

PEDIATRIC ASTHMA

Young Infants with Recurrent Wheezing and Positive Asthma Predictive Index Have Higher Levels of Exhaled Nitric Oxide

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Objective. The aim of this post hoc analysis was to establish the relationship between FE_{NO} levels and the asthma predictive index (API) among infants with recurrent wheezing. **Methods.** Infants with recurrent wheezing (three or more episodes) were recruited consecutively and online FE_{NO} tests at tidal breathing with multiple breaths were performed. **Results.** Twenty-seven (84%) out of 32 infants (median age of 12 months) who met the inclusion criteria for this post hoc analysis, successfully performed the FE_{NO} determinations. Eighteen (66%) infants were classified with positive stringent API. FE_{NO} levels were significantly higher among patients with positive API than those with negative (median [IQR] of 12.3 [14.8] ppb vs. 4.1 [7.9] ppb, respectively, $p = .016$). Furthermore, FE_{NO} and positive API had a significant correlation (Spearman's rho, $\rho = 0.4741$, $p = .0125$). After logistic regression analysis including FE_{NO} levels, gender, age, and use of controller therapy, FE_{NO} was the only variable that was marginally related to API (OR = 1.12, 95% CI: 0.99–1.27, $p = .07$). **Conclusion.** Infants with recurrent wheezing who had a positive stringent API already had higher FE_{NO} levels than those with a negative API. This finding needs to be corroborated in a larger prospective study.

Keywords asthma predictive index, early childhood asthma, fraction of exhaled nitric oxide, infants, recurrent wheezing

INTRODUCTION

Asthma is the most prevalent chronic disease among children and in the vast majority of the cases it starts before the age of five (1). However, it continues to be one of the most difficult disorders for physicians to diagnose in infants and preschoolers. This is partly because clinical symptoms of asthma are variable and non-specific, given that other wheezing disorders co-exist (e.g., transient wheezing) (2, 3). No accurate screening tests using genetic or single biochemical markers have as yet been developed to determine which young children with recurrent wheezing will develop asthma (4). Therefore, the diagnosis and management of asthma in young children are still primarily based on subjective clinical features and findings from medical examinations. One of the clinical tools most commonly proposed for early prediction of asthma in young children with recurrent wheezing is the asthma predictive index (API) (5).

On the other hand, neither airflow limitation nor airway inflammation, the main pathologic hallmarks of asthma, can be assessed routinely in infants. Recently, a study of preschoolers showed higher markers of airway inflammation (IL-2, -4, -8, and -10 and sICAM) measured in exhaled breath condensate among children with a persistent wheezing phenotype than in those without wheezing (6). The

fraction of exhaled nitric oxide (FE_{NO}), as a surrogate marker of eosinophilic inflammation of the airways, has been tested in several studies of asthmatic school-age children (7–9). FE_{NO} was reported to be higher both among preschoolers with recurrent wheezing bronchitis compared with controls (10) and among preschoolers with atopic compared with non-atopic wheezing (11). FE_{NO} also correlated well with preschoolers with probable asthma (12).

Recently, we showed that the determination of online FE_{NO} can be successfully performed in infants (13). Using those data, we conducted this post hoc analysis in order to determine whether recurrent wheezing infants with a positive API (high risk to develop asthma) already had evidence of airway inflammation (as measured by FE_{NO}) at a young age. Our hypothesis is that infants with a positive API have higher levels of FE_{NO} than those with a negative API.

METHODS

The original study was of a cross-sectional design and was carried out in the pediatric pulmonary clinic at the Hospital Universitario Donostia, Spain (13). Briefly, a consecutive sample of 38 patients aged less than 24 months with recurrent wheezing (three or more episodes of confirmed wheezing in the last year) were enrolled in the study. Exclusion criteria for this post hoc analysis included the presence of other chronic respiratory conditions

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(e.g., cystic fibrosis, bronchopulmonary dysplasia, primary ciliary dyskinesia, post-infectious bronchiolitis obliterans, interstitial lung disease, pulmonary malformations, and gastroesophageal reflux), cardiac malformations, neurological abnormality, and prematurity (<37 weeks of gestational age).

A potsprandial FE_{NO} was determined at tidal breathing over multiple breaths without sedation. An online system was used with a stationary chemiluminescence analyzer (CLD 88 sp; Eco Physics AG, Duernten, Switzerland). A mask was used to separate airflow from the oral and nasal cavities (Hans Rudolph Inc, Kansas City, MO, USA), with an expiratory flow between 40 and 60 ml/s (14). The mask was connected to a disposable antibacterial antiviral filter. Mean FE_{NO} was obtained during the spontaneous respiratory cycles (inspiration–expiration) of the subjects over a 60-s period. FE_{NO} was measured at the end of the respiratory cycle, in the stable plateau phase when the expiration of 60–80% of the total tidal volume had been exhaled. All subjects performed three valid determinations with a variability ratio of less than 10% among them, and the mean calculated from these measurements. The determinations ranged between 0.1 and 5000 parts per billion (ppb). The determinations were carried out with NO-free (<5 ppb) room air to avoid environmental contamination. Daily calibration of flow and volume verified the precision of these measurements, as did a calibration of NO to zero. Likewise, the NO gas was calibrated monthly (13).

Data on demographic characteristics, use of controllers, parental medical diagnosis of asthma, medical diagnosis of dermatitis and rhinitis, episodes of wheezing apart from colds and peripheral blood eosinophils (determined in a venous blood sample by a fully automated blood cell counter and expressed as the percentage of total leukocyte count) was taken during the first visit. API was based on the original definition using the stringent index (5), which requires recurrent episodes of wheezing (≥ 3 episodes/year) during the first 3 years of age and one of two major criteria (physician-diagnosed eczema or parental asthma) or two of three minor criteria (physician-diagnosis allergic rhinitis, wheezing without colds, or peripheral eosinophilia $\geq 4\%$) (5).

The study was approved by the hospital's Ethics and Research Committee and written informed consent and permission were obtained from both parents and/or guardians.

Data Analysis

Given that there is limited reliable data on methodology and the lack of standardization of FE_{NO} determination techniques, both in general and for infant populations, no sample size was determined a priori. A univariate analysis of the positive and negative API groups was conducted, as well as a chi-square test for associations among categorical data. The distribution of numerical variables was evaluated using the Shapiro–Wilk test and described as median and interquartile range (IQR). Spearman's coefficient between FE_{NO} and API was calculated. Logistic regression analysis was used to test

for significant associations between API and factors known to influence FE_{NO} levels (age, sex, and the use of controller therapy). Two-tailed *p* values of <.05 were considered significant. STATA 8.0 (Stata Corp, College Station, Texas, USA) statistical software package was used for the analysis.

RESULTS

Twenty-seven (84%) of the 32 original infants who successfully performed the FE_{NO} determinations met the inclusion/exclusion criteria for this post hoc analysis. The median (IQR) age was 12 (2.5) months, 64.8% were males, and the median (IQR) expiratory flow of FE_{NO} was 62 (4.4) ml/s.

There were 18 (66%) patients with positive stringent API. Among this group the prevalence of parental doctor asthma diagnosis, atopic dermatitis, allergic rhinitis, wheezing without viral infections, and eosinophilia were 44%, 56%, 0%, 70%, and 44%, respectively. There were no significant differences in gender, weight, antiasthmatic drug use, number of reproducible maneuvers, and FE_{NO} determinations between infants with positive and negative API (Table 1). However, those in the positive API group were significantly older than negative API group: median (IQR) of 13.5 (6.3) months versus 11 (8) months, respectively, *p* = .033 (Table 1).

Infants with positive API had significantly higher FE_{NO} levels than those with negative API: median (IQR) of 12.3 (14.8) ppb versus 4.1 (7.9) ppb, respectively, *p* = .016 (Table 1 and Figure 1). Furthermore, FE_{NO} and positive API were significantly correlated (Spearman's rho, ρ = .4741, *p* = .0125). Logistic regression analysis of FE_{NO}, gender, and age and the use of controller therapy showed that FE_{NO} was the only variable marginally related to API (OR = 1.12, 95% CI: 0.99–1.27, *p* = .07).

TABLE 1.—Characteristics of the infants with positive and negative original asthma predictive indices (API).

	Positive API <i>n</i> = 18	Negative API <i>n</i> = 9	<i>p</i> -Value
Males (%)	75	54.6	.268
Age (months)	13.5 (6.3)	11 (8)	.033*
Weight (kg)	10 (6.3)	8.5 (4.3)	.605
Controller therapy (%):			.272
None	61.1	88.9	
ICS	22.2	0	
LTRA	5.5	11.1	
ICS and LTRA	11.1	0	
FE _{NO} (ppb)	12.3 (14.8)	4.1 (7.9)	.016*
Expiratory flow (ml/s)	61.7 (8.8)	62 (7.5)	.817
Reproducible maneuvers (%):			.08
Two	27.8	0	
Three	72.2	100	

Note: Numbers are expressed as % or median (interquartile range).

ICS, inhaled corticosteroids; LTRA, leukotriene receptor antagonist; FE_{NO}, fraction of exhaled nitric oxide; ppb, parts per billion.

**p* < 0.05.

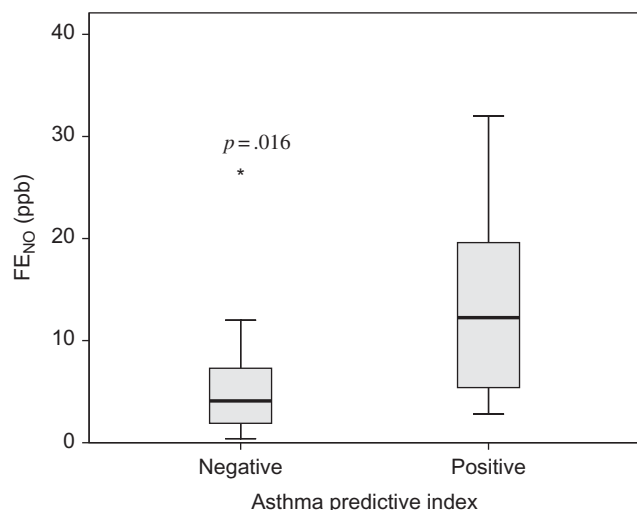


FIGURE 1.—Boxplot comparing exhaled nitric oxide (FE_{NO}) levels between the two groups of subjects with negative and positive original asthma predictive indices.

Note: ppb, parts per billion.

DISCUSSION

This study demonstrates that small infants (median age of 12 months) with recurrent wheezing and high risk to developed asthma (positive API) showed higher, although not significant, levels of FE_{NO} (a surrogate marker of airway inflammation) than those with negative API. A statistically significant correlation between FE_{NO} and positive API was found ($\rho = 0.4741$, $p = .0125$).

Previous studies have demonstrated the utility of FE_{NO} determinations in monitoring asthma therapy and predicting the course of the condition in older children (15–17). In a cohort study of 107 schoolchildren (mean age: 14.7 ± 2.3 years), FE_{NO} > 7 ppb was reported to have 47% sensitivity and 93% specificity for an asthma diagnosis (18). In another study of 96 children (aged 3.8–7.5 years) with asthma symptoms, FE_{NO} discriminated between those with probable asthma and healthy controls with 86% sensitivity and 92% specificity (12). Moeller et al. (19) reported higher offline FE_{NO} levels in preschoolers (median age: 23 months) with positive original stringent API than in controls (11.65 vs. 6.5 ppb, respectively, $p = .001$). In the present study, infants with a positive API had significantly higher FE_{NO} levels (median [IQR] of 12.3 [14.8] ppb) than those with a negative API (4.1 [7.9] ppb). Although the absolute difference is small, it was significant. FE_{NO} values in both the groups were below the standard cut point recommended by the ATS (20). However, that recommendation (20 ppb) was made for older children, and the guidelines “recommend accounting for age as a factor affecting FENO in children younger than 12 years of age (strong recommendation and high quality of evidence).” Further, the measurement of FE_{NO} in infants has been only recently validated. Therefore, studies on FE_{NO} in a large number of healthy and wheezing infants are required to allow confident interpretation of these results.

In the present study, more of the infants in the positive API group were males than females (although not

significantly so) and of significantly older age, both factors that are associated with higher FE_{NO}. However, logistic regression did not find an association between these two characteristics and FE_{NO} levels, perhaps due to the small study population.

It has been demonstrated, using bronchoalveolar lavage, that eosinophilic inflammation is present in children with recurrent wheezing as early as a median age of 14.9 months (21). That is in agreement with our results in that FE_{NO} levels (a surrogate marker of airway inflammation) were marginally higher in recurrent wheezing infants (median age of 12 months) with positive original stringent API than those with negative API. In addition, Keklikian et al. (22) reported that another component of asthma (alteration of lung function) was also present in small infants (mean age 11.9 months) with positive stringent API. These last two findings suggest that in small infants with recurrent wheezing who have positive stringent API early therapy with controllers may be warranted to decrease airway inflammation and improve lung function. A previous trial done in preschoolers with a positive API (mean age 3.0 ± 0.6 years) demonstrated that inhaled corticosteroid improved their symptoms, decreased inflammation, and improved lung function while it was taken, but failed to alter the natural history of the disease (23). It is possible that the results would be different if therapy with inhaled corticosteroids was started earlier (e.g., in infants less than 18 months old with positive stringent API). Further trials with controllers need to be done at this early age.

The present post hoc study has some limitations. First, adequate standardization of measurement technique and establishment of normal values for FE_{NO} measured online during tidal breathing, with multiple breaths in non-sedated infants/toddlers has not been done. However, the number of reproducible maneuvers and expiratory flows were similar between our two groups. Second, the small sample limits statistical power, though we speculate that with more subjects the difference found here would be greater. Studies with large samples at this age need to be done in order to confirm our findings. Third, other potential confounders that could influence the FE_{NO} (e.g., passive tobacco exposure and nitrate-containing foods) were not taken into account.

CONCLUSIONS/KEY FINDINGS

Young infants with recurrent wheezing and a positive stringent API had higher FE_{NO} levels than those with negative API, suggesting that airway inflammation is already present at early age in this group of infants. This finding needs to be corroborated in a larger prospective study.

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DECLARATION OF INTEREST

The authors have no conflicts of interest to declare.

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