achieving this measurement in infants and young children (1). In bronchial asthma, FeNO levels are increased, well

correlated to eosinophil inflammatory markers (2), and modulated by anti-inflammatory medications. In children, the persistence of elevated levels of exhaled nitric oxide has been shown to predict an asthmatic exacerbation (3), but the usefulness of FeNO measurement in the monitoring of asthma therapy remains controversial (4).

Although airway inflammation in wheezy infants is mainly mediated by neutrophil cells (5), FeNO level assessment may differentiate various airway diseases in this age-group population (6). In infants, several factors might

influence the production of FeNO as atopic dermatitis (7), smoking exposure (8), birth weight (9), maternal atopy (10), and the administration of anti-inflammatory medications (11, 12).

The aim of this study was to evaluate the feasibility and reproducibility of FeNO off-line measurement in infants and very young children with recurrent wheezing and to examine whether the control of the respiratory disease correlates with FeNO levels.

Patients and methods

Patients

This prospective study was conducted at the pediatric respiratory division of Charles Nicolle Children's University Hospital, Rouen, France. The purpose of this study and the technique of off-line FeNO measurement were explained to parents, and oral consents were obtained as recommended by the hospital ethics committee. From July 2008 to September 2009, the patients at the respiratory clinic with the following inclusion criteria were recruited: aged ≤ 3 yr old, with a history of severe recurrent wheeze that started before the age of 2, treated with ICS for at least 3 months duration, and followed up at the respiratory clinic since ≥ 6 months. Severe recurrent wheeze was defined by more than 3 exacerbations within one winter or persistent symptoms between two acute episodes (14). At the time of the attendance, the children were clinically stable and having no acute airway infections.

The studied population was divided into 2 groups regarding the clinical control of the respiratory disease. The patients with optimal control (group C) were defined as follows: absence of wheeze exacerbations, diurnal, nocturnal, and exercise-induced symptoms during the last 3 months. The others belong to the uncontrolled group (group NC). Demographic characteristics, personal or family history of atopy, tobacco exposure, respiratory symptoms and treatment, and FeNO levels were collected in an anonymous form. A positive family history of atopy was defined if at least one parent has bronchial asthma and/or allergic rhinitis. A positive personal history of atopy was defined if the patient has food and/or respiratory allergy, diagnosed by a positive prick test ≥ 3 mm or specific IgE, and/or atopic dermatitis.

FeNO collection and measurement

Exhaled air samples were collected with a face-mask placed over the infant's nose and mouth

during a quite tidal breathing; at least 10 breaths were required to obtain a sufficient sample. The child was not sedated, in a sitting position held by the parents. The facemask was connected to a two-way non-rebreathing valve (Hans Rudolph Silicon Rubber Face Mask, Hans Rudolph Inc Kansas, USA) that allows inspiration of NO-free air. Exhaled breath samples were collected twice into 2 mylar balloons (Jurjen de Vries, Leeuwarden, the Netherlands) fitted with the expiratory port. FeNO measurement was performed off-line by a NO analyzer (Endono; Seres, Aix en Provence, France) within the next 30 min. Two measures were obtained for each patient, and the arithmetic mean was taken as a definitive value.

Statistical analysis

Results were expressed as median (interquartile 25th–75th). The statistical analysis was performed by using statistical software (STATISTICA version 8, Statsoft, Maison-Alfort ; France). The reproducibility of FeNO measurements was tested by linear correlation. The qualitative comparison was performed by Chi-squared test or Fischer's test according to the studied variables. The association between continuous variables was searched by non-parametric correlation test of Spearman (Rho) and then tested by linear correlation test (r). The quantitative comparison was carried out by non-parametric test of Mann and Whitney. A p-value < 0.05 was considered statistically significant.

Results

Eighty consecutive patients (51 boys) participated: 40 patients with well-controlled infantile wheeze, median age 14 months (6–29) and 40 patients with severe persistent wheezy respiratory symptoms, median age 14.5 months (6–36). The main characteristics of the population are displayed in Table 1.

Reproducibility of FeNO measurements

Two measurements were realized in each patient and were strongly reproducible: $r = 0.95$, $p < 0.0001$, (Fig. 1).

Correlation between FeNO levels and clinical control of infantile wheeze

FeNO levels were significantly elevated in patients with persistent wheezy respiratory symptoms, median FeNO level was 19.8 (2.5–99.3) ppb in group NC vs. 7.7 (0.6–29.5) ppb in group

Table 1. Fractional exhaled nitric oxide (FeNO) levels regarding possible affecting factors

		N (%)		FeNO ppb*		p
		80	13 (0.6–99.3)			
Total						
Sex	M	51 (64)	13.4 (0.6–86.5)	NS		
	F	29 (36)	11.2 (1.8–99.3)			
Clinical control of wheezy symptoms	Yes	40 (50)	7.7 (0.6–29)	<0.0001		
	Non	40 (50)	19.8 (2.5–99.3)			
Atopic father or mother	Yes	18 (23)	7 (1.9–40)	0.02		
	No	62 (77)	13.5 (0.6–99.3)			
Positive personal history of atopy	Yes	10 (13)	17.4 (7–99.3)	NS		
	No	70 (87)	11.5 (0.6–86.5)			
Passive tobacco smoking	Yes	16 (20)	15.5 (4.3–86.5)	NS		
	No	64 (80)	11 (0.6–99.3)			
Inhaled corticoids dose (fluticasone equivalent, µg/day)*	200–500	33 (41)	12.8 (1.9–99.3)	NS		
	1000	47 (49)	12.6 (0.7–86.5)			

*Results are expressed in median (25th–75th) interquartiles.

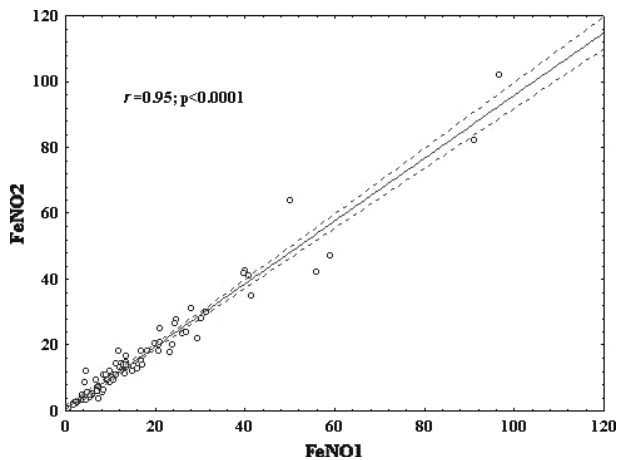


Fig. 1. Reproducibility of FeNO measurements. Two successive samples (FeNO1 and FeNO2) were collected for each patient.

C, $p < 0.0001$. (Table 1 and Fig. 2). We had found an association between FeNO value > 15 ppb and the absence of clinical control of infantile wheeze with a positive predictive value of 65% and a high negative predictive value of 90%.

FeNO levels and other potent conditions that might influence FeNO production

Regarding the literature, atopy, dose of inhaled steroid treatment, and tobacco exposure were analyzed (Table 1).

FeNO and ICS dosage. We found no correlation between the administered ICS dose and FeNO levels ($Rho = 0.08$, $p = 0.46$). Conversely, median ICS dose (fluticasone equivalent) was significantly lower in group C than in NC: 200

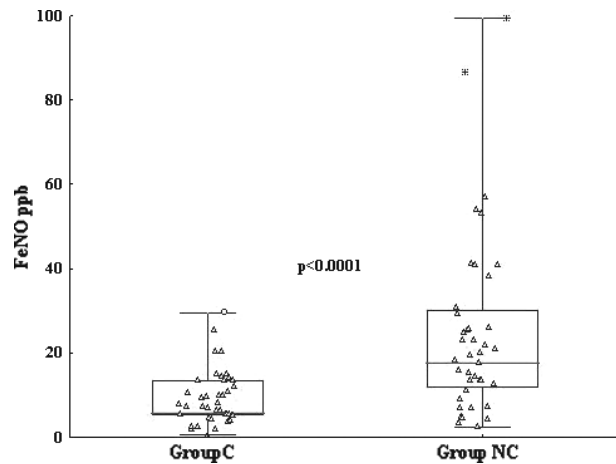


Fig. 2. FeNO levels and clinical control of respiratory symptoms. FeNO was assessed in young children with either optimal control (group C, $n = 40$) or uncontrolled respiratory wheeze symptoms (group NC, $n = 40$).

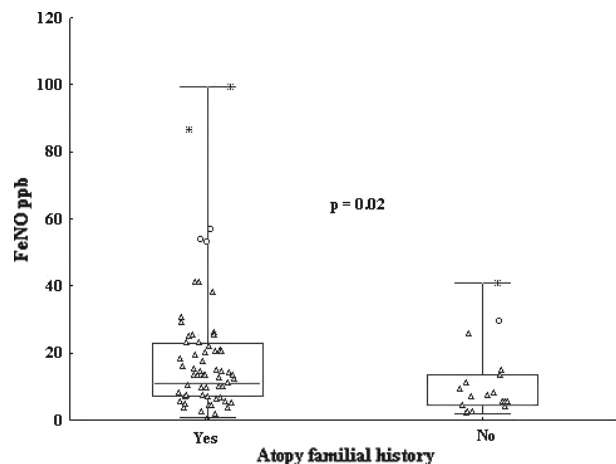


Fig. 3. FeNO levels and familial atopy history. This was defined if at least one parent has bronchial asthma and/or allergic rhinitis ($n = 18$), and 62 children had negative familial atopy history.

(200–1000) µg/day vs. 1000 (200 1000) µg/day, $p = 0.003$.

FeNO and atopy. FeNO was significantly decreased in patients with a positive family history of atopy (Figure 3). No difference was observed between patients with positive or negative personal history of atopy (Table 1).

We did not find any influence of smoking exposure, child age, sex, weight, and height on the FeNO levels.

Discussion

We have measured the exhaled nitric oxide fractions in recurrent wheezy young children

aged less than 3 yr and receiving maintenance treatment by ICS. We reported that FeNO measurement in this young population was easy and reproducible and that optimal control of the respiratory disease was strongly associated with the control of bronchial inflammation.

Although the online measurement of FeNO is well standardized, the off-line method has been stated to be achieved in infants and young children (1). We collected consecutively exhaled breath in two balloons applying the methods previously described (14). We found an accurate reproducibility between the two measures, arguing for a fairly interpretation for clinical practice. Few studies reported normal values of fractional exhaled nitric oxide in infants and young children: 8.4 (7–10.1) ppb (9) and 10.4 ppb (9.1–12) (14), and these were correlated to gestational age and birth weight (9). In our population, FeNO levels were not influenced by the age, weight, or height of the patients.

Interestingly, ninety percent of these infants who acquired an optimal control of the disease had an associated FeNO level similar to that observed in healthy infants (14). The fact that the control of the symptoms reflected the control of the underlying inflammation enhanced the current guidelines on the treatment of preschool asthma, which has yet been based on clinical symptoms. Indeed, this sustained the recommendation to step-down the dosing of ICS when the clinical control of the disease has been obtained (<http://www.ginasthma.com>). Conversely, the median FeNO level was augmented in uncontrolled infants despite higher doses of ICS administered. However, these levels varied within a wide range in this group, and a lack of bronchial inflammation was observed in 40% of these infants. Consequently, the positive predictive value of a FeNO level >15 ppb appeared low in this population of infants under inhaled steroid treatment for at least 3 months. Such difficulties to correlate FeNO levels with the severity or no control of asthma have otherwise been encountered in children, adolescents, and adults. Thus, Piacentini et al. (15) reported that only 33% of uncontrolled children had high exhaled nitric oxide level, and these authors suggested that ICS treatment might partly explained this discrepancy. On the other hand, Malmberg et al. (16) found a good correlation between FeNO levels and exercise-induced bronchoconstriction when the analysis was restricted to the subgroup of atopic children. This highlights the complex interpretation of exhaled NO in asthma. Because of the small size of similar subgroups in

our study, we were not able to test these analyses. However, our findings agreed with the recent systematic review (4), which concluded that exhaled nitric oxide measurement could not be currently recommended for the management of asthma.

Previous studies demonstrated that exhaled nitric oxide was a marker of bronchial inflammation in wheezy infants. In fact, elevated FeNO levels were observed during acute exacerbations (17) and varied in recurrent wheezing from 15.6 ppb (18) to 31.8 ppb (19). Moreover, some data supported that FeNO might be a marker of early childhood asthma. Moeller et al. (20) found increased FeNO levels in young children with a stringent index of asthma at school age in comparison with those having a loose index or no wheezy respiratory symptoms. Gabriele et al. (6) observed that FeNO might differentiate various airway diseases in infants. FeNO levels were higher in wheezy infants (18.4 ppb) than in control (10.4 ppb), broncho-pulmonary dysplasia (11.7 ppb), and cystic fibrosis infants (5.9 ppb). In our study, the median FeNO level in uncontrolled infants was comparable with that reported in these studies. Conversely, the FeNO levels measured in controlled infants under ICS treatment were similar to that obtained in healthy infants (6, 14).

Nevertheless, the pathophysiology associated with an increased FeNO production in infants remained uncertain. In fact, FeNO is recognized as a marker of eosinophilic inflammation, and conversely, current data argued for a predominant neutrophilic lung inflammation in infants with recurrent wheezes (5). FeNO levels have been also shown to reflect small airway disease in infants, which commonly is associated with a neutrophil-related inflammation phenotype (6). In opposite, diminished FeNO levels have been mostly reported in respiratory diseases where inflammation was mainly related to neutrophil cell recruitment in the lung (8, 21–23). In adults, both active and passive smoking decreased exhaled nitric oxide fractions (21). In childhood, the findings have been controversial and might depend on the time of smoking exposure: pre-natal tobacco exposure was associated with lower FeNO (8) and post-natal exposure with higher (22) or lower (8) FeNO levels. In our study, FeNO levels were not modified regarding tobacco exposure. In cystic fibrosis, FeNO levels were commonly decreased, but, however, correlated positively with the disease severity (23). On the other hand, we and others reported high concentrations of eosinophil cationic protein in broncho-alveolar lavage from infants with severe

recurrent wheezing (24, 25). In addition, eosinophils and basement membrane thickening were definitely detected in bronchial tissue from young asthmatic children (26) and this appeared independent of atopy (17). Therefore, bronchial eosinophilic inflammation may be underestimated in infants, and FeNO as a marker of eosinophilic pathway should be yet considered, although this eosinophilic inflammation remained inconstantly observed (26, 27).

Other factors should be considered in the interpretation of FeNO levels. It is known that atopy increases exhaled NO even in asymptomatic children (28). In our study, personal or family history of atopy did not elevate FeNO levels, in agreement with other reports (10, 16, 17, 29) but not with Marchand et al. (7). We cannot exclude that patients with atopic family history were better ICS responders (30) but the limited number of patients in this subgroup restricted any definite conclusion. The other main factor was the administered dose of ICS. No correlation was, however, observed between the given dose and FeNO levels. Moreover, the given dose of ICS was highest in uncontrolled infants where FeNO levels were more augmented.

In conclusion, exhaled nitric oxide is a non-invasive marker of bronchial inflammation in wheezy infants, and FeNO off-line measurement in those very young children is feasible, reproducible, and well accepted. Additionally, optimal clinical control is strongly associated with FeNO levels in this age group. These findings suggest that optimal clinical control of wheezy respiratory symptoms in infants also reflect the control of airway inflammatory process, as FeNO levels were less than 15 ppb. Our study provides an objective evidence that a step-down of inhaled steroids could be considered in asymptomatic patients. Further studies are required to determine the exact place of this measurement in clinical follow-up and daily practice. FeNO assessment also might help to understand the pathophysiology of wheeze syndrome in infants and very young children.

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