Lung function decline and variable airway inflammatory pattern: Longitudinal analysis of severe asthma

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Background: Eosinophilic airway inflammation measured by using induced sputum is an important treatment stratification tool in patients with severe asthma. In addition, sputum eosinophilia has been shown to be associated with severe exacerbations and airflow limitation.

Objectives: We sought to identify whether eosinophilic inflammation in sputum is associated with FEV_1 decrease in patients with severe asthma and whether we could identify subgroups of decrease behavior based on the variation of eosinophilic airway inflammation over time.

Methods: Ninety-seven patients with severe asthma from the Glenfield Asthma Cohort were followed up with scheduled 3-month visits; the median duration of follow-up and number of visits was 6 years (interquartile range, 5.6-7.6 years) and 2.7 visits per year. Induced sputum was analyzed for eosinophilic inflammation at scheduled visits. Linear mixed-effects models were used to identify variables associated with lung function and overall decrease. In addition, using individual patients' mean and SD sputum eosinophil percentages over time, a 2-step cluster analysis was performed to identify patient clusters with different rates of decrease.

Results: FEV_1 decrease was −25.7 mL/y in the overall population. Postbronchodilator FEV_1 was also dependent on exacerbations, age of onset, height, age, sex, and $\log_{10}$ sputum eosinophil percentages ($P < .001$). Three decrease patient clusters were identified: (1) noneosinophilic with low variation (mean decrease, −14.0 mL/y), (2) eosinophilic with high variation (mean decrease, −40.9 mL/y), and (3) hypereosinophilic with low variation (mean decrease in lung function, −19.2 mL/y).

Conclusion: The amplitude of sputum eosinophilia was associated with postbronchodilator FEV_1 in asthmatic patients. In contrast, high variability rather than the amplitude at baseline or over time of sputum eosinophils was associated with accelerated FEV_1 decrease. (J Allergy Clin Immunol 2014;134:287-94.)

Key Words: *Severe asthma, airway inflammation, lung function decline, eosinophilia, induced sputum*

Asthma is a common disease that affects approximately 300 million persons worldwide and has a prevalence of between 7% and 15% in European nations.[1] A proportion of patients with asthma (≤10%) have severe persistent disease and are at a particularly high risk of exacerbations, hospitalization, and death and often have severely impaired quality of life.[2] Although this group represents a relatively small proportion of the asthmatic population, they consume two thirds of the health care costs attributed to asthma.[3]

Eosinophilic airway inflammation is an important biomarker in patients with severe asthma. Sputum eosinophilia is associated with a favorable response to both inhaled and oral corticosteroids in asthmatic patients.[4] A number of previous reports have identified that strategies aimed at optimizing eosinophil counts in sputum with corticosteroids significantly reduce the frequency of asthma exacerbations.[5,6] More recently, targeting eosinophilic airway inflammation in sputum selectively with an mAb to IL-5 (mepolizumab) has been shown to significantly reduce the frequency of asthma exacerbations and might allow downtitration of oral corticosteroids in patients with refractory asthma.[7-9] In addition, we have identified phenotypes of severe asthma enriched for eosinophilic airway inflammation that might respond to therapies directed toward T_H2 immune pathways. Therefore induced sputum eosinophils are important stratification and risk biomarkers in patients with severe asthma.[10]

A number of previous studies have identified that severe asthma is associated with an accelerated rate of lung function, lung function decline with exacerbations,[11] disease duration, cigarette smoking,[12] sex, inhaled corticosteroid therapy,[13] and $CD8^+$ T-cell infiltration[14] of the airway wall being associated with

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Abbreviations used
BDP: Beclomethasone dipropionate
BIC: Bayesian information criterion
LME: Linear mixed effects

lung function decline in adult patients. Immunopathologic studies in patients with severe asthma have demonstrated that the presence of sputum or tissue eosinophilia is associated with remodeling of the airway wall and airflow limitation.[15,16] In addition, 2 reports have identified sputum eosinophilia cross-sectionally as being associated with impaired lung function.[17,18]

However, the presence of eosinophils in sputum might be highly variable and reflect fluctuation in immune activation, natural biological variation, and/or the rate of passage from the tissue compartment to the luminal airway compartment. This is important because airway inflammation measured based on induced sputum[19] and exhaled nitric oxide[20] in asthmatic patients is known to undergo temporal oscillations, which might influence important clinical outcomes, such as exacerbations. Therefore it is possible that therapeutic strategies targeting T_H2 inflammation in asthmatic patients could benefit from optimizing both the amplitude and variability of eosinophilic airway inflammation over time. It is currently unknown whether either the amplitude or variation over time in patients with sputum eosinophilia is associated with lung function decline in patients with severe asthma. Furthermore, the rate of lung function decline in phenotypic subgroups of the population with severe asthma stratified by airway inflammation has not been clearly examined.

We hypothesized that (1) lung function in patients with severe asthma is associated with eosinophilic airway inflammation when accounting for common cofactors, such as exacerbations, that are known to influence lung function decline trajectories and (2) phenotyping patients based on both the amplitude and variation in sputum eosinophil counts over time would identify populations associated with a differential rate of FEV_1 decrease. We tested these hypotheses in a population with severe asthma derived from patients attending the Glenfield Hospital Difficult Asthma Clinic.

We selected postbronchodilator FEV_1 percent predicted as the *a priori* lung function trait of interest to evaluate airway structural changes rather than lung function effects that might have been confounded by airway smooth muscle tone, as would be expected with prebronchodilator spirometry.

METHODS

Patients

We identified patients from our Leicester Difficult Asthma Database cohort at Glenfield Hospital who met the following screening criteria: (1) a physician's diagnosis of asthma with objective evidence (≥1 peak flow variation of ≥20% over a 2-week period, bronchodilator reversibility of ≥12%/200 mL, or airway hyperresponsiveness [methacholine PC_{20} ≤8 mg/mL]); (2) a minimum of 5 years of follow-up with scheduled 3-month visits assessing airway inflammation based on induced sputum and postbronchodilator spirometric results; (3) a minimum of 3 or more visits with sputum cell counts and postbronchodilator FEV_1 measurements over the follow-up period; and (4) complete baseline demographic data (Fig 1).

All patients had a less than 10 pack-year smoking history.

Our Difficult Asthma Database population consisted of 908 patients with asthma (Table I). From this population, 97 patients had 5 or more years of follow-up and at least 3 visits with sputum and postbronchodilator lung function measurements. To benchmark our population to a reference population with refractory asthma, we extracted data from patients who met the American Thoracic Society workshop consensus criteria[21] for refractory asthma (minus the lung function decline population) as a comparator group (Table I). Our lung function decline population was matched for body mass index, sex, and all lung function metrics to the American Thoracic Society refractory asthma group; this is important with respect to the potential for selection bias of lung function traits. In contrast, our lung function decline population was typical of the population that would be recruited into phase 2/phase 3 studies of T_H2-targeted strategies (eg, mepolizumab[7,8] and lebrikizumab[22]) and patients described with refractory asthma in a recent United Kingdom registry population.[23] Specifically, the population was a mixture of patients with early- and later-onset eosinophilic asthma, primarily never smokers, receiving high-dose inhaled corticosteroids and in some cases systemic steroids and add-on therapy. Therefore it would appear that our lung function decline population differed from the refractory asthma database cohort with respect to these parameters, which is not surprising in view of our sputum management protocol, which aims to optimize eosinophilic airway inflammation and therefore might selectively retain such patients.

Approval from the Leicestershire, Northamptonshire, and Rutland research ethics committee was obtained for data analysis of the clinical database for patients attending the Glenfield Hospital Difficult Asthma Clinic.

Asthma management protocol

Patients were managed with a protocol directed at normalizing eosinophilic airway inflammation measured in induced sputum, which was adopted by our clinical service after the report of Green et al.[5] Scheduled clinical assessments with sputum and lung function occurred at 3-month intervals over the lung function decline follow-up period. In the sputum management group decisions about anti-inflammatory treatment were made in accordance with an algorithm based on maintenance of a sputum eosinophil count of less than 3% with a minimum of anti-inflammatory therapy. The threshold of 3% was chosen because it identifies patients with corticosteroid-responsive asthma.[4] If the sputum eosinophil count was less than 1%, we reduced anti-inflammatory treatment irrespective of asthma control. If the sputum eosinophil count was 1% to 3%, no changes were made to anti-inflammatory treatment, and if it was greater than 3%, we increased anti-inflammatory treatment. Modification of individual bronchodilator therapy was performed according to traditional assessments of symptoms, peak expiratory flow, and use of β-agonists.

Baseline investigations

Patients were phenotyped[10] by undergoing extensive evaluation at baseline, including a full medical history, height, weight, allergy, smoking status, and lung function testing with bronchial challenge using methacholine, where appropriate, and sputum inflammometry.[24]

Allergy testing

Atopic status was assessed by using both skin prick tests to the common airborne allergens cat, dog, house dust mite, and *Aspergillus fumigatus* (ALK-Abelló, Hørsholm, Denmark) and total and specific IgE testing. Sensitization was defined as having positive skin test results with a wheal of 3 mm or greater or a positive IgE level of 0.35 kU/L or greater by using the UniCAP250 system (Pharmacia, Milton Keynes, United Kingdom) in patients taking regular antihistamines.

Pulmonary function testing

For each clinic visit, patients underwent standard spirometry (Vitalograph, Maids Moreton, United Kingdom), according to American Thoracic Society/European Respiratory Society guidelines,[25] with reversibility after 15 minutes by using 200 μg of inhaled salbutamol administered through a spacer.

Sputum induction

Sputum induction was performed with previously described methods.[24] Before testing, patients inhaled 200 μg of salbutamol. Patients were asked to blow the nose and rinse the mouth with water. Sputum induction was undertaken with normal saline administered through an ultrasonic nebulizer,