Exhaled nitric oxide: Not associated with asthma, symptoms, or spirometry in children with sickle cell anemia

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Background: The significance of fractional exhaled nitric oxide (FENO) levels in children with sickle cell anemia (SCA) is unclear, but increased levels can be associated with features of asthma and thus increased morbidity.

Objectives: We sought to determine factors associated with FENO and whether FENO levels are associated with increased rates of acute chest syndrome (ACS) and pain.

Methods: All participants had SCA, were part of the prospective observational Sleep and Asthma Cohort study, and had the following assessments: FENO levels, spirometry, blood samples analyzed for hemoglobin, white blood cell counts, eosinophil counts and total serum IgE levels, questionnaires about child medical and family history, and review of medical records.

Results: The analytic sample included 131 children with SCA (median age, 11.2 years; age range, 6-18 years) followed for a mean of 16.2 years, including a mean of 5.1 years after baseline FENO data measurements. In multivariable analyses higher FENO levels were associated with ln(IgE) levels ($P < .001$) and the highest quartile of peripheral eosinophil counts ($P = .03$) but not wheezing symptoms, baseline spirometric indices, or response to bronchodilator. Multivariable analyses identified that the incident rate of ACS was associated with ln(FENO) levels ($P = .03$), as well as male sex ($P = .025$), wheezing causing shortness of breath ($P = .002$), and ACS at less than 4 years of age ($P < .001$). FENO levels were not associated with future pain episodes.

Conclusions: Steady-state FENO levels were not associated with an asthma diagnosis, wheezing symptoms, lung function measures, or prior sickle cell morbidity but were associated with markers of atopy and increased risk of future ACS events. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: *Sickle cell disease, exhaled nitric oxide, asthma, airway inflammation, acute chest syndrome*

Respiratory disorders are a major cause of morbidity and mortality for patients with sickle cell disease (SCD).[1,2] In particular, asthma has been associated with increased morbidity and premature death among children with SCD.[3-6] Several features associated with asthma in the general population, such as wheezing,[7,8] lower airway obstruction,[9] and markers of atopy, including increased IgE levels[10] and positive skin test responses to aeroallergens,[11] have themselves been associated with SCD morbidity.

Measured fractional exhaled nitric oxide (FENO) is a noninvasive biomarker of airway inflammation.[12] In school-aged children and adolescents FENO measurement is reproducible[13] and has been associated with several features of atopy and asthma, including peripheral blood eosinophilia, total serum IgE levels, FEV_1/forced vital capacity (FVC) ratio, and airway hyperresponsiveness.[14-16] The significance of FENO levels in patients with SCD is not well understood. Prior small studies have demonstrated FENO levels in children with SCD to be higher, lower, and the same as those in healthy control subjects.[17-20] Furthermore, relationships between FENO levels and current and/or prior SCD morbidity are inconsistent.[18,19]

Given the association between asthma features and SCD morbidity[9,10,21] juxtaposed with the lack of consistent findings regarding the significance of FENO levels among patients with SCD, a more in-depth exploration of FENO levels among children with SCD is warranted. The aim of the current study was to investigate whether FENO levels are associated with SCD-specific factors, asthma-related factors, or both and whether this noninvasive clinical test has the potential to predict future morbidity among children with the severe form of SCD: sickle cell anemia (SCA; used herein to refer to the HbSS and HbSβ^0 genotypes only). We tested the hypotheses that (1) FENO levels would be correlated with other biomarkers of asthma and atopy among children with SCA and (2) higher steady-state FENO levels would be associated with increased prospective rates of pain and acute chest syndrome (ACS).

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Abbreviations used
ACS: Acute chest syndrome
ATS: American Thoracic Society
FENO: Fractional exhaled nitric oxide
FVC: Forced vital capacity
SAC: Sleep and Asthma Cohort Study
SCA: Sickle cell anemia (refers to HbSS and HbSβ^0 only)
SCD: Sickle cell disease (refers to all sickle cell disease genotypes)

METHODS

Study design

Participants in the Sleep and Asthma Cohort Study (SAC) were aged 4 to 19 years with SCA (HbSS or HbSβ^0). Participants with complete FENO levels, spirometric data, and SCD morbidity data from birth were included in this analysis. SAC is a National Heart, Lung, and Blood Institute–funded prospective, observational cohort study designed to evaluate the contribution of asthma and sleep abnormalities to SCA-related morbidity. Children were enrolled from 2006-2008 without regard to past morbidity or a physician's diagnosis of asthma. Children receiving chronic transfusion therapy or participating in a clinical trial evaluating hydroxyurea therapy at the time of recruitment were excluded, although if they were prescribed chronic transfusion or hydroxyurea therapy during the course of the follow-up period, they remained in the study. Institutional approval was obtained from participating sites in St Louis, Missouri; Cleveland, Ohio; and London, United Kingdom. Written informed consent was obtained from parents, and assent was obtained from children on enrollment, according to institutional policies.

Serum IgE levels were obtained on study entry. Participants also performed measurement of FENO levels followed by prebronchodilator and postbronchodilator spirometry. Given that we enrolled children as young as 4 years old, those who could not perform quality FENO measurements, spirometric measurements, or both at study entry repeated the procedure every 6 months until valid measures were obtained. Lung function data included in the current analysis represent the first valid FENO measurement and spirometry obtained on the same date. Procedures described below for spirometry and FENO measurement were modified from methods used in the National Heart, Lung, and Blood Institute's Childhood Asthma Research and Education Network.[14] Clinically obtained steady-state complete blood count data on the date closest to the pulmonary function date and medications used at the time of pulmonary function testing were obtained from the medical record.

Questionnaires

SAC-certified research coordinators administered a standardized questionnaire to participating parents and children that included questions about medical history; family medical history, including asthma; and respiratory symptoms from the American Thoracic Society (ATS)–Division of Lung Diseases questionnaire.[22] The ATS–Division of Lung Diseases questionnaire was administered at baseline and during all subsequent follow-up visits, and thus we were able to match respiratory symptoms with dates of the matching FENO measurement and spirometry.

Exhaled nitric oxide

Online FENO measurement with the NIOX system (Aerocrine AB, Stockholm, Sweden) was performed according to ATS guidelines.[23] FENO measurement used a resistive device that provided a constant low expiratory flow rate and vellum closure. Participants were required to exhale to residual volume; a mouthpiece was then inserted, and the participant was asked to inhale to total lung capacity. Thereafter, the child exhaled for 10 seconds at a constant flow rate of 0.05 L/s ± 10%. After a 30-second relaxation period, the exhalations were repeated until 3 FENO levels were obtained that varied less than 10% or 2 that varied less than 5%. If a subject did not manage to keep the flow or pressure within the required ranges over the 10 seconds of exhalation, the user profile was changed to 6 seconds per ATS guidelines, and the test was repeated.

Spirometry

After completion of FENO measurements, spirometry was performed by SAC-certified pulmonary function technicians according to ATS standards,[24] as previously described.[25] Appropriate prediction equations for FEV_1, FVC, and FEV_1/FVC ratio were used, taking into account age, sex, height, and ethnicity.[26] To measure bronchodilator response, technicians administered 4 inhalations of albuterol to participants using an AeroChamber (Forest Pharmaceuticals, New York, NY). Spirometry was repeated 15 minutes after albuterol. An increase of 12% or greater in FEV_1 after albuterol was considered a positive bronchodilator response.[27]

Overreading of spirometric and FENO results

Results were reviewed by a single investigator (R.C.S.) to ensure ATS criteria were met across the 3 participating sites for spirometry and FENO measurement; invalid tests were excluded from analyses.

Allergy skin testing

Allergy skin testing was performed by SAC-certified technicians using the Multi-Test II (Lincoln Diagnostics, Decatur, Ill). Ten aeroallergens (Greer Laboratories, Lenoir, NC) were used for skin testing: dust mite (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), cockroach (American and German), cat (standardized), dog (mixed breeds), *Alternaria alternata*, *Aspergillus fumigatus*, grass (standardized southern mix), tree (eastern 8 tree mix), weed (national mix), and mouse. Skin tests were administered with histamine (positive) and saline (negative) controls. Test results were considered positive when the mean diameter of the wheal was 3 mm or greater.

Morbidity data: Definitions of vaso-occlusive pain episode and ACS

A vaso-occlusive pain episode was defined as an episode directly associated with SCA that required hospitalization and opioid treatment. Headaches that required admission to the hospital and were treated with opioids were not considered a vaso-occlusive pain episode.

ACS was defined as an episode of acute respiratory distress requiring a new radiodensity on chest roentgenography, temperature greater than 38°C, and increased respiratory effort with a decrease in oxygen saturation or increase in respiratory rate documented in the medical record. Pneumonia was included in the definition.

Data quality

To ensure a uniform definition of pain and ACS in this multicenter study, the charts of all patients given a diagnosis of ACS or a vaso-occlusive pain episode requiring hospitalization for pain in the chest, extremities, or other areas of the body were reviewed by a single investigator at each of the participating sites after training by the principal investigator and, if necessary, discussed with the site investigators.

Statistical analysis

FENO levels were not normally distributed in our study participants but had a long right tail. FENO levels, total serum IgE levels, and eosinophil counts were natural log-transformed for all regression analyses to accommodate nonnormal distributions. Clinical and biomarker features were tested for their association with FENO levels by using Spearman correlations for continuous variables and Wilcoxon rank sum tests for categorical variables. Multiple linear regression was used to build a model of factors associated with the steady-state ln(FENO) level as the dependent variable. Covariates used in screening multivariable models of ln(FENO) included SCA-specific factors

TABLE I. Characteristics of the study population (n = 131)*

Characteristic	All patients (n = 131)	No asthma (n = 93 [71%])	Asthma† (n = 38 [29%])	*P* value
Age (y), mean (SD)	11.2 (3.6)	11.1 (3.5)	11.3 (3.8)	.81
Total follow-up time from birth (y), mean (SD)	16.2 (3.9)	16.2 (3.7)	16.4 (4.2)	.73
Total follow-up time after eNO was obtained (y), mean (SD)	5.1 (1.1)	5.0 (1.1)	5.1 (1.3)	.65
Male sex (%)	55.0	49.5	68.4	.048
Hemoglobin (g/dL), mean (SD)	8.4 (1.3)	8.5 (1.3)	8.2 (1.1)	.24
White blood cell count (10^9/L), mean (SD)	11.6 (3.7)	11.3 (3.9)	12.3 (3.0)	.15
FENO (ppb), median (IQR)	9.0 (7.6)	8.9 (7.3)	9.9 (9.0)	.60§
FENO ≥25 ppb (%)	8.4	7.5	10.5	.73
IgE (IU/mL), median (IQR), n = 127	46.6 (133.3)	47.6 (121.6)	63.6 (152.8)	.77§
Eosinophils (total count), median (IQR)	354.0 (492.0)	320.0 (451.2)	456.0 (606.2)	.35§
Had ≥2 positive skin test results (%), n = 121	28.9	20.5	47.4	.002
FVC (% predicted), mean (SD)	93.5 (14.1)	93.8 (13.7)	92.8 (15.0)	.71
FEV_1 (% predicted), mean (SD)	88.8 (13.3)	89.8 (13.2)	86.4 (13.5)	.19
FEV_1/FVC ratio, mean (SD)	0.85 (0.07)	0.85 (0.07)	0.83 (0.08)	.13
FEV_1/FVC ratio (% predicted), mean (SD)	94.9 (7.7)	95.6 (7.0)	93.3 (9.3)	.14
FEV_1/FVC ratio < LLN (%)	19.8	15.1	31.6	.03
Percentage with bronchodilator response ≥12%‡	16.8	11.8	28.9	.02
Retrospective rate of pain episodes per year, median (IQR)	0.3 (0.6)	0.3 (0.7)	0.3 (0.6)	.88§
Retrospective rate of ACS episodes per year, median (IQR)	0.1 (0.3)	0.1 (0.2)	0.2 (0.3)	.04§
Prospective rate of pain episodes per year, median (IQR)	0.6 (1.3)	0.5 (1.3)	0.6 (1.3)	.80§
Prospective rate of ACS episodes per year, median (IQR)	0.2 (0.3)	0.0 (0.3)	0.2 (0.5)	<.001§
Receiving hydroxyurea at the time FENO was obtained (%)	11.5	9.7	15.8	.37
Receiving inhaled corticosteroids at the time FENO was obtained (%)	21	2	66	<.001
PC_{20} ≤8.0 (%), n = 66	63.6	65.1	60.9	.73

eNO, Exhaled nitric oxide; *IQR*, interquartile range; *LLN*, lower limit of normal.
*Means and SDs are presented for normally distributed variables. Medians and interquartile ranges are presented for nonnormally distributed variables.
†Participants were classified as having asthma if there had ever been a physician's diagnosis of asthma and current use of an asthma medication at the time of FENO measurement.[7]
‡(Post-FEV_1 − Pre-FEV_1)/Pre-FEV_1 ≥ 0.12.
§Mann-Whitney *U* test.

of interest (sex, WBC, hemoglobin, retrospective history of ACS or pain <4 years of age [herein termed ACS <4 years or pain <4 years], and use of hydroxyurea at the time of FENO measurement) and asthma/atopy factors of interest (IgE levels, eosinophil counts, FEV_1/FVC percent predicted, bronchodilator responsiveness [yes/no], history of wheezing causing shortness of breath, and use of inhaled corticosteroids at time of exhaled nitric oxide measurement). Because age is accounted for in FEV_1/FVC percent predicted values and because age and height were highly correlated ($\rho = 0.91$), we did not include age or height in the screening model. A separate multivariable model was built for the smaller subset of patients who underwent allergy skin testing using a similar approach; in this model having 2 or more positive skin test results was added as a covariate.

Negative binomial regression was then used to test associations between steady-state FENO levels (independent variable) and future rates of pain and ACS (dependent variables). Multivariable models were built in 2 steps. First, all potential covariates of interest were included in a screening model. Initial covariates we considered to be potentially associated with the prospective rates of ACS included sex, SCA-specific factors (hemoglobin, WBC, and ACS < 4 years), and atopy and airway inflammation features (FENO level, IgE level, having ≥2 positive skin test results, FEV_1/FVC percent predicted, and history of wheezing causing shortness of breath). Covariates we considered to be potentially associated with prospective rates of pain included age, sex, SCA-specific factors (hemoglobin, WBC, and retrospective rate of pain), and atopy and airway features (FENO levels, IgE levels, eosinophil counts, FEV_1/FVC percent predicted, and wheeze causing shortness of breath). All covariates meeting the significance criteria of a *P* value of less than .20 were subsequently included in the final model for each of our outcomes of interest. We selected history of wheezing causing shortness of breath for our multivariable models of FENO and rates of ACS and pain versus other wheezing items because of this symptom's association with asthma in children with SCA[6] and with SCA morbidity in prior studies.[8,11] Analyses were conducted with Stata statistical software (version 12; StataCorp, College Station, Tex) and IBM SPSS Statistics (version 22; IBM, Chicago, Ill).

RESULTS

Of 252 participants with SCA in the SAC study, 188 had pain and ACS data available from birth for a mean of 16.2 years (SD, 3.9 years) of follow-up. Of those, the final analytic sample included 131 who had acceptable FENO levels obtained on the same day as successful spirometry. The clinical characteristics of the sample are summarized in Table I.[7] In brief, the mean age in this sample at the time of FENO testing was 11.2 years (SD, 3.6 years), 55% of participants were male, and participants were followed prospectively after FENO/spirometric measurements were obtained for a mean of 5.1 years (SD, 1.1 years).

There was a wide range of FENO levels among study participants (2.7-86.5 ppb). The median FENO level was 9.0, with quarter 1 and quarter 3 values of 6.1 and 13.7 ppb, respectively. Children without acceptable FENO data, spirometric data, or both and therefore excluded from the analysis were younger, had a higher percentage of mothers with asthma, and had lower rates of pain (likely a function of age) but were otherwise similar to those with acceptable FENO levels (see Table E1 in this article's Online Repository at www.jacionline.org).

Factors associated with FENO levels

As shown in Table II, in unadjusted analyses FENO levels were positively associated with age, height, total serum IgE levels, having 2 or more positive skin test results, and blood eosinophil counts. The associations with IgE levels and skin test results were present for children with and without asthma; once the

TABLE II. Associations between FENO levels and participants' characteristics

Categorical variables			
	Median FENO level		
Covariate	No	Yes	Wilcoxon *P* value
Male sex	8.4	10.3	.11
Parent has asthma	8.7	10.6	.14
Participant has asthma	8.9	9.9	.60
Receiving hydroxyurea at the time of FENO measurement	8.7	11.4	.06
Receiving inhaled corticosteroids at the time of FENO measurement	8.8	11.7	.25
Has >12% improvement in FEV_1 after bronchodilator	9.0	10.0	.40
Has ≥2 positive skin test results (n = 121)	8.3	12.4	.001
Wheeze with cold	8.7	10.0	.22
Wheeze without cold	9.0	10.4	.30
Wheeze with SOB	9.0	9.4	.70
Wheeze after exercise	8.9	9.8	.66
Had an ACS event before 4 y of age	9.8	9.0	.45
Had a pain event before 4 y of age	8.9	9.6	.95

Continuous variables		
Covariate	Spearman ρ	*P* value
Age	0.28	.001
Height	0.34	<.001
FEV_1 (% predicted)	−0.07	.46
FVC (% predicted)	−0.04	.63
FEV_1/FVC ratio (actual)	−0.09	.32
FEV_1/FVC ratio (% predicted)	0.00	.95
IgE	0.28	.001
Eosinophils (total no. of cells/m^3)	0.20	.02
White blood cell count	−0.08	.35
Hemoglobin (g/dL)	0.02	.84
Retrospective rate of ACS before FENO measurement	−0.03	.78
Retrospective rate of pain before FENO measurement	−0.03	.76
Prospective rate of ACS after FENO measurement	0.07	.42
Prospective rate of pain after FENO measurement	0.01	.87

SOB, Shortness of breath.

cohort was stratified into smaller "asthma" and "no asthma" subgroups, the association with eosinophils was no longer significant (see Table E2 in this article's Online Repository at www.jacionline.org). Neither wheezing symptoms, spirometric results, nor a diagnosis of asthma were associated with FENO levels. There was no difference in FENO levels between those using and not using inhaled corticosteroids. A multivariable linear regression screening model for ln(FENO) levels was built including SCA-specific factors of interest and asthma/atopy factors of interest. Male sex, IgE levels, blood eosinophil counts, history of wheezing causing shortness of breath, and history of ACS at less than 4 years met the criteria for inclusion in a second model. The final model is shown in Table III with (ln)IgE levels, the highest quartile of eosinophil counts, and male sex independently associated with (ln)FENO levels. As shown in Table II, as well as in a separate multivariable model, which included the subset of 121 participants who had allergy skin testing, having 2 or more positive skin test results was also significantly associated with ln(FENO) levels (adjusted $\beta = 0.27$, $P = .003$).

TABLE III. Final multivariable model of factors associated with (ln)FENO levels among children with SCA

Covariates	β Estimate (SE)	*P* value
Male sex	0.24 (0.11)	.04
White blood cell count	−0.02 (0.015)	.19
(ln)IgE	0.12 (0.04)	.001
Eosinophils (quartile 1 = reference)	—	—
Quartile 2	−0.11 (0.15)	.48
Quartile 3	0.13 (0.15)	.40
Quartile 4	0.34 (0.15)	.03
History of wheezing that caused shortness of breath	−0.17 (0.13)	.20
ACS episode before 4 y of age	−0.16 (0.11)	.16

Association between baseline FENO levels and prospective morbidity

We explored whether steady-state FENO levels would be associated with prospective rates of ACS and pain. An initial screening model for prospective ACS rate found that ln(FENO) levels met the criteria for inclusion in a final model ($P = .04$), as did sex, wheezing leading to shortness of breath, and ACS at less than 4 years. In the reduced model all were significantly associated with prospective ACS (Table IV). In an analysis stratified by asthma status, ln(FENO) levels remained associated with prospective rates of ACS in the larger "no asthma" group but was no longer significant in the "asthma" group (see Tables E2, E3, and E4 for all results stratified by the participants' asthma status in this article's Online Repository at www.jacionline.org).

In the initial screening model for prospective rate of pain, ln(FENO) levels were not significant ($P = .49$).

DISCUSSION

A diagnosis of asthma is a risk factor for future ACS episodes in children with SCA,[6,10,28] but making this diagnosis is challenging because of the overlap with respiratory symptoms in patients with SCA without a comorbid condition of asthma. An objective test would be helpful in identifying the subgroup of children with the highest risk of future ACS symptoms. For the first time, we have demonstrated that higher FENO levels are associated with higher future rates of ACS. Although histories of wheeze causing shortness of breath and ACS in the first 4 years of life appear to be stronger predictors of ACS, our results suggest that FENO measurement might serve as a tool to aid physicians and researchers in stratifying those at the highest risk for future ACS events. Furthermore, the association of FENO levels with future ACS indicates for the first time the role of airway inflammation in the risk of this important outcome among children with SCA.

Although studies have clearly linked a diagnosis of asthma and atopy with SCD-related morbidity,[3,7,21] no studies have evaluated the association between a measure of airway inflammation and pulmonary characteristics commonly associated with asthma, such as wheezing symptoms and airway obstruction, among children with SCA. In our study although FENO levels were associated with IgE levels, having 2 or more positive skin test results, and peripheral blood eosinophilia, they were not associated with a doctor's diagnosis of asthma, wheeze symptoms, airway obstruction, or response to bronchodilator. Although FENO levels have been shown to be correlated with

TABLE IV. Final multivariable model of prospective rate of ACS in Children with SCA*

Covariate	IRR	95% CI	*P* value
ln(FENO)	1.44	1.04-1.99	.03
Male sex	0.59	0.38-0.93	.02
History of wheezing causing shortness of breath	2.34	1.38-3.98	.002
ACS episode before age 4 y	2.79	1.81-4.31	<.001

IRR, Incidence rate ratio.
*Negative binomial regression models with adjustment for overdispersion by using robust SEs. Two-tailed significance values are shown.

eosinophilic airway inflammation in the general population and among asthmatic children,[29-31] they have also been shown to correlate with lymphocytic airway inflammation in patients following lung transplantation,[32] with both neutrophilic[33] and eosinophilic[34] airway inflammation in patients with chronic obstructive pulmonary disease, and with lymphocytic airway inflammation in early bronchopulmonary involvement in patients with Crohn disease[35] and murine models of systemic sclerosis.[36] Future studies in SCD should include direct examination of inflammatory cell types in the sputum.

Previous studies have been conflicting about relationships between FENO levels and SCD complications. Two studies found FENO levels were lower in patients with SCD with a history of ACS compared with those without ACS,[18,37] whereas 2 other studies, similar to our study, found no differences between those with and without a prior ACS episode.[19,38] Pawar et al[19] noted that FENO levels among patients during an acute vaso-occlusive crisis pain episode were no different than among those at steady state. A recent study of FENO levels measured at variable flow rates[39] found increased alveolar nitric oxide concentration and production among patients with SCD compared with healthy race-matched control subjects. They also found significant positive correlations between alveolar nitric oxide and pulmonary blood flow in the SCD group, suggesting that alveolar nitric oxide production is related to the chronic hyperdynamic circulation found in patients with SCD. Furthermore, FENO levels measured at 50 mL/s were positively correlated with pulmonary blood flow but were not correlated with measures of airway obstruction or resistance, suggesting that some component of the FENO of a patient with SCD is due to increased alveolar nitric oxide production resulting from chronic anemia rather than airway inflammation from asthma.[39] In contrast, using flow-independent methods, Radhakrishnan et al[17] were able to determine that increased FENO levels among nonatopic children with SCD were higher compared with those in healthy control subjects, but their results showed that increases in FENO levels originated in the bronchial tree and not from alveolar sources. Further studies of FENO in larger cohorts of patients with SCD of varying ages and disease severity, with and without atopy, might clarify the relative contribution of airway and alveolar FENO and possible associations with other markers of disease severity, such as endothelial dysfunction and markers of pulmonary hypertension.

This study has a number of strengths, including ascertainment of FENO levels by using standardized ATS criteria, use of objective measurements of lung function with centralized standardized overreading of lung function, and prospective ascertainment of SCA-specific morbidity.

Although this is the largest study to date of FENO in patients with SCA, a limitation is measuring FENO levels at one flow rate.[17,40] SAC chose to measure FENO levels according to current ATS guidelines because this is the method widely available to clinicians rather than using methods available only as part of a research protocol. Our study only included children with SCA, and thus our results cannot be generalized to children with milder forms of SCD. Lastly, this study was not powered to allow us to definitely test whether FENO levels offer useful prognostic information specifically among children with history of early-life ACS because we were unable to identify a cutoff value of FENO associated with ACS risk with the sample size we had. A FENO level of 25 ppb appears to be the upper limit of normal for the general pediatric population[13] and has been associated with a favorable response to inhaled corticosteroids among children without SCD but with asthma[41]; however, we had very few children in our cohort with FENO levels of greater than this cutoff.

In conclusion, this study found that FENO levels were correlated with features of atopy (IgE levels, skin test reactivity, and peripheral blood eosinophil counts), but not respiratory symptoms, airway obstruction, response to bronchodilator, or asthma diagnosis, among children with SCA and that FENO levels did not reflect prior morbidity. Although steady-state FENO levels did not predict future risk of pain, it was associated with future risk of ACS. Based on our preliminary findings, evaluation of FENO as a biomarker for prospective morbidity represents an area for future study. More importantly, this study provides strong evidence that mechanisms for airway inflammation and associated respiratory symptoms in patients with SCD are different from what we see in the general population with asthma. With improved understanding of the pathophysiology of sickle cell airway disease, we will be able to offer individualized targeted therapies to our patients.

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Key messages

- **Higher FENO levels were not associated with typical respiratory features of asthma, including a doctor's diagnosis, respiratory symptoms, or airway obstruction, among children with SCA.**
- **FENO levels were associated with atopy features (eosinophilia, higher serum IgE levels, and having ≥2 positive skin test results) and prospective rates of ACS in children with SCA.**
- **These findings provide insight into mechanisms of pulmonary inflammation in children with SCD.**

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TABLE E1. Characteristics of the SAC population stratified by whether FENO measurements were obtained

Variable	Without FENO measurements (n = 57)	With FENO measurements (n = 131)	*P* value
Age (y)	6.7	10.6	<.001
WBC	12.4	12.0	.49
Hb (g/dL)	8.2	8.3	.53
Reticulocyte (%)	11.2	11.0	.80
Eosinophil (median)	2.5	3.0	.87
IgE (median)	69.6	49.6	.60
Male sex (%)	50.9	55.0	.61
Maternal history of asthma (%)	23.2	10.3	.02
History of inhaled corticosteroid use (ever [%])	14.0	20.6	.29
History of hydroxyurea use (ever [%])	38.6	32.8	.44
History of wheezing leading to shortness of breath (%)	28.1	24.4	.60
History of asthma (%)	28.1	29.0	.90
Rate of ACS before baseline (%), median	0.20	0.12	.10
Rate of pain before baseline (%), median	0.14	0.29	.03
Prospective rate of ACS after baseline (%), median	0.00	0.00	.42
Prospective rate of pain after baseline (%), median	0.14	0.49	<.01

Hb, Hemoglobin.

TABLE E2. Associations between FENO levels and participants' characteristics stratified by asthma status

Categorical variables

	All subjects (n = 131)			No asthma (n = 93)			Has asthma (n = 38)		
	Median FENO level			Median FENO level			Median FENO level		
Covariate	No	Yes	*P* value*	No	Yes	*P* value	No	Yes	*P* value
Male sex	8.4	10.3	.11	8.3	9.9	.13	8.6	11.2	.63
Parent has asthma	8.7	10.6	.14	8.3	11.8	<.05	9.9	8.7	.65
Participant has asthma	8.9	9.9	.60						
Receiving hydroxyurea at the time of FENO measurement	8.7	11.4	.06	8.7	18.2	.16	9.2	11.4	.65
Receiving inhaled corticosteroids at the time of FENO measurement	8.8	11.7	.25	8.9	8.4	.62	9.0	17.4	.05
Has ≥12% improvement in FEV_1 after bronchodilator	9.0	10.0	.50	9.0	6.9	.34	9.0	17.4	.05
Had ≥2 positive skin test results (n = 121)	8.3	12.4	.001	8.1	11.8	.01	8.8	12.6	.03
Wheeze with cold	8.7	10.0	.22	8.9	8.2	.98	9.1	11.4	.28
Wheeze without cold	9.0	10.4	.30	9.8	7.8	.14	7.9	12.5	.07
Wheeze with SOB	9.0	9.4	.70	8.9	8.0	.58	8.4	11.2	.29
Wheeze after exercise	8.9	9.8	.66	9.4	8.7	.43	11.8	9.2	.47
Had an ACS event before 4 y of age	9.8	9.0	.45	8.1	10.1	.28	13.3	8.7	.12
Had a pain event before 4 y of age	8.9	9.6	.95	8.3	9.9	.13	8.6	11.2	.63

Continuous variables

	All subjects		No asthma		Has asthma	
Covariate	ρ†	*P* value	ρ	*P* value	ρ	*P* value
Age	0.28	.001	0.27	.009	0.31	.06
Height	0.34	<.001	0.30	.004	0.43	.007
FEV_1 (% predicted)	−0.07	.46	−0.10	.33	0.02	.89
FVC (% predicted)	−0.04	.63	−0.11	.31	0.07	.67
FEV_1/FVC ratio (actual)	−0.09	.32	−0.01	.90	−0.23	.17
FEV_1/FVC ratio (% predicted)	0.00	.95	0.05	.62	−0.08	.65
IgE	0.28	.001	0.25	.02	0.37	.02
Eosinophils (total no. of cells/mm^3)	0.20	.02	0.18	.08	0.23	.17
White blood cell count	−0.08	.35	−0.07	.51	−0.11	.51
Hemoglobin (g/dL)	0.02	.84	0.15	.16	−0.25	.13
Retrospective rate of ACS before FENO measurement	−0.03	.78	−0.02	.84	−0.17	.32
Retrospective rate of pain before FENO measurement	−0.03	.76	0.05	.62	−0.18	.27
Prospective rate of ACS after FENO measurement	0.07	.42	0.07	.53	0.03	.87
Prospective rate of pain after FENO measurement	0.01	.87	−0.02	.82	0.04	.79

SOB, Shortness of breath.
*Wilcoxon rank sum *P* value.
†Spearman correlation coefficient.

TABLE E3. Multivariable model of factors associated with (ln)FENO levels among children with SCA stratified by asthma status

	No asthma		Asthma	
Covariates	**β estimate (SE)**	***P* value**	**β estimate (SE)**	***P* value**
Male sex	0.24 (0.13)	.07	−0.02 (0.23)	.94
White blood cell count	−0.02 (0.02)	.15	−0.03 (0.04)	.50
(ln)IgE	0.10 (0.04)	.03	0.15 (0.07)	.03
Eosinophils (quartile 1 = reference)				
Quartile 2	0.03 (0.17)	.88	−0.69 (0.35)	.05
Quartile 3	0.35 (0.19)	.07	−0.45 (0.27)	.10
Quartile 4	0.45 (0.18)	.01	−0.17 (0.34)	.61
History of wheezing that caused shortness of breath	−0.40 (0.18)	.03	0.19 (0.24)	.42
ACS episode before 4 y of age	−0.07 (0.15)	.63	−0.26 (0.20)	.22

TABLE E4. Multivariable model of prospective rates of ACS in children with SCA stratified by asthma status*

	No asthma			Has asthma		
Covariate	**IRR**	**95% CI**	***P* value**	**IRR**	**95% CI**	***P* value**
ln(FENO)	1.64	1.06-2.54	.03	1.24	0.80-1.93	.34
Male sex	0.56	0.30-1.05	.07	0.61	0.31-1.20	.15
History of wheezing causing shortness of breath	2.42	1.06-5.49	.04	2.02	1.13-3.62	.02
ACS episode before age 4 y	1.71	0.92-3.18	.03	5.52	2.85-10.71	<.001

IRR, Incidence rate ratio.

*Negative binomial regression models with adjustment for overdispersion using robust SEs. Two-tailed significance values are shown.