Biomarker-based asthma phenotypes of corticosteroid response

Douglas C. Cowan, BMedSci, MBChB, MRCP, PhD,[a] D. Robin Taylor, MD, DSc, FRCP (Ed),[a] Laura E. Peterson, BA,[b] Jan O. Cowan,[a] Rochelle Palmay,[a] Avis Williamson,[a] Jef Hammel, MS,[b] Serpil C. Erzurum, MD,[b,c] Stanley L. Hazen, MD, PhD,[d] and Suzy A. A. Comhair, PhD[b]

Dunedin, New Zealand, and Cleveland, Ohio

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

From [a]the Dunedin School of Medicine, University of Otago, and the Departments of [b]Pathobiology and [d]Cellular and Molecular Medicine, Lerner Research Institute, and [c]the Respiratory Institute, Cleveland Clinic.

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Corresponding author: Suzy A. A. Comhair, PhD, Cleveland Clinic, Lerner Research Institute, Department of Pathobiology, NC2-130, 9500 Euclid Ave, Cleveland, OH 44195. E-mail: comhais@ccf.org.

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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