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Biomarker Surrogates Do Not Accurately Predict Sputum Eosinophils and Neutrophils in Asthma

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Abstract

Background—Sputum eosinophils (Eos) are a strong predictor of airway inflammation, exacerbations, and aid asthma management, whereas sputum neutrophils (Neu) indicate a different severe asthma phenotype, potentially less responsive to T_H2 -targeted therapy. Variables such as blood Eos, total IgE, fractional exhaled nitric oxide (FeNO) or FEV₁% predicted, may predict airway Eos, while age, FEV₁% predicted, or blood Neu may predict sputum Neu. Availability and ease of measurement are useful characteristics, but accuracy in predicting airway Eos and Neu, individually or combined, is not established.

Objectives—To determine whether blood Eos, FeNO, and IgE accurately predict sputum eosinophils, and age, FEV₁% predicted, and blood Neu accurately predict sputum neutrophils (Neu).

Methods—Subjects in the Wake Forest Severe Asthma Research Program (N=328) were characterized by blood and sputum cells, healthcare utilization, lung function, FeNO, and IgE. Multiple analytical techniques were utilized.

Results—Despite significant association with sputum Eos, blood Eos, FeNO and total IgE did not accurately predict sputum Eos, and combinations of these variables failed to improve prediction. Age, FEV₁% predicted and blood Neu were similarly unsatisfactory for prediction of sputum Neu. Factor analysis and stepwise selection found FeNO, IgE and FEV₁% predicted, but not blood Eos, correctly predicted 69% of sputum Eos <or 2%. Likewise, age, asthma duration and blood neutrophils correctly predicted 64% of sputum Neu <or 40%. A model to predict both sputum Eos and Neu accurately assigned only 41% of samples.

Conclusion—Despite statistically significant associations FeNO, IgE, blood Eos and Neu, FEV₁% predicted, and age are poor surrogates, separately and combined, for accurately predicting sputum eosinophils and neutrophils.

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Keywords

asthma phenotypes; sputum eosinophils and neutrophils; inflammatory biomarker surrogates; T_H2 biomarkers; fractional exhaled nitric oxide

INTRODUCTION

All levels of asthma severity, show evidence of airway inflammation, with both eosinophils and neutrophils contributing to this pathology¹⁻⁵. We recently reported that asthma subjects with both increased sputum eosinophils and neutrophils had the lowest lung function, worse asthma control, and increased symptoms and healthcare requirements⁶. Although induced sputum eosinophilic or neutrophilic phenotypes have been shown to predict asthma exacerbations⁷⁻⁹ and responsiveness to corticosteroids, or corticosteroid reduction with anti-IL5 therapy^{3,10,11,12}, sputum induction and processing is technically difficult and not readily available in most clinical settings. Clearly, readily accessible biomarkers which accurately predict airway inflammation are needed for managing difficult-to-treat asthma, and to serve as response endpoints in clinical trials testing and registering new biologic therapies for asthma. Thus, more accessible biomarkers are employed as surrogates for sputum inflammatory cell measurements in clinical studies without first establishing their accuracy across the full range of asthma or severity subphenotypes¹³⁻¹⁴.

Eosinophils are a primary cellular component in studies that focus on airway inflammation in asthma. FeNO, blood eosinophils, FEV₁%predicted and IgE have been suggested as potential predictive biomarkers for sputum eosinophils or bronchial mucosal eosinophils¹³⁻²⁰. However, other studies raise questions regarding the accuracy of these biomarker associations with sputum eosinophils^{5,21-23}, and their clinical usefulness in predicting asthma exacerbations²⁴. The association of FeNO with sputum eosinophils has been more widely examined, but cutpoints to predict sputum Eos vary extensively and are confounded by exposure to tobacco smoke and inhaled corticosteroid 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 neutrophils²⁶⁻²⁸, although this granulocyte is implicated in severe asthma²⁹⁻³², associated with reduced FEV₁²⁶, irreversible airflow obstruction³², and loss of control by rapid withdrawal of inhaled corticosteroid¹¹. Increased neutrophils, reduced FEV₁ 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 neutrophils, and ongoing T_H2 responses supports a causal role for neutrophils in airway hyperresponsiveness^{34, 35}. A recent report suggested that asthmatics might be stratified by both neutrophils and eosinophils in blood³⁶. Thus, previous studies and our report⁶ support the importance and need to assess both sputum eosinophils and neutrophils 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 eosinophils and neutrophils, we analyzed the accuracy of biomarkers that may 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 severity. Our hypotheses were that variables associated with T_H2 inflammation, FeNO, IgE, and blood eosinophils, should accurately

predict sputum eosinophils, while separately, age, reduced FEV₁ and blood neutrophils, should accurately predict sputum neutrophils. We also examined more complex models to predict both sputum eosinophils and neutrophils. 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

Patients with mild to severe asthma were comprehensively characterized according to the SARP phenotype protocol at Wake Forest School of Medicine as previously described³⁷. Briefly, non-smoking subjects (<5 packyears) who met ATS 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 (BHR), allergen skin prick tests, total serum IgE level, collection of blood, exhaled NO measurement, sputum induction⁶, and administration of questionnaires that characterized asthma symptoms, quality of life, medications and healthcare utilization^{33,37}.

Subjects

SARP subjects had severe (N=71, as defined by the ATS Workshop on Refractory Asthma³⁸) or nonsevere asthma (N=257, asthmatics not meeting “severe” criteria and were classified as “mild” asthma with FEV₁% predicted ≥80%, or “moderate” asthma with FEV₁% predicted <80%)^{33,37}. A portion of these subjects were studied in our report of subject stratification by sputum granulocytes and inflammatory proteins⁶; (previous data in repository Table E1 and E2).

Demographics and clinical characteristics for all Wake Forest subjects with sputum samples matched those previously reported for subjects from all SARP sites³⁷ (Table 1).

Sputum Induction and Processing

The sputum induction method was adopted from the NHLBI Asthma Clinical Research Network³⁹. Sputum was processed immediately; cell cytopins were stained for differential counts. The sputum cell differential counts were adequate for further analyses (i.e. <80% squamous^{6,39}) from 254 of the 328 subjects with sputum samples. Previous studies have considered sputum eosinophils 1-3% as representing an “eosinophilic” phenotype^{3,9,40,41} and sputum neutrophils as high as 61%^{3,5,40}. Many factors contribute to variable neutrophil %, including age, air pollution, environmental endotoxin levels, different induction procedures, and exercise^{42,43}. In this and our previous study⁶, we utilized sputum eosinophils ≥2% and neutrophils ≥40% as the cutpoints because our cohort of subjects with severe asthma⁶ had median values of 1.7% eosinophils and 39% neutrophils, which correspond to reports for subjects with asthma of similar age and smoke exposure^{18,26,41} (additional discussion in online repository).

Statistics

Demographic, and biomarker data are presented as means ± standard deviations, or medians (25%-75% quartiles) for continuous variables, and as % positive for categorical variables. Measures not meeting 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 observed before log transformation¹⁶. Continuous variables were tested by parametric; or non-parametric tests (SAS 9.2, or Sigmastat 3.1). Analyses with a significant

difference were further explored by post-hoc pairwise tests (Tukey). Categorical variables were analyzed using Chi-square tests.

Initial analyses considered potential T_H2 biomarker variables reported for association with sputum Eos¹³⁻²⁰: FeNO, IgE, and blood eosinophils; and variables reported for association with sputum Neu^{26, 27, 32}: age, FEV₁%predicted and blood neutrophils. 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 Eos or Neu. ROC analyses provide information to determine optimal models for potential biomarker predictor(s) as binary classifiers, comparing the true positive rate to the false positive rate. A ROC AUC of 0.5 indicates inability of a biomarker to predict a subject's correct classification. Factor analysis with 19 or 20 variables (table in online repository) confirmed those variables associated with sputum eosinophil % or neutrophil %. These variables were included in stepwise selection and discriminant analysis for prediction of combined sputum Eos < or 2% and sputum Neu < or 40%, or each granulocyte separately. Variables with a p value <0.05 were accepted as significant.

RESULTS

Subject Demographics

There were significant differences in lung function, bronchodilator reversibility, symptoms and healthcare utilization for subjects with severe compared to those with mild and moderate asthma (Table 1). 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, frequency of positive skin tests, number of positive skin tests, serum eosinophils, or FeNO.

Subjects with mild, moderate or severe asthma did not have substantial differences in sputum cell counts (Table 2). However as previously demonstrated⁶, subjects stratified by combined sputum Eos < or 2% and Neu < and 40% had significant differences in lung function, atopic measures and healthcare utilization (Table E1 in Online Repository at www.jacionline.org). Analyses for contribution by combined Eos and Neu, or each granulocyte individually, to the observed differences were assessed (Table E2 in online repository). The results confirm that sputum Eos and Neu, both combined and individually, were significantly associated with asthma severity characteristics including reduced lung function, and increased healthcare utilization.

Distribution of Sputum Granulocytes by Potential Biomarker Predictors

Sputum eosinophil %s were significantly associated with blood eosinophils ($R=0.191$, $p=0.002$) and with FeNO ($R=0.321$, $p<0.001$) (Figure 1A and 1B, respectively), but sputum Eos% were widespread across the range of blood Eos and FeNO, 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 Eos% among these asthma severity groups (Table 2). Youden index cutpoints for blood eosinophils=300/ μ l and for FeNO=30ppb (maximum sensitivity and specificity derived from ROC curve analyses in the section below) were graphed with the sputum Eos cutpoint < or 2%. Despite significant positive associations between these biomarkers, many subjects with sputum Eos 2% would be misclassified by criteria requiring blood Eos 300/ μ l (false negative rate=41%), or FeNO 30ppb (false negative rate=35%), and likewise, many subjects with sputum Eos<2% would also be misclassified based on the same blood Eos and FeNO criteria (false positive rates=35%, and 33%, respectively). Therefore, both subjects with higher or lower sputum Eos would be misclassified by the surrogate biomarkers of blood Eos, or FeNO.

Similarly, sputum neutrophil % (Figure 2A and B) was positively associated with age ($R=0.40$, $p<0.001$) and negatively associated with baseline pre-bronchodilator $FEV_1\%$ predicted ($R=0.16$, $p=0.013$), but were broadly spread across age and $FEV_1\%$ predicted ranges, and the R values were low (<0.5), although significant. Subjects with mild, moderate or severe asthma were distributed throughout the age range, but only severe subjects were spread across the $FEV_1\%$ predicted range (mild and moderate were classified by baseline $FEV_1\%$ predicted $\geq 80\%$ and $< 80\%$, respectively). Youden index cutpoints for age=40yr and for $FEV_1\%$ predicted=90% were graphed with the Neu cutpoint $< 40\%$. As observed for sputum Eos above, many subjects with sputum Neu $\geq 40\%$ would be misclassified as $<40\%$ (false negative rate of 41% for age, and 45% for $FEV_1\%$ predicted), whereas others with actual sputum Neu $<40\%$ would be misclassified as $\geq 40\%$ (false positive rate of 27% for age, and 38% for $FEV_1\%$ predicted at respective Youden cutpoints). As observed with sputum Eos, both subjects with higher or lower sputum Neu would be misclassified by the surrogate biomarkers of age and $FEV_1\%$ predicted.

ROC Curves for Predicting Sputum Eos $< \text{or} \geq 2\%$ and Predicting Sputum Neu $< \text{or} \geq 40\%$

ROC analyses to predict sputum Eos and Neu were performed to determine optimum cutpoints, accuracy and positive predictive values. The ROC curves for blood eosinophils, FeNO or IgE for predicting sputum Eos $< \text{or} \geq 2\%$, and for age, $FEV_1\%$ predicted, and blood Neu for predicting sputum Neu $< \text{or} \geq 40\%$ are presented in Figure 3A & 3B, respectively. Although statistically significant, the AUC show fair to poor accuracy and positive predictive values (summarized in Table 3).

Additional ROC analyses were performed to determine if alternative criteria improved prediction of sputum Eos; for example, to predict sputum Eos $< \text{or} \geq 3\%$, or to predict Eos $< \text{or} \geq 2\%$ in subjects with severe asthma only (a group only on 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 Eos $< \text{or} \geq 2\%$ with minimal or no improvement in sensitivity, specificity and accuracy (Table 3) (see Figures E1, E2 and E3 in the Online Repository).

Combinations of variables, as suggested¹⁴ and employed^{13, 41}, were examined for improved prediction of sputum eosinophils. ROC curve of blood Eos in combination with FeNO ≥ 30 ppb or with IgE ≥ 100 IU/ml showed no enhancement in AUCs or accuracy for combined variables over blood Eos alone (Table 3). Nor was the combination of FeNO, either with blood Eos $\geq 300/\mu\text{l}$ or with IgE ≥ 100 IU/ml superior to FeNO alone (Table 3).

Likewise, combining lower reversibility ($<15\%$) with $FEV_1\%$ predicted to examine whether a characteristic of more fixed airflow obstruction better predicted sputum Neu $< \text{or} \geq 40\%$, failed to provide any improvement (Table 3).

Factor Analysis Variables Associated with Sputum Eosinophils and Neutrophils

In order to determine whether other variables may better predict sputum Eos and Neu, factor analysis was performed for 19 variables including gender, age, asthma duration, race, lung function (baseline prebronchodilator $FEV_1\%$ predicted, FVC% predicted, and FEV_1/FVC), T_H2 associated variables (FeNO, and IgE), and measures of exacerbations (emergency department visits, hospitalizations, and ICU admission)(Table E2, online repository). A separate factor analysis including corticosteroid use (20 variables) did not differ, nor was corticosteroid use associated with either sputum Eos% or Neu%. Similarly, adding maximum $FEV_1\%$ reversibility to the factor analysis did not alter those variables associated with either sputum Eos% or Neu%.

Sputum Eos% showed associations with FeNO and blood Eos, whereas sputum Neu% showed associations with age and asthma duration. These 4 variables and 1-2 variables from each factor group (BMI, gender, race, IgE, blood neutrophils, and baseline FEV₁%predicted) were included in stepwise discriminate analysis to predict combined sputum Eos+Neu. FeNO ($p<0.001$), age ($p<0.001$) and blood neutrophils ($p=0.04$) were significant for predicting the 4 sputum subgroups, Eos $\geq 2\%$ +Neu $\geq 40\%$, Eos $\geq 2\%$ +Neu $<40\%$, Eos $<2\%$ +Neu $\geq 40\%$, or Eos $<2\%$ +Neu $<40\%$ (Figure E4A-D in online repository). However, only 41% of sputum samples were correctly assigned to the 4 sputum groups.

FeNO ($p<0.001$), baseline FEV₁%predicted ($p=0.018$), and IgE ($p=0.034$) were significant for predicting sputum Eos $< \geq 2\%$ (Figure 4). Sixty-nine percent of subjects were correctly assigned to $< \geq 2\%$ sputum Eos. Although identified by factor analysis, blood Eos were not significant in stepwise discriminant analysis for prediction of sputum Eos. Moreover, subjects stratified by blood Eos (online repository Table E2) had smaller differences in lung function and asthma symptoms, and no significant differences in measures of healthcare use.

Age ($p<0.001$), absolute blood neutrophil ($p=0.007$), and asthma duration ($p=0.03$) were significant for prediction of sputum Neu $< \geq 40\%$ (Figure 5). Sixty-four % of subjects were correctly assigned to $< \geq 40\%$ sputum Neu. Although FEV₁%predicted was significant for negative association with sputum Neu%, as observed here and in previous reports^{26, 32}, this variable was more strongly associated with sputum Eos%.

DISCUSSION

Sputum eosinophilia and neutrophilia are characteristics of bronchial inflammation in asthma, reflect underlying airway pathology and are useful in predicting responses to therapeutic interventions^{3, 7-12}. Investigators have explored alternative biomarkers as surrogates due to 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 endpoints for clinical trials and subsequent registration of new biologic therapies for asthma that target specific airway inflammation. However, before employing surrogate biomarkers, whether individually or combined, sensitivity, specificity, and accuracy of prediction compared to 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 Eos, FeNO, and total serum IgE, are associated with sputum Eos¹³⁻²⁰, while age and FEV₁%predicted are associated with sputum Neu^{26, 32}. Despite these reported associations, accuracy of a biomarker to predict sputum cells requires specific demonstration in individual subjects with asthma. In general, Eos rather than Neu sputum inflammation has been more frequently investigated. The NHLBI Severe Asthma Research Program (SARP) reported that 495 subjects stratified by asthma severity and FeNO $< \geq 35$ ppb showed differences in sputum eosinophils²⁵. In a separate study of 546 subjects with asthma, FeNO was significantly correlated with total IgE levels, blood and sputum eosinophils¹⁹. Moreover, anti-IgE therapy reduces not only IgE, but also FeNO, blood and sputum eosinophils⁴⁴, suggesting interactions between these T_H2 biomarkers. But neither the Dweik²⁵ nor Gruchalla¹⁹ studies addressed the accuracy for FeNO, IgE or blood Eos to predict sputum cells in individual subjects. McGrath et al.⁴¹ examined a subpopulation with mild-moderate asthma and showed ROC analyses for blood Eos (N=361) and FeNO (N=756) to predict sputum Eos $< \geq 2\%$. These results had 72% sensitivity, 69% specificity at 220/ μ l blood Eos, and 64% sensitivity and 73% specificity at 20ppb FeNO. Although accuracy values were not given, the areas under the curves (0.77 and

0.72, respectively) are similar to those we report, but at the slightly higher cutpoints observed in our cohort of subjects that included subjects with more severe asthma. A recent study on serum periostin in 59 subjects with corticosteroid refractory asthma reported AUC for FeNO= 0.79, blood eosinophils= 0.71, and IgE= 0.62, but did not provide ROC accuracy predicting a “composite” eosinophil variable (tissue and sputum)⁴⁵; these values are similar to the AUC reported here for these individual biomarkers. Other studies raise concerns regarding the specificity and clinical utility of the association between FeNO and airway Eos²¹⁻²⁴.

Studies in small groups of patients with asthma have reported different conclusions regarding the correlation of FeNO and Eos either in sputum or in bronchial biopsies^{5,15, 21-23, 46}. The differences among these studies may be due to the small numbers of subjects, differences in severity of asthma, 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, from 8.3ppb to nearly 10-fold higher at 73ppb^{16,18,20,46,48}. SARP subjects were non-smokers and therefore unaffected by smoking. ROC curve analyses for FeNO prediction of < or ≥ 2%Eos in subgroups of subjects with severe asthma only, all on high dose corticosteroids, or in subjects without corticosteroid treatment, were equivalent to ROC analyses in the full SARP cohort of mild to severe asthma, suggesting that severity of asthma, and treatment with corticosteroids minimally impacted FeNO prediction of sputum Eos. Furthermore, corticosteroid use was not associated with either sputum Eos or Neu in factor analysis, supporting small effect of continuous corticosteroid use on sputum Eos or Neu. Even without these confounding effects, FeNO had only 66% accuracy, and 54% positive predictive value for sputum eosinophils, 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 Eos and Neu; FeNO, IgE and FEV₁% predicted were identified for sputum Eos, and age, asthma duration and blood Neu for sputum Neu. Nevertheless, these combined variables were less successful, only 41% correct, for predicting four sputum granulocytic groups than predicting two groups, either < or ≥ 2%Eos, 69% correct, or < or ≥ 40%Neu, 64% correct. These results confirm the similarly weak values for accuracy (ranging from 54-72%) observed in ROC curve analyses either for individual or combinations of surrogate biomarkers.

Inflammatory phenotype groups in asthma distinguished by blood Eos, either with³⁶ or without Neu¹⁷, have been investigated and recently employed in a clinical trial¹³. In the study with Lebrikizumab, subjects were initially characterized for T_H2 phenotypes based on combined blood Eos and IgE. The combined blood Eos + IgE stratification scheme was derived from IL13-responsive gene expression levels (periostin, serpin B2 and CLCA1) in a study of 42 patients with asthma⁴⁹. Subsequent stratification by < or >median levels of serum periostin performed better than blood Eos+IgE in predicting the response to anti-IL13 treatment. Nevertheless, stratification by < or >median FeNO performed equally well as stratification by serum periostin. In a separate study in corticosteroid refractory asthma, FeNO correlated equally as well as serum periostin with sputum eosinophil%, and better than periostin with tissue and blood eosinophils⁴⁵. Our results indicate that blood Eos, although associated with T_H2 inflammatory parameters, are poor predictors of sputum eosinophilic inflammation, lung function and healthcare utilization. The fact that blood eosinophils transmigrate quickly into tissue in response to localized inflammation suggests that association of blood eosinophils with airway inflammation may be transient⁵⁰, and as observed here, lacking strong positive correlation. Measurements of multiple biomarkers, including sputum cells, serum proteins such as periostin, and other biochemical markers,

should be examined in future studies to determine the best approach for characterizing asthma severity, healthcare utilization and responses to biologic therapies.

Despite FeNO predicting change in FEV1 as well as periostin to anti-IL13 therapy¹³, the use of FeNO to guide treatment in asthma has produced conflicting results^{24, 51-53}. In contrast, trials employing sputum eosinophils to guide treatment and predict asthma control have reported more positive results^{7-9, 12, 24, 54}. Trials focused on neutrophils for management in refractory asthma have used antibiotics with mixed effectiveness⁵⁵⁻⁵⁷, but a recent trial of a CXCR2 antagonist in patients with severe asthma successfully reduced sputum neutrophils and mild asthma exacerbations⁵⁸. We confirm that increased sputum Eos+Neu are both associated with increased frequency of asthma exacerbations and healthcare utilization which may have a pathophysiologic basis when both T_H2 eosinophilic and T_H17 neutrophilic airway inflammation³⁴⁻³⁵ coexist in asthma.

In conclusion, this study confirms associations of FeNO, IgE and blood Eos with sputum Eos%, and age, FEV₁%predicted, and blood Neu with sputum Neu%. However, the results of this study clearly demonstrate the poor accuracy and ability of these biomarkers, alone or in combination, to predict sputum Eos and Neu 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 Eos+Neu appears to be the best minimally invasive indicator of airway inflammation and is significantly correlated with asthma severity and healthcare utilization. Further investigation of novel biomarkers, such as periostin^{13,59-60}, panels of serum proteins, or allergen reactivity profiles⁶¹ may in the future provide relevant phenotypes that reflect inflammatory cells in the airways.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AUC	area under the curve
Eos	eosinophils
FeNO	fractional exhaled nitric oxide
ICS	inhaled corticosteroids
Neu	neutrophils
ROC	receiver operating characteristic
SARP	Severe Asthma Research Program (of NHLBI)
BHR	bronchial hyperresponsiveness

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Key Messages

- Blood eosinophils, FeNO, FEV₁%predicted or IgE, alone or in combination, lack sufficient sensitivity and specificity for accurately predicting sputum eosinophils in asthma, or serving as reliable biomarkers of more severe asthma, exacerbations, or healthcare utilization.
- Similarly, age, FEV₁%predicted, and blood neutrophils, alone or in combination lack sufficient sensitivity and specificity for accurately predicting sputum neutrophils.

Figure 1A and 1B.

Subject Distribution by Sputum Eos% and Blood Eos count/μl (1A) or FeNO ppb (1B) for mild, moderate and severe asthma. Sputum Eos > 2% and blood Eos < 300/μl (1A) or sputum Eos > 2% and FeNO < 30 ppb (1B) are false negative predictions indicated by crosshatched areas. Sputum Eos < 2% and blood Eos > 300/ml (1A) or sputum Eos < 2% and FeNO > 30 ppb (1B) are false positive predictions indicated by shaded areas.

Figure 2A and 2B.

Subject Distribution by Sputum Neu%, < or ≥ 40% and Age (2A) and FEV₁% predicted (2B) for subjects with mild, moderate and severe asthma. Sputum Neu ≥ 40% are indicated by crosshatched area for false negative prediction by age < 40yr and by FEV₁% predicted > 90%. Sputum Neu < 40% are indicated by shaded area for false positive prediction by age > 40yr and by FEV₁% predicted < 90%.

Figure 3A and 3B.

ROC curves for blood Eos, FeNO or IgE individually to predict sputum Eos < or > 2% (3A), or for age, FEV₁%predicted or blood Neu to predict sputum Neu < or > 40% (3B). Areas under the curves were similar for all (Table III).

Figure 4.

Three dimensional distribution of subjects' FeNO, IgE, and FEV₁%predicted levels for those with <2% sputum Eos (filled circles) or ≥2% sputum Eos (open circles). Subjects with <2% sputum Eos are shifted to the left with lower FeNO and higher FEV₁%predicted levels, whereas subjects with ≥2% sputum Eos are shifted to the right with higher FeNO levels although not necessarily lower FEV₁%predicted.

Figure 5.

Three dimensional distribution of subjects' age, asthma duration and blood Neu levels for subjects with sputum Neu < 40% (filled circles) or ≥ 40% (open circles). Subjects with < 40% sputum Neu are shifted left with younger age, shorter asthma duration and lower blood Neu, whereas subjects with ≥ 40% Neu, are shifted right with older age, longer asthma duration and more blood Neu.

Table I**Subject Demographics ***

	Mild	Moderate	Severe	P-Value
	N=134	N=123	N=71	
Sex (% female)	80%	64%	58%	0.001
Age	33 ± 11	39 ± 14	45 ± 12	<0.0001
Age at onset	14 ± 13	17 ± 16	20 ± 17	0.20
Duration	18 ± 12	23 ± 14	25 ± 17	0.015
Baseline FEV ₁ % predicted	93 ± 9	66 ± 12	66 ± 23	<0.0001
Baseline FVC % predicted	100 ± 10	79 ± 14	77 ± 20	<0.0001
FEV ₁ / FVC	0.79 ± 0.06	0.69 ± 0.1	0.67 ± 0.12	<0.0001
Maximum FEV ₁ % predicted	103 ± 8	81 ± 12	78 ± 21	<0.0001
FEV ₁ %Reversibility to 2 puffs B-agonist	12 ± 11	23 ± 22	19 ± 17	<0.0001
Log PC ₂₀ (geometric mean)	1.32 (N=128) **	0.79 (N=98) **	0.87 (N=42) **	0.047
FeNO	42 ± 48	38 ± 33	36 ± 38	0.43
No. + skin tests	4.1 ± 2.8	4.0 ± 2.9	3.7 ± 3.4	0.37
Blood Eos (K/ml)	0.24 ± 0.19	0.27 ± 0.2	0.26 ± 0.19	0.52
Blood Neu (K/ml)	3.7±0.002	3.6±0.001	4.1±0.001	0.10
IgE(IU/ml, geometric mean)	115	107	100	0.9
Frequency of Wheezing †	65/17/18	51/23/26	51/17/32	0.080
Frequency Nighttime Symptoms	68/16/16	64/13/23	49/20/31	0.055
Health Care Utilization, Past year %				
One or more urgent healthcare visits for asthma	30%	32%	51%	0.009
Emergency Department visits	15%	18%	39%	<0.0001
Hospitalized for asthma	2%	6%	17%	<0.0001
Health Care Utilization, Ever %				
Daily use B-agonist	30%	40%	49%	0.019
Symptoms worse if corticosteroid reduced	36%	36%	86%	<0.0001
Emergency Department visits	56%	66%	77%	0.01
Hospitalized for asthma	27%	37%	56%	<0.0001
Hospitalized in ICU	6%	11%	23%	0.002

* Data presented as mean±sd, median (25% to 75% quartiles), or % positive response. P values meeting significance in boldface

† Three categories, left to right, indicate % positive response for “never to <2x/week”, “>2x/week but <1x/day”, “daily”

** Subjects with FEV₁%predicted <50% did not undergo methacholine challenge.

Table II

Sputum Differential Cell Counts for Subjects Stratified by Asthma Severity *

	Mild (N=105)	Moderate (N=96)	Severe (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)	0.28
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)	0.32
WBC %	60 (38-83)	70 (41-81)	63 (46-82)	0.95
Bronchial Epith. %	2 (1-4.3)	4 (1-9)	3 (1-8.3)	0.02
Squamous Epith. %	37 (14-59)	26 (14-49)	26 (13-49)	0.42
Macrophage %	57 $\pm$ 2.6	50 $\pm$ 2.6	46 $\pm$ 3.7	0.028
Lymphocyte %	1.4 (0.58-2.80)	1.3 (0.60-2.90)	1.5 (0.40-2.85)	1.0
Neutrophil %	33.7 (13-59)	39 (22-63)	47 (23-68)	0.057
Eosinophil %	0.7 (0.2-3.3)	1.5 (0.4-4.8)	1.9 (0.3-5)	0.10

* Data presented as median (25% to 75% quartiles). P values meeting significance in boldface.

Table III

Summary ROC Curve Analyses:

	Youden J Index	Cutpoint	Sensitivity	Specificity	Accuracy	Positive Pred.Value	AUC	P value
<i>To Predict Sputum Eos <or 2%</i>								
Blood Eos (254) *	0.237	300	59%	65%	63%	50%	0.66	<0.001
FeNO (238)	0.323	30 ppb	65%	67%	66%	54%	0.72	<0.001
IgE (243)	0.277	120	66%	61%	63%	51%	0.64	0.037
Blood Eos+FeNO (107)	0.121	400	45%	67%	55%	62%	0.58	0.24
Blood Eos+IgE (144)	0.215	300	66%	55%	60%	57%	0.63	0.04
FeNO+ Blood Eos (103)	0.302	35 ppb	73%	58%	65%	63%	0.69	0.003
FeNO+ IgE (136)	0.216	30 ppb	69%	53%	60%	56%	0.66	0.003
<i>To Predict Sputum Neu<or 40%</i>								
Age (254)	0.318	40 yr	59%	73%	67%	64%	0.71	<0.001
Blood Neu (254)	0.132	3000	77%	37%	55%	50%	0.60	0.006
FEV₁%predicted (254)	0.164	90%	55%	62%	58%	63%	0.56	0.049
FEV₁%predicted+ maxFEV₁% reversal<15% (159)	0.191	90%	52%	67%	59%	64%	0.60	0.048
<i>To Predict Sputum Eos <or 3%</i>								
Blood Eos (238)	0.292	300	63%	66%	65%	47%	0.69	<0.001
FeNO (238)	0.287	30 ppb	65%	64%	64%	46%	0.71	<0.001
<i>To Predict Severe Sputum Eos <or 2%</i>								
Blood Eos (53)	0.418	300	63%	79%	72%	71%	0.75	0.004
FeNO (49)	0.378	30 ppb	61%	77%	69%	70%	0.77	0.014
<i>To Predict no ICS Sputum Eos <or 2%</i>								
Blood Eos (92)	0.243	200	82%	42%	54%	38%	0.62	0.13
FeNO (85)	0.346	40 ppb	63%	72%	69%	47%	0.74	0.002

* Number of subjects given in parentheses.