
Effect of elevated exhaled nitric oxide levels on the risk of respiratory tract illness in preschool-aged children with moderate-to-severe intermittent wheezing

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Background: The fractional concentration of exhaled nitric oxide (FeNO) is a noninvasive marker for airway inflammation but requires further study in preschool-aged children to determine its clinical relevance.

Objective: To determine whether the risk of respiratory tract illnesses (RTIs), disease burden, and atopic features are related to FeNO in preschool-aged children with moderate-to-severe intermittent wheezing.

Methods: We determined FeNO using the off-line tidal breathing technique in 89 children, aged 12 to 59 months, with moderate-to-severe intermittent wheezing. The risk of RTI was determined by comparing participants with a baseline FeNO of greater than the 75th percentile (24.4 ppb) with those with a baseline FeNO at the 75th percentile or lower using Cox regression analysis.

Results: The risk of RTI was significantly higher in children with an FeNO of greater than 24.4 ppb relative to those with lower FeNO values (adjusted relative risk, 3.80; 95% confidence interval, 1.74–8.22; $P < .001$). FeNO levels of greater than 24.4 ppb were associated with more positive skin test results to aeroallergens ($P = .03$) but not with other atopic characteristics or historic parameters of illness burden.

Conclusions: An elevated FeNO in preschool-aged children with moderate-to-severe intermittent wheezing was associated with an increased risk of RTI during a 1-year follow-up. In addition, a higher FeNO was associated with aeroallergen sensitization.

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INTRODUCTION

The fractional concentration of exhaled nitric oxide (FeNO) is a noninvasive biomarker reflecting eosinophilic airway inflammation.¹ The interest in adding FeNO measurement to the routine evaluation of patients with asthma derives from the fact that this test requires little effort from the patient, can be performed even by young children, is noninvasive, and is safe.²

Levels of FeNO in school-aged children with mild-to-moderate persistent asthma correlate with features of atopy and asthma, including peripheral blood eosinophilia, IgE levels, number of positive aeroallergen skin test results, and airway hyperresponsiveness,³ as well as disease severity and disease activity.^{4,5} Furthermore, monitoring airway inflammation by FeNO levels may be useful in optimizing inhaled steroid dose adjustment both in adults and school-aged children.^{6–9}

Data on the utility of FeNO determination in preschool-aged children are limited to 2 reports suggesting that FeNO may help to diagnose asthma.^{10,11} To provide additional evidence on the role of FeNO in characterizing preschool-aged children with moderate-to-severe intermittent wheezing, we determined whether FeNO levels are associated with the risk of respiratory tract illnesses (RTIs), disease burden, and atopic features in this population.

METHODS

Study Population

The cohort described in these analyses is a subgroup of children, aged 12 to 59 months, recruited into the Acute

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Intervention Management Strategies (AIMS) clinical trial of the Childhood Asthma Research and Education (CARE) Network. AIMS was a multicenter, double-blind, randomized, placebo-controlled, double-dummy, parallel-group comparison of 3 treatment regimens (inhaled corticosteroid, leukotriene receptor antagonist, or β_2 -agonists alone) used episodically at the early signs of RTIs in children with recurrent episodes of moderate-to-severe wheezing.¹² After institutional review board approval, written informed consent was obtained from the parent of each participant.

Children aged 12 to 59 months were included in the trial if they had at least 2 episodes of moderate-to-severe wheezing in the context of an upper respiratory tract infection during the 12 months before screening. At least 1 episode had to be documented by a health care provider, and at least 1 episode had to be within 6 months before screening. These criteria were established to maximize the likelihood of occurrence of moderate-to-severe wheezing episode(s) during the trial. "Moderate to severe" is an overall descriptor of the severity of prior wheezing episodes (as defined by the inclusion criteria). In addition, children were considered to have intermittent disease based on a clinical history of intermittent illness in the prior year and experiencing symptoms (nocturnal cough, daytime cough, wheezing, difficulty breathing, or symptoms interfering with activities) and/or requiring albuterol use on average fewer than 4 days per week in the 2-week observation period before the randomization visit. Children were excluded if, during the prior 12 months, they received more than 6 courses of systemic corticosteroids, had more than 2 hospitalizations for wheezing illnesses, or were treated with controller medications for more than 4 months or within the 2 weeks before screening. Comprehensive allergy and asthma questionnaires were administered at enrollment. At the randomization visit, the following studies were performed: allergy skin prick testing to a panel of 8 aeroallergens (house dust mite mix, cat, dog, tree mix, grass mix, weed mix, mold mix, and cockroach mix) and 3 food allergens (milk, egg, and peanut), serum total IgE level, peripheral blood eosinophil counts, assessments of quality of life,¹³ and completion of the Juniper Pediatric Asthma Caregiver's Quality of Life Questionnaire.¹⁴

FeNO Measurements

Of the 280 children, 110 (39.3% of the entire AIMS study cohort) attempted the FeNO collection procedure, of which 89 (80.9%) had determinations that were considered acceptable by prespecified standards (requirements for adequate FeNO collection are described later). The determination of FeNO in only 39.3% of the entire AIMS cohort was due to initiating FeNO collections after the commencement of the trial; trial recruitment began in February 2004, and FeNO measurements began to be obtained in June 2004. FeNO was measured either at the study randomization visit ($n = 57$) or (for participants enrolled before June 2004) before any study medication was administered ($n = 32$). Thus, FeNO was only measured for participants who had not been treated with

asthma controller medication for at least 4 weeks preceding the measurement, because it is established that inhaled corticosteroid therapy can reduce FeNO levels.^{5,15,16}

FeNO was measured according to American Thoracic Society/European Respiratory Society recommendations¹ by an off-line reservoir technique using the NIOX system (Aerocrine AB, Stockholm, Sweden). Children were seated on their mother's lap and breathed through a face mask that was gently placed on their face. The face mask was attached to a 2-way valve that allowed the inspiration of NO-free air to prevent contamination with ambient air. After 5 breaths of NO-free air, 5 breaths of exhaled air were collected in triplicate from each participant in a nondiffusing (polyethylene) 0.5-L gas collection bag (Hans Rudolph, Inc, Shawnee, Kansas) during quiet and regular tidal breathing. Nasal contamination was prevented by closing the velum by exhaling against 5-cm H₂O oral pressure, achieved by a resistor between the collection bag and the expiratory side of the valve. If a child did not cooperate (crying or irregular breathing) or if a leak was detected, the collection was repeated after several minutes. If more than 3 attempts did not allow FeNO collection, as previously specified, the child was not included in the FeNO substudy. The FeNO concentration in the bags was analyzed by the NIOX off-line analyzer within 3 hours of collection. The final FeNO level represents the average of these triplicates.

Outcomes and Statistical Analysis

The parameters of demographics, atopic characterization, and disease burden (number of emergency department [ED] visits in the past year, number of physician office visits, number of days of missed school or day care in the past year, asthma controller medication use in the past year, number of courses of oral corticosteroids in the past year, and scores on 2 quality-of-life questionnaires [Pediatric Asthma Caregiver's Quality of Life Questionnaire and PedsQL (Pediatric Quality of Life Inventory)]) were compared between quartiles of FeNO levels using 1-way analysis of variance or Kruskal-Wallis tests for continuous data and χ^2 tests for categorical data. These analyses included data from all 89 children with acceptable FeNO measurements.

Survival analysis methods (Kaplan-Meier curves and Cox proportional hazards regression) were used to characterize risk of RTI, during a 1-year follow-up period, according to FeNO level. An RTI describes the constellation of respiratory symptoms that, by the parents' perception, would be expected to precede and signal the development of an episode of wheezing. As previously described, 32 patients had FeNO measurements obtained after the randomization visit. The inclusion of these participants in the survival analysis would cause selection bias toward patients with a decreased risk of RTI because those who had an early RTI were excluded from FeNO measurements at a visit after randomization because they had used study medication. To eliminate this bias, these analyses included only data from the 57 patients with FeNO measurements collected at randomization. Kaplan-Meier

curves for time to first RTI were stratified according to baseline FeNO levels dichotomized at the 75th percentile (24.4 ppb). We chose these FeNO levels as the cutoff for our analysis based on the fact that similar FeNO levels were reported by Meyts et al¹⁷ as the upper limit of the 95% confidence interval (CI) in preschool-aged children with a history of recurrent wheezing.

Cox proportional hazards regression was used to estimate the relative risk of RTI associated with high FeNO levels. This analysis was performed with and without adjustment for potential confounders, including Asthma Predictive Index (API) status,¹⁸ 1 or more positive aeroallergen skin test results, number of courses of oral corticosteroids in the prior year, and number of ED visits for acute wheezing in the prior year. All statistical analyses were performed using SAS, version 9.1, statistical software (SAS Institute Inc, Cary, North Carolina); graphs were created using Splus 8.0 (Insightful Corp, Seattle, Washington).

RESULTS

Between June and November 2004, FeNO levels were measured successfully in 89 (81%) of 110 children. The reasons for the inability to measure FeNO levels were as follows: uncooperative child ($n = 15$), child or parent refused ($n = 3$), or equipment failure ($n = 3$). The subgroup of the uncooperative children included mainly younger children (13 of the 15 children in this subgroup were <36 months old).

The distribution of FeNO (Fig 1) is skewed toward the right, with a median (quartile 1-quartile 3) of 14.0 (10.5–24.4) ppb. FeNO levels were measured with high intraindividual reproducibility, as evident by 86.5% of the children having at least 2 FeNO measurements within 5 ppb or 10% of each other.¹⁹ Median (quartile 1-quartile 3) FeNO levels did not differ between children who were recruited to the study in the spring ($n = 18$) (15.50 [11.17–26.67] ppb), summer ($n = 51$) (13.03 [9.97–21.40] ppb), or fall ($n = 20$) (17.40 [11.68–

33.48]) ($P = .25$). No children were recruited during the winter.

FeNO levels by quartile were not associated with age or sex (Table 1). Children with FeNO levels in the highest 2 quartiles were significantly more likely to be of Hispanic or Latino ethnicity than those in the lower quartiles ($P = .004$). However, FeNO levels did not differ between whites and nonwhites. Higher FeNO levels were associated with more positive skin test results to aeroallergens ($P = .03$) but not with other atopic characteristics, including number of positive skin test results to food allergens, IgE level, eosinophil count, presence of eczema, positive modified API status,¹⁸ or parental asthma (Table 1). FeNO levels were not associated with parameters of illness burden during the year before enrollment, including number of emergency department visits, number of days of missed school or day care, number of courses of oral corticosteroids, or asthma quality-of-life scores (Table 2).

The risk of experiencing an RTI was evaluated in the 57 children from whom FeNO was collected at the randomization visit. This subgroup was comparable to the 32 children who had FeNO measurement after randomization, except for having significantly more whites (49 [86.0%] vs 16 [50.0%]; $P < .001$). The risk of RTI was significantly higher in children with FeNO levels at the 75th percentile (24.4 ppb) or higher relative to those with FeNO levels lower than 24.4 ppb (relative risk [RR], 3.00; 95% CI, 1.50–6.01; $P = .002$) (Fig 2). This elevated risk of RTI was not associated with any other baseline characteristics, as described in Tables 1 and 2. Furthermore, after adjustment for potential confounders, including API status, 1 or more positive aeroallergen skin test results, number of courses of oral corticosteroids in the prior year, and number of ED visits for acute wheezing in the prior year, the risk for RTI remained significantly elevated (RR,

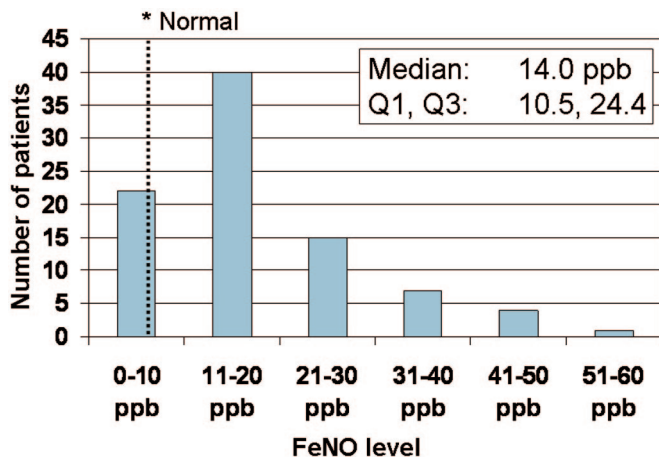


Figure 1. Distribution of the fractional concentration of exhaled nitric oxide (FeNO) levels. * Upper limit of normal, from Baraldi et al.²¹

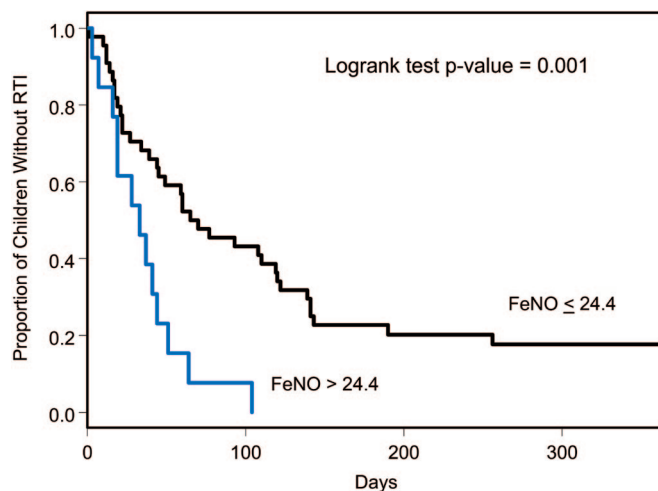


Figure 2. Kaplan-Meier estimates of the time to a first episode of respiratory tract illness (RTI) by fractional concentration of exhaled nitric oxide (FeNO) level at randomization.

Table 1. Relationships Between FeNO Levels and Demographic and Atopic Characteristics^a

Characteristics	FeNO quartiles ^b				P value
	1 (n = 22)	2 (n = 23)	3 (n = 21)	4 (n = 23)	
Demographics					
Age, y ^c	3.2 (0.8)	3.4 (1.1)	3.4 (0.9)	3.3 (1.2)	.60
Male sex	16 (73)	17 (74)	13 (62)	15 (65)	.44
Race					
White	17 (77)	16 (70)	14 (67)	18 (78)	.98
Nonwhite	5 (23)	7 (30)	7 (33)	5 (22)	.98
Hispanic or Latino ethnicity	2 (9)	5 (22)	9 (43)	10 (43)	.004
Season of recruitment					
Spring	4 (18)	3 (13)	5 (24)	6 (26)	.12
Summer	13 (59)	18 (78)	10 (48)	10 (43)	
Fall	5 (23)	2 (9)	6 (29)	7 (30)	
Atopic features					
No. of positive skin test results per child ^c					
To aeroallergens (maximum, 8)	0.7 (0.9)	0.7 (1.1)	1.5 (1.8)	1.4 (1.6)	.03
To food allergens (maximum, 3)	0.6 (0.9)	0.2 (0.5)	0.6 (0.9)	0.3 (0.8)	.62
IgE level, kU/L ^d	44.8 (4.0)	52.7 (5.1)	89.9 (4.0)	55.9 (6.6)	.44
Eosinophils, % ^d	3.8 (2.0)	3.2 (1.9)	3.8 (1.8)	5.0 (2.0)	.12
Presence of eczema	1 (5)	2 (9)	1 (5)	3 (13)	.39
Parental asthma	7 (32)	10 (43)	7 (33)	9 (39)	.26
Positive API	16 (73)	13 (57)	11 (52)	13 (57)	.27

Abbreviations: API, Asthma Predictive Index; FeNO, fractional concentration of exhaled nitric oxide.

^a Data are expressed as number (percentage) of children in each quartile unless otherwise noted.^b The range for quartile 1 is 10.5 ppb or less; 2, 10.6 to 14.0 ppb; 3, 14.1 to 24.4 ppb; and 4, greater than 24.4 ppb.^c Data are given as mean (SD).^d Data are given as geometric mean (coefficient of variation).Table 2. Association of FeNO Levels With Pretrial Illness Burden^a

Variable	FeNO quartiles ^b				P value
	1 (n = 22)	2 (n = 23)	3 (n = 21)	4 (n = 23)	
Disease burden in the past year					
No. of ED visits	0.8 (1.2)	1.0 (2.0)	2.0 (5.2)	1.1 (1.6)	.53
No. of office visits	3.1 (1.5)	4.4 (3.7)	4.6 (5.1)	3.6 (3.0)	.63
No. of days of missed school or day care	8.9 (21.4)	4.5 (5.8)	4.1 (6.6)	2.9 (4.0)	.11
Medication use in the past year ^c					
Inhaled corticosteroid	10 (45)	7 (30)	6 (29)	8 (35)	.62
Montelukast	2 (9)	2 (9)	1 (5)	3 (13)	.65
Any controller (including oral corticosteroids)	17 (77)	13 (57)	17 (81)	14 (61)	.56
No. of courses of oral corticosteroids in the past year ^c					
0	10 (45)	10 (43)	6 (29)	14 (61)	
1	5 (23)	3 (13)	5 (24)	5 (22)	
2	6 (27)	4 (17)	6 (29)	3 (13)	.40
3	1 (5)	2 (9)	4 (19)	0 (0)	
≥4	0 (0)	4 (17)	0 (0)	1 (4)	
Quality-of-life scores					
PACQLQ overall score (maximum, 7)	6.62 (0.66)	6.60 (0.60)	6.50 (0.80)	6.50 (0.80)	.44
PedsQL total score (maximum, 100)	0.29 (0.25)	0.40 (0.40)	0.40 (0.22)	0.50 (0.30)	.17

Abbreviations: ED, emergency department; PACQLQ, Pediatric Asthma Caregiver's Quality of Life Questionnaire; PedsQL, Pediatric Quality of Life Inventory.

^a Data are expressed as mean (SD) unless otherwise noted.^b The range for quartile 1 is 10.5 ppb or less; 2, 10.6 to 14.0 ppb; 3, 14.1 to 24.4 ppb; and 4, greater than 24.4 ppb.^c Data are given as number (percentage) of children in each quartile.

3.80; 95% CI, 1.74–8.22; $P < .001$). To further examine possible confounding between API status and baseline FeNO levels, we repeated this analysis including the interaction between FeNO and API status. The interaction was not significant ($P = .95$), and the FeNO effect remained significant within both API strata (RR, 3.56 [95% CI, 1.45–8.76] for API positive; and RR, 3.74 [95% CI, 1.26–11.10] for API negative).

DISCUSSION

We have demonstrated that FeNO levels can be measured effectively and with high reproducibility in most preschool-aged children who participated in this clinical trial. To our knowledge, this study investigated the association between baseline FeNO levels and the parameters of disease burden, atopic characteristics, and risk for RTI in the largest cohort of preschool-aged children with moderate-to-severe intermittent wheezing. Although their disease was intermittent in nature, as evidenced by experiencing asthma-like symptoms on approximately 25% of days (or an average of 1.75 days per week) during the trial,²⁰ these children exhibited a high level of pretrial disease morbidity, reflected by high rates of health care utilization and oral corticosteroid use in the year before enrollment.¹² High FeNO levels (>24.4 ppb) observed in these preschool-aged children were associated with a 3-fold greater risk of RTI during the subsequent 12 months. This finding was not due to different patterns or degree of aeroallergen sensitivity nor was it related to baseline characteristics. To the best of our knowledge, this is the first report on the association between elevated FeNO levels and the constellation of respiratory symptoms that, by the parents' perception, would be expected to precede and signal the development of an episode of wheezing.

Among this cohort, the median FeNO level at baseline was 14.0 ppb, a level higher than seen in similarly aged nonatopic healthy controls by the off-line technique (mean [SD], 5.9 [0.7] ppb²¹; and mean [95% CI], 3.9 [3.5–4.2] ppb²²). The differences in FeNO levels between these cohorts might be explained by the higher prevalence of atopy and the history of recurrent wheezing (one of the major inclusion criteria) in our cohort. Although the elevated median FeNO levels measured in our study reflect the high prevalence of atopy in this population, they were not associated with the pretrial disease activity of these children. The relationship between FeNO levels and markers of atopy,^{23,24} or between FeNO levels and the diagnosis of atopic dermatitis,²⁵ has been noted previously and may be partially explained by polymorphisms in the NO synthase gene that were found in association with both elevated FeNO and IgE levels.²³

In addition, a recent study²⁶ supports the proposed association between elevated FeNO levels and history of recurrent wheezing episodes, because preschool-aged children with recurrent wheezing episodes and stringent APIs had a significantly higher FeNO when compared with children with chronic persistent or recurrent cough but no history of wheeze. These authors suggest that FeNO is not only a

marker for allergic inflammation but may have a role in the pathogenesis of respiratory disease. Their suggestion could be supported by the finding that higher FeNO levels, measured when asymptomatic and during the first month of life among infants born to atopic and/or smoking mothers, were associated with a significantly increased risk of subsequent respiratory symptoms during the first year of life.²⁷

The only demographic characteristic that was associated with higher baseline FeNO was Hispanic/Latino ethnicity, a finding that has not been reported previously. How this relates to the higher prevalence of asthma among Hispanics in the United States^{28,29} is not clear. However, we are cautious in the interpretation of this finding because of the small number of Hispanic children in our cohort; more studies are needed for confirmation. Ethnic differences in FeNO levels for "healthy" children have been reported previously: Asian children, especially boys, have significantly higher FeNO levels compared with white children.³⁰ These differences may be related to genetic variations in the neuronal nitric oxide synthase gene.^{31,32} It was also reported by Kovesi et al³¹ that African American children have higher FeNO levels than white children, but the small sample size of African Americans in the study by Kovesi et al results in wide CIs that make definitive conclusion inappropriate.

In summary, in this cohort of preschool-aged children with moderate-to-severe intermittent wheezing, high FeNO levels at randomization were associated with an increased likelihood of a subsequent RTI. Furthermore, in this same cohort, FeNO levels were elevated compared with reported norms in healthy preschool-aged children. FeNO levels in asymptomatic children were related to the degree of aeroallergen sensitization and Hispanic ethnicity, but not with other atopic or demographic characteristics. Our results suggest that FeNO levels might be used as an additional tool in the evaluation and development of treatment strategies in young children with moderate-to-severe intermittent wheezing. The detection of a high FeNO level may serve as a clinical indicator for a child being at greater risk for an RTI during the next 12 months. Whether the use of asthma controller medications or other strategies are effective in the prevention of wheezing episodes, which are commonly triggered by RTI in children with elevated FeNO levels, requires further study.

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