Biomarker-based asthma phenotypes of corticosteroid response

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Background: Asthma is a heterogeneous disease with different phenotypes. Inhaled corticosteroid (ICS) therapy is a mainstay of treatment for asthma, but the clinical response to ICSs is variable.
Objective: We hypothesized that a panel of inflammatory biomarkers (ie, fraction of exhaled nitric oxide [FENO], sputum eosinophil count, and urinary bromotyrosine [BrTyr] level) might predict steroid responsiveness.
Methods: The original study from which this analysis originates comprised 2 phases: a steroid-naive phase 1 and a 28-day trial of ICSs (phase 2) during which FENO values, sputum eosinophil counts, and urinary BrTyr levels were measured. The response to ICSs was based on clinical improvements, including a 12% or greater increase in FEV_1, a 0.5-point or greater decrease in Asthma Control Questionnaire score, and 2 doubling dose or greater increase in provocative concentration of adenosine 5′-monophosphate causing a 20% decrease in FEV_1 ($PC_{20}AMP$). Healthy control subjects were also evaluated in this study for comparison of biomarkers with those seen in asthmatic patients.
Results: Asthmatic patients had higher than normal FENO values, sputum eosinophil counts, and urinary BrTyr levels during the steroid-naive phase and after ICS therapy. After 28-day trial of ICSs, FENO values decreased in 82% of asthmatic patients, sputum eosinophil counts decreased in 60%, and urinary BrTyr levels decreased in 58%. Each of the biomarkers at the steroid-naive phase had utility for predicting steroid responsiveness, but the combination of high FENO values and high urinary BrTyr levels had the best power (13.3-fold, $P < .01$) to predict a favorable response to ICS therapy. However, the magnitude of the decrease in biomarker levels was unrelated to the magnitude of clinical response to ICS therapy.
Conclusion: A noninvasive panel of biomarkers in steroid-naive asthmatic patients predicts clinical responsiveness to ICS therapy. (J Allergy Clin Immunol 2015;135:877-83.)

Key words: *Asthma, inhaled corticosteroids, biomarker, clinical outcome, sputum eosinophils, urinary bromotyrosine, fraction of exhaled nitric oxide*

Inhaled corticosteroids (ICSs) are the mainstay of treatment for asthma. However, a considerable proportion of asthmatic patients do not respond to ICSs based on lung function,[1] other clinical outcomes, or both. The variability in response is attributed to different mechanisms underlying the airway inflammation.[2-4] Biomarkers relevant to the underlying pathophysiologic process, the response to treatment, or both would be useful in personalizing care of the asthmatic patient.[5]

Over the last decade, fraction of exhaled nitric oxide (FENO) values and sputum eosinophil counts have been used as biomarkers of airway inflammation and predictors of steroid responsiveness. FENO values are correlated with airway eosinophilia[6] and associated with airway hyperresponsiveness.[7] FENO values in healthy and asthmatic populations overlap, but FENO values are higher in asthmatic patients compared with those in healthy subjects.[8,9] Furthermore, studies indicate that high FENO values in asthmatic patients indicate an at-risk phenotype for exacerbation and predict clinical response to ICSs or oral corticosteroids.[9]

Eosinophils are important effector cells in asthmatic patients, and increased numbers in the sputum and peripheral blood are well recognized as biomarkers of active atopic inflammation.[10] Levels of eosinophilia identify clinical asthma phenotypes, such as eosinophilic and noneosinophilic asthma.[10] There is a relationship between sputum eosinophil counts and exacerbation on withdrawal of steroids.[2,11] Thus measurement of sputum eosinophilia represents a possible tool for adjusting asthma therapy to reduce exacerbations and is related to measures of airflow obstruction and bronchial hyperresponsiveness.[12] On activation, eosinophils undergo respiratory burst, generating high levels of reactive oxygen species,[13] eicosanoids, platelet-activating factor, and cytokines. Eosinophil peroxidase is unique in its ability to convert

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Abbreviations used
ACQ: Asthma Control Questionnaire
AMP: Adenosine 5′-monophosphate
AUC: Area under the curve
BrTyr: Bromotyrosine
FENO: Fraction of exhaled nitric oxide
ICS: Inhaled corticosteroid
LOC: Loss of control
m/z: Mass/charge ratio
OR: Odds ratio
$PC_{20}AMP$: Provocative concentration of adenosine 5′-monophosphate causing a 20% decrease in FEV_1

respiratory burst–generated hydrogen peroxide into hypobromous acid, a reactive brominating oxidant that modifies protein tyrosine residues forming urinary bromotyrosine (BrTyr).[13,14] Thus BrTyr is a biochemical fingerprint of eosinophil activation, and this highly stable product can be detected in blood and urine.

Despite many studies evaluating biomarkers in asthmatic patients, none have specifically set out to compare the variance of biomarkers in response to treatment or evaluated the utility of biomarkers in combination to define treatment response phenotypes. Several studies describe correlations between FENO values and sputum eosinophil counts.[6,7,15] However, others show that the sensitivity and specificity of the FENO value as a predictor of sputum eosinophilia are modest, and indeed, the relationship between FENO values and eosinophilia appears to be independent of asthma control.[16] Furthermore, anti–IL-5 therapy decreases sputum eosinophil counts but does not affect FENO values, suggesting that biomarkers can provide unique information about clinical responsiveness and mechanisms of inflammation.[5,17] In this context the combination of high urinary BrTyr levels and FENO values were found to be associated with greater odds of asthma, but only urinary BrTyr levels were associated with prediction of future asthma exacerbations in both pediatric and adult cohorts.[18,19] Altogether, current data indicate that the information provided by the biomarkers FENO, sputum eosinophils, and urinary BrTyr is not necessarily duplicative. They provide distinct insights into the underlying pathophysiologic mechanisms of disease and effects of treatment but, perhaps more importantly, might provide for biomarker-based phenotyping of clinical responders to treatment.

The purpose of this study was to determine whether a panel of inflammatory biomarkers (FENO values, sputum eosinophil counts, and/or urinary BrTyr levels) might accurately predict clinical responsiveness to ICSs. Biomarkers were measured in steroid-naive asthmatic patients in comparison with those in healthy control subjects and again after ICS therapy. Cut points for each biomarker and combinations of biomarkers that predict steroid responsiveness were determined. Finally, the change in each biomarker in response to ICS therapy was evaluated to investigate the correlation of the steroid effect on biomarkers and clinical response.

METHODS

Study population

Data and samples originated from 46 patients with stable persistent asthma between 18 and 75 years old who were enrolled in a previously described study.[20] Forty healthy control subjects were enrolled for comparison of baseline values of inflammatory biomarkers. Exclusion criteria for enrollment included respiratory tract infection in the preceding 4 weeks, a greater than 10 pack year smoking history or smoking in the previous 3 months, use of oral prednisone in the previous 3 months, history of life-threatening asthma, FEV_1 less than 50% of predicted value, other pulmonary disease, significant comorbidity likely to influence the conduct of the study, pregnancy, and breast-feeding.

Study design

The original study,[20] from which this secondary analysis originates, comprised 2 phases: a steroid-naive phase (phase 1) and an inhaled steroid phase (phase 2), an open-label trial of inhaled fluticasone (500 μg twice daily for 28 days).

Phase 1: Steroid-naive phase. ICSs and long-acting β-agonists were withdrawn from asthmatic patients for 28 days or until loss of control (LOC) occurred to achieve a steroid-naive state. Individualized criteria for LOC was based on modification of the criteria developed by Jones et al[15] and included any one of the following criteria: (1) decrease in mean morning peak expiratory flow by 10% or greater, (2) decrease in 2 consecutive morning or evening peak expiratory flows by greater than 20%, (3) increase in average daily bronchodilator requirement by 4 or more puffs, (4) increase of 2 or more nights in nocturnal wakening because of asthma, or (5) experience of asthma symptoms that are distressing/intolerable.[14] At LOC or after 28 days, whichever came sooner, all asthmatic patients underwent evaluation based on measurement of lung function,[21] bronchial hyperresponsiveness to adenosine 5′-monophosphate (AMP),[22] Asthma Control Questionnaire (ACQ) scores,[23] and biomarker values (FENO values,[24] sputum eosinophil counts,[25] and urinary BrTyr levels[18,19]).

Phase 2: Steroid treatment. During the steroid phase, asthmatic patients were given 500 μg of fluticasone (Flixotide; GlaxoSmithKline, Greenford, United Kingdom) twice daily by means of inhalation through a spacer for a period of 28 or more days, during which they completed a daily diary. After steroid treatment, subjects underwent evaluation by measurement of lung function,[21] bronchial hyperresponsiveness to AMP,[22] ACQ scores,[23] and biomarker values (FENO values,[24] sputum eosinophil counts,[25] and urinary BrTyr levels[18,19]).

Defining clinical responsiveness to ICS therapy. Steroid clinical responsiveness was defined as 1 or more of the following: 12% or greater increase in FEV_1,[26] 0.5-point or greater decrease in ACQ score,[23] or 2 doubling dose or greater increase in provocative concentration of AMP causing a 20% decrease in FEV_1 ($PC_{20}AMP$).[22]

Study procedures

A shortened 6-item version of the ACQ, a validated questionnaire for assessing asthma control that excluded measurement of FEV_1, was used.[23,27] Each item was scored on a 7-point scale (0-6), and a minimal clinically important change of 0.5 in the mean of the 6 items would justify a change in the patient's treatment (in the absence of undue side effects or excessive costs).[23]

Spirometry was performed with a rolling seal spirometer (SensorMedics, Yorba Linda, Calif) in accordance with American Thoracic Society/European Respiratory Society guidelines.[21]

Bronchial hyperresponsiveness to AMP was performed by using the standardized protocol of Polosa et al.[22] Briefly, on each challenge day, AMP doses (range, 0.59-300 mg/mL) were freshly prepared. Increasing doubling concentrations of AMP were delivered through a nebulizer connected to a breath-activated dosimeter (Morgan, Kent, United Kingdom) at 5-minute intervals, and spirometry was performed. $PC_{20}AMP$ values were determined by means of linear interpolation of the dose-response curve. AMP challenges in which a 20% decrease in FEV_1 was not achieved were assigned a $PC_{20}AMP$ value of 1200 mg/mL.

FENO values were measured with a chemiluminescence analyzer (NiOX MINO; Aerocrine, Stockholm, Sweden) before any forced expiratory maneuvers according to current guidelines at an exhaled flow rate of 50 mL/s.[24]

After sputum induction,[28] sputum eosinophil counts were obtained by using the standardized protocol of Fahy et al.[25] Briefly, total cell differentials were obtained by counting 400 nonsquamous cells. All cell counts were read and confirmed by 2 trained observers. A cut point of 2% or greater was used to define eosinophilic asthma, and a cut point of less than 2% was used to define noneosinophilic asthma.[3]

BrTyr levels were assayed, as previously reported, by using stable isotope dilution HPLC with online electrospray ionization tandem mass

spectrometry.[18,19] Briefly, synthetic [$^{13}C_6$]-BrTyr was used as an internal standard, and [$^{13}C_9$,$^{15}N_1$]-tyrosine was included to simultaneously monitor for potential artificial generation of analyte. Amino acids in urine were separated over a Prodigy C18 column (150 × 2.0 mm, 5 mm; Phenomenex, Torrance, Calif) at a flow rate of 0.2 mL/min by using a discontinuous gradient generated with mobile phases comprised of 0.2% formic acid in water (solvent A) and 0.2% formic acid in acetonitrile (solvent B). The mass spectrometer was operated in positive ionization mode. Multiple-reaction monitoring was used to detect unique precursor product transitions of both ^{79}Br and ^{81}Br isotopologues of each bromotyrosine (natural abundance and its isotopologues) by using unique mass/charge ratios (m/z) for the molecular cation $[MH]^+$ precursor and product ions as follows: [^{79}Br]Bromotyrosine, m/z 260→214; [^{81}Br]Bromotyrosine, m/z 262→216; [^{79}Br,$^{13}C_6$]Bromotyrosine, m/z 266→220; [^{81}Br,$^{13}C_6$]Bromotyrosine, m/z 268→222; [^{79}Br,$^{13}C_9$,$^{15}N_1$]Bromotyrosine, m/z 270→223; and [^{81}Br,$^{13}C_9$,$^{15}N_1$]Bromotyrosine, m/z 272→225. We adjusted for differences in urinary dilution based on spot urinary creatinine concentration, with BrTyr levels reported in BrTyr nanograms per milligram of creatinine. Under the conditions used for the assay, no artificial bromination was detected, and average spike and recovery was 101% (range, 98% to 105%); assay precision of less than 7% was noted across all concentration ranges examined.[18,19]

Atopy was determined by using skin prick testing and defined as at least 1 positive reaction (a weal of >2 mm) to the following allergens: cat pelt, grass mix, and house dust mite (Hollister-Stier Laboratories, Spokane, Wash).

Whole-blood IgE levels were measured by means of fluorescent Enzyme Immunoassay ImmunoCAP total IgE (Thermo Scientific, www.phadia.com) by Canterbury Healthy Laboratories (Christchurch, New Zealand).

Study measurements and statistical analyses

The characteristics of the study population are described as means and SEs. The paired *t* test was used to compare values obtained during the steroid-naive and steroid phases to evaluate significant changes in urinary BrTyr levels, sputum eosinophil counts, or FENO values in response to ICS treatment. Cut points were used to evaluate urinary BrTyr levels, sputum eosinophil counts, and FENO values as biomarkers for ICS clinical responsiveness. At the steroid-naive phase, cut points of sputum eosinophils of 3% or greater[29] and FENO values of 35 ppb or greater[30] were based on published data. A cut point for urinary BrTyr of 0.45 ng/mg of creatinine was selected as that maximizing the significance of the 2-group Wilcoxon rank sum test comparison (based on values at the steroid-naive and steroid phases). Clinical responsiveness was defined as significant improvements in ACQ, FEV_1, and PC_{20}AMP values based on cut points obtained from international guidelines. A comparison of clinical responsiveness between subjects defined by the biomarker cut points was made by using the Wilcoxon rank sum test. To further describe the relationships observed in the 2-group comparisons (2 groups: lower or higher than the biomarker's cut point), each clinical responsiveness outcome was assessed with respect to a threshold value, yielding a dichotomous form of the outcome. Logistic regression analyses were then used for each biomarker to estimate the odds ratio (OR) for the association between the biomarker level and the likelihood of achieving a clinical response at or greater than the threshold level. Receiver operating characteristic curves and their corresponding areas under the curve (AUCs) described visually the relationships between dichotomized forms of responsiveness with respect to thresholds of 1, 2, and/or 3 clinical outcomes and the continuous versions of urinary BrTyr levels, sputum eosinophil counts, and FENO values.

Ethical considerations and patient safety

Ethics approval was obtained from the Lower South Regional Ethics Committee of New Zealand (LRS/06/11/056, LRS/06/12/059).[20]

RESULTS

Subjects' characteristics

This study included a subset of 46 asthmatic patients from a prior cohort who had completed the steroid withdrawal and steroid phases (ICS treatment). In addition, healthy control subjects were studied. Baseline characteristics are shown in Table I. In this cohort 80.4% of the asthmatic patients had atopic asthma (Table I), and 65.2% were classified as having eosinophilic asthma (Table I and see Table E1 in this article's Online Repository at www.jacionline.org). Age, sex, weight, and height were not significantly different between control subjects and asthmatic patients (Table I).

TABLE I. Study population

	Asthmatic patients (steroid-naive phase)	Healthy control subjects
Sex (F/M)	29/17	21/19
Age (y)	39.8 (2.1)	35.4 (1.7)
Height (m)	1.70 (0.01)	1.72 (0.01)
Weight (kg)	78.5 (2.5)	86.7 (4.1)
Duration of asthma (y)	22.4 (2.4)	NA
On ICS at baseline (no/yes)	16/30	NA
BDP equivalent (μg)	1007.7 (93.5)	
Atopy (Y/N)*	37/9	11/29†
Serum IgE level (kU/mL)	451 (163)	86 (21)†
Eosinophilic asthma (Y/N)	30/16	
Lung function		
FEV_1 (L)	2.54 (0.12)	3.38 (0.12)†
FEV_1 (%)	76.2 (3.0)	95.0 (2.0)†
FVC (L)	3.97 (0.15)	4.2 (0.2)†
FVC (%)	98.5 (2.1)	97.1 (2.3)
FEV_1/FVC ratio	0.66 (0.02)	0.82 (0.01)†
PC_{20}AMP (mg/mL)	143.48 (51.8)	NA
Asthma questionnaire		
ACQ score	1.60 (0.15)	NA

Results are presented as means (SEs). Eosinophilic asthma was defined as more than 2% sputum eosinophils in the steroid-naive phase.
BDP, Beclomethasone dipropionate; *F*, female; *FVC*, forced vital capacity; *M*, male; *NA*, not applicable.
*Atopy is defined by at least 1 positive reaction (wheal, >2 mm) to the following allergens: cat pelt, grass mix, and house dust mite.
†$P < .05$ between control subjects and asthmatic patients.

Effect of ICSs on inflammatory biomarkers

Urinary BrTyr levels, FENO values, and sputum eosinophil counts were measured at the steroid-naive and steroid phases. The inflammatory biomarkers (urinary BrTyr and FENO) were significantly higher in all steroid-naive asthmatic patients compared with those in control subjects ($P < .05$ for all comparisons, Table II). The asthmatic patients had variable changes in biomarker levels with ICS treatment: 82% of the asthmatic patients had decreased FENO values, 60% had decreased sputum eosinophil counts, and 58% had decreased urinary BrTyr levels after ICS therapy. The asthmatic patients whose biomarker values did not decrease had baseline FENO values and urinary BrTyr levels greater than those seen in healthy control subjects, indicating that the lack of decrease in biomarkers was not due to having "normal" levels.

The changes in biomarkers in asthmatic patients treated with ICSs were not concordant (Fig 1). More than 50% of asthmatic patients had decreases in both FENO values and sputum eosinophil counts, but 30% of asthmatic patients had a significant decrease in FENO values but no change in sputum eosinophil counts (Fig 1, *A*). There was less concordance between FENO values and urinary BrTyr levels, but most asthmatic patients had decreases in one of the 2 of these biomarkers with ICS therapy (ie, only 3.8% did not have decreased in either FENO values or urinary BrTyr levels; Fig 1, *B*). Urinary BrTyr levels and sputum eosinophil counts had the least concordant change (Fig 1, *C*).

TABLE II. Measurements of FENO values, sputum eosinophil counts, and urinary BrTyr levels in steroid-naive and steroid-treated asthmatic patients and control subjects

		All asthmatic patients			Asthmatic patients with decreases in biomarker values				Asthmatic patients with no decrease in biomarker values		
	Control subjects	Steroid naive	Steroid treatment	Paired *t* test	Steroid naive	Steroid treatment	Paired *t* test	Responder (%)	Steroid naive	Steroid treatment	Paired *t* test
FENO value (ppb)	15.5 (1.1)	56.5 (5.6)*	25.8 (2.3)	<.0001	61.9 (6.5)*	22.7 (1.7)	<.0001	82	29.9 (7.2)*	39.8 (9.5)	.03
BrTyr level (ng/mg of creatinine)	0.28 (0.04)	0.53 (0.1)*	0.49 (0.06)	.43	0.63 (0.2)*	0.45 (0.1)	.006	58	0.44 (0.1)*	0.56 (0.07)	.006
Eosinophil count (%)	NA	18.9 (3.3)	9.9 (2.6)	.012	26.5 (4.6)	9.0 (2.7)	<.0001	60	4.8 (2.2)	12.8 (4.9)	.08

Results are presented as means (SEs).
NA, Not applicable.
*$P < .05$ for all comparisons with healthy control subjects, including steroid-naive and steroid-treated patients with or without a decrease in biomarker values.

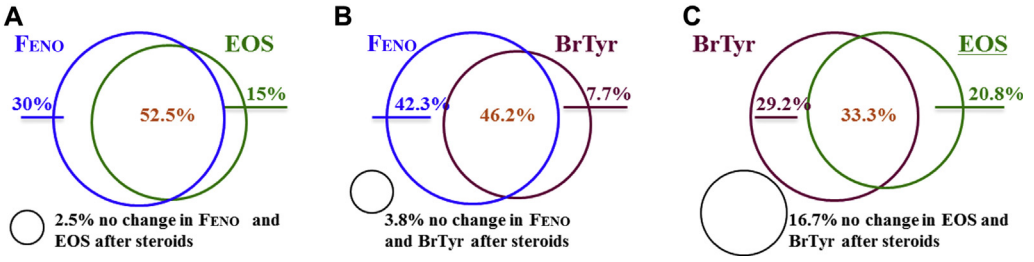

FIG 1. Changes in biomarker values in asthmatic patients treated with ICSs. The percentage of asthmatic patients with decreases in biomarker values with ICS treatment for 28 days is shown proportionately by the size of the *colored circles* (*blue*, FENO values; *green*, sputum eosinophil counts *[EOS]*; and *red*, urinary BrTyr levels), and the percentage of asthmatic patients who do not have decreases in values of either of the 2 biomarkers in each panel is shown by *black circles*.

Biomarkers as predictors of clinical response to ICS therapy

Receiver operating characteristic curve analyses were used to assess the utility of biomarker measurements during the steroid-naive phase for the prediction of clinical responsiveness to steroid. Clinical responsiveness was defined as improvements in clinical outcomes (≥12% increase in FEV_1 and/or ≥0.5-point decrease in ACQ score and/or ≥2 doubling dose increase in PC_{20}AMP value). Fig 2 shows the receiver operating characteristic curves for FENO values, sputum eosinophil counts, and urinary BrTyr levels as predictors of steroid response based on improvement in at least 2 of the 3 clinical outcomes. Furthermore, likelihood ratio tests confirmed that clinical responsiveness to steroid was associated with FENO values ($P = .004$, Wilcoxon test), sputum eosinophil counts ($P = .001$, Wilcoxon test), and urinary BrTyr levels ($P = .03$, Wilcoxon test). The ORs for dichotomized steroid responsiveness with improvement in 2 or 3 clinical outcomes with respect to baseline sputum eosinophil counts of 3% or greater were 9.20 (improvement in 2 clinical outcomes: 95% CI, 2.31-43.09; $P = .001$) and 10.50 (improvement in 3 clinical outcomes: 95% CI, 1.73-203.7; $P = .002$), respectively. Asthmatic patients with baseline FENO values of 35 ppm or greater had 10.5-fold greater likelihood of response to inhaled steroids, as measured by improvement in at least 3 clinical outcomes (2 clinical improvements: OR, 3.43; 95% CI, 0.93-413.54; $P = .004$; 3 clinical outcomes: OR, 10.50; 95% CI, 1.73-203.7; $P = .014$). The ORs with respect to a baseline urinary BrTyr level of 0.45 ng/mg of creatinine or greater were 6.22 (improvement in 2 clinical outcomes: 95% CI, 1.22-47.94; $P = .031$) and 1.5 (improvement in 3 clinical outcomes: 95% CI, 0.3-7.3; $P = .619$), respectively. Asthmatic patients with high FENO values (≥35 ppm) and high urinary BrTyr levels (≥0.45 ng/mg of creatinine) were 13 times as likely to have a positive response to steroid treatment, as measured by 2 clinical outcomes (2 clinical outcomes: OR, 13.3; 95% CI, 2.1-130.6; $P = .007$; 3 clinical outcomes: OR, 7.2; 95% CI, 0.9-153.5; $P = .076$; Fig 3).

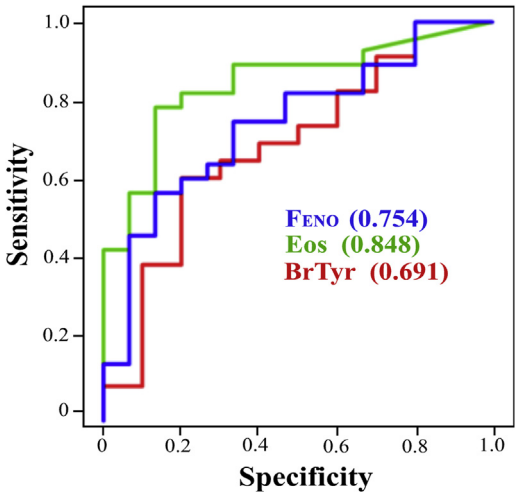

FIG 2. Receiver operating characteristic analysis describes the ability of each biomarker (ie, FENO values, sputum eosinophil counts, and urinary BrTyr levels) to classify asthmatic patients who respond to ICS therapy, as measured by 2 of 3 clinical outcomes (≥12% increase in FEV_1 and/or ≥0.5-point decrease in ACQ score and/or ≥2 doubling dose increase in PC_{20}AMP value).

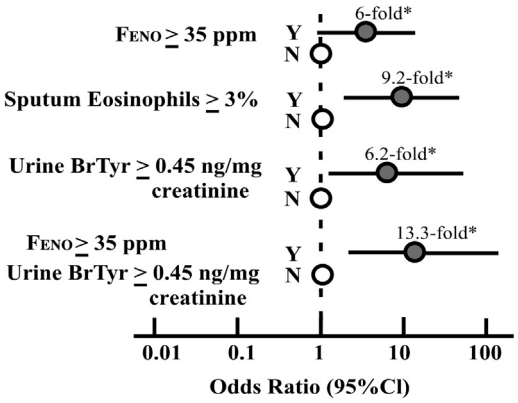

FIG 3. ORs and 95% CIs for the association between a high biomarker value (FENO value, ≥35 ppm; sputum eosinophil count, ≥3%; and urinary BrTyr level, ≥0.45 ng/mg of creatinine) or biomarker combination (FENO and urinary BrTyr) and 2 positive clinical outcomes in response to ICS therapy. Results shown represent the ORs *(solid circles)* and 95% CIs *(lines)* of having a steroid response versus having no steroid response. *Asterisks* indicate a *P* value of less than .05, as determined by using the likelihood ratio χ^2 test.

To evaluate whether the mechanism of clinical response might be attributed to a biomarker inflammation pathway, we evaluated the numbers of asthmatic patients within subgroups defined by levels greater than the cut points that are clinically relevant to each steroid response and who also experienced a decrease in biomarker levels. Interestingly, 90% of the steroid-naive asthmatic patients with FENO values of 35 ppm or greater had decreased FENO values after ICS therapy, 85% with sputum eosinophils of 3% or greater had decreased sputum eosinophil counts after ICS therapy, and 59% with urinary BrTyr levels of 0.45 ng/mg of creatinine or greater had decreased urinary BrTyr levels after ICS therapy. Although the majority of asthmatic patients experience a decrease in biomarker levels, there was no association between the magnitude of decrease in biomarker levels and the magnitude of clinical response. However, the study might be underpowered to detect this type of association, and a larger study may confirm such an association.

DISCUSSION

This is the first study to compare the utility of a panel of biomarkers that identify the presence of atopic inflammation and oxidative stress for prediction of clinical response to steroids. The effect of ICSs on inflammatory biomarkers (ie, sputum eosinophil counts, FENO values, and urinary BrTyr levels) was not uniformly concordant, although there were substantial parallel decreases among biomarkers. Each of the biomarkers had utility for predicting steroid responsiveness; the combination of high FENO values and high urinary BrTyr levels had particular power to predict a favorable clinical response to ICS therapy with either improvement in ACQ score, FEV_1, or airway reactivity.

This study allows direct comparison among biomarkers in response to ICS treatment. The majority of asthmatic patients experienced a decrease in FENO values with ICS therapy, and this was by far the most consistent biomarker to decrease compared with either sputum eosinophil counts or urinary BrTyr levels. FENO values are traditionally considered a surrogate marker of eosinophilic asthma.[6] Likewise, the end products of protein bromination, such as BrTyr, are considered to reflect eosinophil activation.[13,14] The concept that the eosinophil plays a role in asthma is long established, as is the clinical benefit of corticosteroids in patients with eosinophilic asthma in association with suppression of eosinophilic inflammation.[10,31] Treatment strategies based on sputum eosinophil counts lead to decreases in exacerbations without increased treatment, at least in adults.[2,32,33] Despite the fact that these biomarkers provide information on atopic inflammation, there was only partial concordance in response to ICSs of the 3 inflammatory biomarkers in this study. This might be related to the fact that FENO values correlate with bronchial mucosal eosinophilia rather than luminal (sputum) eosinophilia.[34] Epithelial inducible nitric oxide synthase has been shown to be the main determinant of FENO,[35] and it is postulated that the decrease in FENO values seen after steroid treatment might be due to an inhibitory effect of steroids on inducible nitric oxide synthase induction, perhaps irrespective of airway eosinophilia. Atopy might be an important contributor to FENO values independent of eosinophils, so that FENO values might remain increased in atopic subjects despite steroid suppression of eosinophilic airway inflammation.[26] Finally, FENO measurement does not allow an estimate of alveolar and airway contributions,[36] such that localization of this inflammatory signal within the lower respiratory tract is not possible. Other studies suggest discordance between biomarkers. Notably, the administration of mepolizumab in patients with refractory eosinophilic asthma led to a decrease in sputum eosinophil counts but no change in FENO values.[17] A study in children found marked discordance in the longitudinal relationship between sputum eosinophil counts and FENO values.[37] Finally, in another study no correlation was found between urinary BrTyr levels and FENO values.[19] Therefore it is possible that sputum eosinophil counts, FENO values, and urinary BrTyr levels are not as closely linked as previously thought and that they might represent biomarkers of different underlying pathophysiologic mechanisms.

Sputum eosinophil counts have utility in the prediction of steroid response[2,6,29,38,39] and LOC[2,40] and in titrating the steroid dose to minimize exacerbations.[33] However, sputum induction is unpleasant for the patient, sputum processing and cell analysis is a time-consuming procedure requiring technical expertise, and the test is not widely available. Furthermore, the performance characteristics of sputum eosinophil counts for the prediction of steroid responsiveness are modest. Combining sputum eosinophil counts and FENO values might improve this prediction. In this study the finding of high sputum eosinophil counts was a good predictor of steroid responsiveness, irrespective of whether defined by improvement in 2 (AUC, 0.848) or 3 (AUC, 0.749) clinical outcomes. Both sputum eosinophil counts and FENO values individually, but not urinary BrTyr levels, had AUCs of greater than 0.7, indicating clinically significant utility in prediction of steroid responsiveness, irrespective of whether defined by improvement in 2 or 3 clinical outcomes. Previously, we have shown that the urinary BrTyr level, a noninvasive marker of oxidant stress and eosinophil activation, is increased in asthmatic patients and predicts exacerbation in both pediatric and adult asthma populations.[18,19] The combination of high FENO values and urinary BrTyr levels was associated with the greatest likelihood of clinical response to ICS therapy. This suggests that these 2 biomarkers used in combination might have superior clinical utility in the prediction of steroid responsiveness, thus avoiding the need for sputum induction, processing, and analysis and the associated discomfort for the patient.

A limitation of this study is that the ICS trial was not placebo controlled. This was for reasons of safety. Seventy percent of participants demonstrated LOC within 28 days after steroid withdrawal. It would have been unethical to allow these subjects

to proceed for a further 28 days beyond the point of LOC on placebo treatment. As a consequence, we conclude that some of the changes seen in association with steroid treatment might be explained to some degree by regression to the mean. In particular, we cannot exclude the possibility that changes seen in sputum eosinophil counts did not reflect regression to the mean or variation with time, although the latter in particular seems unlikely given the reproducibility of sputum cell measurements over time.[41] In addition, we were unable to assess the predictive power of biomarkers for other important outcomes, such as risk of exacerbations. Previous studies suggest that sputum eosinophil counts,[42] FENO values,[9] and, at least in children, urinary BrTyr levels[19] might have a role in assessing the risk of exacerbation in asthmatic patients. Finally, although the majority of asthmatic patients experienced a decrease in biomarker levels, there was no association between the magnitude of decrease in biomarker values and magnitude of clinical response. However, the study might be underpowered to detect this type of association, and a larger study could confirm such an association. Future studies will focus on evaluation of biomarker panels for assessment of exacerbation risk and whether the magnitude of change in biomarker values might predict the magnitude of clinical benefit with treatments.

Key messages

- **Asthmatic patients have variable and nonconcordant decreases in sputum eosinophil counts, FENO values, and urinary BrTyr levels in response to ICS therapy.**
- **Each biomarker at baseline was predictive of clinical steroid responsiveness, but the combination of high FENO values and high urinary BrTyr levels had the greatest power to predict a favorable response to ICSs.**

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TABLE E1. Baseline characteristics at the end of the steroid-naive phase for all patients who completed the steroid phase based on eosinophilic asthma

	Eosinophilic asthma (n = 30)	Noneosinophilic asthma (n = 15)
Sex (F/M)	18/12	11/4
Age (y)	41.3 (2.4)	37.8 (3.9)
Height (m)	1.69 (0.01)	1.71 (0.02)
Weight (kg)	78.5 (3.0)	78.9 (4.6)
Duration of asthma (y)	23.8 (2.9)	19.7 (4.4)
On ICS at baseline (no/yes)	6/24	5/10 ACQ*
BDP equivalent (μg)	783.3 (113.6)	313.3 (147.6)*
Atopy (no/yes)*	7/24	4/11
Serum IgE level (kU/mL)	516.2 (229.9)	322.5 (133.8)
Lung function		
FEV_1 (L)	2.37 (0.14)	2.87 (0.21)*
FEV_1 (%)	72.1 (3.4)	85.0 (5.2)*
FVC (L)	3.98 (0.21)	3.93 (0.21)
FVC (%)	100.3 (2.5)	95.1 (3.8)
FEV_1/FVC ratio	0.63 (0.02)	0.72 (0.02)*
PC_{20}AMP (mg/mL)	75.1 (46.9)	270.6 (114.9)
Asthma questionnaire		
ACQ score	1.82 (0.18)	1.02 (0.17)*
Urinary BrTyr (ng/mg of creatinine)	0.60 (1.2)	0.43 (0.06)
F_{ENO}	65.0 (7.5)	38.1 (6.0)*
Sputum eosinophil (%)	28.1 (3.9)	0.6 (0.2)*

BDP, Beclomethasone dipropionate; *F*, female; *FVC*, forced vital capacity; *M*, male.
*Atopy is defined by at least 1 positive reaction (wheal, >2 mm) to the following allergens: cat pelt, grass mix, and house dust mite. $P < .05$ between control subjects and asthmatic patients.