Original article

Fractional exhaled nitric oxide measurements are most closely associated with allergic sensitization in school-age children

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Background: Factors affecting fractional exhaled nitric oxide (FeNO) in early childhood are incompletely understood.
Objective: To examine the relationships between FeNO and allergic sensitization, total IgE, atopic dermatitis, rhinitis, asthma, and lung function (spirometry) in children.
Methods: Children at high risk of asthma and other allergic diseases because of parental history were enrolled at birth and followed prospectively. FeNO was measured by an online technique at ages 6 and 8 years. Relationships among FeNO, various atopic characteristics, and asthma were evaluated.
Results: Reproducible FeNO measurements were obtained in 64% (135/210) of 6-year-old and 93% (180/194) of 8-year-old children. There was seasonal variability in FeNO. Children with aeroallergen sensitization at ages 6 and 8 years had increased levels of FeNO compared with those not sensitized (geometric mean; 6 years, 10.9 vs 6.7 parts per billion [ppb], $P < .0001$; 8 years, 14.6 vs 7.1 ppb, $P < .0001$). FeNO was higher in children with asthma than in those without asthma at 8 years but not 6 years of age (6 years, 9.2 vs 8.3 ppb, $P = .48$; 8 years, 11.5 vs 9.2 ppb, $P = .03$). At 8 years of age, this difference was no longer significant in a multivariate model that included aeroallergen sensitization ($P = .33$). There were no correlations between FeNO and spirometric indices at 6 or 8 years of age.
Conclusion: These findings underscore the importance of evaluating allergen sensitization status when FeNO is used as a potential biomarker in the diagnosis and/or monitoring of atopic diseases, particularly asthma. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: *Fractional exhaled nitric oxide (FeNO), asthma, allergic sensitization, atopic dermatitis, lung function, children, seasonality, atopy*

Fractional exhaled nitric oxide (FeNO) is frequently measured in both research and clinical settings as a potential biomarker for the diagnosis and treatment of asthma. Exhaled nitric oxide is thought to be a sensitive marker of ongoing eosinophilic airway inflammation,[1,2] and FeNO levels decrease with anti-inflammatory therapy.[2] FeNO is particularly attractive for use in children, because it can be measured by using noninvasive, standardized methods and yields reproducible, real-time results.[3-5] Indeed, FeNO measurement has shown potential promise as a noninvasive objective tool for use in the prediction of persistent wheezing[6] and diagnosis of asthma in preschool children.[7] Studies in both adults and children suggest that elevated FeNO can effectively predict response to inhaled corticosteroids.[5,8,9] Measurement of FeNO has also shown potential utility in guiding anti-inflammatory therapy in both adults[10,11] and children[12] with asthma, but its ability to do so has recently been demonstrated not to be superior to guideline-based strategies.[13]

Thus, although FeNO certainly has shown some promise as a biomarker in asthma diagnosis and therapy, many questions remain. Previous studies have consistently demonstrated strong correlations between elevated FeNO and atopy,[4,14-16] but there have been inconsistent results when comparing wheezing phenotypes and FeNO levels in young children.[6,14,17] Levels of FeNO in childhood are also known to increase with age,[3] but normal values in early school-age children have not been well established. Thus, a more complete understanding of the factors that affect FeNO levels in early school-age children is critical to proper interpretation of its measurement.

Children enrolled in the Childhood Origins of ASThma (COAST) study, a birth cohort study designed to investigate the host and environmental factors involved in the development of asthma and allergic diseases in children at high risk because of

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Abbreviations used
AD: Atopic dermatitis
COAST: Childhood Origins of ASThma
FeNO: Fractional exhaled nitric oxide
FVC: Forced vital capacity
ppb: Parts per billion
$FEV_{0.5}$: Forced expiratory volume in 0.5 seconds
FEV_1: Forced expiratory volume in 1 second

parental history, were therefore evaluated to delineate better the relationship between FeNO and other markers of asthma and allergic disease. The longitudinal, observational nature of the study provides a means to examine the natural course of FeNO levels throughout childhood in relation to the onset, persistence, and remittance of various atopic phenotypic characteristics. The following report describes these relationships in this high-risk cohort of children at 6 and 8 years of age.

METHODS

Study subjects

A total of 289 children were enrolled in the COAST study at birth as previously described,[18] and 254 were followed through age 8 years. For a child to qualify, at least 1 parent was required to have a history of physician-diagnosed asthma and/or respiratory allergies, with the latter defined by 1 or more positive aeroallergen skin prick tests. Informed consent was obtained from the parents, and the Human Subjects Committee at the University of Wisconsin approved the study.

FeNO measurement

Fractional exhaled nitric oxide was measured during scheduled study visits at 6 and 8 years of age using the NIOX system (Aerocrine, Stockholm, Sweden) according to American Thoracic Society online measurement standards adapted for children.[19] The expiratory flow rate was 0.05 L/s. Exhalation times were at least 6 seconds with a 2-second analysis period. Children were required to have 3 measurements within 10% or 2 measurements within 5% for acceptability. Measurements were made before the performance of spirometry or impulse oscillometry.

Pulmonary function testing

Spirometry ($FEV_{0.5}$, FEV_1, and FEV_1/forced vital capacity [FVC]) was performed at 6 and 8 years of age with the Jaeger Masterscope computer system (Jaeger-Toennies GmbH, Hoechberg, Germany) using protocols described by the Childhood Asthma Research and Education Network.[4] Because the American Thoracic Society Standardization of Spirometry 1994 Update does not address recommendations for children specifically,[20] modified criteria published by Eigen et al[21] were used to define standards for maneuver acceptability.

Total IgE and allergen-specific IgE

Blood was collected at 6 years of age, and total IgE and specific IgE to dog, cat, cockroach, ragweed, birch, timothy grass, *Alternaria alternata*, *Dermatophagoides farinae, Dermatophagoides pteronyssinus,* peanut, and egg, were measured by using automated fluoroenzyme immunoassays (Unicap 100; Pharmacia and Upjohn Diagnostics, Kalamazoo, Mich) as previously described.[22] Allergen-specific IgE values of 0.35 kU/L (class I) or greater were considered positive, and the sensitivity for detection of total IgE was 2 kU/L.

Clinical definitions

Atopic dermatitis (AD) was defined as physician-diagnosed, either documented by a health care provider in the medical record or by parental report of physician-diagnosed AD on historical questionnaires. As previously described,[23] current asthma was diagnosed at 6 and 8 years of age on the basis of the documented presence of 1 or more of the following characteristics in the previous year: (1) physician diagnosis of asthma, (2) use of albuterol for coughing or wheezing episodes (prescribed by physician), (3) use of a daily controller medication, (4) step-up plan including use of albuterol or short-term use of inhaled corticosteroids during illness, and (5) use of prednisone for asthma exacerbation. Rhinitis was defined as routinely or seasonally having frequent sneezes and/or itchy/runny nose, and was ascertained by parental report on historical questionnaires.

Statistical analysis

Relationships between the years 6 and 8 FeNO outcomes (log-transformed) and season, sex, asthma, AD, total and specific IgE, skin prick test, peripheral blood eosinophils, and pulmonary function tests were examined by using linear regression models. Because FeNO measurements were found to vary by the season of measurement, season was included as a covariate in these models. The strengths of association between FeNO and total IgE, peripheral blood eosinophils, and pulmonary function tests were summarized by using the Pearson partial correlation coefficient adjusting for season. FeNO, total IgE, and eosinophil measurements were log-transformed for analysis, and FeNO levels were summarized by using the geometric mean. A 2-sided P value of .05 was regarded as statistically significant.

RESULTS

Reproducible FeNO measurements were obtained in 64% (135/210) of 6-year-old children and 93% (180/194) of 8-year-old children. There were no differences in sex, aeroallergen sensitization, food sensitization, asthma, rhinitis, or AD in children who performed reproducible FeNO versus those who did not. The geometric mean FeNO increased from 8.6 parts per billion (ppb) at 6 years of age to 9.9 ppb at 8 years of age ($P = .01$). There were no differences in FeNO based on sex at either age (6 years, girls 8.4 vs boys 8.7 ppb, $P = .80$; 8 years, girls 10.3 vs boys 9.6 ppb, $P = .42$). However, there was seasonal variability in FeNO measurement at both 6 and 8 years of age, with higher FeNO in summer and fall than winter and spring (Fig 1; 6 years, $P = .04$; 8 years, $P = .01$). Therefore, all subsequent analyses adjust for season of FeNO measurement. This adjustment did not alter any of the relationships described, nor did adjustment for asthma controller medication use.

FeNO and atopy

Children with aeroallergen sensitization, defined as at least 1 positive aeroallergen RAST at age 6 years, had increased levels of FeNO compared with those not sensitized to aeroallergens (Table I; 6 years, 10.9 vs 6.7 ppb, $P < .0001$; 8 years, 14.6 vs 7.1 ppb, $P < .0001$). Similar results were obtained when aeroallergen sensitization was assessed at ages 1 and 3 years by RAST and age 5 years by skin prick testing (data not shown). Children sensitized to foods, defined as at least 1 positive food allergen RAST at age 6 years, also had higher levels of FeNO than those without food sensitization (Table I; 6 years, 10.9 vs 8.0 ppb, $P = .02$; 8 years, 14.0 vs 8.9 ppb, $P = .0001$).

There was a significant positive correlation between total IgE and FeNO (6 years, $r = +0.36$, $P < .0001$; 8 years, $r = +0.46$, $P < .0001$). There was also a weak positive correlation between

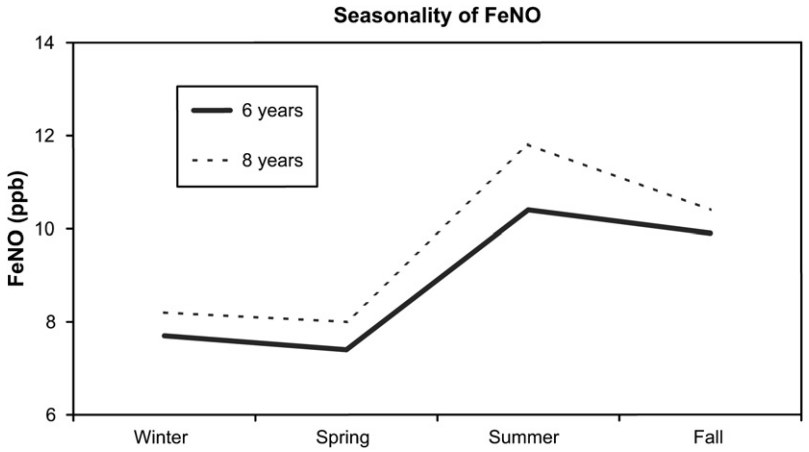

FIG 1. Seasonality of FeNO measurement at 6 and 8 years of age. Geometric mean measurements were higher in summer and fall than in winter and spring (6 years, $P = .04$; 8 years, $P = .01$).

TABLE I. FeNO (geometric mean [25th, 75th percentile]) at 6 and 8 years of age in children of varying phenotypes

		N	FeNO, 6 y (ppb)	*P* value	N	FeNO, 8 y (ppb)	*P* value
Aeroallergen RAST, age 6 y	−	63	6.7 (5.0, 9.0)	<.0001	87	7.1 (5.3, 8.4)	<.0001
	+	59	10.9 (6.7, 17.9)		70	14.6 (8.3, 27.1)	
Food RAST, age 6 y	−	95	8.0 (5.3, 10.1)	.02	123	8.9 (6.1, 10.6)	.0001
	+	26	10.9 (5.9, 14.8)		33	14.0 (7.1, 29.6)	
Current rhinitis	−	70	7.3 (5.1, 9.3)	.0006	84	7.6 (5.4, 9.1)	<.0001
	+	65	10.2 (6.6, 14.8)		93	12.4 (7.5, 20.3)	
Current AD	−	91	8.1 (5.2, 10.8)	.13	133	9.2 (6.0, 12.4)	.002
	+	44	9.5 (6.2, 11.4)		47	12.4 (7.8, 20.8)	
Current asthma	−	92	8.3 (5.6, 10.2)	.48	115	9.2 (6.1, 11.3)	.03
	+	43	9.2 (5.4, 13.6)		65	11.5 (7.4, 20.3)	

peripheral blood eosinophils and FeNO (6 years, $r = +0.19$, $P = .04$; 8 years, $r = +0.23$, $P = .005$).

FeNO and rhinitis

Children with current rhinitis had significantly higher FeNO levels than those without rhinitis at ages 6 and 8 years (Table I; 6 years, 10.2 vs 7.3 ppb, $P = .0006$; 8 years, 12.4 vs 7.6 ppb, $P < .0001$). FeNO was highest in children with rhinitis who also demonstrated aeroallergen sensitization (Table II).

FeNO and AD

Children with current AD had significantly higher FeNO levels than those without AD at 8 years, but not at 6 years of age (Table I; 6 years, 9.5 vs 8.1 ppb, $P = .13$; 8 years, 12.4 vs 9.2 ppb, $P = .002$). However, this relationship was no longer significant after stratification by allergic sensitization (Table II).

FeNO and asthma

Similarly, children with current asthma had higher FeNO levels than those without asthma at age 8 years, but not at 6 years of age (Table I; 6 years, 9.2 vs 8.3 ppb, $P = .48$; 8 years, 11.5 vs 9.2 ppb, $P = .03$). Once again, this relationship was no longer significant after stratification by allergic sensitization (Table II).

FeNO and spirometry

Reproducible spirometry measurements were obtained in 70% (95/135) of 6-year-old children and 88% (158/180) of 8-year-old children with reproducible FeNO measurements. There were no significant correlations between FeNO and any measure of pulmonary function ($FEV_{0.5}$, FEV_1, FEV_1 % predicted, FVC, FVC % predicted, $FEV_{0.5}$/FVC, or FEV_1/FVC).

DISCUSSION

Despite efforts over the past decade to understand better the relationships between FeNO and the development of asthma and allergic disease, normal FeNO levels in early school-age children are not well established. We report FeNO measurements obtained with an online technique in a large cohort of children at 6 and 8 years of age, allowing for effective comparisons with previously published studies. We clearly show that in our high-risk birth cohort, FeNO was most significantly associated with atopic (the presence of allergic sensitization) status. In fact, FeNO was elevated only in children with asthma and AD who also demonstrated allergic sensitization. Interestingly, rhinitis without detectable allergic sensitization was significantly associated with elevations in FeNO at age 8 years, albeit less so than in children with both rhinitis and demonstrable allergic sensitization.

Our overall rates of obtaining successful FeNO measurements at 6 years of age (64%) and 8 years of age (93%) by online measurement were similar to previously published data.[3,24]

TABLE II. Multivariable comparison of FeNO measurements (geometric mean [25th, 75th percentile]) at 6 and 8 years of age

Allergic sensitization	Current rhinitis	N	FeNO, 6 y (ppb)	*P* value	N	FeNO, 8 y (ppb)	*P* value
−	−	43	6.6 (5.0, 8.2)	Rhinitis: .054	53	6.7 (5.1, 8.2)	Rhinitis: .03
−	+	17	7.2 (4.1, 11.0)		28	7.5 (6.5, 9.1)	
+	−	17	8.6 (5.1, 10.8)	RAST: 0.001	22	10.4 (7.0, 14.6)	RAST: <0.0001
+	+	45	11.6 (7.4, 22.2)		52	16.0 (8.6, 29.9)	

Allergic sensitization	Current asthma	N	FeNO, 6 y (ppb)	*P* value	N	FeNO, 8 y (ppb)	*P* value
−	−	44	7.1 (5.3, 9.7)	Asthma: .88	59	6.9 (5.3, 8.3)	Asthma: .37
−	+	16	5.9 (4.3, 7.9)		23	7.3 (5.5, 8.6)	
+	−	39	8.6 (6.7, 14.3)	RAST: <.0001	39	13.4 (7.3, 22.1)	RAST: <.0001
+	+	23	10.3 (6.8, 26.2)		36	15.0 (8.1, 28.1)	

Allergic sensitization	Current AD	N	FeNO, 6 y (ppb)	*P* value	N	FeNO, 8 y (ppb)	*P* value
−	−	42	6.6 (4.7, 9.0)	AD:.21	67	6.9 (5.3, 8.3)	AD:.24
−	+	18	7.0 (5.6, 9.1)		15	7.7 (6.7, 8.5)	
+	−	38	9.8 (6.1, 14.8)	RAST: <.0001	48	13.9 (7.5, 28.0)	RAST: <.0001
+	+	24	12.2 (7.4, 23.5)		27	14.6 (8.8, 22.6)	

Successful measurement in two thirds of 6-year-olds and more than 90% of 8-year-olds confirms the appeal of FeNO measurement in this age group as a reliable, noninvasive test that yields real-time results.

Another important finding of this study is that FeNO measurements varied by season, with summer and fall yielding the highest FeNO measurements. This is similar to recent findings reported from another cohort in which FeNO was highest in fall.[25] One potential explanation for this finding could be greater exposure to allergens, such as dust mites and viruses (rhinovirus in particular), during the summer and fall, respectively. Importantly, controlling for season of measurement did not alter any of the relationships seen between atopic status and FeNO; however, season of measurement still should be considered when interpreting FeNO measurements in a clinical or research setting.

Although Buchvald and Bisgaard[17] reported no association between FeNO and atopy as measured by RAST testing in children 2 to 5 years old, Brussee et al,[14] in a significantly larger cohort of 4-year-old children, reported a small but statistically significant elevation of FeNO in atopic individuals as determined by RAST testing. In this article, we report greater differences in FeNO in atopic versus nonatopic children at age 8 years compared with age 6 years. A significantly more pronounced elevation of FeNO in older atopic children has been demonstrated by many researchers,[3,26,27] which suggests that although normal FeNO values have previously been shown to increase with age, there also appears to be a larger discrepancy between normal and abnormal values as individuals progress through childhood. The small difference between normal and abnormal FeNO seen in early school-age children makes it difficult to foresee widespread successful use of FeNO for diagnosis in this age group.

In this study, we found a significant relationship between FeNO and asthma only in those children with concomitant allergic sensitization. This is consistent with at least 1 pediatric[26] and 1 adult study,[16,26] but not with others.[14,28] This discrepancy may be secondary to the use of many different methods for classification of history of wheezing and asthma throughout the studies, in addition to the various ages of the populations studied, because a greater percentage of teenagers and young adults, compared with early school-age children, have atopic asthma. Whether a stronger relationship between asthma and elevated levels of FeNO will develop over time in our cohort remains to be seen.

Although there is much agreement that there is a strong relationship between elevated FeNO and atopy, there have been mixed results when comparing measurements of lung function and FeNO. Several groups have demonstrated a correlation between spirometric evidence of airway obstruction and elevated FeNO in children[4]; however, most studies have not shown any correlation between elevated FeNO and impairment of FEV_1 or FEV_1/FVC.[28,29] In this study, we found no significant correlation between FeNO and any measurement of lung function at 6 or 8 years of age. This confirms the notion that FeNO measures a different aspect of atopic airway disease than spirometry, and is potentially a more sensitive test for allergic airway disease in this age group,[6,7,30] in which the vast majority of children with asthma have normal lung function.[31]

There are several limitations to our study. First, COAST is a cohort of children at high risk for the development of asthma and other allergic diseases, which could limit the generalizability of our results. However, despite the high-risk status of the COAST cohort, the geometric mean FeNO measurements were comparable to those previously published in an unselected population of early school-age children.[3] Second, because of the observational nature of COAST, treatment regimens varied among children. Some individuals with asthma were taking inhaled corticosteroids, which are known to decrease FeNO. However, after adjustment for controller medication use, the relationship between asthma and FeNO did not change.

In summary, in this cohort of children at 6 and 8 years of age at high risk for the development of asthma and allergic disease, elevations of FeNO were strongly and significantly correlated with allergic sensitization. Although these data add to the growing evidence of a strong relationship between elevated FeNO and atopy in children, the relationship of FeNO and asthma in early school-age children is much less clear. These

findings underscore the importance of evaluating allergen sensitization status when FeNO is used as a potential biomarker in the diagnosis and/or monitoring of atopic diseases, particularly asthma.

Clinical implications: When FeNO is used as a biomarker for the diagnosis and/or monitoring of atopic diseases such as asthma, the presence or absence of allergic sensitization should be carefully considered.

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