Exhaled Nitric Oxide: Not Associated with Asthma, Symptoms, or Spirometry in Sickle Cell Anemia

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Background: Significance of exhaled nitric oxide (FeNO) levels in children with sickle cell anemia (SCA) is unclear, but increased levels may be associated with features of asthma and thus increased morbidity.

Objectives: 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, spirometry, blood samples analyzed for hemoglobin, white blood cell count, eosinophils and total serum IgE, 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 (range 6-18) followed for a mean of 16.2 years, including a mean 5.1 years after the baseline FeNO data measurements. In multivariable analyses higher FeNO was associated with ln(IgE) ($p<0.001$), and the highest quartile of peripheral eosinophil count ($p=0.03$), but not wheezing symptoms, baseline spirometry indices, or response to bronchodilator. Multivariable analyses identified that incident rate of ACS was associated with ln(FeNO) ($p=0.03$) as well as male gender ($p=0.025$), wheezing causing shortness of breath ($p=0.002$), and ACS <4 years of age ($p <0.001$). FeNO was not associated with future pain episodes.

Conclusions: Steady state FeNO was not associated with an asthma diagnosis, wheezing symptoms, lung function measures, or prior sickle cell morbidity, but was associated with markers of atopy and increased risk of future ACS events.

KEY MESSAGES:

- Higher FeNO levels were not associated with typical respiratory features of asthma including MD diagnosis, respiratory symptoms, or airway obstruction among children with sickle cell anemia (SCA).
- FeNO levels were associated with atopy features (eosinophilia, higher serum IgE levels, and having 2 or more positive skin tests) and prospective rates of ACS in children with SCA.

  These findings provide insight into mechanisms of pulmonary inflammation in children with sickle cell disease.

CAPSULE SUMMARY

Higher FeNO levels were not associated with prior morbidity, asthma, respiratory symptoms, or airway obstruction but were associated with features of atopy (eosinophilia, higher serum IgE levels, and having 2 or more positive skin tests) and prospective rate of ACS in children with sickle cell anemia.

KEY WORDS: Sickle cell disease, exhaled nitric oxide, asthma, airway inflammation, acute chest syndrome

ABBREVIATIONS

ACS: Acute Chest Syndrome

ATS-DLD: American Thoracic Society Division of Lung Diseases

CARE: Childhood Asthma Research and Education

FeNO: fractional concentration of exhaled nitric oxide

FEV_1/FVC: forced expiratory volume in 1 second/forced vital capacity

HU: hydroxyurea

NHLBI: National Heart Lung Blood Institute

ppb: parts per billion

SAC: Sleep and Asthma Cohort Study

SCA: sickle cell anemia (refers to HbSS and $HbS\beta^0$ only)

SCD: sickle cell disease (refer to all sickle cell disease genotypes)

INTRODUCTION

Respiratory disorders are a major cause of morbidity and mortality for patients with sickle cell disease (SCD).[1,2] Asthma in particular 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 elevated IgE levels[10] and positive skin tests to aeroallergens[11] have themselves been associated with SCD morbidity.

The measured fractional concentration of exhaled nitric oxide (FeNO) is a non-invasive biomarker of airway inflammation.[12] In school-age children and adolescents FeNO is reproducible[13] and has been associated with several features of atopy and asthma including peripheral blood eosinophilia, total serum IgE, a reduced forced expiratory volume in 1 second/forced vital capacity (FEV_1/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 healthy controls.[17-20] Furthermore, the 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 is associated with SCD-specific factors and/or asthma-related factors, and whether this non-invasive 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 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).

METHODS

Study design

Participants in the Sleep and Asthma Cohort (SAC) study were ages 4 to 19 years with SCA (HbSS or HbSβ^0). Participants with complete FeNO, spirometry data, and SCD morbidity data from birth were included in this analysis. SAC is a National Heart Lung Blood Institute (NHLBI)-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 physician diagnosis of asthma. Children receiving chronic transfusion therapy or participating in a clinical trial evaluating hydroxyurea (HU) therapy at the time of recruitment were excluded, although if they were prescribed chronic transfusion or HU therapy during the course of the follow-up period they remained in the study. Institutional approval was obtained from participating sites in St. Louis, MO, Cleveland, OH and London, UK. Written informed consent was obtained from parents and assent was obtained from children upon enrollment according to institutional policies.

Serum IgE was obtained upon study entry. Participants also performed measurement of exhaled nitric oxide (FeNO) followed by pre- and post-bronchodilator spirometry. Given that we enrolled children as young as 4 years old, those who could not perform quality FeNO and/or spirometry measurements 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 were modified from methods used in the NHLBI Childhood Asthma Research and Education (CARE) 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 the questions about medical history, family medical history including asthma, and respiratory symptoms from the American Thoracic Society and Division of Lung Diseases (ATS-DLD) questionnaire.[22] The ATS-DLD was administered at baseline and during all subsequent follow-up visits, thus we were able to match respiratory symptoms with the dates of the matching FeNO and spirometry sessions.

Exhaled nitric oxide

Online FeNO using the NIOX system (Aerocrine AB, Stockholm, Sweden) was performed according to ATS guidelines.[23] Measurement of FeNO 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%. Following a 30-second relaxation period, the exhalations were repeated until 3 FeNO values were obtained that varied <10% or 2 varying<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 as per ATS guidelines and the test repeated.

Spirometry

Following 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 were used taking into account age, gender, height, and ethnicity.[26] To measure bronchodilator response, technicians administered 4 inhalations of albuterol using an AeroChamber (Forest Pharmaceuticals, New York, NY) to participants. Spirometry was repeated 15 minutes post-albuterol. An increase of $\geq$12% in FEV_1 following albuterol was considered a positive bronchodilator response.[27]

Over-reading of spirometry and FeNO

To ensure ATS criteria were met across the three participating sites spirometry and FeNO, results were reviewed by a single investigator (RCS); invalid tests were excluded from analyses.

Allergy skin testing

Allergy skin testing was performed by SAC-certified technicians using Multi-test II (Lincoln Diagnostics, Decatur, IL). Ten aeroallergens (Greer Laboratories, Lenoir, NC) were used for skin testing: dust mite (*Dermatophagoides pteronyssinus* and *D garinae*), cockroach (American and German), cat (standardized), dog (mixed breeds), *Alternaria alternans*, *Aspergillus fumigates*, 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. Tests were considered positive when the mean diameter of the wheal was $\geq$ 3 mm.

Morbidity Data: Definitions of vaso-occlusive pain episode and acute chest syndrome

- A vaso-occlusive pain episode was defined as an episode directly associated with SCA, which 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 roentgenogram, temperature greater than 38^0 Celsius and increased respiratory effort with a decrease in oxygen saturation or increased 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 multi-center study, the charts of all patients diagnosed with 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 was not normally distributed in our study participants, but had a long right tail. To accommodate non-normal distributions, FeNO, total serum IgE, and eosinophil count were natural-log transformed for all regression analyses. Clinical and biomarker features were tested for their association with FeNO 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 steady state ln(FeNO) as the dependent variable. Covariates used in screening multivariable models of ln(FeNO) included SCA-specific factors of interest (gender, WBC, Hb, retrospective history of ACS or pain under 4 years of age [herein termed ACS <4years or pain <4years], and use of HU at time of FeNO); and asthma/atopy factors of interest (IgE, eosinophils, FEV_1/FVC % predicted, bronchodilator responsiveness [Y/N], history of wheezing causing shortness of breath, and use of inhaled corticosteroids at time of eNO).

Because age is accounted for in the FEV_1/FVC% predicted values and because age and height were highly correlated (ρ=.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 had allergy skin testing using a similar approach; in this model having 2 more positive skin tests was added as a covariate.

Negative binomial regression was then used to test associations between steady state FeNO (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: gender, SCA specific factors (Hb, WBC, and retrospective rate of ACS), as well as atopy and airway inflammation features (FeNO, IgE level, having 2 or more positive skin tests, FEV_1/FVC % predicted, and history of wheezing causing shortness of breath). Covariates we considered to be potentially associated with prospective rates of pain included: age, gender, SCA specific factors (Hb, WBC, retrospective rate of pain), and atopy and airway features (FeNO, IgE, eosinophils, FEV1/FVC%, wheeze causing SOB). All covariates meeting significance criteria of $p<0.20$ 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 using Stata statistical software (Version 12, College Station, TX: StataCorp LP) and IBM SPSS Statistics (Version 22, Chicago, IL, IBM).

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 values for FeNO obtained on the same day as a successful spirometry session. The clinical characteristics of the sample are summarized in Table 1. 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 the FeNO/spirometry 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 parts per billion (ppb). The median was 9.0 with Q1 and Q3 6.1 and 13.7 ppb, respectively. Children without acceptable FeNO and/or spirometry data, and therefore excluded from the analysis, were younger, had a higher percentage of mothers with asthma, had lower rates of pain (likely a function of age), but were otherwise similar to those with acceptable FeNO values (Supplementary table 1).

Factors Associated with FeNO

As shown in table 2, in unadjusted analyses FeNO was positively associated with age, height, total serum IgE, having 2 or more positive skin tests, and blood eosinophils. The associations with IgE and skin tests were present for children with and without asthma; once the cohort was stratified into smaller asthma and no asthma subgroups, the association with eosinophils was no longer significant (Supplementary Table 2). Neither wheezing symptoms, spirometry results, nor a diagnosis of asthma were associated with FeNO. There was no difference in FeNO levels between those using and not using inhaled corticosteroids. A multivariable linear regression screening model for ln(FeNO) was built including SCA-specific factors of interest and

asthma/atopy factors of interest. Male gender, IgE, blood eosinophils, history of wheezing causing shortness of breath, and history of ACS <4years met criteria for inclusion in a second model. The final model is shown in table 3 with (ln)IgE, the highest quartile of eosinophil count, and male gender independently associated with (ln)FeNO. As shown in Table 2 as well as in a separate multivariable model which included the 121 participants who had allergy skin testing, having 2 or more positive skin tests was also significantly associated with ln(FeNO) (adjusted β=0.27, p=0.003).

Association between baseline FeNO 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) met criteria for inclusion in a final model (p=0.04), as did gender, wheezing leading to shortness of breath, and ACS<4 years. In the reduced model all were significantly associated with prospective ACS (Table 4). In an analysis stratified by asthma status, ln(FENO) remained associated with prospective rates of ACS in the larger "no asthma" group but was no longer significant in the "asthma group" (Supplementary Table 3.)

In the initial screening model for prospective rate of pain, ln(FeNO) was not significant (p=0.49).

DISCUSSION

A diagnosis of asthma is a risk factor for future ACS episodes in children with SCA,[10,28,29] but making this diagnosis is challenging because of the overlap with respiratory symptoms in individuals with SCA without a co-morbid 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 higher levels of FeNO are associated with higher future

rates of ACS. While 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 may serve as a tool to aid physicians and researchers in stratifying those at the highest risk for future ACS events. Further, the association of FeNO with future ACS indicates for the first time the role of airway inflammation in risk of this important outcome among children with SCA.

While studies have clearly linked a diagnosis of asthma and atopy with SCD 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, while FeNO was associated with IgE, having 2 or more positive skin tests, and peripheral blood eosinophilia, it was not associated with doctor diagnosis of asthma, wheeze symptoms, airway obstruction, or response to bronchodilator. While FeNO has been shown to be correlated with eosinophilic airway inflammation in the general population and among children with asthma,[30-32] it has also been shown to correlate with lymphocytic airway inflammation in lung transplant patients,[33] with both neutrophilic[34] and eosinophilic[35] airway inflammation in COPD, and with lymphocytic airway inflammation in early bronchopulmonary involvement in Crohn's disease[36] and in murine models of systemic sclerosis.[37] Future studies in SCD should include direct examination of inflammatory cell types in the sputum.

Previous studies have been conflicting about relationships between FeNO and SCD complications. Two studies found FeNO levels were lower in SCD patients with a history of ACS compared to those without ACS[18,38] while 2 other studies – similar to our study - found no differences between those with and without a prior ACS episode.[19,39] Pawar et al noted that FeNO levels among patients during an acute VOC pain episode were no different than among

those at steady state.[19] A recent study of FeNO measured at variable flow rates[40] found elevated alveolar NO concentration and production among SCD patients compared to healthy race-matched controls. They also found significant positive correlations between alveolar NO and pulmonary blood flow in the SCD group, suggesting that alveolar NO production is related to the chronic hyperdynamic circulation found in patients with SCD. Furthermore, FeNO measured at 50ml/sec was positively correlated with pulmonary blood flow but was not correlated with measures of airway obstruction or resistance, suggesting that some component of the FeNO of a SCD patient is due to increased alveolar NO production resulting from chronic anemia rather than airway inflammation from asthma.[40] In contrast, utilizing flow-independent methods, Radhakrishnan et al. were able to determine that elevated FeNO levels among non-atopic children with SCD were higher compared to healthy controls, but their results showed that increase in FeNO originated in the bronchial tree and not from alveolar sources.[17] Further studies of FeNO in larger cohorts of SCD patients of varying ages and disease severity, with and without atopy, may 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 had a number of strengths, including the ascertainment of FeNO using standardized ATS criteria, use of objective measurements of lung function with centralized standardized over-reading of lung function, and prospective ascertainment of SCA-specific morbidity. While this is the largest study to date of FeNO in individuals with SCA, a limitation is measuring FeNO at one flow rate. [17,41] SAC chose to obtain FeNO according to current ATS guidelines, as this is the method widely available to clinicians, rather than utilizing methods available only as part of a research protocol. Our study only included children with SCA, 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, as we were unable to identify a cutoff value of FeNO that was 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 non-SCD children with asthma;[42] however, we had very few children in our cohort with FeNO above this cutoff.

In conclusion, this study found that FeNO was correlated with features of atopy (IgE, skin test reactivity, and peripheral blood eosinophils) – 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. While steady state FeNO 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 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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Sleep Asthma Cohort (SAC) investigative team

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Robyn T. Cohen analyzed and interpreted the data, drafted and revised of the manuscript, and approved the version to be published.

Robert C. Strunk conceived and designed the study, participated in acquisition and interpreting the data, drafting and revision of the manuscript, and approved the version to be published. JJF contributed to interpretation of the data, drafting and revision of the manuscript, and final approval of the version to be published.

Carol L. Rosen and Fenella J. Kirkham contributed to conception and design of the study, acquisition of the data, critical revision of the manuscript, and approved the version to be published.

Jane Kirkby contributed to conception and design of the study, interpretation of the data, critical revision of the manuscript, and approved the version to be published.

Mark J. Rodeghier analyzed and interpreted the data, drafted and revised the manuscript, and approved the version to be published.

Michael R. DeBaun conceived and designed of the study, participated in acquisition of the data, interpretation of the data, drafting and revision of the manuscript, and approved the version to be published.

388 **Table 1. Characteristics of the study population (N=131)[a]**

	All patients N=131	No asthma N= 93 (71%)	Asthma [b] N= 38 (29%)	P Value
Characteristic				
Age, years (mean, SD)	11.2 (3.6)	11.1 (3.5)	11.3 (3.8)	0.81
Total follow up time from birth, years (mean, SD)	16.2 (3.9)	16.2 (3.7)	16.4 (4.2)	0.73
Total follow-up time after eNO was obtained, years (mean, SD)	5.1 (1.1)	5.0 (1.1)	5.1 (1.3)	0.65
Male (%)	55.0	49.5	68.4	0.048
Hemoglobin, g/dl (mean, SD)	8.4 (1.3)	8.5 (1.3)	8.2 (1.1)	0.24
White blood cell count, 10^9/L (mean, SD)	11.6 (3.7)	11.3 (3.9)	12.3 (3.0)	0.15
FeNO, ppb (median, IQR)	9.0 (7.6)	8.9 (7.3)	9.9 (9.0)	0.60[d]
FeNO ≥ 25 ppb (%)	8.4%	7.5	10.5	0.73
IgE, IU/ml (median, IQR) (n=127)	46.6 (133.3)	47.6 (121.6)	63.6 (152.8)	0.77[d]
Eosinophils, total count (median, IQR)	354.0 (492.0)	320.0 (451.2)	456.0 (606.2)	0.35[d]
Had 2 or more positive skin tests (%, n=121)	28.9%	20.5%	47.4%	0.002
FVC, % predicted (mean, SD)	93.5 (14.1)	93.8 (13.7)	92.8 (15.0)	0.71
FEV_1, % predicted (mean, SD)	88.8 (13.3)	89.8 (13.2)	86.4 (13.5)	0.19
FEV_1/FVC (mean, SD)	0.85 (.07)	0.85 (0.07)	0.83 (0.08)	0.13
FEV_1/FVC, % predicted (mean, SD)	94.9 (7.7)	95.6 (7.0)	93.3 (9.3)	0.14
FEV_1/FVC < LLN (%)	19.8	15.1	31.6	0.03
Percent with + bronchodilator response[c]	16.8	11.8	28.9	0.02
Retrospective rate of pain episodes per year (median, IQR)	0.3 (0.6)	0.3 (0.7)	0.3 (0.6)	0.88[d]
Retrospective rate of ACS episodes per year (median, IQR)	0.1 (0.3)	0.1 (0.2)	0.2 (0.3)	0.04[d]
Prospective rate of pain episodes per year (median, IQR)	0.6 (1.3)	0.5 (1.3)	0.6 (1.3)	0.80[d]
Prospective rate of ACS episodes per year (median, IQR)	0.2 (0.3)	0.0 (0.3)	0.2 (0.5)	<0.001[d]
On hydroxyurea at the time FeNO was obtained (%)	11.5	9.7	15.8	0.37
On inhaled corticosteroids at the time FeNO was obtained	21%	2%	66%	<0.001

$PC_{20} \leq 8.0$ (%) (n=66)	63.6	65.1	60.9	0.73

Abbreviations: SD=standard deviation; FeNO=exhaled nitric oxide; ppb=parts per billion; IQR=interquartile range; FVC=forced vital capacity; FEV_1=forced expiratory volume in 1 second; ACS=acute chest syndrome

[a]Means and SD are presented for normally distributed variables, Medians and IQR's are presented for non-normally distributed variables

[b]Participants were classified as having asthma if had ever been a physician diagnosis of asthma and current use of an asthma medication at the time of the FeNO measurement.[7]

[c]$(\text{Post } FEV_1 - \text{Pre } FEV_1)/\text{Pre-}FEV_1 \geq 0.10$

[d]Mann-Whitney U test

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Table 2. Associations between FeNO and Participant Characteristics			
Categorical variables			
	Median FeNO		
Covariate	**No**	**Yes**	**Wilcoxon P value**
Male gender	8.4	10.3	0.11
Parent has asthma	8.7	10.6	0.14
Participant has asthma	8.9	9.9	0.60
On hydroxyurea at the time of FeNO	8.7	11.4	0.06
On inhaled corticosteroids at the time of FeNO	8.8	11.7	0.25
Has >12% improvement in FEV1 after bronchodilator	9.0	10.0	0.40
Has ≥ 2 positive skin tests (N=121)	8.3	12.4	0.001
Wheeze with cold	8.7	10.0	0.22
Wheeze without cold	9.0	10.4	0.30
Wheeze with SOB	9.0	9.4	0.70
Wheeze after exercise	8.9	9.8	0.66
Had an ACS event prior to 4 years of age	9.8	9.0	0.45
Had a pain event prior to 4 years of age	8.9	9.6	0.95
Continuous variables			
Covariate	**Spearman's ρ**		**P value**
Age	.28		0.001
Height	.34		<0.001
FEV_1% predicted	-.07		0.46
FVC% predicted	-.04		0.63
FEV_1/FVC (actual)	-.09		0.32
FEV_1/FVC (% predicted)	.00		0.95
IgE	.28		0.001
Eosinophils, total no. of cells/cu.mm	.20		0.02
White blood cell count	-.08		0.35
Hemoglobin (g/dL)	.02		0.84
Retrospective rate of ACS prior to FeNO	-.03		0.78
Retrospective rate of pain prior to FeNO	-.03		0.76
Prospective rate of ACS after FeNO	.07		0.42
Prospective rate of pain after FeNO	.01		0.87

Abbreviations: FeNO=exhaled nitric oxide; ppb=parts per billion; FVC=forced vital capacity; FEV_1=forced expiratory volume in 1 second; ACS=acute chest syndrome

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Table 3. Final multivariable model of factors associated with (ln)FeNO among children with SCA		
Covariates	**β Estimate (SE)**	**P value**
Male gender	.24 (.11)	0.04
White blood cell count	-.02 (0.015)	0.19
(ln) IgE	.12 (.04)	0.001
Eosinophils (quartile 1=reference)	..	..
Quartile 2	-0.11 (.15)	0.48
Quartile 3	.13 (.15	0.40
Quartile 4	.34 (.15)	0.03
History of wheezing that caused shortness of breath	-.17 (.13)	0.20
ACS episode prior to 4 years of age	-.16 (.11)	0.16

Abbreviations: FeNO=exhaled nitric oxide; SCA= sickle cell anemia; SE=standard error; ACS=acute chest syndrome

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Table 4. Final multivariable model of prospective rate of ACS in Children with SCA[a]

Covariate	IRR	95% CI	P value
ln (FeNO):	1.44	1.04-1.99	0.03
Male gender	0.59	0.38-0.93	0.02
History of wheezing causing shortness of breath	2.34	1.38-3.98	.002
ACS episode prior to age 4 years	2.79	1.81-4.31	<0.001

Abbreviations: ACS=acute chest syndrome ; SCA=sickle cell anemia; IRR=incidence rate ratio; CI=confidence interval; FeNO=exhaled nitric oxide; SCA= sickle cell anemia; SE=standard error;

[a]Negative Binomial regression models with adjustment for over-dispersion, using robust standard errors. Two-tailed significance values.

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