

Biomarker surrogates do not accurately predict sputum eosinophil and neutrophil percentages in asthmatic subjects

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Background: Sputum eosinophil percentages are a strong predictor of airway inflammation and exacerbations and aid asthma management, whereas sputum neutrophil percentages indicate a different severe asthma phenotype that is potentially less responsive to T_H2 -targeted therapy. Variables, such as blood eosinophil counts, total IgE levels, fraction of exhaled nitric oxide (FENO) levels, or FEV₁ percent predicted, might predict airway eosinophil percentages, whereas age, FEV₁ percent predicted, or blood neutrophil counts might predict sputum neutrophil percentages. Availability and ease of measurement are useful characteristics, but accuracy in predicting airway eosinophil and neutrophil percentages either individually or combined is not established.

Objectives: We sought to determine whether blood eosinophil counts, FENO levels, and IgE levels accurately predict sputum eosinophil percentages and whether age, FEV₁ percent predicted, and blood neutrophil counts accurately predict sputum neutrophil percentages.

Methods: Subjects in the Wake Forest Severe Asthma Research Program (n = 328) were characterized by blood and sputum cell counts, health care use, lung function, FENO levels, and IgE levels. Multiple analytic techniques were used.

Results: Despite significant association with sputum eosinophil percentages, blood eosinophil counts, FENO levels, and total IgE levels did not accurately predict sputum eosinophil percentages, and combinations of these variables did not improve prediction. Age, FEV₁ percent predicted, and blood neutrophil counts were similarly unsatisfactory for the prediction of sputum neutrophil percentages. Factor analysis and stepwise selection found FENO levels, IgE levels, and FEV₁ percent predicted, but not blood eosinophil counts, correctly predicted 69% of sputum eosinophil percentages of less than 2% or 2% and greater. Likewise, age,

asthma duration, and blood neutrophil counts correctly predicted 64% of sputum neutrophil percentages of less than 40% or 40% and greater. A model to predict both sputum eosinophil and neutrophil percentages accurately assigned only 41% of samples.

Conclusion: Despite statistically significant associations, FENO levels, IgE levels, blood eosinophil and neutrophil counts, FEV₁ percent predicted, and age are poor surrogates, both separately and combined, for accurately predicting sputum eosinophil and neutrophil percentages. (J Allergy Clin Immunol 2013;■■■:■■■-■■■.)

Key words: Asthma phenotypes, sputum eosinophils and neutrophils, inflammatory biomarker surrogates, T_H2 biomarkers, fraction of exhaled nitric oxide

All levels of asthma severity show evidence of airway inflammation, with both eosinophils and neutrophils contributing to this pathology.¹⁻⁵ We recently reported that asthmatic patients with both increased sputum eosinophil and neutrophil percentages had the lowest lung function, worse asthma control, and increased symptoms and health care requirements.⁶ Although induced sputum eosinophilic or neutrophilic phenotypes have been shown to predict asthma exacerbations⁷⁻⁹ and responsiveness to corticosteroids or corticosteroid reduction with anti-IL-5 therapy,^{3,10-12} sputum induction and processing is technically difficult and not readily available in most clinical settings. Clearly, readily accessible biomarkers that accurately predict airway inflammation are needed for managing difficult-to-treat asthma and to serve as response end points in clinical trials testing and registering new biologic therapies for asthma. Thus more accessible biomarkers are used as surrogates for sputum inflammatory cell measurements in clinical studies without first establishing their accuracy across the full range of asthma or severity subphenotypes.^{13,14}

Eosinophils are a primary cellular component in studies that focus on airway inflammation in asthma. Fraction of exhaled nitric oxide (FENO), blood eosinophil numbers, FEV₁ percent predicted, and IgE levels have been suggested as potential predictive biomarkers for sputum eosinophil or bronchial mucosal eosinophil numbers.¹³⁻²⁰ However, other studies raise questions regarding the accuracy of these biomarker associations with sputum eosinophil percentages^{5,21-23} and their clinical usefulness in predicting asthma exacerbations.²⁴ The association of FENO levels with sputum eosinophil percentages has been more widely examined, but cut points to predict sputum eosinophil percentages vary extensively and are confounded by exposure to tobacco smoke and inhaled corticosteroid (ICS) therapy.^{16,18,20,25} Moreover, some studies have reported small numbers of subjects or did not evaluate subjects across the entire spectrum of asthma severity, restricting their clinical predictive value.

Fewer asthma studies have focused on sputum neutrophil percentages,²⁶⁻²⁸ although this granulocyte is implicated in patients

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Supported by the National Heart, Lung, and Blood Institute Severe Asthma Research Program Awards HL69167, U10HL109164, and RC2HL101487.

Disclosure of potential conflict of interest: A. T. Hastie, W. C. Moore, B. M. Rector, and D. A. Meyers have received grants from the National Heart, Lung, and Blood Institute (NHLBI). R. M. Pascual has consultant arrangements with United Therapeutics and has received grants from Actelion. S. P. Peters has received a grant and travel support from the National Institutes of Health (NIH)/NHLBI; has served on advisory boards for AstraZeneca, Aerocrine, Merck, Targacept, and TEVA; has received payment for lectures from Merck and Integrity CE; and has received royalties from UpToDate. E. R. Bleeker has received grants and travel support from the NIH/NHLBI. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication December 7, 2012; revised March 5, 2013; accepted for publication March 13, 2013.

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0091-6749/\$36.00

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<http://dx.doi.org/10.1016/j.jaci.2013.03.044>

Abbreviations used

AUC: Area under the curve
 FENO: Fraction of exhaled nitric oxide
 ICS: Inhaled corticosteroid
 ROC: Receiver operating characteristic
 SARP: Severe Asthma Research Program

with severe asthma²⁹⁻³² and associated with reduced FEV₁,²⁶ irreversible airflow obstruction,³² and loss of control by rapid withdrawal of ICSs.¹¹ Increased neutrophil numbers, reduced FEV₁ levels and irreversible airflow obstruction are generally thought to characterize chronic obstructive pulmonary disease, but increasing evidence has identified these characteristics in subgroups of patients with severe asthma.^{26,32,33} Moreover, synergy between airway sensitization, IL-17-induced neutrophil numbers, and ongoing T_H2 responses supports a causal role for neutrophils in airway hyperresponsiveness.^{34,35} A recent report suggested that asthmatic patients can be stratified based on both neutrophil and eosinophil counts in blood.³⁶ Thus previous studies and our report⁶ support the importance and need to assess both sputum eosinophil and neutrophil percentages associated with abnormalities in lung function, exacerbations, and more severe asthma.

Because of inconsistencies and lack of information for potential surrogate markers for both sputum eosinophil and neutrophil percentages, we analyzed the accuracy of biomarkers that might be associated with sputum eosinophils and neutrophils, individually and in combination, to predict sputum granulocytic inflammation in a large, comprehensively characterized cohort of subjects with a broad spectrum of asthma severities. Our hypotheses were that variables associated with T_H2 inflammation, FENO levels, IgE levels, and blood eosinophil counts should accurately predict sputum eosinophil percentages, whereas age, reduced FEV₁ levels, and blood neutrophil counts should accurately predict sputum neutrophil percentages. We also examined more complex models to predict both sputum eosinophil and neutrophil percentages. The comprehensive approach of considering both eosinophilic and neutrophilic inflammation in the context of other biomarkers is a novel approach with important implications for understanding interrelationships among these biomarkers.

METHODS

Subjects with mild-to-severe asthma were comprehensively characterized according to the Severe Asthma Research Program (SARP) phenotype protocol at Wake Forest School of Medicine, as previously described.³⁷ Briefly, nonsmoking subjects (<5 pack years) who met American Thoracic Society criteria for the diagnosis of asthma (enriched for severe asthma) provided informed consent (Wake Forest Institutional Review Board–approved protocol, BG01-425). Evaluation included spirometry, bronchodilator reversibility and bronchial responsiveness to methacholine (bronchial hyperresponsiveness), allergen skin prick tests, total serum IgE measurement, blood collection, exhaled nitric oxide measurement, sputum induction,⁶ and administration of questionnaires that characterized asthma symptoms, quality of life, medications, and health care use.^{33,37}

Subjects

SARP subjects had severe asthma ($n = 71$), as defined by the American Thoracic Society Workshop on Refractory Asthma,³⁸ or nonsevere asthma ($n = 257$; ie, asthmatic patients not meeting severe asthma criteria and classified as having mild asthma with FEV₁ of $\geq 80\%$ of predicted value or moderate

asthma with FEV₁ of <80% of predicted value).^{33,37} A portion of these subjects were studied in our report of subject stratification by sputum granulocyte and inflammatory protein levels (previous data overlap with current data shown in Tables E1 and E2 in this article's Online Repository at www.jacionline.org).⁶

Demographics and clinical characteristics for all Wake Forest subjects with sputum samples matched those previously reported for subjects from all SARP sites (Table I).³⁷

Sputum induction and processing

The sputum induction method was adopted from the National Heart, Lung, and Blood Institute's Asthma Clinical Research Network.³⁹ Sputum was processed immediately, and cell cytospin preparations were stained for differential counts. The sputum cell differential counts were adequate for further analyses (ie, <80% squamous^{6,39}) from 254 of the 328 subjects with sputum samples. Previous studies have considered sputum eosinophil levels of 1% to 3% as representing an eosinophilic phenotype^{3,9,40,41} and sputum neutrophil levels as high as 61% as a neutrophilic phenotype.^{3,5,40} Many factors contribute to variable neutrophil percentages, including age, air pollution, environmental endotoxin levels, different induction procedures, and exercise.^{42,43} In this and our previous study,⁶ we used sputum eosinophil counts of 2% and greater and neutrophil counts of 40% and greater as cut points because our cohort of subjects with severe asthma⁶ had median values of 1.7% eosinophils and 39% neutrophils, which correspond to reports for asthmatic subjects of similar age and smoke exposure (additional Methods and Results are provided in this article's Online Repository at www.jacionline.org).^{18,26,41}

Statistics

Demographic and biomarker data are presented as means $\pm$ SDs or medians (25% to 75% quartiles) for continuous variables and as percent positive values for categorical variables. Measures not meeting the Kolmogorov-Smirnov test for normal distribution were transformed to log or square root values. A zero in cell differentials was replaced with a value half of the lowest value observed before log transformation.¹⁶ Continuous variables were tested by using parametric or nonparametric tests with SAS 9.2 (SAS Institute, Cary, NC) or Sigmasat 3.1 (SyStat Software, San Jose, Calif) software. Analyses with a significant difference were further explored by using *post hoc* pairwise tests (Tukey). Categorical variables were analyzed with χ^2 tests.

Initial analyses considered potential T_H2 biomarker variables reported for association with sputum eosinophil counts (ie, FENO levels, IgE levels, and blood eosinophil counts)¹³⁻²⁰ and variables reported for association with sputum neutrophil counts (ie, age, FEV₁ percent predicted, and blood neutrophil counts).^{26,27,32} Receiver operating characteristic (ROC) curve analyses (sensitivity, specificity, area under the curve [AUC], accuracy, and positive predictive value) tested individual or combined biomarker prediction of sputum eosinophil or neutrophil percentages. ROC analyses provide information to determine optimal models for potential biomarker predictors as binary classifiers, comparing the true-positive rate with the false-positive rate. An ROC AUC of 0.5 indicates inability of a biomarker to predict a subject's correct classification. Factor analysis with 19 or 20 variables (see the Results section in the Online Repository) confirmed those variables associated with sputum eosinophil or neutrophil percentages. These variables were included in stepwise selection and discriminant analysis for prediction of combined sputum eosinophil counts of less than 2% or 2% and greater and sputum neutrophil counts of less than 40% or 40% and greater or each granulocyte separately. Variables with a *P* value of less than .05 were accepted as significant.

RESULTS**Subjects' demographics**

There were significant differences in lung function, bronchodilator reversibility, symptoms, and health care use for subjects with severe asthma compared with those with mild and moderate asthma (Table I). Age and duration of asthma were increased in subjects with more severe asthma, but there were no differences in atopic measures, including total serum IgE levels, frequency

TABLE I. Subjects' demographics*

	Mild asthma (n = 134)	Moderate asthma (n = 123)	Severe asthma (n = 71)	P value
Sex (% female)	80	64	58	.001
Age (y)	33 ± 11	39 ± 14	45 ± 12	<.0001
Age at onset (y)	14 ± 13	17 ± 16	20 ± 17	.20
Duration (y)	18 ± 12	23 ± 14	25 ± 17	.015
Baseline FEV ₁ (% predicted)	93 ± 9	66 ± 12	66 ± 23	<.0001
Baseline FVC (% predicted)	100 ± 10	79 ± 14	77 ± 20	<.0001
FEV ₁ /FVC ratio	0.79 ± 0.06	0.69 ± 0.1	0.67 ± 0.12	<.0001
Maximum FEV ₁ (% predicted)	103 ± 8	81 ± 12	78 ± 21	<.0001
FEV ₁ (% reversibility to 2 puffs β-agonist)	12 ± 11	23 ± 22	19 ± 17	<.0001
Log PC ₂₀ (geometric mean)	1.32 (n = 128) [†]	0.79 (n = 98) [†]	0.87 (n = 42) [†]	.047
FENO (ppb)	42 ± 48	38 ± 33	36 ± 38	.43
No. of positive skin test results	4.1 ± 2.8	4.0 ± 2.9	3.7 ± 3.4	.37
Blood eosinophil count (K/μL)	0.24 ± 0.19	0.27 ± 0.2	0.26 ± 0.19	.52
Blood neutrophil counts (K/μL)	3.7 ± 0.002	3.6 ± 0.001	4.1 ± 0.001	.10
IgE (IU/mL), geometric mean	115	107	100	.9
Frequency of wheezing [‡]	65/17/18	51/23/26	51/17/32	.080
Frequency of nighttime symptoms [‡]	68/16/16	64/13/23	49/20/31	.055
Health care use in past year (%)				
≥1 Urgent health care visit for asthma	30	32	51	.009
Emergency department visits	15	18	39	<.0001
Hospitalized for asthma	2	6	17	<.0001
Health care use ever (%)				
Daily β-agonist use	30	40	49	.019
Symptoms worse if corticosteroid reduced	36	36	86	<.0001
Emergency department visits	56	66	77	.01
Hospitalized for asthma	27	37	56	<.0001
Hospitalized in ICU	6	11	23	.002

ICU, Intensive care unit.

*Data are presented as means ± SDs, medians (25% to 75% quartiles), or percentage positive response. P values meeting significance are shown in boldface.

[†]Subjects with FEV₁ of less than 50% of predicted value did not undergo methacholine challenge.[‡]Three categories, left to right, indicate percentage of positive response for "never to less than 2 times/week," "more than 2 times/week but less than 1 time/day," and "daily."

of positive skin test results, number of positive skin test results, serum eosinophil counts, or FENO levels.

Subjects with mild, moderate, or severe asthma did not have substantial differences in sputum cell counts (Table II). However, as previously demonstrated,⁶ subjects stratified by combined sputum eosinophil counts of less than 2% or 2% and greater and neutrophil counts of less than 40% or 40% and greater had significant differences in lung function, atopic measures, and health care use (see Table E1). Analyses for contribution by combined eosinophils and neutrophils or each granulocyte individually to the observed differences were assessed (see Table E2). The results confirm that sputum eosinophil and neutrophil percentages, both combined and individually, were significantly associated with asthma severity characteristics, including reduced lung function and increased health care use (in contrast to stratification by blood granulocytes; see Tables E3 and E4 in this article's Online Repository at www.jacionline.org).

Distribution of sputum granulocytes by potential biomarker predictors

Sputum eosinophil percentages were significantly associated with blood eosinophil counts ($R = 0.191$, $P = .002$) and FENO levels ($R = 0.321$, $P < .001$; Fig 1) but were widespread across the range of blood eosinophil counts and FENO levels, and the R

values were low (<0.5), although significant. Subjects with mild, moderate, or severe asthma were distributed throughout, agreeing with the lack of difference in sputum eosinophil percentages among these asthma severity groups (Table II). Youden index cut points for blood eosinophil counts (300/μL) and FENO levels (30 ppb; maximum sensitivity and specificity derived from ROC curve analyses in the section below) were graphed with the sputum eosinophil percentage cut points of less than 2% or 2% and greater. Despite significant positive associations between these biomarkers, many subjects with sputum eosinophil counts of 2% and greater would be misclassified by criteria requiring blood eosinophil counts of 300/μL and greater (false-negative rate, 41%) or FENO levels of 30 ppb and greater (false-negative rate, 35%), and likewise, many subjects with sputum eosinophil counts of less than 2% would also be misclassified based on the same blood eosinophil count and FENO level criteria (false positive rate, 35% and 33%, respectively). Therefore both subjects with higher or lower sputum eosinophil percentages would be misclassified by the surrogate biomarkers of blood eosinophil counts or FENO levels.

Similarly, sputum neutrophil percentages (Fig 2) were positively associated with age ($R = 0.40$, $P < .001$) and negatively associated with baseline prebronchodilator FEV₁ percent predicted ($R = 0.16$, $P = .013$) but were broadly spread across age and FEV₁ percent predicted ranges, and the R values were low (<0.5), although

TABLE II. Sputum differential cell counts for subjects stratified by asthma severity*

	Mild asthma (n = 105)	Moderate asthma (n = 96)	Severe asthma (n = 53)	P value
Total cell count ($\times 10^6/\text{mL}$)	1.96 (1.2-3.4)	2.3 (1.4-3.6)	2.1 (1.3-4.7)	.28
WBC count ($\times 10^6/\text{mL}$)	1.05 (0.50-2.15)	1.25 (0.65-2.46)	1.47 (0.55-2.75)	.32
WBC (%)	60 (38-83)	70 (41-81)	63 (46-82)	.95
Bronchial epithelium (%)	2 (1-4.3)	4 (1-9)	3 (1-8.3)	.02
Squamous epithelium (%)	37 (14-59)	26 (14-49)	26 (13-49)	.42
Macrophages (%)	57 $\pm$ 2.6	50 $\pm$ 2.6	46 $\pm$ 3.7	.028
Lymphocytes (%)	1.4 (0.58-2.80)	1.3 (0.60-2.90)	1.5 (0.40-2.85)	1.0
Neutrophils (%)	33.7 (13-59)	39 (22-63)	47 (23-68)	.057
Eosinophils (%)	0.7 (0.2-3.3)	1.5 (0.4-4.8)	1.9 (0.3-5)	.10

*Data are presented as medians (25% to 75% quartiles). P values meeting significance are shown in boldface. WBC, White blood cell.

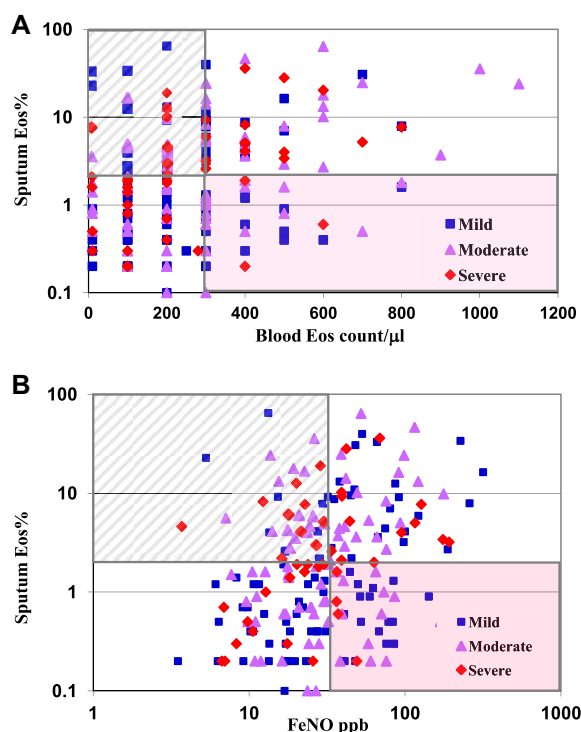


FIG 1. Subject distribution by sputum eosinophil percentages and blood eosinophil counts per microliter (A) or FeNO levels (in ppb; B) for subjects with mild, moderate, and severe asthma. Sputum eosinophil percentages of greater than 2% and blood eosinophil counts of less than 300/ μL (Fig 1, A) or sputum eosinophil percentages of greater than 2% and FeNO levels of less than 30 ppb (Fig 1, B) are false-negative predictions (indicated by crosshatched areas). Sputum eosinophil percentages of less than 2% and blood eosinophil counts of greater than 300/ μL (Fig 1, A) or sputum eosinophil percentages of less than 2% and FeNO levels of greater than 30 ppb (Fig 1, B) are false-positive predictions (indicated by shaded areas).

significant. Subjects with mild, moderate, or severe asthma were distributed throughout the age range, but only subjects with severe asthma were spread across the FEV₁ percent predicted range (mild and moderate asthma were classified by baseline FEV₁ of $\geq 80\%$ and $<80\%$ of predicted value, respectively). Youden index cut points for age (40 years) and FEV₁ percent predicted (90%) were graphed with the neutrophil percentage cut points of less than 40% or 40% and greater. As observed for sputum eosinophil counts above, many subjects with sputum neutrophil counts of 40% and greater would be misclassified as having counts of less than 40% (false-negative rate, 41% for age and 45% for FEV₁ percent predicted), whereas others with actual sputum neutrophil percentage

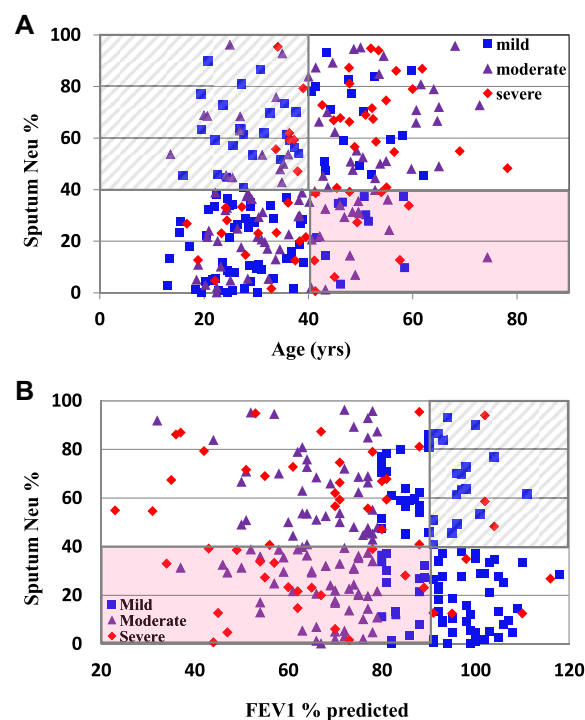


FIG 2. Subject distribution by sputum neutrophil percentages of less than 40% or 40% and greater and age (A) and FEV₁ percent predicted (B) for subjects with mild, moderate, and severe asthma. Sputum neutrophil percentages of 40% and greater are indicated by crosshatched areas for false-negative prediction by age less than 40 years and by FEV₁ percent predicted greater than 90%. Sputum neutrophil percentages of less than 40% are indicated by shaded areas for false-positive prediction by age greater than 40 years and FEV₁ percent predicted less than 90%.

of less than 40% would be misclassified as having percentages of 40% and greater (false-positive rate, 27% for age and 38% for FEV₁ percent predicted at respective Youden cut points). As observed with sputum eosinophil percentages, both subjects with higher or lower sputum neutrophil percentages would be misclassified by the surrogate biomarkers of age and FEV₁ percent predicted.

ROC curves for predicting sputum eosinophil counts of less than 2% or 2% and greater and predicting sputum neutrophil counts of less than 40% or 40% and greater

ROC analyses to predict sputum eosinophil and neutrophil percentages were performed to determine optimum cut points,

accuracy, and positive predictive values. The ROC curves for blood eosinophil counts, FENO levels, or IgE levels for predicting sputum eosinophil percentages of less than 2% or 2% and greater and for age, FEV₁ percent predicted, and blood neutrophil counts for predicting sputum neutrophil percentages of less than 40% or 40% and greater are presented in Fig 3, respectively. Although statistically significant, the AUC shows fair-to-poor accuracy and positive predictive values (summarized in Table III).

Additional ROC analyses were performed to determine whether alternative criteria improved prediction of sputum eosinophil counts, such as to predict sputum eosinophil counts of less than 3% or 3% and greater or to predict eosinophil percentages of less than 2% or 2% and greater in subjects with severe asthma only (a group only receiving high-dose inhaled or oral corticosteroids) or only in subjects without ICS treatment to examine potential confounding by corticosteroid exposure. The results for each of these were nearly identical to ROC curves for predicting sputum eosinophil percentages of less than 2% or 2% and greater, with minimal or no improvement in sensitivity, specificity, and accuracy (Table III and see Figs E1-E3 in this article's Online Repository at www.jacionline.org).

Combinations of variables, as previously suggested¹⁴ and used,^{13,41} were examined for improved prediction of sputum eosinophil percentages. The ROC curve of blood eosinophil counts in combination with a FENO level of 30 ppb and greater or with IgE levels of 100 IU/mL and greater showed no enhancement in AUCs or accuracy for combined variables over blood eosinophil counts alone (Table III), nor was the combination of FENO levels, either with blood eosinophil counts of 300/ μ L and greater or IgE levels of 100 IU/mL and greater, superior to FENO levels alone (Table III).

Likewise, combining lower reversibility (<15%) with FEV₁ percent predicted to examine whether a characteristic of more fixed airflow obstruction better predicted sputum neutrophil counts of less than 40% or 40% and greater did not provide any improvement (Table III).

Factor analysis variables associated with sputum eosinophil and neutrophil counts

To determine whether other variables might better predict sputum eosinophil and neutrophil percentages, factor analysis was performed for 19 variables, including sex, age, asthma duration, race, lung function (baseline prebronchodilator FEV₁ percent predicted, FVC percent predicted, and FEV₁/FVC ratio), T_H2-associated variables (FENO and IgE levels), and measures of exacerbations (emergency department visits, hospitalizations, and intensive care unit admission, see Table E5). A separate factor analysis including corticosteroid use (20 variables) did not differ, nor was corticosteroid use associated with either sputum eosinophil or neutrophil percentages. Similarly, adding maximum FEV₁ percentage reversibility to the factor analysis did not alter those variables associated with either sputum eosinophil or neutrophil percentages.

Sputum eosinophil percentages showed associations with FENO and blood eosinophil counts, whereas sputum neutrophil percentages showed associations with age and asthma duration. These 4 variables and 1 to 2 variables from each factor group (body mass index, sex, race, IgE level, blood neutrophil count, and baseline FEV₁ percent predicted) were included in stepwise discriminant analysis to predict combined sputum eosinophil and neutrophil percentages. FENO levels ($P < .001$), age ($P < .001$), and blood neutrophil counts ($P = .04$) were significant for predicting the 4

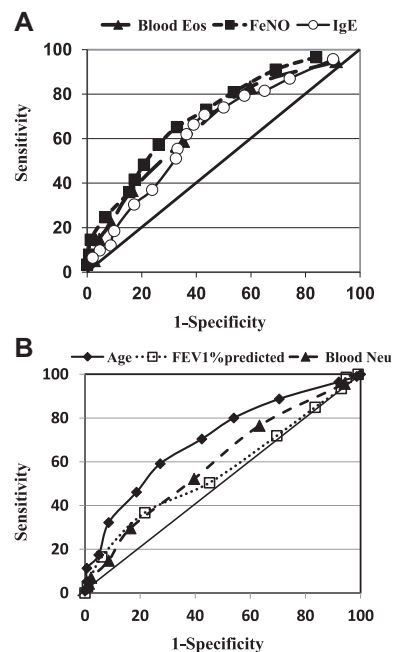


FIG 3. ROC curves for blood eosinophil counts, FENO levels, or IgE levels individually to predict sputum eosinophil percentages of less than 2% or 2% and greater (A) or for age, FEV₁ percent predicted, or blood neutrophil counts to predict sputum neutrophil percentages of less than 40% or 40% and greater (B). AUCs were similar for all subjects (Table III).

sputum subgroups of (1) eosinophil count of 2% and greater plus neutrophil count of 40% and greater, (2) eosinophil count of 2% and greater plus neutrophil count of less than 40%, (3) eosinophil count of less than 2% plus neutrophil count of 40% and greater, and (4) eosinophil count of less than 2% plus neutrophil count of less than 40% (see Fig E4 in this article's Online Repository at www.jacionline.org). However, only 41% of sputum samples were correctly assigned to the 4 sputum groups.

FENO levels ($P < .001$), baseline FEV₁ percent predicted ($P = .018$), and IgE levels ($P = .034$) were significant for predicting sputum eosinophil percentages of less than 2% or 2% and greater (Fig 4). Sixty-nine percent of subjects were correctly assigned to the groups with less than 2% or 2% and greater sputum eosinophil percentages. Although identified by means of factor analysis, blood eosinophil counts were not significant in stepwise discriminant analysis for prediction of sputum eosinophil percentages. Moreover, subjects stratified based on blood eosinophil counts (see Table E2) had smaller differences in lung function and asthma symptoms and no significant differences in measures of health care use.

Age ($P < .001$), absolute blood neutrophil counts ($P = .007$), and asthma duration ($P = .03$) were significant for prediction of sputum neutrophil percentages of less than 40% or 40% and greater (Fig 5). Sixty-four percent of subjects were correctly assigned to the groups with less than 40% or 40% and greater sputum neutrophil percentages. Although FEV₁ percent predicted was significant for negative association with sputum neutrophil percentages, as observed here and in previous reports,^{26,32} this variable was more strongly associated with sputum eosinophil percentages.

DISCUSSION

Sputum eosinophilia and neutrophilia are characteristics of bronchial inflammation in asthmatic patients, reflect underlying

TABLE III. Summary ROC curve analyses

	Youden J index	Cut point	Sensitivity	Specificity	Accuracy	Positive predictive value	AUC	P value
To predict sputum eosinophil percentages of <2% or ≥2%								
Blood eosinophil count (n = 254)*	0.237	300	59%	65%	63%	50%	0.66	<.001
FENO level (n = 238)	0.323	30 ppb	65%	67%	66%	54%	0.72	<.001
IgE level (n = 243)	0.277	120	66%	61%	63%	51%	0.64	.037
Blood eosinophil count + FENO level (n = 107)	0.121	400	45%	67%	55%	62%	0.58	.24
Blood eosinophil count + IgE level (n = 144)	0.215	300	66%	55%	60%	57%	0.63	.04
FENO level + blood eosinophil count (n = 103)	0.302	35 ppb	73%	58%	65%	63%	0.69	.003
FENO level + IgE level (n = 136)	0.216	30 ppb	69%	53%	60%	56%	0.66	.003
To predict sputum neutrophil percentages <40% or ≥40%								
Age (n = 254)	0.318	40 yr	59%	73%	67%	64%	0.71	<.001
Blood neutrophil count (n = 254)	0.132	3000	77%	37%	55%	50%	0.60	.006
FEV ₁ % predicted (n = 254)	0.164	90%	55%	62%	58%	63%	0.56	.049
FEV ₁ % predicted + maximum FEV ₁ reversal <15% (n = 159)	0.191	90%	52%	67%	59%	64%	0.60	.048
To predict sputum eosinophil percentages <3% or ≥3%								
Blood eosinophil count (n = 238)	0.292	300	63%	66%	65%	47%	0.69	<.001
FENO level (n = 238)	0.287	30 ppb	65%	64%	64%	46%	0.71	<.001
To predict severe sputum eosinophil percentages <2% or ≥2%								
Blood eosinophil count (n = 53)	0.418	300	63%	79%	72%	71%	0.75	.004
FENO level (n = 49)	0.378	30 ppb	61%	77%	69%	70%	0.77	.014
To predict no ICS sputum eosinophil percentages <2% or ≥2%								
Blood eosinophil count (n = 92)	0.243	200	82%	42%	54%	38%	0.62	.13
FENO level (n = 85)	0.346	40 ppb	63%	72%	69%	47%	0.74	.002

P values meeting significance are shown in boldface.

*Number of subjects is provided in parentheses.

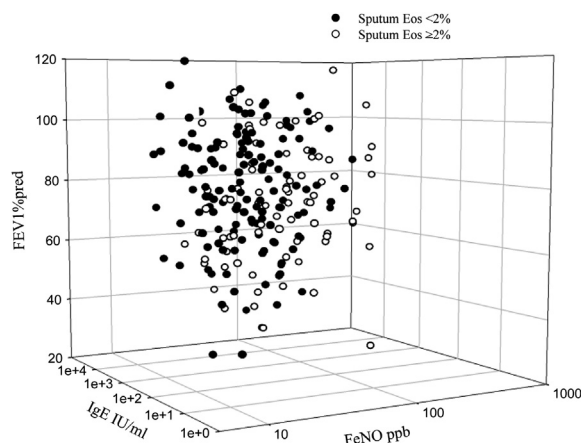


FIG 4. Three-dimensional distribution of subjects' FENO levels, IgE levels, and FEV₁ percent predicted for those with less than 2% sputum eosinophil counts (solid circles) or 2% and greater sputum eosinophil counts (open circles). Subjects with less than 2% sputum eosinophil percentages are shifted to the left, with lower FENO levels and higher FEV₁ percent predicted, whereas subjects with 2% and greater sputum eosinophil percentages are shifted to the right, with higher FENO levels, although not necessarily lower FEV₁ percent predicted.

airway pathology, and are useful in predicting responses to therapeutic interventions.^{3,7-12} Investigators have explored alternative biomarkers as surrogates because of the time-intensive and technically demanding methods for sputum induction, processing, and analysis in clinical settings. The potential usefulness of readily available predictive biomarkers for sputum eosinophils and neutrophils includes not only the management of difficult-to-treat asthma but also serve as important end points for clinical trials and subsequent registration of new biologic therapies for

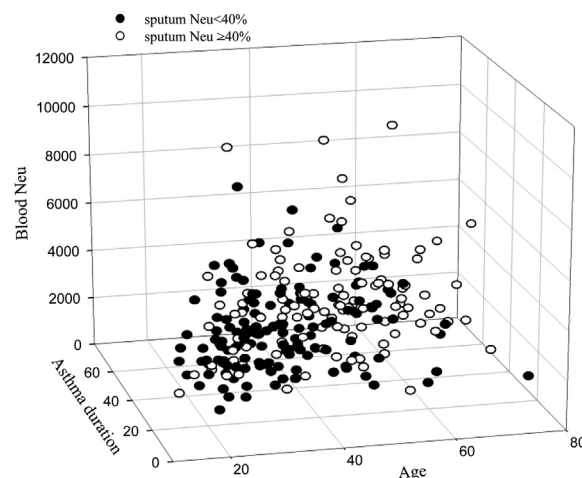


FIG 5. Three-dimensional distribution of subjects' age, asthma duration, and blood neutrophil counts for those with sputum neutrophil counts of less than 40% (solid circles) or 40% and greater (open circles). Subjects with less than 40% sputum neutrophil percentages are shifted left, with younger age, shorter asthma duration, and lower blood neutrophil counts, whereas subjects with 40% and greater neutrophil percentages are shifted to the right, with older age, longer asthma duration, and higher blood neutrophil counts.

asthma that target specific airway inflammation. However, before using surrogate biomarkers, whether individually or combined, sensitivity, specificity, and accuracy of prediction compared with actual sputum counts must be demonstrated across the full range of asthma severity or shown to be applicable to specific asthma subphenotypes only.

Readily accessible biomarkers, including blood eosinophil counts, FENO levels, and total serum IgE levels, are associated with sputum eosinophil percentages,¹³⁻²⁰ whereas age and

FEV₁ percent predicted are associated with sputum neutrophil percentages.^{26,32} Despite these reported associations, the accuracy of a biomarker to predict sputum cell percentages requires specific demonstration in individual asthmatic subjects. In general, eosinophilic rather than neutrophilic sputum inflammation has been more frequently investigated. The National Heart, Lung, and Blood Institute's SARP reported that 495 subjects stratified by asthma severity and FENO levels of less than 35 ppb or 35 ppb and greater showed differences in sputum eosinophil percentages.²⁵ In a separate study of 546 asthmatic subjects, FENO levels were significantly correlated with total IgE levels and blood and sputum eosinophil counts.¹⁹ Moreover, anti-IgE therapy reduces not only IgE levels but also FENO levels and blood and sputum eosinophil counts,⁴⁴ suggesting interactions between these T_H2 biomarkers. However, neither the Dweik et al²⁵ nor Gruchalla et al¹⁹ studies addressed the accuracy of FENO levels, IgE levels, or blood eosinophil counts to predict sputum cell percentages in individual subjects. McGrath et al⁴¹ examined a subpopulation with mild-to-moderate asthma and showed ROC analyses for blood eosinophil percentages (n = 361) and FENO levels (n = 756) to predict sputum eosinophil percentages of less than 2% or 2% and greater. These results had 72% sensitivity and 69% specificity at a blood eosinophil count of 220/ μ L and 64% sensitivity and 73% specificity at a FENO level of 20 ppb. Although accuracy values were not given, the AUCs (0.77 and 0.72, respectively) are similar to those we report, although at the slightly higher cut points observed in our cohort of subjects, which included subjects with more severe asthma. A recent study on serum periostin levels in 59 subjects with corticosteroid-refractory asthma reported AUCs for FENO levels of 0.79, blood eosinophil counts of 0.71, and IgE levels of 0.62 but did not provide ROC accuracy predicting a "composite" eosinophil variable (tissue and sputum)⁵; these values are similar to the AUCs reported here for these individual biomarkers. Other studies raise concerns regarding the specificity and clinical utility of the association between FENO levels and airway eosinophil percentages.²¹⁻²⁴

Studies in small groups of asthmatic subjects have reported different conclusions regarding the correlation of FENO levels and eosinophil numbers in either sputum or bronchial biopsy specimens.^{5,15,21-23,46} The differences among these studies might be due to the small numbers of subjects, differences in asthma severity, possible effects of varying doses of corticosteroid therapy,^{23,26} or confounding by exposure to tobacco smoke.^{16,20,47} In addition, widely varying thresholds are proposed for FENO levels, from 8.3 ppb to nearly 10-fold higher at 73 ppb.^{16,18,20,46,48} SARP subjects were nonsmokers and therefore unaffected by smoking. ROC curve analyses for FENO prediction of eosinophil percentages of less than 2% or 2% and greater in subgroups of subjects with severe asthma only, all of whom were receiving high-dose corticosteroids, or in subjects without corticosteroid treatment were equivalent to ROC analyses in the full SARP cohort of subjects with mild-to-severe asthma, suggesting that asthma severity and treatment with corticosteroids minimally affected FENO prediction of sputum eosinophil percentages. Furthermore, corticosteroid use was not associated with either sputum eosinophil or neutrophil percentages in factor analysis, supporting a small effect of continuous corticosteroid use on sputum eosinophil or neutrophil percentages. Even without these confounding effects, FENO measurement had only 66% accuracy and a 54% positive predictive value for sputum eosinophil percentages, which is little better than random chance.

In the present report factor analysis and stepwise discriminate analysis were used to determine which variables were significantly associated with sputum eosinophil and neutrophil percentages: FENO levels, IgE levels, and FEV₁ percent predicted were identified for sputum eosinophil percentages, and age, asthma duration, and blood neutrophil counts were identified for sputum neutrophil percentages. Nevertheless, these combined variables were less successful (only 41% correct) for predicting 4 sputum granulocytic groups than for predicting 2 groups, either less than 2% or 2% and greater eosinophil counts (69% correct) or less than 40% or 40% and greater neutrophil percentages (64% correct). These results confirm the similarly weak values for accuracy (range, 54% to 72%) observed in ROC curve analyses either for single or combinations of surrogate biomarkers.

Inflammatory phenotype groups in asthmatic subjects distinguished by blood eosinophil counts either with³⁶ or without¹⁷ neutrophil counts have been investigated and recently used in a clinical trial.¹³ In the study with lebrikizumab, subjects were initially characterized for T_H2 phenotypes based on combined blood eosinophil counts and IgE levels. The combined blood eosinophil count plus IgE level stratification scheme was derived from IL-13-responsive gene expression levels (periostin, serpin B2, and CLCA1) in a study of 42 asthmatic subjects.⁴⁹ Subsequent stratification by less than or greater than median levels of serum periostin performed better than blood eosinophil counts plus IgE levels in predicting the response to anti-IL-13 treatment. Nevertheless, stratification by less than or greater than the median FENO level performed equally well as stratification by serum periostin level. In a separate study in subjects with corticosteroid-refractory asthma, FENO levels correlated equally as well as serum periostin levels with sputum eosinophil percentages and better than periostin levels with tissue and blood eosinophil counts.⁴⁵ Our results indicate that blood eosinophil counts, although associated with T_H2 inflammatory parameters, are poor predictors of sputum eosinophilic inflammation, lung function, and health care use. The fact that blood eosinophils transmigrate quickly into tissue in response to localized inflammation suggests that association of blood eosinophil counts with airway inflammation might be transient⁵⁰ and, as observed here, lacking strong positive correlation. Measurements of multiple biomarkers, including sputum cells, serum proteins (eg, periostin), and other biochemical markers, should be examined in future studies to determine the best approach for characterizing asthma severity, health care use, and responses to biologic therapies.

Despite FENO levels predicting change in FEV₁, as well as periostin levels, to anti-IL-13 therapy,¹³ the use of FENO levels to guide treatment in asthmatic subjects has produced conflicting results.^{24,51-53} In contrast, trials using sputum eosinophil percentages to guide treatment and predict asthma control have reported more positive results.^{7-9,12,24,54} Trials focused on neutrophils for management in subjects with refractory asthma have used antibiotics with mixed effectiveness,⁵⁵⁻⁵⁷ but a recent trial of a CXCR2 antagonist in subjects with severe asthma successfully reduced sputum neutrophil counts and mild asthma exacerbations.⁵⁸ We confirm that increased sputum eosinophil and neutrophil percentages are both associated with increased frequency of asthma exacerbations and health care use, which might have a pathophysiologic basis when both T_H2 eosinophilic and T_H17 neutrophilic airway inflammation^{34,35} coexist in asthmatic subjects.

In conclusion, this study confirms associations of FENO levels, IgE levels, and blood eosinophil counts with sputum eosinophil

percentages and age, FEV₁ percent predicted, and blood neutrophil counts with sputum neutrophil percentages. However, the results of this study clearly demonstrate the poor accuracy and ability of these biomarkers, alone or in combination, to predict sputum eosinophil and neutrophil percentages in individual subjects across the spectrum of asthma severity. Accuracy was not improved by restriction to specific asthma subgroups. These are important observations, particularly for studies relying on surrogate biomarkers of airway inflammation, because without direct assessment of induced sputum, subjects with asthma will be misclassified. At present, assessment of sputum eosinophil plus neutrophil percentages appears to be the best minimally invasive indicator of airway inflammation and is significantly correlated with asthma severity and health care use. Further investigation of novel biomarkers (eg, periostin),^{13,59,60} panels of serum proteins, or allergen reactivity profiles,⁶¹ might provide relevant phenotypes that reflect inflammatory cells in the airways.

We thank Jeffrey Krings, RN, and Regina Smith, clinical coordinators, for their essential contributions.

Key messages

- Blood eosinophil counts, FENO levels, FEV₁ percent predicted, or IgE levels, alone or in combination, lack sufficient sensitivity and specificity for accurately predicting sputum eosinophil percentages in asthmatic subjects or serving as reliable biomarkers of more severe asthma, exacerbations, or health care use.
- Similarly, age, FEV₁ percent predicted, and blood neutrophil counts, alone or in combination, lack sufficient sensitivity and specificity for accurately predicting sputum neutrophil percentages.

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METHODS

Rationale for selection of sputum eosinophil count and neutrophil count cut points

Previous studies have considered sputum eosinophil percentages of 1% or greater to 3% as representing an eosinophilic phenotype^{E1-E3} and sputum neutrophil percentages as high as 61%^{E1,E3,E4} as representing neutrophilic phenotype; in this and a previous study^{E5} we used sputum eosinophil percentages of 2% and greater and neutrophil percentages of 40% and greater as cut points that represented the equivalent medians for sputum eosinophil percentages (1.7%) and neutrophil percentages (39%) in subjects with severe asthma.^{E5} Our selection of the neutrophil percentage cut point considered previous reports, including confounders. Balbi et al^{E6} stated that the normal range for sputum neutrophil counts “remains a matter for evaluation” because many factors influence neutrophil inflammation in the airways, including age, air pollution, environmental endotoxin levels, genetics, and even variable sputum induction methods (eg, length of induction and variable hypotonic saline concentrations). In addition, moderate exercise might significantly increase neutrophil percentages in healthy and asthmatic subjects.^{E7} Although others have reported neutrophil percentages greater than our median of 39%, asthmatic subjects in former studies have included substantial numbers of current smokers or exsmokers (range, 25% to 50% of a group with a ≥ 10 pack year history).^{E8,E9} Exposure to tobacco smoke has been shown to increase sputum neutrophil percentages^{E10,E11} to greater than the levels in non-smokers, and neutrophil-associated markers apparently do not decrease, even after 6 months smoking cessation.^{E12} Being a nonsmoker was an inclusion criterion in our study. Moreover, studies reporting sputum neutrophil percentages increased to greater than our 40% cut point examined subject groups with mean or median ages of 50 years or greater in addition to smoke exposure.^{E9,E13} Sputum neutrophil percentages increase in both healthy and asthmatic subjects with age,^{E14-E16} which might contribute to the higher neutrophil percentages designated as neutrophilic in other studies. The median sputum neutrophil percentage previously for our severe cohort was 39% (interquartile range, 16% to 62%),^{E5} which is comparable with reports with similar age groups and smoke exposure in asthmatic subjects.^{E14-E20} Finally, we considered the effect of corticosteroids^{E14,E21} on sputum eosinophil and neutrophil percentages in our group of asthmatic subjects. As reported previously^{E5} and currently observed in factor analysis, corticosteroid use was not associated with either sputum eosinophil or neutrophil percentages and did not result in significant sputum eosinophil or neutrophil percentage differences when subjects were stratified by corticosteroid use ($P = .12$ for eosinophil percentages and $P = .37$ for neutrophil percentages). Therefore we considered less than 2% or 2% and greater eosinophil percentages and less than 40% or 40% and greater neutrophil percentages to be reasonable cut points for stratification of our subjects with asthma by using sputum granulocytic inflammation.

RESULTS

Subject stratification by sputum or blood inflammatory cell counts

We have previously reported on differences between subjects stratified into 4 groups by sputum granulocyte counts.^{E5} The subject group with combined increased eosinophil and neutrophil percentages had the lowest lung function, worse asthma control, and increased health care requirements. Those findings were confirmed by the expanded data set of subjects with adequate sputum samples ($n = 254$) in this report (Table E1). Analyses of primary importance for the combination of sputum eosinophil and neutrophil percentages or eosinophil percentages or neutrophil percentages individually to the differences in lung function and health care use were determined for subjects stratified based on both sputum eosinophil percentages and neutrophil percentages (4 groups), eosinophil percentages (2 groups), or sputum neutrophil percentages (2 groups). In comparisons between models, 4 groups versus 2 groups, either eosinophil or neutrophil percentages were examined (Table E2). The results confirm that both eosinophilic and neutrophilic inflammation associate with the loss of lung function and increased health care use.

Stratification by blood eosinophil and blood neutrophil counts

Inflammatory phenotype groups have been distinguished based on blood eosinophil and neutrophil counts^{E22}; therefore subjects were stratified based on blood eosinophil counts ($<300/\text{mL}$ or $>300/\text{mL}$, Youden J cut point from ROC analysis) to investigate whether this proposed surrogate for sputum eosinophils distinguished groups with greater health care use, exacerbations, and severity (Table E3). Although there were significant differences in methacholine bronchial hyperresponsiveness (log PC_{20}), FENO levels, and IgE levels and small but significant differences in FEV₁ percent predicted and FEV₁/FVC ratio, there were no differences in any variable related to overall asthma health care use or frequency and severity of exacerbations. Adding blood neutrophil counts ($<4000/\text{mL}$ or $>4000/\text{mL}$; midpoint selected between calculated Youden J cut point [3000/mL] and a previously reported [5000/mL] cut point^{E22}) to the stratification scheme did not improve the ability of blood granulocyte differentials to predict asthma severity or any distinctions in health care requirements (Table E4). Race, which was not significant for blood eosinophil counts of less than 300/mL or 300/mL and greater, became significant with additional stratification by blood neutrophil counts ($P = .004$). The substantially lower frequency of African American subjects with increased blood neutrophil counts ($\geq 4000/\text{mL}$) combined with increased blood eosinophil counts contrasts with the lack of any difference for race in subjects stratified based on sputum granulocyte percentages (Table E1).

Additional ROC analyses

Others have reported on the use of FENO measurement to predict sputum eosinophil percentages of 3% and greater, with a derived FENO level of 41 ppb and greater (65% sensitivity and 79% specificity) selective for sputum eosinophilia.^{E23} Therefore ROC curves were generated for FENO levels or blood eosinophil counts to predict sputum eosinophil percentages of less than 3% or 3% and greater (FENO level: AUC, 0.71 [$P < .0001$]; blood eosinophil counts: AUC, 0.69 [$P < .0001$]; Fig E1). The results were nearly identical to ROC curves for predicting sputum eosinophil percentages of less than 2% or 2% and greater (Fig 3, A, and see the print Results section) with only minimal improvement for blood eosinophil counts and no improvement for FENO levels. Accuracy slightly improved for blood eosinophil counts but not for FENO levels; positive predictive value decreased for both (Table III).

In addition, ROC curve analyses were performed for FENO measurement or blood eosinophil count prediction of sputum eosinophil percentages of less than 2% or 2% and greater in subjects with severe asthma only (Fig E2). This smaller subgroup, all receiving high-dose inhaled or oral corticosteroids, had the same FENO level and blood eosinophil count cut points (30 ppb and 300/mL, respectively) as those for the full range of asthma severity. Blood eosinophil count had an AUC of 0.75 ($P = .004$), and for the cut point of 300/mL and greater, there was a sensitivity of 63%, specificity of 79%, accuracy of 0.72, and positive predictive value of 0.71. FENO level had an AUC of 0.77 ($P = .014$), and for a threshold of 30 ppb and greater, there was a sensitivity of 61%, specificity of 77%, accuracy of 0.69, and positive predictive value of 0.70. However, for both blood eosinophil counts and FENO levels, there were substantial false-negative rates (38% and 39%, respectively), which indicate more than a third of subjects with severe asthma with 2% and greater eosinophil percentages would be

misclassified based on blood eosinophil counts or FENO levels. These findings are consistent with the lack of association between FENO levels and sputum eosinophil percentages observed in subjects with severe or prednisolone-dependent asthma.^{E24,E25}

Finally, to test whether ICSs limited the utility of FENO level or blood eosinophil count prediction of sputum eosinophil percentages of less than 2% or 2% and greater,^{E14,E17,E21} ROC curves for these biomarkers were examined in subjects with non-severe asthma without ICS treatment (Fig E3). Blood eosinophil counts had an AUC of 0.62 ($P = .13$), and for a cut point of 200/mL and greater, there was a sensitivity of 82%, specificity of 42%, accuracy of 0.54, and positive predictive value of 0.38. FENO levels had an AUC of 0.74 ($P = .0024$), and for a cut point of 40 ppb and greater, there was a sensitivity of 63%, specificity of 72%, accuracy of 0.69, and positive predictive value of 0.47. These analyses differed very little in AUC and accuracy compared with the ROC curve analyses for all subjects, both untreated and ICS treated, and compared with the ROC curve analyses for subjects with severe asthma, all receiving high-dose ICSs or oral corticosteroids (Table III). Positive predictive value was decreased, which might be due to the smaller number of subjects. Nevertheless, these data suggest that long-term high-dose ICS treatment does not substantially alter prediction of sputum eosinophil percentages in the larger population based on FENO levels and blood eosinophil counts.

Factor analysis to determine variables associated with sputum granulocytes

Factor analysis to determine which variables were associated with sputum granulocytes included standard variables, such as age, sex, race, asthma duration, body mass index, lung function (baseline prebronchodilator FEV₁ percent predicted, FVC percent predicted, and FEV₁/FVC ratio), T_H2-associated variables (FENO and IgE levels), and measures of exacerbations (emergency department visits, hospitalizations, and intensive care unit admission selected from Table E1). The results of factor analysis with 19 variables are shown in Table E5. A separate factor analysis that included corticosteroid use to test for its effect (20 variables)^{E14,E17,E21,E26} did not substantially differ in results from those obtained for the 19 variables, nor was corticosteroid use associated with either sputum eosinophil or neutrophil percentages. Similarly, adding maximum FEV₁ percentage reversibility to the factor analysis did not alter those variables associated with either sputum eosinophil or neutrophil percentages. A final selected group of 10 variables (boldface text in Table E5) were used for stepwise discriminate analysis.

FENO levels ($P < .001$), age ($P < .001$), and blood neutrophil counts ($P = .04$) were significant for predicting combined eosinophil and neutrophil percentages in 4 sputum groups: (1) eosinophil percentage of 2% and greater plus neutrophil percentage of 40% and greater, (2) eosinophil percentage of 2% and greater plus neutrophil percentage of less than 40%, (3) eosinophil percentage of less than 2% plus neutrophil percentage of 40% and greater, or (4) eosinophil percentage of less than 2% plus neutrophil percentage of less than 40% by using stepwise discriminate analysis (Fig E4). However, only 41% of sputum samples were correctly assigned to the 4 sputum groups. Stepwise discriminant analysis correctly assigned 69% of subjects in predicting 2 sputum groups (ie, eosinophil counts of <2% or ≥2%) and 64% of subjects in predicting 2 sputum groups (ie, neutrophil counts of <40% or ≥40%).

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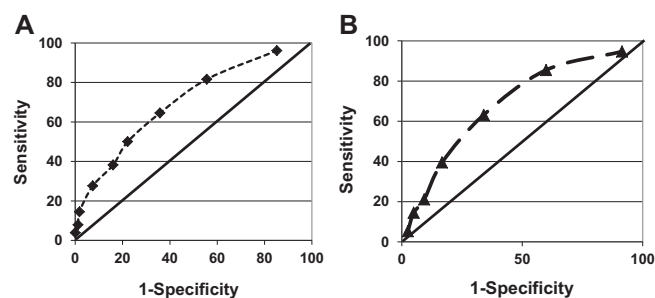


FIG E1. ROC curve for FENO levels (**A, left**) or blood eosinophil counts (**B, right**) to predict sputum eosinophil percentages of less than 3% or 3% and greater. FENO level had an AUC of 0.71 ($P < .0001$). Blood eosinophil count had an AUC of 0.69 ($P < .0001$). These results were nearly identical to the curves for predicting sputum eosinophil percentages of less than 2% and 2% or greater.

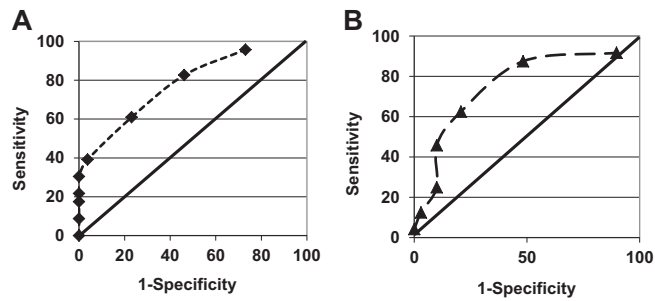


FIG E2. ROC curve for FENO levels (**A, left**) or blood eosinophil counts (**B, right**) to predict sputum eosinophil percentages of less than 2% or 2% and greater in subjects with severe asthma. The FENO level AUC was 0.773 ($P = .01$), and the blood eosinophil count AUC was 0.756 ($P = .004$). These are slightly better results than for subjects with asthma of all severities, but false-negative rates (38% and 39%, respectively) indicate misclassification of more than one third of the subjects with severe asthma as having sputum eosinophil percentages of less than 2%.

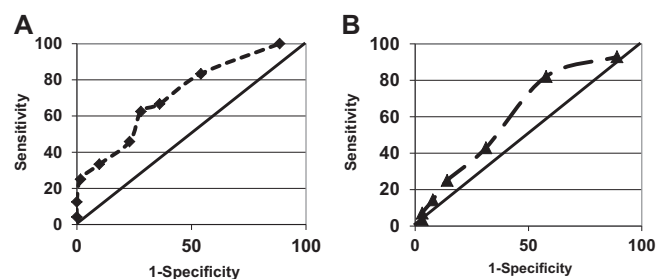


FIG E3. ROC curve for FENO levels (**A, left**) or blood eosinophil counts (**B, right**) to predict sputum eosinophil counts of less than 2% or 2% and greater in subjects with nonsevere asthma without ICS treatment. FENO level had an AUC of 0.738 ($P = .0024$), and blood eosinophil count had an AUC of 0.618 ($P = .13$). These results suggest that ICSs do not confound FENO level or blood eosinophil count prediction of sputum eosinophil percentages in the larger population treated and untreated with ICSs.

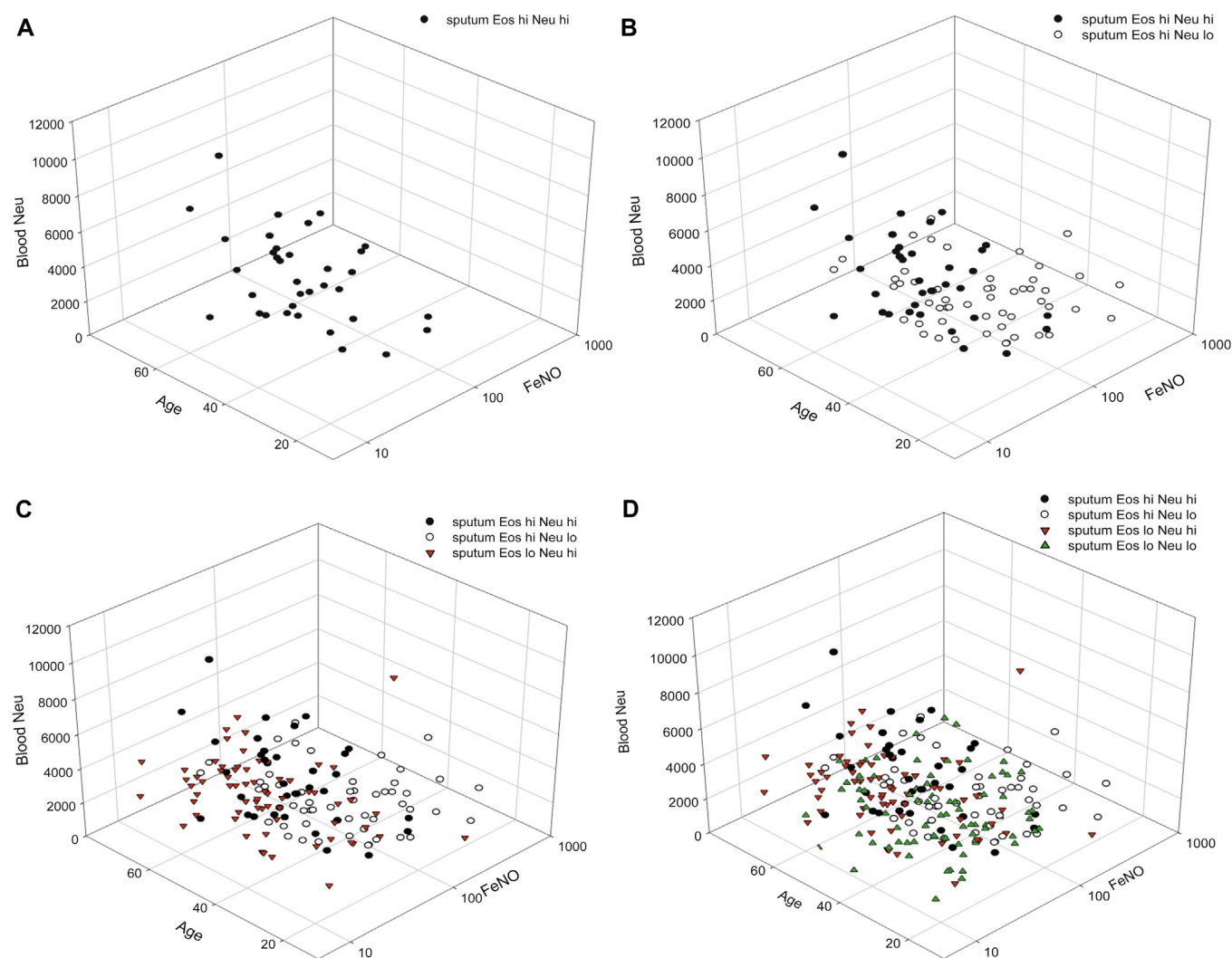


FIG E4. Subject distribution for FeNO levels, age, and blood neutrophil counts as sputum eosinophil percentage of 2% and greater plus neutrophil percentage of 40% and greater (*solid circles, A*), sputum eosinophil percentage of less than 2% plus neutrophil percentage of 40% and greater (*open circles, B*), sputum eosinophil percentage of less than 2% plus neutrophil percentage of 40% and greater (*inverted red triangles, C*), and sputum eosinophil percentage of less than 2% plus neutrophil percentage of less than 40% (*upright green triangles, D*). Generally, subjects with neutrophil percentage of 40% and greater with or without eosinophil percentages of greater than 2% were mainly older than 40 years, whereas subjects with sputum eosinophil percentages of greater than 2% plus neutrophil percentages of less than 40% were younger than 40 years, but subjects with eosinophil percentages of less than 2% plus neutrophil percentages of less than 40% were broadly distributed overall.

TABLE E1. Subjects stratified by sputum eosinophil plus neutrophil percentages*

	Eosinophil count <2% + neutrophil count <40%	Eosinophil count <2% + neutrophil count ≥40%	Eosinophil count ≥2% + neutrophil count <40%	Eosinophil count ≥2% + neutrophil count ≥40%	<i>P</i> value
No.	82	77	57	38	
Sex (% female)	73	70	67	63	.68
Race (%), white/African American/other	57/34/9	65/34/1	53/40/7	50/45/5	.36
Age at asthma onset (y)	16 ± 15	17 ± 16	15 ± 14	20 ± 15	.28
Asthma duration (y)	18 ± 12	26 ± 16	18 ± 11	24 ± 15	.002
Baseline predrug FEV ₁ (% predicted)	83 ± 18	78 ± 16	76 ± 18	71 ± 20	.013
Baseline predrug FVC (% predicted)	92 ± 15	85 ± 15	90 ± 17	82 ± 18	.005
Baseline predrug FEV ₁ /FVC ratio	0.75 ± 0.1	0.75 ± 0.1	0.7 ± 0.1	0.69 ± 0.1	.006
Log PC ₂₀ to methacholine (geometric mean)†	1.15 (n = 75)	1.35 (n = 66)	0.78 (n = 46)	0.42 (n = 26)	.016
No. of positive skin test results	3.9 ± 3.0	3.6 ± 2.9	4.0 ± 3.0	4.7 ± 3.0	.27
Blood eosinophil count (K/mL)	0.24 ± 0.22	0.21 ± 0.13	0.34 ± 0.22	0.32 ± 0.22	.0002
FENO level (ppb)	25 ± 2	19 ± 2	48 ± 3	34 ± 2	<.001
IgE level (IU/mL), geometric mean	100	63	158.5	158.5	.0006
Frequency of wheezing (%)‡	61/20/19	65/17/18	53/19/28	34/26/40	.065
Nighttime symptoms (%)‡	65/14/21	67/16/17	49/28/23	58/13/29	.207
Health care use in past year (%)					
≥1 Urgent health care visits for asthma in past year	31	40	23	61	.002
Emergency department visits	16	21	18	41	.027
Hospitalized for asthma	4	5	9	16	.099
Health care use ever (%)					
Daily use of β-agonist	24	34	54	47	.002
Symptoms worse if corticosteroids reduced	48	45	44	70	.047
Emergency department visits	52	70	67	82	.011
Hospitalized for asthma	26	38	42	57	.010
Hospitalized in ICU	7	10	21	22	.040

ICU, Intensive care unit.

*Data are presented as means ± SDs, medians (25% to 75% quartiles), or percentage positive response. *P* values meeting Bonferroni correction are shown in boldface.†Subjects with FEV₁ of less than 50% of predicted value did not undergo methacholine challenge.

‡Three categories, left to right, indicate percentage positive response for “never to less than 2 times/week,” “greater than 2 times/week but less than 1 time/day,” and “daily.”

TABLE E2. Comparison of *P* values for differences in variables based on stratification by sputum eosinophil percentages (2 groups), sputum neutrophil percentages (2 groups), both sputum eosinophil and neutrophil percentages (4 groups), and between models (4 groups vs 2 groups, either eosinophil percentages or neutrophil percentages)

	<i>P</i> value, eosinophil percentages	<i>P</i> value, neutrophil percentages	<i>P</i> value, eosinophil and neutrophil percentages	<i>P</i> value diff, (eosinophil plus neutrophil percentages vs eosinophil percentages)	<i>P</i> value diff, (eosinophil plus neutrophil percentages vs neutrophil percentages)	Comment
Baseline predrug FEV ₁ (% predicted)	.0076	.048	.0024	.026	.004	Both
Baseline predrug FVC (% predicted)	.49	.0003	.0010	.0003	.31	Neutrophil percentages
Baseline predrug FEV ₁ /FVC ratio	.00024	.98	.0012	.78	.0002	Eosinophil percentages
Log PC ₂₀ methacholine	.0068	.93	.024	.71	.006	Eosinophil percentages
No. of positive skin test results	.16	.85	.37	.78	.16	Neither
Blood eosinophil counts (K/μL)	5.28×10^{-5}	.18	.0002	.30	7.85×10^{-5}	Eosinophil percentages
FENO levels (ppb)	4.67×10^{-8}	.005	1.21×10^{-8}	.0099	8.96×10^{-8}	Both
Log IgE levels (IU/mL)	.008	.076	.009	.12	.013	Eosinophil percentages
Daily use of β-agonist (% positive response)	.0004	.8	.0015	.57	.0003	Eosinophil percentages
Asthma symptoms worsen if corticosteroids reduced (% positive response)	.22	.26	.22	.22	.18	Neither
Emergency department visits ever (%)	.061	.0096	.0044	.0057	.034	Both
Emergency department visits in past year (%)	.13	.038	.028	.026	.08	Neutrophil percentages
≥1 Urgent health care visits for asthma in past year (%)	.62	.0018	.0058	.0015	.45	Neutrophil percentages
Hospitalized for asthma ever (%)	.0096	.061	.0044	.033	.005	Both
Hospitalized for asthma in past year (%)	.037	.37	.065	.27	.03	Eosinophil percentages
Hospitalized in ICU ever (%)	.0063	.8	.021	.61	.0006	Eosinophil percentages

ICU, Intensive care unit.

TABLE E3. Stratification of subjects based on blood eosinophil counts of less than 300/mL or 300/mL and greater*

	Blood eosinophil counts <300/mL	Blood eosinophil counts ≥300/mL	<i>P</i> value
No.	194	134	
Sex (% female)	73	64	.11
Race, white/African American/other (%)	56/39/5	58/37/5	.95
Age at asthma onset (y)	16 ± 15	17 ± 16	.88
Asthma duration (y)	21 ± 15	22 ± 13	.38
Baseline predrug FEV ₁ (% predicted)	79 ± 19	74 ± 20	.01
Baseline predrug FVC (% predicted)	88 ± 16	86 ± 19	.12
Baseline predrug FEV ₁ /FVC ratio	0.74 ± 0.1	0.71 ± 0.1	.002
Log PC ₂₀ to methacholine, geometric mean	1.41 (n = 163) [†]	0.65 (n = 105) [†]	.0003
No. of positive skin test results	3.6 ± 2.9	4.5 ± 2.9	.015
IgE (IU/mL), geometric mean	79	159	.0002
FENO (ppb)	30 ± 32.6	52.9 ± 47.1	<.0001
Frequency of wheezing [‡]	49/27/24	62/14/24	.011
Frequency of nighttime symptoms [‡]	64/18/18	61/14/24	.34
Health care use in past year (%)			
≥1 Urgent health care visits for asthma	35	35	1
Emergency department visits	20	23	.58
Hospitalized for asthma	6	8	.65
Health care use ever (%)			
Daily use of β-agonist	36	40	.49
Symptoms worse if corticosteroids reduced	46	48	.82
Emergency department visits	62	67	.48
Hospitalized for asthma	34	41	.24
Hospitalized in ICU	9	15	.11

ICU, Intensive care unit.

*Data are presented as means ± SDs, medians (25% to 75% quartiles), or percentage positive response. *P* values meeting significance are shown in boldface.[†]Subjects with FEV₁ of less than 50% of predicted value did not undergo methacholine challenge.[‡]Three categories, left to right, indicate percentage positive response for “never to less than 2 times/week,” “greater than 2 times/week but less than 1 time/day,” and “daily.”

TABLE E4. Subjects stratified by blood eosinophil plus neutrophil counts*

	Eosinophil count <300/mL + neutrophil count <4000/mL	Eosinophil count <300/mL + neutrophil count ≥4000/mL	Eosinophil count ≥300/mL + neutrophil count <4000/mL	Eosinophil count ≥300/mL + neutrophil count ≥4000/mL	<i>P</i> value
No.	109	85	65	69	
Sex (% female)	69	78	55	72	.032
Race, white/African American/other (%)	52/46/2	61/29/10	48/49/3	68/25/7	.004
Age at asthma onset (y)	17 ± 15	16 ± 14	17 ± 16	17 ± 16	.95
Asthma duration (y)	20 ± 14	23 ± 16	19 ± 13	24 ± 13	.13
Baseline predrug FEV ₁ (% predicted)	79 ± 19	79 ± 19	74 ± 21	74 ± 20	.080
Baseline predrug FVC (% predicted)	88 ± 16	88 ± 17	87 ± 19	85 ± 20	.38
Baseline predrug FEV ₁ /FVC ratio	0.73 ± 0.12	0.74 ± 0.1	0.7 ± 0.09	0.71 ± 0.11	.013
Log PC ₂₀ methacholine (geometric mean)†	1.38 (n = 91)	1.45 (n = 72)	0.65 (n = 52)	0.65 (n = 53)	.004
No. of positive skin test results	3.6 ± 3.0	3.7 ± 2.9	4.8 ± 2.8	4.2 ± 3.0	.054
Sputum eosinophil counts (%)	2.9 ± 6.2	3.2 ± 8.8	6.4 ± 11.4	6.7 ± 10.2	.0003
FENO levels (ppb)	23 ± 2	19 ± 2	46 ± 2	33 ± 2	<.001
IgE levels (IU/mL), geometric mean	79.4	79.4	200	126	.0004
Frequency of wheezing (%)‡	64/15/21	59/13/28	48/28/24	51/26/23	.102
Frequency of nighttime symptoms (%)‡	69/14/17	52/15/33	63/18/19	65/17/18	.132
Health care use in the past year (%)					
≥1 Urgent health care visits for asthma	32	39	35	35	.84
Emergency department visits	17	25	23	23	.49
Hospitalized for asthma	6	5	5	10	.54
Health care use ever (%)					
Daily use of β-agonist	31	36	38	42	.87
Symptoms worse if corticosteroids reduced	42	52	45	51	.52
Emergency department visits	63	61	65	68	.84
Hospitalized for asthma	37	31	40	42	.51
Hospitalized in ICU	9	8	16	15	.37

ICU, Intensive care unit.

*Data are presented as means ± SDs, medians (25% to 75% quartiles), or percentage positive response. *P* values meeting Bonferroni correction are shown in boldface.†Subjects with FEV₁ of less than 50% of predicted value did not undergo methacholine challenge.

‡Three categories, left to right, indicate percentage positive response for “never to less than 2 times/week,” “greater than 2 times/week but less than 1 time/day,” and “daily.”

TABLE E5. Factor analysis for selected variables

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Age	−18	79	1	−27	9	5
Asthma duration	−7	66	13	24	−4	−14
BMI	−13	−5	26	−10	−15	73
Sex	61	15	18	−25	7	17
Race background	1	−38	27	12	−1	−11
Number of positive skin test results	−17	−2	−1	80	16	−2
Baseline FEV₁ (% predicted)	86	−23	−25	11	−8	−18
Baseline FVC	61	−36	−16	37	−2	−29
Baseline FEV ₁ /FVC ratio	73	−12	−21	−25	−12	9
Blood eosinophil counts	−10	0	−6	18	68	10
Blood neutrophil counts	10	11	−19	4	8	74
Sputum eosinophil counts (%)	3	3	10	−2	72	0
Sputum neutrophil counts (%)	−4	63	7	14	−23	12
Feno levels	−4	−30	13	−1	61	−29
IgE levels	1	7	33	63	0	0
β-Agonist use	−12	−23	52	5	18	17
Emergency department visit	25	30	49	0	−5	25
Ever hospital stay for breathing	−11	21	76	1	1	−9
Ever ICU for asthma	−19	−7	72	19	5	−8

Values are multiplied by 100 and rounded to the nearest integer. Boldface type indicates a value of greater than 0.4. Variables in boldface were selected for stepwise discriminate analysis.

BMI, Body mass index; *ICU*, intensive care unit.