

Relationship of exhaled nitric oxide to clinical and inflammatory markers of persistent asthma in children

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Background: Exhaled nitric oxide (eNO) is a noninvasive test that measures airway inflammation. Insufficient information is available concerning correlations between eNO and biologic, physiologic, and clinical characteristics of asthma in children currently not taking controller medications.

Objective: The aim of this study was to find correlations between eNO and other characteristics of children with mild to moderate asthma currently not taking medications.

Methods: Children aged 6 to 17 years with mild to moderate persistent asthma, taking only albuterol as needed, were characterized during 2 visits 1 week apart before being randomly assigned into a clinical trial. At the screening visit, online measurements of eNO, spirometry before and after bronchodilator, and biomarkers of peripheral blood eosinophils, serum eosinophil cationic protein, total serum IgE, and urinary leukotriene E₄ were obtained. During a week characterization period before randomization, symptoms were recorded on a diary and peak expiratory flows were measured twice daily using an electronic device. At the randomization visit, eNO was repeated followed by a methacholine challenge and aeroallergen skin testing. Correlations and rank regression analyses between eNO and clinical characteristics, pulmonary function, and biomarkers were evaluated.

Results: eNO was significantly correlated with peripheral blood eosinophils ($r = .51$, $P < .0001$), IgE ($r = .48$, $P < .0001$), and serum eosinophil cationic protein ($r = .31$, $P = .0003$) but not with urinary leukotriene E₄ ($r = .16$, $P = .08$). A moderate correlation was found between eNO and the number of positive aeroallergen skin tests ($r = .45$, $P < .0001$). eNO did not correlate with FEV₁ % predicted but was weakly correlated

with FEV₁/forced vital capacity ($r = -.19$, $P = .032$), bronchodilator response ($r = .20$, $P = .023$), and FEV₁ PC₂₀ methacholine ($r = -.31$, $P = .0005$). No significant correlations were found between eNO and clinical characteristics or morning or evening peak expiratory flow measurements. The rank regression analysis demonstrated that 5 variables accounted for an R square of .52 (eosinophils [$P < .0001$], IgE [$P = .0023$], age [$P < .0001$], months of inhaled corticosteroid use in the year before study entry [$P = .01$], and FEV₁ PC₂₀ [$P = .0061$]).

Conclusions: These findings suggest that eNO provides information about the asthmatic state consistent with information from other markers of inflammation. It is a noninvasive technique that could be used in decisional management of children with asthma. (*J Allergy Clin Immunol* 2003;112:883-92.)

Key words: Childhood asthma, exhaled nitric oxide, biomarkers, pulmonary function test measurements, clinical characteristics, eosinophils, IgE, immediate hypersensitivity

Asthma control is monitored by means of symptoms using pulmonary function tests, particularly FEV₁ and FEV₁/forced vital capacity (FVC), to supplement the clinical information. There is growing interest in determining the role of biomarkers in asthma care. Most of these studies have been conducted in adults. Peripheral blood eosinophils^{1,2} and sputum eosinophils³ correlate with response to oral corticosteroids. Studies in adults have shown that improved asthma control can be attained by use of airway responsiveness to methacholine⁴ and sputum eosinophils⁵ to modulate asthma therapy rather than waiting for clinical symptoms to appear or pulmonary functions to deteriorate. Because the application of biomarkers to assessment of asthma management has become more widespread, exhaled nitric oxide (eNO) has been studied to predict the clinical course with reductions in or response to medications. eNO levels correlate with response to inhaled corticosteroids (ICSs), in that patients with higher levels have better responses.⁶ Jones et al demonstrated that increases of eNO have a positive predictive value for loss of control of asthma as ICSs are withdrawn, providing information about control of asthma equal to induced sputum eosinophils and methacholine responsiveness.⁷ eNO is a particularly attractive biomarker, because the test requires little effort from the patient, can be measured even in young children,⁸ and the results of the test can be immediately available.⁹ A

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Abbreviations used

CARE:	Childhood Asthma Research and Education
CLIC:	Characterizing the Response to a Leukotriene Receptor Antagonist and an Inhaled Corticosteroid
eNO:	Exhaled nitric oxide
FVC:	Forced vital capacity
ICS:	Inhaled corticosteroid
NHLBI:	National Heart, Lung, and Blood Institute
PEF:	Peak expiratory flow
Q1 and Q3:	Upper and lower quartiles

device to measure eNO is now Food and Drug Administration approved for use. Clinicians are questioning the applicability of measuring eNO in clinical practice, as well as the interpretation of results.

This study takes advantage of a cohort of children with mild to moderate persistent asthma enrolled in a clinical trial to determine characteristics responsible for differential response to an ICS and a leukotriene receptor antagonist. These children were thoroughly characterized when taking no asthma controller medication. This cohort offers an opportunity to study correlations between eNO and measurements of both pulmonary physiology and biomarkers considered to be relevant to allergic asthma airway inflammation. Evaluation of these characterization measures before randomization into treatment arms provides the unique opportunity of assessing correlations between the measures in the relationship to the baseline disease process itself without interference of controller medications that might alter eNO levels after treatment initiation (eg,^{10,11}).

METHODS

Study population

Participants aged 6 to less than 18 years were successfully screened, characterized, and randomly assigned into the clinical trial, Characterizing the Response to a Leukotriene Receptor Antagonist and an Inhaled Corticosteroid (CLIC), at the 5 clinical centers of the Childhood Asthma Research and Education (CARE) Network funded by the National Heart, Lung, and Blood Institute (NHLBI). CLIC was designed to determine characteristics of children with asthma who had favorable responses to both a leukotriene receptor antagonist and an ICS, one but not the other medication, or neither of the medications. Asthma phenotype was defined by several biologic and physiologic measures in preparation for the comparison of these outcomes to changes of FEV₁ during a double-blind crossover protocol (Fig 1). Inclusion and exclusion criteria for participation are shown in Table I.

Clinical monitoring during characterization period

At the first visit, albuterol metered dose inhaler with Aerochamber (Trudell Medical International, London, Canada) technique was reviewed, and medications (placebo forms of both study medications, a Diskus and oral tablet) were dispensed. An electronic peak flow meter (AM1; Jaeger-Toennies GmbH, Hoechberg, Germany) was dispensed after instruction in appropriate technique. A diary was

provided for recording twice-daily entries of asthma symptoms, nocturnal awakenings, rescue albuterol use, and study medication use. The importance of these entries was emphasized. At the randomization visit, the diary was reviewed, and the electronic peak flow meter was downloaded. Children with less than 80% compliance in the 1-week diary documentation of peak expiratory flow (PEF) measurements, symptom scores, and albuterol use were excluded.

Pulmonary function testing

Pulmonary function tests were performed by CARE Network–certified pulmonary function technicians by use of a pneumotachygraph-type spirometer interfaced with a personal computer system (Jaeger-Toennies GmbH, Hoechberg, Germany). Equipment specifications and testing procedures for the maximal expiratory flow volume maneuvers met or exceeded American Thoracic Society 1994 spirometry standards.^{12,13} Age, sex, and ethnicity appropriate prediction equations were used to calculate the percent of predicted values for FEV₁ and FVC (Wang, 1993 #1366). Modified after techniques described by Eigen et al,¹⁴ spirometry was performed at least 4 hours after the last use of a short-acting bronchodilator. Maximal postbronchodilator spirometry was performed 15 minutes after a total of 6 to 8 inhalations of albuterol. Baseline spirometry was compared with post (4 inhalations of albuterol) spirometry values. An additional 2 inhalations of albuterol were given, and spirometry was then repeated and compared with previous results. This scenario was continued until there was < 5% change in FEV₁ or 8 total inhalations of albuterol were administered.

Methacholine airway challenge procedure

A CARE Network–certified pulmonary function technician measured airway responsiveness by the decrease in FEV₁ after administering increasing concentrations of methacholine (Provocholine, Methapharm, Coral Springs, Fla) delivered by the small-volume nebulizer-tidal breathing technique (Wright nebulizer, Medi-tech Ltd, Montreal, Quebec) according to a standardized procedure¹⁵ and American Thoracic Society 2000 methacholine guidelines.¹⁶ The test was performed at least 4 hours after the last use of a short-acting bronchodilator and/or caffeine consumption and at least 4 weeks after oral corticosteroid use or respiratory tract infection. Baseline FEV₁ value equaled or exceeded 70% of predicted. A diluent step was performed and used as the reference value for the calculation of the PC₂₀.

eNO measurement

eNO was measured using the online technique recommended by the American Thoracic Society¹⁷ with the NIOX system (Aerocrine AB, Stockholm, Sweden). This technique used a resistive device that provided a constant low expiratory flow rate and vellum closure. Patients were seated (with no nose clip), exhaled to residual volume, inserted mouthpiece, inhaled to total lung capacity, then exhaled for 10 seconds at a constant flow rate of 0.05 L/s (BTPS) ± 10%. Visual incentives provided feedback for flow rate compliance. The end point of measurement occurred when a plateau for 4 seconds was observed. The plateau variance criterion was 10% or 5 ppb. Exhalations were repeated after a 30-second relaxation period until the performance of 3 eNO values varied less than 10% or 2 values varied less than 5%. The measurement of eNO was obtained before each measurement of spirometry, including those that preceded the beginning of bronchodilator or methacholine challenge procedures.

Other biomarker measurements

Peripheral blood eosinophil counts were done by automated assay at each center. Total serum IgE (Pharmacia CAP system) and eosinophil cationic protein (Pharmacia CAP system) were mea-

TABLE I. Inclusion and exclusion criteria for enrollment into CLIC

Inclusion criteria	
1.	Ability to perform reproducible spirometry
2.	History of combination of asthma symptoms or rescue bronchodilator use on an average of 3 or more days per week during the previous 4 wk
3.	History of prior clinical varicella or varicella vaccine
4.	Nonsmoker including no use of smokeless tobacco products in the past year
5.	Ability of parent to provide informed consent, as evidenced by signing a copy of the consent form approved by the institutional review board of the subject's respective study institution, with assent from the child
6.	Demonstrate airway lability defined as $\geq 12\%$ improvement in FEV ₁ after the maximal bronchodilator testing procedure with albuterol metered dose inhaler or methacholine responsiveness with an FEV ₁ PC ₂₀ ≤ 12.5 mg/mL
Exclusion criteria	
1.	Received any oral or inhaled glucocorticoid treatment for any condition within 4 wk before enrollment with the exception of nasal corticosteroids administered on a continuous basis
2.	Received leukotriene modifiers, theophylline derivatives, or mast cell stabilizers for asthma within 2 wk before enrollment
3.	Severe asthma as indicated by: received 4 or more oral corticosteroid bursts for asthma exacerbations within the past year; required intubation or experienced seizures as a result of an asthma exacerbation ever; required 2 or more hospitalizations for asthma in the past year; FEV ₁ < 70% predicted; during characterization phase: albuterol > 4 times (8 inhalations) per day on average (excluding pre-exercise use), nighttime awakenings from asthma 2 or more times per wk, or PEF variability of 30% or more
4.	Received treatment within the previous 4 wk with medications known to significantly interact with either study medication
5.	Significant medical illness other than asthma, including other chronic or active lung disease
6.	History of respiratory tract infection within 4 wk of enrollment
7.	Receiving allergen hyposensitization other than established maintenance
8.	Pregnant or lactating or child-bearing potential with failure to practice acceptable birth control
9.	Morbidly obese
10.	History of adverse reactions to study medications

sured centrally on serum from blood clotted at room temperature and then frozen and shipped. Urine was frozen and sent to a central laboratory for analysis (immunoaffinity purification/immunoassay quantitation) of leukotriene E₄.¹⁸

Aeroallergen skin testing

Skin tests were performed with 8 common aeroallergens (mixtures for dust mite [*Dermatophagoides pteronyssinus* and *D farinae*], cockroach [American and German], mold [mix #1], grass [standardized Southern mix], tree [eastern 8 tree mix], weed [national mix], dog [mixed breeds], and cat [standardized] [Greer Laboratories, Lenoir, NC]) using Multitest II (Lincoln Diagnostics, Decatur, Ill) on children in all the clinical centers. Testing was performed by CARE Network-certified personnel in accordance with a study specific protocol.^{19,20} A test was considered positive if the skin test resulted in a wheal with mean diameter (mean of maximum and 90-degree mid-point diameters) of at least 3 mm greater than a saline control.

Statistical methods

Spearman correlation coefficients were used to assess the relationships among the biomarkers, clinical and pulmonary function measures, and allergy skin test reactivity. In this manner, potential predictors of eNO were identified. PC₂₀ was analyzed on the log base 2 scale because of doubling doses in the methacholine challenge procedure. The eNO measurements and many of the biomarkers displayed a skewed distribution, and these were analyzed on the logarithmic scale. Geometric means and coefficients of variation were computed for these measurements, along with medians and quartiles on the log scale. Other continuous measurements were summarized using arithmetic mean and SD, along with median and first and third quartiles (Q1 and Q3). Categorical measurements were summarized by percentage.

By use of potential predictors identified through the Spearman correlations, a model-building process was performed that used stepwise rank regression analysis to determine which potential predictors were significant in explaining the variability in eNO measurements in the presence of the other variables. These potential

predictors include average days per week with symptoms, morning PEF (% predicted), PEF variability, FVC (% predicted), FEV₁ (% predicted), FEV₁/FVC, maximum bronchodilator response, PC₂₀, absolute eosinophils, ECP, IgE, urinary leukotrienes, number of positive aeroallergen skin tests (of 8 possible), months ICS use in the year before study entry, use of ICS in past year, age, age at onset of asthma, duration of asthma, sex, race, ethnicity, minority status, and height. Although several of these explanatory variables are correlated, the stepwise selection process prevents problems with multicollinearity in the model. A *P* value of .10 was required for a variable to enter the model; likewise, a *P* value of .10 was required for variables in the model to stay in the model.

A complete cohort of randomly assigned CLIC subjects was used for the model building. The cohort was revised according to variables in the final model to include all possible patients. All summary statistics and analyses were performed by means of SAS Version 8 statistical software (SAS Institute, Cary, NC). Significance was established at *P* < .05, 2-tailed.

Protocol review

The protocol was approved by each center's Institutional Review Board and by an NHLBI Protocol Review Committee and an NHLBI Data Safety Monitoring Board.

RESULTS

Demographics and lung function results

Demographic characteristics and lung function results obtained during screening of the children entered into the trial are shown in Table II. The 144 participants had a mean age of 11 years, with 41% females and 48% minorities. Forty-one percent had used a controller medication, and 28% had used an ICS for a mean of 2.5 ± 3.5 months in the 12 months before entry but not in the last month before trial enrollment. Spirometry results at base-

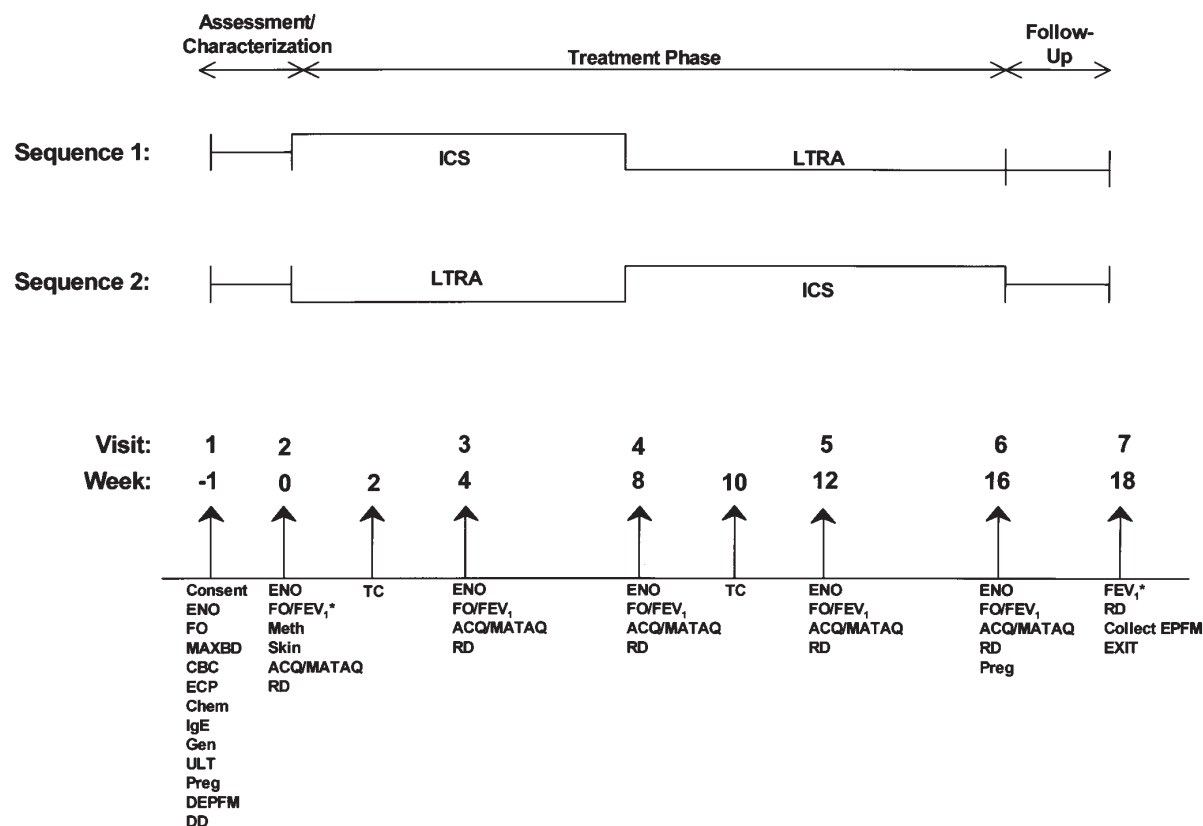


FIG 1. Study design for the National Heart, Lung, and Blood Institute's Childhood Asthma Research and Education Network study entitled "Characterizing the Response to a Leukotriene Receptor Antagonist and an Inhaled Corticosteroid." ACQ, Asthma Control Questionnaire; MATAQ, Modified Asthma Therapy Assessment Questionnaire; CBC, complete blood count, total eosinophil count; *Consent*, obtain informed consent; ECP, plasma eosinophilic cationic protein; *Chem*, chemistry; *IgE*, serum IgE; *Preg*, pregnancy test in those reaching menarche; ENO, exhaled nitric oxide; *DD*, dispense diary; *DEPFM*, dispense electronic peak flow meter; *RD*, review symptom diary; *FO/FEV₁*, forced oscillation and spirometry before and after bronchodilator treatment (* indicates no bronchodilator testing at this visit); *ULT*, urinary leukotriene measurement; *max BD*, maximal bronchodilator response; *Gen*, genetics analysis; *Skin*, allergen skin tests; *TC*, telephone call; *EXIT*, completion and discharge from study. Treatments: *ICS*, Inhaled corticosteroid. Inhaled fluticasone propionate (Flovent Diskus 100 µg per inhalation) or corresponding placebo administered as 1 inhalation twice daily. *LTRA*, Leukotriene receptor antagonist. Montelukast tablet (5 mg for those 6-14 years and 10 mg for those 15-18 years) or corresponding placebo administered as 1 tablet once daily at night.

TABLE II. Characteristics of the 144 CLIC participants: Demographics, pulmonary function test and clinical characteristics, and skin test reactivity

Female	41%		
Minority	48%		
	Mean ± SD	Median	Lower and upper quartiles
Age, y	11.4 ± 3.4	11.42	8.0, 14.3
Symptoms during characterization, days per wk	4.1 ± 2.5	4.4	2.0, 6.2
Spirometry: forced vital capacity, % predicted	105.3 ± 11.8	104.8	97.4, 113.0
FEV ₁ , % predicted	95.0 ± 12.9	95.3	85.8, 103.1
FEV ₁ /forced vital capacity, %	79.4 ± 8.5	81.0	72.0, 86.0
Bronchodilator reversibility, % change in FEV ₁ from baseline	15.3 ± 9.4	14.4	10.0, 19.9
PC ₂₀ , mg/mL	1.2, 3.8*	1.1†	0.45†, 3.4†
AM PEF, % predicted	77.2 ± 12.7	77.5	68.4, 85.4
PEF variability, %‡	9.5 ± 5.8	8.4	5.6, 11.9
Skin test reactivity, No. positive tests of 8 tested	2.8 ± 2.3	3.0	1.0, 4.0

*Geometric mean, coefficient of variation.

†Median and 1st and 3rd quartiles are reported on the log₂ scale.

‡AM PEF–PM PEF/[(AM PEF + PM PEF)/2] × 100.

TABLE III. Biomarker results

Biomarker	Geometric mean	Median*	Lower and upper quartiles*
eNO, parts per billion	24.4	26.3	10.9, 52.3
IgE, kU/L	139.5	154.3	53.5, 409.0
Peripheral blood eosinophils (cells per mm ³)	255.7	266.6	150.0, 476.0
ECP, mg/L	15.2	15.9	8.8, 25.7
Urinary leukotriene E ₄ , pg/mL	102.2	105.0	71.0, 134.0

*Median and 1st and 3rd quartiles are reported on the log scale.

Normal values: eNO: between 10 and 20 ppb (Aerocrine); IgE increases by age to 10 y; normals for age 10 y and older are mean = 22 kU/L with = 1 SD = 85 (PharmaciaDiagnostics); peripheral blood eosinophils: 50-350/mm³ (*Nelson Textbook of Pediatrics*); ECP: Median 5.9 µg/L (90th percentile 12.7 µg/L, 95th percentile 18.1 µg/L) (PharmaciaDiagnostics); urinary leukotriene E₄: 103 ± 9 pg LTE/mg creatinine.¹⁸

TABLE IV. Rank correlations between biomarkers and clinical and pulmonary function test characteristics and allergy skin reactivity

	eNO	No. positive skin tests	Symptoms	AM PEF	PF var	FVC % pred	FEV ₁ % pred	FEV ₁ /forced vital capacity %	FEV ₁ % change	PC ₂₀	Eos	ECP	IgE	uLTE4
eNO	—	0.45§	0.06	0.04	0.11	0.002	-0.13	-0.19*	0.20*	-0.31‡	0.51§	0.31‡	0.48§	0.16
eos	0.51§	0.32§	0.15	-0.12	0.19*	-0.06	-0.13	-0.16	0.18*	-0.29‡	—	0.54§	0.39§	0.36§
ECP	0.31‡	0.20*	0.23‡	-0.27‡	0.07	-0.02	-0.14	-0.18*	0.05	-0.14	0.54§	—	0.23‡	0.04
IgE	0.48§	0.52§	0.05	0.03	0.19*	0.04	-0.12	-0.12	0.17*	-0.33‡	0.39§	0.23‡	—	0.32‡
Ult	0.16	0.16	-0.08	0.04	0.12	0.01	0.02	-0.04	0.03	-0.10	0.36§	0.04	0.32‡	—

**P* < .05.

‡*P* < .01.

‡*P* < .001.

§*P* < .0001.

line demonstrated values for FVC and FEV₁ % predicted within the normal range, but a substantial percentage of children had values for FEV₁/FVC below the normal range (approximately 77%²¹). Three percent and 33% of participants had only bronchodilator reversibility or methacholine responsiveness, respectively, and 64% demonstrated both. Of the participants with bronchodilator reversibility, 69% achieved at least 12% increase with 4 puffs of albuterol, 30% needed 6 puffs, and 1% required 8 puffs. Most participants were allergic, with more than 78% having at least 1 positive skin test to an aeroallergen. Of the 8 aeroallergen extracts tested, a positive test to cat was most prevalent (49%).

Clinical characteristics

Clinical symptoms recorded on diary cards and PEF measurements downloaded from the electronic peak flow meter during characterization are shown in Table II. On average, the participants had 4.1 days of symptoms in the week of observation before randomization into the trial, with 50% having 2.0 to 6.2 days of symptoms per week, consistent with the history of symptoms obtained at the screening visits. Mean morning PEF was 77% of predicted normal with PEF variability of 9.5% (Table II), both consistent with mild to moderate persistent asthma. Average number of days per week with symptoms correlated with PEF variability significantly, but only weakly (*r* = .24, *P* = .004) and not with any other clinical or pulmonary function measure, including FEV₁ PC₂₀ methacholine and bronchodilator reversibility. PEF variability correlated weakly with morning PEF % predicted (*r* = -.30, *P* = .0002), maximum bronchodilator response (*r* = .26, *P* = .002), and FEV₁ PC₂₀ methacholine (*r* = -.22, *P* = .010).

Biomarkers results

Results of biomarkers are presented in Table III. All biomarkers were elevated relative to normal values, and each had a wide range.

Baseline eNO values are presented in Table III, with median of 26.3 ppb and Q1 and Q3 of 10.9 and 52.3, respectively. The mean was 41.1, with an SD of 46.8, a range of 0.3 to 335.6, and a 95% CI of 33.0, 49.1. These values are elevated compared with normal values²² and consistent with values found in other populations of children^{23,24} and adults with asthma.^{25,26} eNO was obtained at both visits 1 and 2. The values for these 2 measurements were highly correlated between the 2 visits (visit 1, median [Q1, Q3] = 26.3 [10.9, visit 2, median [Q1, Q3] = 26.9 [10.6, 53.9]; *r* = .91, *P* < .0001). The correlation coefficient for variability between eNO at the 2 visits (*r* = .91) was higher than for any of the spirometry measures at the 2 visits, *r* = .84 for FVC % predicted, *r* = .71 for FEV₁ % predicted, and *r* = .76 for FEV₁/FVC.

eNO correlated significantly with each of the biomarkers except urinary leukotriene E₄ (Table IV, Fig 2). A significant correlation also was found between eNO and the number of positive aeroallergen skin tests (Table IV).

eNO did not correlate with clinical characteristics and had only a weak correlation to FEV₁/FVC ratio among the spirometry characteristics. The correlations between eNO and tests of airway lability were only weak, although significant (for bronchodilator reversibility *r* = .20, *P* = .02, and for methacholine responsiveness *r* = -.31, *P* = .005).

In general, significant, but only weak to modest, correlations were found among the 5 biomarkers. The bio-

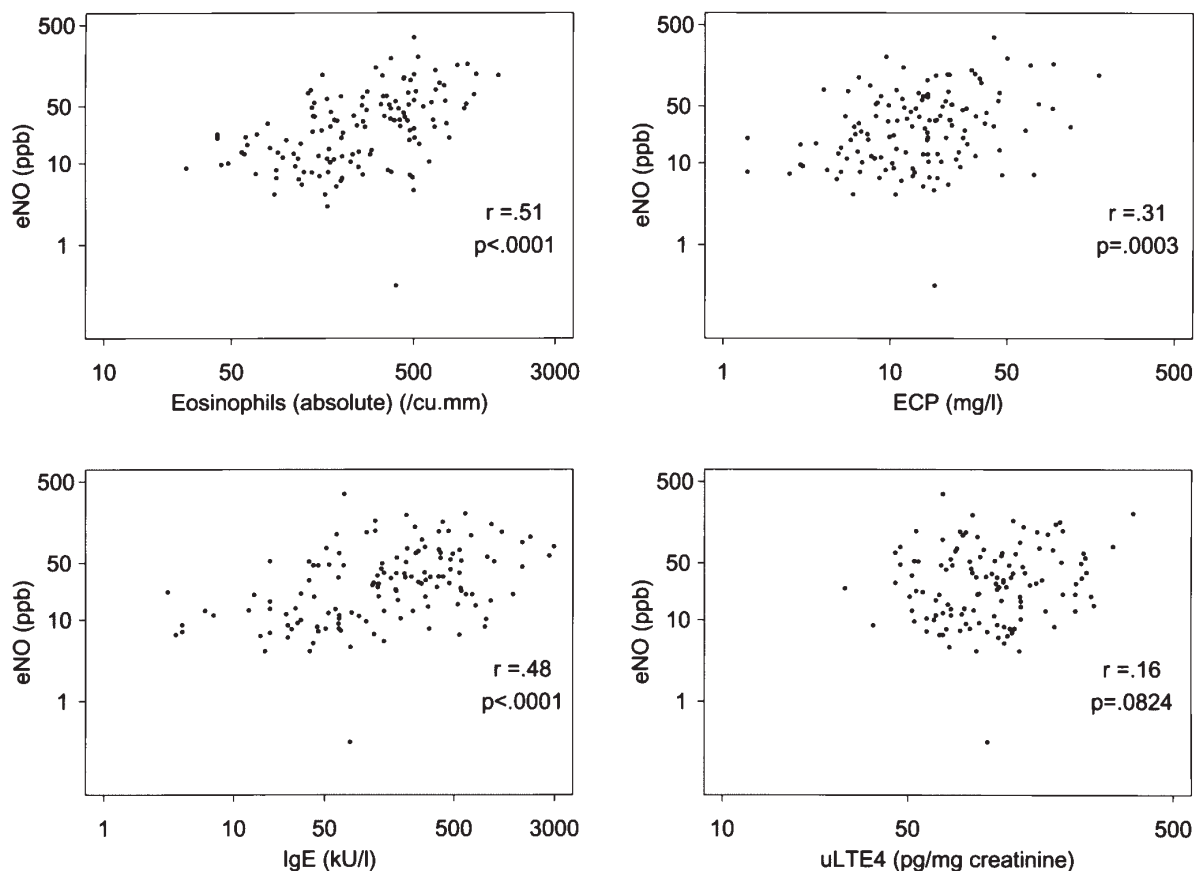


FIG 2. Scattergrams of exhaled nitric oxide (visit 1) by peripheral blood eosinophils, IgE, plasma eosinophilic cationic protein, and urinary leukotriene measurement E_4 (on the log scale).

marker with the best correlations to the other 4 biomarkers is peripheral blood eosinophils, with each correlation significant at the $< .001$ level. IgE had 3 of the 4 correlations significant at this level. Urinary leukotriene E_4 had 2 of 4 correlations significant at the $< .001$ level and was the only marker without a significant correlation to eNO. All the biomarkers except urinary leukotriene E_4 were significantly correlated with the number of positive aeroallergen skin tests.

Predictor variables

Stepwise rank regression analysis was performed to determine which potential predictors were significant in explaining the variability in eNO measurements in the presence of the other variables. Eosinophils, IgE, age, months of ICS use in the year before study entry, and FEV_1 PC_{20} entered and remained in the model (Table V). No other variables were reached at the .10 level of significance specified for model entry in the presence of these 5 predictors, and each of these 5 predictors is significant in the model at the more stringent .05 level. The model shows positive relationships for eosinophils, IgE, and age, but negative relationships for PC_{20} and months of ICS use in the year before study entry.

DISCUSSION

Although there have been many studies of eNO in asthma, the group of children enrolled in the CLIC trial offers several advantages in further defining the role of eNO in assessing asthma control and airway inflammation. The 144 children have mild to moderate persistent asthma clinically and by pulmonary function criteria (Table II) but differ from other groups of children in whom eNO has been studied in that they had not received oral or inhaled corticosteroids for at least 1 month and no other controller medications for at least 2 weeks. The CLIC cohort has many similarities to the larger cohort of children with mild to moderate asthma enrolled in the Childhood Asthma Management Program. Both groups had spirometry results indicating well-maintained FVC and FEV_1 but with the mean FEV_1 /FVC ratio at the borderline of normal, indicating airflow obstruction in almost 50% of the population. Eosinophil and IgE levels were similarly elevated,²⁷ and in both populations there were modest, but highly significant, correlations between the eosinophil and IgE levels and FEV_1 PC_{20} methacholine.²⁸ Thus, the CLIC population seems representative of many of the characteristics of other childhood populations of mild to moderate asthma.

TABLE V. Stepwise rank regression results, with outcome rank of eNO

Predictor*	Univariable results						Multivariable results ($R^2 = .52$)			
	β_0	Standard error	P value	β_1	Standard error	P value	R^2	β	Standard error	P value
Intercept (β_0)	—	—	—	—	—	—	—	25.48	9.57	.009
Rank of peripheral blood eosinophils (cells per mm ³)	31.90	5.69	< .0001	.49	.08	< .0001	.24	.36	.07	< .0001
Rank of IgE, kU/L	32.71	5.74	< .0001	.48	.08	< .0001	.23	.23	.07	.002
Rank of age	36.73	5.95	< .0001	.42	.08	< .0001	.17	.42	.07	< .0001
Rank of No. of months corticosteroid use in year before study entry	63.15	7.82	< .0001	-.002	.11	.9829	.00	-.21	.08	.010
Rank of PC ₂₀	83.21	6.20	< .0001	-.32	.09	.0003	.10	-.20	.07	.006

*Other candidate variables include ranks for each of average days per week with symptoms, morning peak flow (% predicted), peak flow variability, FVC (% predicted), FEV₁ (% predicted), FEV₁/forced vital capacity, maximum bronchodilator response, ECP, urinary leukotrienes, number of positive aeroallergen skin tests, use of inhaled corticosteroids in past year, age at onset of asthma, asthma duration, sex, race, ethnicity, minority status, and height. None of these were significant at $\alpha = .10$ in the presence of the 5 reported variables, and they did not enter the model.

The results of the baseline characteristics of the CLIC cohort indicate that eNO correlates moderately with peripheral blood eosinophils and IgE levels (Table IV). The correlation with degree of allergic sensitization was also moderate. In contrast to relationships between eNO and the other biomarkers, correlations between eNO with spirometry were insignificant for % predicted FEV₁ and FVC and weak for airway obstruction as represented by FEV₁/FVC, bronchodilator reversibility and methacholine responsiveness (Table IV). Although there were many significant correlations by univariate testing, the multiple regression accounting for all the variables with significant relationships indicated that only peripheral blood eosinophils, IgE, age, months of ICS use in the year before study entry, and PC₂₀ remained in the model (Table V). These results indicate that eNO is reflecting primarily inflammation related to each of these factors. Correlations with clinical symptoms and PEF measurements were not significant. These findings suggest that eNO is detecting information about the asthmatic state not accessed by clinical characteristics and that the correlations between eNO and pulmonary function test characteristics (other than PC₂₀) are probably caused by the impact of eosinophils, IgE, age, and corticosteroid use on pulmonary functions.

The effect of prior use of ICSs on eNO levels is surprising, given that all children had been off ICSs for at least 1 month before enrollment. Prior studies have demonstrated that levels of eNO return to baseline as early as 2 weeks after discontinuation of ICSs.^{11,29} The effect of ICS use in the past year on eNO levels in this study is therefore possibly a marker of disease severity and might be a more sensitive measure of severity than spirometry or clinical characteristics in patients with mild to moderate persistent asthma who had taken no oral or inhaled corticosteroids for at least a month.

Age was significantly associated with eNO levels in the stepwise rank regression. Age was associated even if height was forced into the model, indicating that the increase with age was not simply due to larger lung size. Narang et al³⁰ and Franklin et al²² studied eNO levels in normal children. Both groups found a similar effect of age on eNO levels. This effect was present even in prepubertal children.

Elevated levels of eNO are thought to result from increased expression and activity of inducible form of nitric oxide synthase in airway epithelial and inflammatory cells.^{31,32} Levels are elevated in individuals with asthma. Levels have been associated with several clinical measures of disease severity in children and adults and can provide information about response to medications both at times of medication initiation and withdrawal.^{7,23,33-37} In general, levels are greater in patients with asthma with aeroallergen sensitivity,^{10,38,39} and levels correlate with the number of positive skin tests among those with allergy even when no asthma is present.²²

Levels of eNO are elevated in children with asthma compared with children with no history of respiratory symptoms. Oral and inhaled corticosteroids decrease eNO levels, either in children with clear exacerbation or in those with stable but persistent asthma symptoms. Studies find that the degree of eNO elevation varies with severity, increasing with allergen or pollution exposure.⁴⁰ Covar et al¹⁰ demonstrated that discontinuation of ICSs in children leads to an increase in eNO levels even after a long period of ICS treatment in the Childhood Asthma Management Program. However, only a few studies have examined relationships between eNO and markers of inflammation.⁴¹⁻⁴⁶ Most of these studies focused on determining correlations between eNO and eosinophils and/or ECP from induced sputum, bronchoalveolar lavage fluid, or bronchial biopsy.

Methacholine challenge has been used as a tool to define the degree of asthma control and to improve clinical outcomes.⁴ Peripheral blood eosinophil levels have been shown to reflect disease activity and are useful in both diagnosis and management of asthma in adults.^{1,2} In children, peripheral blood eosinophil levels are significantly correlated with airway lability as determined by reversibility to bronchodilator, responsiveness to methacholine, and variability of PEF.^{10,28} Both methacholine challenge and eosinophil counts require time for testing procedures and analysis and therefore have limitations in widespread use in clinical practice. The results of this study indicate that eNO provides information about the asthmatic state consistent with information from other markers of inflammation. eNO is the first noninvasive technique with the capacity to obtain information about a clinically important biomarker in real time that could be used in decisional management of children with asthma. Most patients with asthma, even children as young as 5 years, can easily perform the measurement. This study does not address changes in eNO with variations in clinical course or in response to medications. However, the ease of measurement and the significant correlations with other markers of inflammation does suggest that eNO levels might predict responses to controller medications and potentially be useful for adjusting medication regimens.

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