

Asthma morbidity among inner-city adolescents receiving guidelines-based therapy: Role of predictors in the setting of high adherence

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Background: With the expanding effort to provide guidelines-based therapy to adolescents with asthma, attention must be directed to evaluating which factors predict future asthma control when guidelines-based management is applied.

Objective: We evaluated the role of fraction of exhaled nitric oxide in parts per billion, markers of allergic sensitization, airway inflammation, and measures of asthma severity in determining future risk of asthma symptoms and exacerbations in adolescents and young adults participating in the Asthma Control Evaluation study.

Methods: Five hundred forty-six inner-city residents, ages 12 through 20 years, with persistent asthma were extensively evaluated at study entry for predictors of future symptoms and exacerbations over the subsequent 46 weeks, during which guidelines-based, optimal asthma management was offered. Baseline measurements included fraction of exhaled nitric oxide in parts per billion, total IgE, allergen-specific IgE, allergen skin test reactivity, asthma

symptoms, lung function, peripheral blood eosinophils, and, for a subset, airway hyperresponsiveness and sputum eosinophils.

Results: The baseline characteristics we examined accounted for only a small portion of the variance for future maximum symptom days and exacerbations—11.4% and 12.6%, respectively. Future exacerbations were somewhat predicted by asthma symptoms, albuterol use, previous exacerbations, and lung function, whereas maximum symptom days were predicted, also to a modest extent, by symptoms, albuterol use, and previous exacerbations, but not lung function.

Conclusion: Our findings demonstrate that the usual predictors of future disease activity have little predictive power when applied to a highly adherent population with persistent asthma that is receiving guidelines-based care. Thus, new predictors need to be identified that will be able to measure the continued fluctuation of disease that persists in highly adherent, well-treated populations such as the one studied. (*J Allergy Clin Immunol* ■■■■;■■■:■■■-■■■.)

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Abbreviations used

ACE: Asthma Control Evaluation

FE_{NO}: Fraction of exhaled nitric oxide in parts per billion

FVC: Forced vital capacity

Asthma is a complex disease of the airways that is characterized by variable and recurring symptoms, airflow obstruction, bronchial hyperresponsiveness, and underlying inflammation. The interrelationships among these various features determine the clinical manifestations, influence the severity of the disease processes, and serve as a target for treatment in asthma.

The most recent Expert Panel Report 3: Guidelines for the Diagnosis and Management of Asthma¹ recommend assessing asthma severity and achieving control as key components to effective asthma care. Assessments of asthma severity and control incorporate 2 important domains, which are distinct but likely interrelated: (1) current impairment—that is, the frequency and intensity of symptoms and functional limitations; and (2) future risk—that is, the likelihood of asthma exacerbations or progressive loss of lung function.² An unresolved question is whether baseline asthma assessments, atopic status, and airway inflammation can predict future asthma control—that is, the level of impairment and exacerbation frequency in the face of guidelines-based management.

This question constitutes the focus of this report. More specifically, we first sought to examine what participant characteristics best predict future asthma symptoms and exacerbations among patients with asthma receiving guidelines-directed treatment who are adherent to this treatment. To answer this question, we evaluated not only baseline measurements of current impairment (asthma symptoms) but also a number of additional objective measurements that reflect airway inflammation (fraction of exhaled nitric oxide in parts per billion [FE_{NO}], sputum and blood eosinophilia), atopic status (total IgE, number of positive skin tests, specific IgE against common aeroallergens), and lung function (FEV₁% predicted, FEV₁/FVC, and postbronchodilator percent change in FEV₁). In addition, we analyzed the relationship among FE_{NO}, asthma symptoms, other markers of inflammation, atopy, and lung function.

The aims of this study were secondary aims of the Asthma Control Evaluation (ACE) study, which was implemented to determine whether guidelines-directed treatment, supplemented by FE_{NO} as a biomarker, would improve asthma outcomes compared with a guidelines-based approach alone in a high-risk inner-city population.³

METHODS

Study population and design

In brief, 546 participants, ages 12 to 20 years, with physician-diagnosed asthma were enrolled at 10 urban locations across the United States. Eligibility was limited to residents of census tracts in which at least 20% of households had incomes below the federal poverty threshold. In addition, individuals had to have evidence of moderate to severe persistent disease. Those receiving long-term control therapy were required to have symptoms of persistent disease or uncontrolled asthma, whereas those who were not on long-term

control therapy had to have symptoms of persistent asthma and evidence of uncontrolled disease.^{1,3} Participants had to sleep at least 4 nights per week in 1 home (to ensure consistent exposure to the same household environment) and had to be nonsmokers for 1 year before recruitment with a urinary cotinine level <100 ng/mL at enrollment. All appropriate institutional review boards approved this study. Written informed consent was obtained from each participant or the parent or legal guardian. Adolescents age 12 to 17 years provided assent.

The ACE study was a randomized, double-blind, parallel-group trial with a 3-week run-in to characterize participants, establish asthma treatment, and evaluate adherence. After run-in, participants were randomized to either a reference group (guidelines-based care) or a FE_{NO} group (exhaled nitric oxide added to guidelines-based care) for a 46-week treatment period. Follow-up visits were conducted every 6 to 8 weeks during the treatment period. Findings presented in the current article relate to data collected at the enrollment visit (week 0), 3 weeks later at the randomization visit (week 3), and during the follow-up period (weeks 9 [visit 3] through 49 [visit 8]). From week 0 to week 49, study participants received guidelines-directed asthma treatment using algorithms prepared by the study investigators.³ Treatment of all participants included inhaled corticosteroids (fluticasone) at various doses with or without long-acting β -adrenergic agonists (salmeterol). More difficult-to-control participants also received cysteinyl leukotriene receptor antagonists (montelukast).

Measurement of FE_{NO}

Fraction of exhaled nitric oxide in parts per billion was measured for all study participants at the randomization visit by using a technique modified after Silkoff et al⁴ and following American Thoracic Society guidelines.⁵ FE_{NO} was measured (flow rate 50 mL/s) with a rapid-response chemiluminescent analyzer (NIOX System; Aerocrine, Solna, Sweden).⁶

Total and allergen-specific serum IgE

At the randomization visit, a venous blood sample was obtained from all study participants for measurement of serum total IgE and allergen-specific IgE to cockroach (*Blattella germanica*), house dust mites (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), cats (cat epithelium and dander), and mold (*Alternaria alternata*). Serum IgE was measured with the Uni-Cap System (Phadia, Uppsala, Sweden).

Skin tests

Skin testing was performed during the randomization visit by the puncture method on the volar surface of the forearm by using a Multi-Test II device (Lincoln Diagnostics, Decatur, Ill). Allergen extracts were obtained from Greer Laboratories (Lenoir, NC). This article's Online Repository at www.jacionline.org lists the 14 extracts, concentrations, and positive and negative controls, and describes the test methods and assessment of results.

Blood eosinophils

At the randomization visit, a venous blood sample was obtained from all study participants for determination of total serum eosinophil count by local clinical laboratories.

Sputum induction and processing

A subset of participants from four research sites (Dallas, Denver, New York, and Tucson) underwent sputum induction at the randomization visit. Sputum was induced by inhalation of hypertonic saline solution (3%) as previously described by Fahy et al⁷ by using a DeVilbiss Ultra-Sonic Pico #3207 nebulizer (Nouvag, Lake Hughes, Calif). Participants with prealbuterol FEV₁ $\geq$ 70% of predicted (206 of 213, 97%; 1 of 206 refused) underwent sputum induction after pretreatment with albuterol. Slides were prepared and stained at the local laboratories. Differential cell counts were performed centrally by a blinded technician. The Online Repository lists for specific details regarding sputum analysis.

TABLE I. Participant characteristics at randomization* (N = 546†)

Demographics	
Age at recruitment (y)	14.4 ± 2.1
Male (%)	52.7
Race/ethnic group (%)	
Black	63.6
Hispanic	22.9
Other or mixed	13.6
Caretaker completed high school (%)	76.5
Household income <\$15,000 (%)	52.2
≥1 Household member employed (%)	82.4
At least 1 smoker in household (%)	40.7
History of hay fever, allergic rhinitis, or eczema (%)	53.5
Asthma-related symptoms at randomization (no. of days/last 2 wks)	
Maximum symptom days	2.3 ± 2.9
Wheezing, cough, tightness in the chest	2.0 ± 2.8
Slow down or stop play or activities	1.1 ± 1.8
Nights awake because of asthma	0.6 ± 1.5
School days missed	0.2 ± 0.8
Days of albuterol use	1.4 ± 2.6
Nights of albuterol use	0.5 ± 1.6
≥1 Exacerbation in year before recruitment (%)‡	78.9
Mean post-randomization asthma-related symptoms (no. of days/last 2 wks)	
Maximum symptom days	2.0 ± 1.7
School days missed	2.0 ± 0.4
Days of albuterol use	1.1 ± 1.7
Nights of albuterol use	0.5 ± 0.9
Exacerbation rate (per y)‡	0.7 ± 1.1
Lung function	
FEV ₁ (% of predicted value)	95.8 ± 15.7
FEV ₁ /FVC	80.1 ± 8.7
Postbronchodilator percent change in FEV ₁ (visit 1)	8.1 (3.9-15.1)
Methacholine PC ₂₀ (mg/mL)§	3.5 (0.8-26.0)
Allergic biomarkers	
Total IgE (kU/L)	262 (100-658)
Allergen-specific IgE ≥0.35 kU _A /L (%)	
<i>Alternaria</i>	41.4
Cat	50.7
Dust mite, <i>D pteronyssinus</i>	47.6
Dust mite, <i>D farinae</i>	47.5
German cockroach	48.3
No. of positive skin tests of 14 total	5 (2-7)
Skin test sensitivity¶	
≥1 Positive skin test (%)	87.9
Cat (%)	58.2
Dog (%)	13.7
Dust mite (%)	46.9
Mold (%)	51.6
Cockroach (%)	61.2
Rodent (%)	38.2
Inflammatory biomarkers	
Blood eosinophils (per μL)	211 (118-373)
Sputum eosinophils (% of WBC)§	0.9 (0.0-2.0)
FE _{NO} (ppb)	20.1 (11.2-40.6)

WBC, White blood cell.

*Plus-minus values are means ± SDs. Interquartile range is provided in parentheses with medians.

†Results were available for the following number of children: 468 for caretaker completed high school, 502 for household income, 477 for smoker in household, 398 for school days missed, 532 for prebronchodilator FEV₁ (% of predicted) and prebronchodilator FEV₁/FVC, 521 for postbronchodilator percent change in FEV₁, 534 for total IgE, 531 for skin test sensitivity, and 533 for blood eosinophils.

‡Exacerbations are a combined measure of prednisone usage, asthma-related unscheduled visits, and hospitalizations.

§Measurements taken at 4 sites only.

Spirometry

Spirometry was performed by certified technicians, according to American Thoracic Society standards,⁸ using a Jaeger Masterscreen (VIASYS Healthcare GmbH, Hoechst, Germany). Spirometry was performed before and after bronchodilator treatment at baseline, using 4 puffs of albuterol metered dose inhaler (MDI) administered via Aerochamber (Forrest Laboratories, Carson City, Nevada). All pulmonary function tests were centrally overread for quality control purposes; the overreading failures, which account for slightly more than 3% (171/5169) of all procedures performed, have been excluded from analysis.

Methacholine challenge

At the randomization visit, a subset of participants from 4 research sites (Dallas, Denver, New York, and Tucson) underwent methacholine challenge testing as previously described by Strunk et al.⁹ Airway responsiveness was measured by determining the concentration of methacholine required to produce a drop in FEV₁ of 20% compared with a control (postdiluent) level (PC₂₀) after the administration of increasing concentrations of methacholine using the small volume nebulizer-tidal breathing technique. The Online Repository lists specific details regarding the methacholine challenge procedure. Individuals who did not reach PC₂₀ (37 of 144; 26%) were assigned an upper limit of detection of 26 mg/mL. All methacholine challenge procedures and interpretations were overread for purposes of quality assurance; the overreading failures (37 of 181; 20%) have been excluded from analysis.

Asthma impairment and risk outcomes

The primary outcomes were maximum symptom days and exacerbations. Maximum symptom days per 2-week recall and exacerbations were assessed at each visit during the 46-week treatment period. Maximum symptom days, as used in previous inner-city asthma studies,^{10,11} were defined as the largest value among the following variables reported over the previous 2 weeks: (1) number of days with wheezing, chest tightness, or cough; (2) number of nights of sleep disturbance; and (3) number of days when activities were affected. This measure allows asthma symptoms to be correctly gauged whether the study participant expresses asthma as reduction in play, sleep disturbance, or wheeze. An asthma exacerbation was defined as a hospitalization, unscheduled visit (including emergency department visits), or prednisone course for asthma.

Statistical analyses

Partial Pearson correlations were calculated for the entire study population to measure the strength of relationships between 2 variables while controlling the effects of site, race, age, and study group. The primary objective of these analyses was the identification of predictors of future asthma control while receiving guidelines-based therapy. Symptoms and FE_{NO} at randomization, not baseline, were used because only at randomization, after the 3-week run-in, were the participants receiving well-characterized standardized asthma medical treatment. Similar analyses were also performed for the reference group (guidelines-based care) only (n = 270), and no significant differences were apparent (data not shown).

Multivariate analyses were carried out on the average maximum symptom days and exacerbations during follow-up. Longitudinal analyses were not possible because postrandomization exacerbation values were sparse. The order in which the variables were entered into the analyses was set *a priori* based on ease and cost of obtaining the clinical measurements. To maximize the sample size for these analyses (N = 477), we excluded variables obtained only on a subset of participants (methacholine PC₂₀ and sputum eosinophils).

||Individuals (N = 37) who did not reach PC₂₀ were assigned an upper limit of detection (26 mg/mL).

¶Dust mite includes participants who are skin test positive to *Dermatophagoides pteronyssinus* or *Dermatophagoides farinae*. Mold includes participants who are skin test positive to *Alternaria tenuis*, *Cladosporium herbarium*, *Aspergillus* mix, or *Penicillium notatum*. Roach includes participants who are skin test positive to American and German cockroach mix or German cockroach. Rodent includes participants who are skin test positive to mouse or rat epithelia.

TABLE II. Pearson correlations of baseline participant characteristics with follow-up symptoms and exacerbations (visits 3-8)*

	N	Maximum symptom days	Exacerbations
Asthma-related symptoms at randomization (no. of days/last 2 wk)			
Maximum symptom days	546	0.25 (<.001)	0.01 (.77)
Wheezing, cough, tightness in the chest	546	0.25 (<.001)	0.00 (.962)
Slow down or stop play or activities	546	0.19 (<.001)	0.05 (.237)
Nights awake because of asthma	546	0.14 (.001)	0.03 (.478)
School days missed	381	0.08 (.116)	0.08 (.128)
Days of albuterol use	546	0.27 (<.001)	0.13 (.003)
Nights of albuterol use	546	0.19 (<.001)	0.21 (<.001)
Lung function			
FEV ₁ (% of predicted value)	532	0.07 (.132)	−0.02 (.698)
FEV ₁ /FVC	532	−0.01 (.798)	−0.10 (.026)
Postbronchodilator percent change in FEV ₁ (visit 1)	521	0.07 (.098)	0.19 (<.001)
Methacholine PC ₂₀ †‡	144	−0.10 (.237)	−0.08 (.354)
Allergic biomarkers			
Total IgE‡	534	0.01 (.765)	0.06 (.192)
Allergen-specific IgE‡			
<i>Alternaria</i>	533	0.05 (.268)	0.04 (.418)
Cat	533	−0.01 (.848)	0.03 (.478)
Dust mite, <i>D pteronyssinus</i>	534	0.02 (.656)	0.06 (.195)
Dust mite, <i>D farinae</i>	533	0.04 (.381)	0.07 (.098)
German cockroach	534	0.04 (.342)	0.06 (.200)
Sum of the 5 allergen-specific IgEs	534	0.01 (.794)	0.06 (.192)
Number of positive skin tests	531	0.01 (.826)	0.03 (.437)
Skin test wheal size (mm)			
<i>Alternaria</i>	531	−0.01 (.776)	−0.03 (.543)
Cat	531	0.00 (.934)	0.04 (.366)
Dust mite, <i>D pteronyssinus</i>	531	−0.04 (.424)	−0.02 (.657)
Dust mite, <i>D farinae</i>	531	0.01 (.824)	0.05 (.291)
German cockroach	531	0.03 (.554)	0.03 (.430)
Mouse	531	0.01 (.823)	0.06 (.162)
Rat	531	−0.02 (.685)	0.01 (.743)
Dog	531	−0.04 (.379)	−0.02 (.710)
Inflammatory biomarkers‡			
Blood eosinophils	533	0.02 (.699)	0.06 (.135)
Sputum eosinophils†	157	−0.03 (.701)	0.05 (.564)
FE _{NO}	546	0.01 (.897)	0.11 (.010)

WBC, White blood cell.

*Values are Pearson correlations adjusted for site, race, age, and study group with *P* values in parentheses.

†Measurements taken at 4 sites only.

‡Log-transformed data were used for determination of Pearson correlations.

The purpose of relative importance is to quantify the relative contribution of an individual variable to the model's total explanatory value.¹² To control for the study design and possible confounders, we adjusted these analyses for study group, site, race, and age. Assessment of relative importance in multivariate analysis is simple when all variables are uncorrelated. Each variable contribution is just the R^2 from univariate regression, and all univariate R^2 values add up to the full model R^2 . When variables are correlated, the order in which the variables are entered into the model affects their relative contribution. The effects of this ordering can be minimized by averaging sequential sums of squares over all possible orderings of variables, decomposing R^2 into nonnegative contributions. These are computer-intensive methods that have been achievable recently as a result of the advances in computational capabilities. To examine further the relation between the individual variables and FE_{NO}, we combined them into *a priori* specific domains and determined 95% CIs through bootstrap (5000 bootstrap samples) to assess the variability of the relative importance and investigate pairwise differences.

Log-transformations of skewed data (FE_{NO}, methacholine PC₂₀, total IgE, allergen-specific IgE, sum of the 5 allergen-specific IgEs, blood and sputum eosinophils) were used for partial correlations and multivariate analyses. A *P* value <.05 was considered statistically significant. All statistical analyses were performed by using SAS statistical software version 9.1 (SAS Institute Inc, Cary, NC) and the R system for statistical computing version 2.7.0.¹³

The calculation of relative importance was conducted using the R add-on package relaimpo.¹⁴

RESULTS

Participant characteristics at randomization

Of the 546 participants enrolled, 53% were male, and 64% were black. Table I describes additional demographic characteristics of the population as well as asthma-related symptoms, lung function, atopic status, and degree of allergic inflammation at randomization. The mean number of maximum symptom days over a 2-week period decreased significantly from 5.6 ± 4.6 at enrollment to 2.3 ± 2.9 after the 3-week run-in period at randomization (mean within-participant reduction, 3.4 days/2 weeks; *P* < .001). Mean FEV₁ (% of predicted value) at randomization was $95.8\% \pm 15.7\%$. Despite the decrease in maximum symptom days between enrollment and randomization, half of the participants (274 of 546) had FE_{NO} levels ≥ 20 ppb and 75% (401 of 534) had serum IgE levels ≥ 100 kU/L at randomization. Moreover, 26% (40 of 157) had sputum eosinophilia defined as an eosinophil

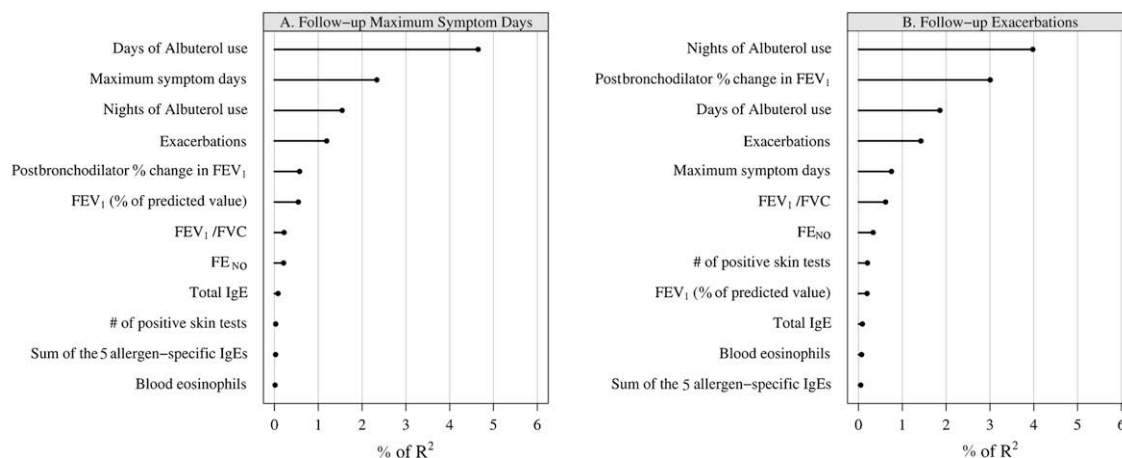


FIG 1. Relative importance of baseline characteristics for predicting maximum symptom days (A) and exacerbations (B) during follow-up (visits 3-8). Combined, these baseline characteristics explain 11.4% and 12.6% of the variation for maximum symptom days and exacerbations, respectively.

percentage of sputum white blood cells $\geq 2\%$.¹⁵ Marked airway hyperresponsiveness also existed, with 62% (89 of 144) having a $PC_{20} < 8$ mg/mL. Finally, the majority of participants had 1 or more positive skin test (88%), with the median number of positive tests being 5 of 14 total tests placed (interquartile range, 2-7). Allergic sensitization was most prevalent to cockroach (61.2%), cat (58.2%), molds (51.6%), and dust mites (46.9%).

In addition to skin testing, allergen-specific IgE levels were measured for 5 allergens (*A alternata*, cat, *D pteronyssinus*, *D farinae*, and German cockroach; Table I). For each of the allergens tested, between 40% and 50% of the participants had allergen-specific IgE levels greater than 0.35 kU_A/L.

Adherence to treatment was 88.6% at randomization and averaged 86.6% during follow-up (visits 3-8). Despite this high level of adherence, 388 of 539 (72%) ACE participants with follow-up data had at least 1 visit with poor asthma control (more than 3 days of symptoms or 1 night of symptoms in 2 weeks or FEV₁ <80% of personal best), of which 137 (25%) had even more severe morbidity (14 days of symptoms or more than 4 nights of symptoms in the last 2 weeks or FEV₁ <70% of personal best).

Importance of different variables (asthma-related symptoms, lung function, allergic biomarkers, and inflammatory biomarkers) in predicting future symptoms and exacerbations

We sought to determine which clinical variables measured at randomization were best associated with future risk of asthma exacerbations and maximum symptom days (both measures of risk were assessed during the follow-up period, ie, study weeks 9-49). As shown in Table II, modest but significant correlations were demonstrated between several asthma-related symptom variables and future maximum symptom days and exacerbations. Maximum symptom days ($r = 0.25$; $P < .001$), days of albuterol use ($r = 0.27$; $P < .001$), and nights of albuterol use ($r = 0.19$; $P < .001$), measured at randomization, all predicted future maximum symptom days. The latter 2 variables (days of albuterol use [$r = 0.13$; $P = .003$] and nights of albuterol use [$r = 0.21$; $P < .001$]) also correlated with future exacerbations, as did postbronchodilator percent change in FEV₁ at enrollment ($r = 0.19$; $P < .001$), FEV₁/FVC ratio ($r = -0.10$; $P = .026$), and FE_{NO} ($r = 0.11$;

$P = .010$) measured at the randomization visit. Neither methacholine sensitivity nor any of the allergic or inflammatory biomarkers (except FE_{NO}, as noted) were associated with maximum symptom days or exacerbations measured during the 46 weeks of follow-up.

When using relative importance measures, asthma symptom variables, especially albuterol use, independently explained most of the variability in future maximum symptom days ($R^2 = 6.2\%$) and exacerbations ($R^2 = 5.8\%$; Fig 1). Postbronchodilator percent change in FEV₁ also was found to play a role in explaining future exacerbations.

Table III presents 3 different models that evaluate the additional contribution of FE_{NO} to various combinations of conventionally measured parameters in predicting future maximum symptom days and asthma exacerbations. For model 1, symptoms, which are a composite of 4 variables and FE_{NO}, were the only predictors considered; for model 2, symptoms, lung function measurements, and FE_{NO} were considered; and for model 3, symptoms, lung function measurements, inflammatory and allergic biomarkers, and FE_{NO} were considered. As shown, asthma-related symptoms alone (days with symptoms, days and nights of albuterol use in the 2 weeks before randomization, and exacerbations in the year before enrollment) explained future maximum symptom days and exacerbations variance significantly ($R^2 = 9.9\%$, $P < .05$, for maximum symptom days; $R^2 = 8.6\%$, $P < .001$, for exacerbations). The addition of lung function measurements to asthma-related symptoms significantly increased the percent of variation explained for future exacerbations (models 2 and 3; $R^2 = 3.3\%$; $P < .001$) but not for maximum symptom days. None of the other variables (inflammatory markers, atopic markers, or FE_{NO}) further contributed to the prediction of either future maximum symptom days or exacerbations.

Correlations between FE_{NO} and asthma-related symptoms, lung function, and allergic and inflammatory biomarkers measured at randomization

We found significant correlations between FE_{NO} and all the parameters evaluated, except for most asthma-related symptom measurements (Table IV). The strongest correlation was between FE_{NO} and PC₂₀ ($r = -0.49$; $P < .001$). Moderate correlations

TABLE III. Percent of variation in follow-up asthma outcomes explained by symptoms, lung function, biomarkers, and FE_{NO} as measured at randomization*

Predictors from randomization§	Follow-up outcomes‡	
	Maximum symptom days	Exacerbations
Model 1 – symptoms + FE _{NO}		
Site, race, age, sex, and study group	10.8	2.2
... + Symptoms	20.7†	10.8†
... + FE _{NO}	20.9	11.4
Model 2 – symptoms and lung function + FE _{NO}		
Site, race, age, sex, and study group	10.8	2.2
... + Symptoms	20.7†	10.8†
... + Lung function	21.7	14.1†
... + FE _{NO}	22.1	14.2
Model 3 – symptoms, lung function, atopy and inflammation + FE _{NO}		
Site, race, age, sex, and study group	10.8	2.2
... + Symptoms	20.7†	10.8†
... + Lung function	21.7	14.1†
... + Inflammation	21.7	14.3
... + Atopy	21.8	14.7
... + FE _{NO}	22.2	14.8

*The order that variables were entered into the model was set *a priori* on the basis of ease and cost of clinical measurements. Values are percent of variation explained (R^2).

†Variable improves R^2 ($P < .05$).

‡Maximum symptom days is the mean of all postrandomization measurements. Exacerbations are a combined measure of the number of asthma-related hospitalizations, unscheduled clinic visits, and prednisone bursts during follow-up.

§Symptoms include 4 variables: days with symptoms, days and nights of albuterol use in the 2 weeks before randomization, and exacerbations in the year before recruitment. Lung function includes 3 variables: FEV₁% predicted and FEV₁/FVC measured at randomization plus postbronchodilator percent change in FEV₁ measured at enrollment. Inflammation represents blood eosinophils. Atopy includes 2 variables: number of positive skin tests and total IgE.

were seen between FE_{NO} and other lung function parameters (postbronchodilator percent change in FEV₁, $r = 0.31$, $P < .001$; FEV₁% predicted, $r = -0.16$, $P < .001$; FEV₁/FVC, $r = -0.29$, $P < .001$) as well as with allergic biomarkers (total IgE, $r = 0.37$, $P < .001$; sum of the 5 allergen-specific IgEs, $r = 0.35$, $P < .001$) and inflammatory biomarkers (blood eosinophils, $r = 0.39$, $P < .001$; sputum eosinophils, $r = 0.38$, $P < .001$). Among the symptom variables, maximum symptom days had a small but significant correlation ($r = 0.09$; $P = .044$) with FE_{NO}, but other variables such as school days missed and days and nights of albuterol use showed no significant correlation.

Relative importance of different parameters for determining FE_{NO} at randomization

Using multivariate analyses, we focused on the relative importance of various parameters in determining FE_{NO}. As shown in Fig 2, A, PC₂₀ ($R^2 = 13.8\%$) and FEV₁/FVC ratio ($R^2 = 8.5\%$) explained most of the variability of FE_{NO}, followed by the number of positive skin tests and sputum/blood eosinophils. All parameters considered together accounted for 50.3% of the FE_{NO} variance. In Fig 2, B, the various parameters were grouped into specific domains: lung function (methacholine PC₂₀, FEV₁/FVC, FEV₁% predicted, and postbronchodilator percent change in FEV₁), inflammation (blood and sputum eosinophils), atopy (number of positive skin tests, total IgE, and sum of the 5 allergen-specific IgEs), and symptoms (maximum symptom days, days of albuterol use, nights of albuterol use, and exacerbations). Once again, the lung function ($R^2 = 26.0\%$) domain explained most of the FE_{NO} variability, followed by the inflammation ($R^2 = 12.7\%$) and atopy ($R^2 = 8.5\%$) domains. There was minimal relationship between FE_{NO} and the asthma symptom domain.

DISCUSSION

Our study reveals that factors often used to predict future asthma risk in poorly controlled populations are of no clinical benefit in predicting future risk in a well treated, highly adherent population of inner-city adolescents and young adults with persistent asthma. We did find that future maximum symptom days and exacerbations could be predicted, but only to a minor extent, by using a combination of asthma-related symptoms and lung function measurements that included FEV₁% predicted, FEV₁/FVC, and postbronchodilator percent change in FEV₁. However, these predictors explained only approximately 12.6% and 11.4% of the variance (Fig 1) for exacerbations and maximum symptom days, respectively. Moreover, the independent contributions of degree of inflammation (as measured by FE_{NO}, sputum and blood eosinophils), and degree of atopy, all measured at randomization, were minimal.

The literature suggests that an increased risk of symptoms and exacerbations may be predicted by numerous factors including recent asthma exacerbations,¹⁶ poor asthma control,^{17,18} severe airway obstruction,^{19,20} history of intensive care admissions or frequent emergency department visits,²¹ elevated FE_{NO} levels,^{20,22} allergen sensitivity and exposure,²³⁻²⁵ depression,²⁶ and poor attitudes about the use of asthma medicines.²⁷ Moreover, numerous studies have examined baseline predictors of treatment response and loss of asthma control. Such predictor variables examined in these studies have included baseline levels of serum IgE and eosinophil cationic protein, FE_{NO}, PC₂₀, pulmonary function measurements, and asthma symptoms. Sputum eosinophils too have been examined in longitudinal studies and have been found to be useful as a predictor of asthma deterioration after inhaled corticosteroid reduction.²⁸⁻³²

Indeed, although predictors of future asthma symptoms and exacerbations have been extensively studied, no single study has

TABLE IV. Pearson correlations of participant characteristics with FE_{NO} at randomization*

	N	FE _{NO} †
Asthma-related symptoms at randomization (no. of days/last 2 wks)		
Maximum symptom days	546	0.09 (.044)
Wheezing, cough, tightness in the chest		0.08 (.055)
Slow down or stop play or activities		0.04 (.316)
Nights awake because of asthma		0.05 (.238)
School days missed	381	0.03 (.581)
Days of albuterol use	546	0.07 (.092)
Nights of albuterol use	546	0.07 (.094)
Lung function		
FEV ₁ (% of predicted value)	532	−0.16 (<.001)
FEV ₁ /FVC	532	−0.29 (<.001)
Postbronchodilator percent change in FEV ₁ (visit 1)	521	0.31 (<.001)
Methacholine PC ₂₀ ‡	144	−0.49 (<.001)
Allergic biomarkers		
Total IgE‡	534	0.37 (<.001)
Allergen-specific IgE‡		
<i>Alternaria</i>	533	0.20 (<.001)
Cat	533	0.23 (<.001)
Dust mite, <i>D pteronyssinus</i>	534	0.23 (<.001)
Dust mite, <i>D farinae</i>	533	0.21 (<.001)
German cockroach	534	0.12 (.006)
Sum of the 5 allergen-specific IgEs	534	0.35 (<.001)
Number of positive skin tests	531	0.29 (<.001)
Skin test wheal size (mm)	531	
<i>Alternaria</i>		0.15 (.001)
Cat		0.18 (<.001)
Dust mite, <i>D pteronyssinus</i>		0.10 (.018)
Dust mite, <i>D farinae</i>		0.13 (.002)
German cockroach		0.14 (.002)
Mouse		0.11 (.015)
Rat		0.12 (.005)
Dog		0.18 (<.001)
Inflammatory biomarkers‡		
Blood eosinophils	533	0.39 (<.001)
Sputum eosinophils†	157	0.38 (<.001)

*Values are Pearson correlations adjusted for site, race, age, and study group with *P* values in parentheses.

†Measurements taken at 4 sites only.

‡Log-transformed data were used for determination of Pearson correlations.

evaluated all of these potential predictive factors together, and none has examined these factors in a well-treated, highly adherent population. Thus, a unique attribute of our work is that, unlike previous studies, an attempt was made to identify predictors of future asthma risk in a highly adherent population of inner-city adolescents and young adults who were receiving optimal care (ie, guidelines-based medical management) for the entire 46 weeks of follow-up. Interestingly, none of 4 major asthma-related factors (symptoms, lung function measurements, allergic biomarkers, and inflammatory biomarkers) measured at baseline were useful in predicting future risk of disease as measured by future asthma symptoms and exacerbations. It must be pointed out, however, that our inability to identify useful predictors was not a result of resolution of disease, because we found that disease activity was not completely eliminated.

Another unique aspect of our study was the type of analyses performed. Like Pharoah et al³³ in analyzing breast cancer outcomes, another polygenic disease, we applied a model that looked at predictor variables in combination, as opposed to applying a

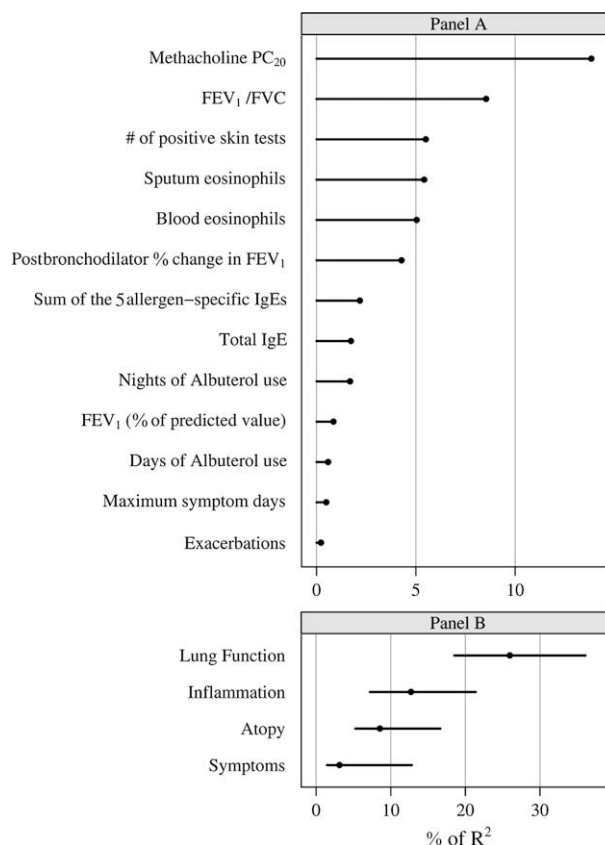


FIG 2. Relative importance of baseline characteristics for predicting baseline FE_{NO}. **A**, Individual variables. **B**, Variables combined into specific domains with 95% CI. Domains are defined as lung function (methacholine PC₂₀, FEV₁/FVC, FEV₁% predicted, and postbronchodilator percent change in FEV₁), inflammation (blood and sputum eosinophils), atopy (number of positive skin tests, total IgE, and sum of the 5 allergen-specific IgEs), and symptoms (maximum symptom days, days of albuterol use, nights of albuterol use, and exacerbations). Combined, these baseline characteristics explain 50.3% of the variation in FE_{NO}.

model that evaluated simple univariate correlations only. In applying this type of model, Pharoah et al³³ found that 7 established common breast cancer susceptibility alleles in combination explained only 5% of the genetic risk for this disease. In comparison, the factors we examined accounted for a little more than 10% of the variance in maximum symptom days and exacerbations (11.4% and 12.6%, respectively). Importantly, we also showed that complex measurements such as methacholine sensitivity and sputum eosinophils, along with IgE measurements and blood eosinophils, did not explain any of the remaining variance of these outcomes.

In our analyses, we found that baseline FE_{NO} levels at randomization were related to future asthma exacerbations (Table II). However, this relationship did not hold up in our multivariate models (Fig 1; Table III). Thus, while baseline FE_{NO} levels were found to be correlated with a number of inflammatory or lung function measurements, this biomarker was not a good predictor of future asthma risk, as defined by future maximum symptom days and asthma exacerbations. Others too have demonstrated relationships between FE_{NO} and allergen sensitivity,^{9,34} methacholine PC₂₀,^{9,34,35} and blood and/or sputum eosinophils.^{9,34-40} In the few studies that have found relationships between FE_{NO} levels and future exacerbations,^{20,22,41,42} the study designs and/or participant populations were quite different from

those of our study. In particular, our study population had very high adherence to a guidelines-based treatment algorithm.

Previous inner-city studies have demonstrated that cockroach sensitivity and exposure are associated with increased asthma-associated morbidity.^{23,24} In the current study, there was no relationship between atopy and future asthma risk, and allergen exposure was not considered in the analysis. Because the purpose of the current study was to identify predictive markers that could be readily measured by the practicing physician, allergen exposure was not analyzed.

In conclusion, we found that when well-treated, adherent populations of patients with persistent asthma are evaluated for determinants of future disease risk, asthma-related symptoms and lung function measurements are only somewhat predictive of future maximum symptoms days and exacerbations. Furthermore, more complex baseline measurements, such as FE_{NO} levels, inflammatory markers, and markers of atopy, are not predictive of future disease risk. These findings highlight the need to identify better clinical predictors, perhaps including indices that are independent of inflammation, for asthma morbidity in treated populations.

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Clinical implications: Markers of allergic sensitization, airway hyperresponsiveness, and airway inflammation do not predict future asthma exacerbations in inner-city adolescents who are receiving guidelines-based therapy and who are adherent to their treatment regimens.

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METHODS

Skin tests

The following allergen extracts were used for skin testing: mouse epithelia, dog epithelia, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, cat hair, rat epithelia, American and German cockroach mix, German cockroach, *Alternaria tenuis*, *Cladosporium herbarium*, *Aspergillus* mix, *Penicillium notatum*, ragweed mix, and timothy grass. All extracts were 1:20 (wt/vol) except for *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, timothy grass, and cat, which were standardized extracts of 10,000 Biologic Allergy Units per milliliter. The resulting wheals were measured after 15 minutes. Wheal sizes were calculated as the average of the longest diameter and its orthogonal midpoint diameter. Skin tests were considered valid if the wheal size of the negative control was 3 mm or smaller and the wheal size of the positive control (histamine) was at least 3 mm larger than the wheal size of the negative control. A skin test response was considered to be positive if the wheal size for the allergen was at least 3 mm larger than that for the negative control. For those analyses treating skin test wheal sizes as continuous data, the wheal size of the negative control was subtracted from the wheal size of each specific allergen.

Sputum induction and processing

Sputum was induced by inhalation of hypertonic saline solution (3%) by using a DeVilbiss Ultra-Sonic Pico #3207 nebulizer (Nouvag, Lake Hughes, Calif). Participants with prealbuterol FEV₁ $\geq$ 70% of predicted (206 of 213, 97%; 1 of 206 refused) underwent sputum induction after pretreatment with

albuterol. A 12-minute sputum induction was performed, during which peak flow was monitored every 2 minutes with a Mini-Wright Standard peak flow meter (Alliance Tech Medical, Inc, Rockdale, Tex). Slides were prepared and stained at the local laboratories. Differential cell counts were performed centrally by a blinded technician. A minimum of 400 cells was counted. Sputum samples with volume <0.3 mL (21 of 205; 10%) were not processed. Slides with $>80\%$ squamous cells, mucus plugs, or poor distribution of cells were excluded (27 of 184; 15%).

Methacholine challenge

Airway responsiveness was measured by determining the concentration of methacholine required to produce a drop in FEV₁ of 20% compared with a control (postdiluent) level (PC₂₀) after the administration of increasing concentrations of methacholine by using the small volume nebulizer-tidal breathing technique. In brief, the participant performed tidal breathing for 2 minutes while inhaling from a nebulizer. After the 2-minute breathing exposure, spirometry was performed using a Jaeger Masterscreen (VIASYS Healthcare GmbH, Hoechberg, Germany). The procedure was repeated with increasing concentrations of methacholine until there was a 20% drop in FEV₁ or the maximum concentration was administered. The following concentrations of methacholine were tested: 0 (diluent only), 0.098, 0.195, 0.391, 0.781, 1.563, 3.125, 6.25, 12.5, and 25.0 mg/mL. The PC₂₀ was calculated by the Jaeger software by linear interpolation with log₁₀ transformation between the last 2 concentrations administered.