

DIAGNOSIS

Endobronchial Biopsy: A Guide for Asthma Therapy Selection in the Era of Bronchial Thermoplasty

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Objective. Bronchial thermoplasty (BT) reduces airway smooth muscle in patients with severe asthma. We developed a novel standardized histologic grading system assessing inflammation and structural remodeling on endobronchial biopsy (EBBx) in severe persistent asthma and evaluated airway structure before and after BT. In addition, we correlated invasive and non-invasive inflammatory markers in severe persistent asthma. **Methods.** Thirty-three patients with severe persistent asthma underwent bronchoscopy, including bronchoalveolar lavage (BAL) and diagnostic EBBx. The control group (N = 41) underwent EBBx for other clinical indications. Biopsies were graded for airway inflammation and epithelial and submucosal structural features. We also evaluated airway histology in three patients before and after BT. **Results.** Compared to the control group, patients with severe persistent asthma more often had intraepithelial eosinophils and lymphocytes (67% vs. 17% and 61% vs. 27%; $p < .001$ and $p = .005$, respectively) and prominent smooth muscle and goblet cell hyperplasia (88% vs. 29% and 47% vs. 22%, $p < .001$ and $p = .004$, respectively). Other features including epithelial denudation and basement membrane thickening were not significantly different. Following BT, airway smooth muscle was no longer prominent due to partial replacement by fibrosis. Increased submucosal eosinophilic inflammation and BAL eosinophilia correlated with exhaled nitric oxide (eNO, $p = .05$ for both). **Conclusions.** We developed a clinically applicable standardized histologic grading system which identified structural but not inflammatory changes before and after BT in severe persistent asthmatics. Additionally, we demonstrated that eNO is representative of submucosal eosinophilia in this population. This semi-quantitative assessment will be useful for practicing pathologists assessing EBBx from severe persistent asthma patients for diagnostic and clinical research purposes.

Keywords bronchoscopy, inflammation, pathology, smooth muscle

INTRODUCTION

Severe persistent asthma remains a challenging clinical problem and treatment requires an evaluation to exclude alternate diagnoses and non-adherence, followed by evidence-based treatment intensification (1). In 2010, the Food and Drug Administration in the United States approved bronchial thermoplasty (BT) for the management of severe asthma (2). BT uses radiofrequency energy delivered during bronchoscopy to the airway walls. Clinical trials have demonstrated improvement in asthma control and reduction in exacerbations following BT (3), and the safety and efficacy of BT have been shown in patients with severe asthma (3–9). However, the mechanism by which BT improves asthma control is not well understood. Pre-clinical data suggest that BT may selectively reduce airway smooth muscle mass, thereby reducing airway hyperreactivity (10). To our knowledge, no

studies have evaluated airway histology in severe persistent asthma patients before and after BT.

Exhaled nitric oxide (eNO), a non-invasive marker of airway inflammation, has been increasingly used in clinical research to guide treatment decisions for asthma, despite reported inconsistencies in its utility (11, 12). Comparisons of eNO with inflammation in airway compartments are lacking. There has also been a recent interest in the inflammatory phenotypes of asthma from induced sputum, however, correlations of inflammation in induced sputum to bronchoalveolar lavage (BAL) or biopsy are also lacking.

While the basic histopathologic changes associated with structural remodeling in asthma (e.g., sub-basement membrane fibrosis, increased smooth muscle content, mucous gland hyperplasia) are widely recognized, there is surprisingly no histopathologic grading system for endobronchial biopsy (EBBx) for use in the clinical setting. A histopathologic grading system for small airways assessment has been described and utilized in the setting of chronic obstructive pulmonary disease, smoking, and other inhaled particulates (13). With the current trend for EBBx in severe asthma assessment, our system will be helpful to pathologists and clinicians collaborating in the care of patients with severe persistent asthma and in research studies in this

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population, including classification of severe asthma phenotypes.

With the diverse treatment options available for severe asthma, appropriate patient selection using EBBx may help guide the selection of interventions addressing the inflammatory or structural abnormalities that occur in this condition, leading to a more personalized approach to therapy. For example, a recent study demonstrated how bronchoscopy could help identify patients who may benefit from omalizumab to suppress eosinophilic airway inflammation (14).

The goal of our study was to develop a novel standardized histologic grading system for the clinical setting to assess both the inflammatory and structural abnormalities in diagnostic EBBx in patients with severe persistent asthma. This will be useful for clinicians interested in personalized medicine for patients with asthma. Exploratory goals included using this system to evaluate airway abnormalities in three patients with severe persistent asthma before and after BT and to describe and correlate patterns of airway inflammation in severe persistent asthma patients using non-invasive (eNO) and invasive measures (BAL and EBBx). This is the first description of the histologic findings in asthma patients before and after BT.

METHODS

Patients aged ≥ 18 years referred to the Refractory Obstructive Lung Disorders Center for severe persistent asthma over 5 consecutive years between January 2007 and December 2011 were assessed by history, physical examination, routine laboratory studies, and spirometry with flow volume loops. Severe persistent asthma was defined using the National Asthma Education and Prevention Program guidelines (1), and required ongoing respiratory symptoms (at least 2 days of respiratory symptoms or at least one nocturnal awakening due to respiratory symptoms over the past 2 weeks) despite at least 80% adherence to medium or high-dose inhaled corticosteroids (fluticasone) plus salmeterol delivered by the Diskus® device (GlaxoSmithKline, Research Triangle Park NC). Adherence was based on objectively measured Diskus® dose counter data over a 2-week period. Exclusion criteria were a smoking history of >10 pack-years, evidence of vocal cord dysfunction by history or flow-volume loops, laboratory screen suggestive of connective tissue disorder, allergic bronchopulmonary aspergillosis, or Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, or radiographic evidence of an alternate pulmonary disorder on high-resolution chest computed tomography (e.g., interstitial lung disease, bronchiectasis) (e-Table 1). Pulmonary function test results and eNO levels, gathered according to standard guidelines (15), were used from the timepoint most immediately prior to the bronchoscopic biopsy.

Patients included in this study underwent a diagnostic bronchoscopy (16) with collection and analysis of BAL fluid and EBBx to exclude alternate diagnoses, including sarcoidosis, airway infection, and hypereosinophilic

syndromes (17). Details of the procedure can be found in the Supplementary Material. Four to six biopsies of the right lower lobe were taken at the carinas between RB8a and RB8b, RB9a and RB9b, and RB8 and RB9. There were no complications due to the EBBx. BT was performed on selected patients ($N = 3$) using the Alair System (Asthmatx, Inc., Sunnyvale CA) as described elsewhere (4, 10, 18). Selection criteria are detailed in the Supplementary Material. The mean (range) of activations per lobe were: right lower lobe, 50 (32–75); left lower lobe, 40 (30–58); and bilateral upper lobes, 54 (45–65). Post-BT EBBx were obtained during a subsequent thermoplasty procedure after an interval of 6 weeks in two patients and 3 weeks in one patient, from prior BT-treated areas in the right lower lobe. One patient had minor bleeding after post-BT EBBx. There were no serious adverse events or complications from the BT procedure or the post-BT EBBx procedure.

A standardized grading synoptic form for EBBx was developed based on inflammatory and airway remodeling histopathologic features thought to be important in asthma, including the presence (grade 1) or absence (grade 0) of intraepithelial inflammation, epithelial changes, basement membrane thickening, prominent airway smooth muscle and submucosal mucous glands, and irregularity of elastic fibers. Submucosal inflammation for each cell type was graded as absent, rare, mild, moderate, or severe (grade 0 through 4, respectively) based on arbitrary cut-offs. The detailed grading synoptic form can be found in the Supplementary Material (e-Figure 1). Diagnostic hematoxylin and eosin (H&E)-stained glass slides from formalin-fixed paraffin-embedded EBBx were systematically assessed and graded by two pulmonary pathologists (IOG and ANH). To determine which parameters of the grading system were most specific for severe persistent asthma patients, a group of control EBBx from a single calendar year performed for any other indication were also evaluated using the same grading system.

Descriptive statistics used means, standard deviations, and proportions. Spearman's ρ , Cuzick's non-parametric trend test, and Fisher's exact test were used to test for bivariate measures of association. A two-tailed p -value of less than .05 defined statistical significance. Computations were performed using STATA version 12.1SE (StataCorp, College Station, TX). The study was approved by the University of Chicago Institutional Review Board and included written informed consent (protocol #09-334B and #15543).

RESULTS

Clinical Characteristics (Table 1)

Study participants with severe persistent asthma ($N = 33$) had a mean age of 47 years and 82% of the participants were women. Histopathologic comparisons were made to a control group of diagnostic EBBx from patients undergoing bronchoscopy for any indication other than asthma ($N = 41$).

TABLE 1.—Clinical characteristics of severe persistent asthma patients and histologic controls.

Clinical diagnosis	Severe persistent asthma	Histologic controls
Number of patients (<i>n</i>)	33	41
Average age at biopsy (years)	47 ± 15	61 ± 13
Female (%)	82	54
Pre-bronchodilator FEV ₁ , % predicted*	64 ± 25	N/A
Post-bronchodilator FEV ₁ , % predicted*	69 ± 23	N/A
Pre-bronchodilator FEV ₁ /FVC, actual*	65 ± 14	N/A
Indication for biopsy		
Severe asthma (%)	100	0
Mass or nodules (%)		66
Rule out sarcoid, amyloid, infection, malignancy (%)		27
Other pulmonary symptoms (%)		7

Note: *Expressed as mean ± SD.

Epithelial Features (Figure 1)

More patients with severe persistent asthma had goblet cell hyperplasia (47% vs. 22%, $p = .004$), intraepithelial eosinophils (67% vs. 17%, $p < .001$), and intraepithelial lymphocytes (61% vs. 27%, $p = .005$). The presence of epithelial denudation, intraepithelial neutrophils, reserve cell hyperplasia, squamous metaplasia, and dysplasia of the epithelium were similar between the groups.

Subepithelial Structural Features (Figure 1)

Patients with severe persistent asthma were more likely to have prominent airway smooth muscle (88% vs. 29%, $p < .001$), but less likely to have prominent submucosal mucous glands (0% vs. 15%, $p = .030$). The presence of irregular elastic fibers and sub-

basement membrane fibrosis was not significantly different between the groups.

Submucosal Inflammation (Figure 2)

Submucosal eosinophilic airway inflammation was more common in patients with severe persistent asthma (79% vs. 54% with more than rare eosinophils, $p < .05$). Two or fewer submucosal eosinophils per high power field (i.e., the upper limit of grade 1) are not specific for severe persistent asthma.

Inflammation Relationships (Table 2 and e-Figure 2 in Supplementary Material)

Average eNO correlated with BAL eosinophilia in severe persistent asthma ($p = .049$) and both were related to submucosal eosinophils ($p = .045$ and $p < .001$,

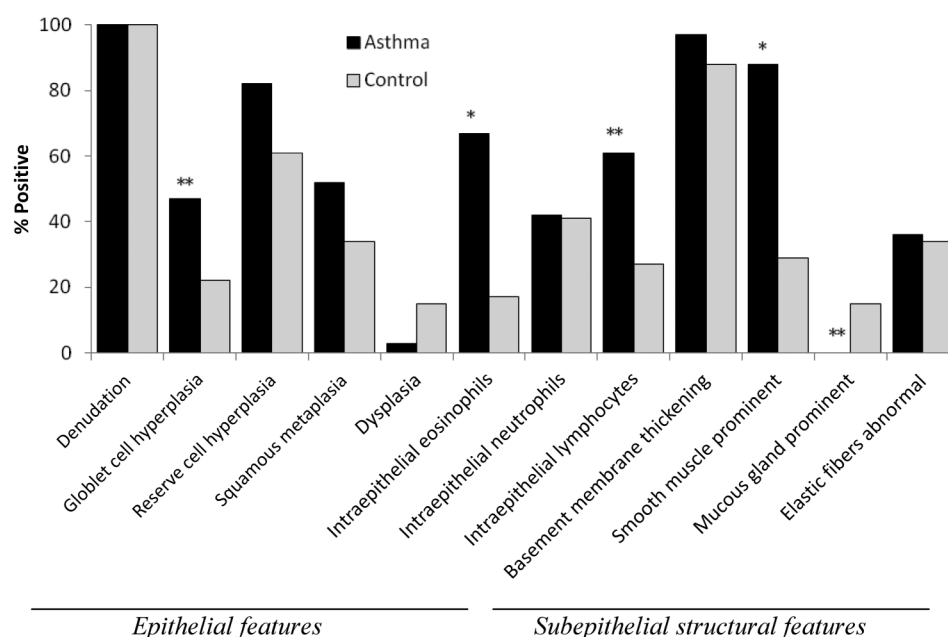


FIGURE 1.—Structural and inflammatory characteristics on endobronchial biopsy. Compared to controls, more severe persistent asthma patients had goblet cell hyperplasia, intraepithelial eosinophils, intraepithelial lymphocytes, and prominent smooth muscle, and fewer severe persistent asthma patients had prominent mucous glands.

Notes: *Significant compared to control ($p < .001$). **Significant compared to control ($p < .05$).

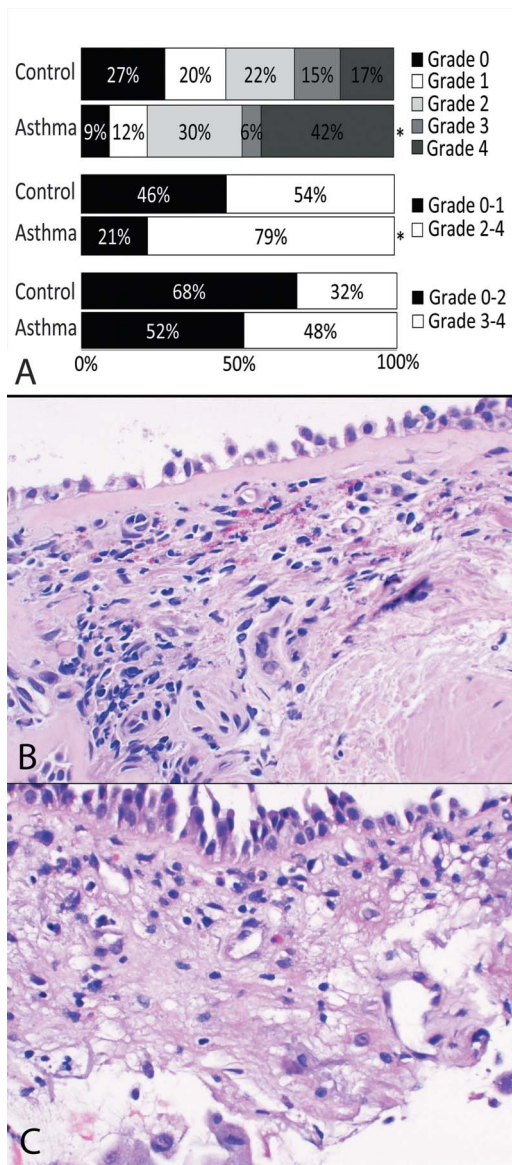


FIGURE 2.—Submucosal eosinophilic inflammation in severe persistent asthma. (A) The more detailed 5-tier grading system can be collapsed into a simpler 2-tier grading system with a cut-off of two submucosal eosinophils (upper limit of grade 1) as within the range of normal.

*Significant compared to control ($p < .05$). Representative (B) severe persistent asthma patient and (C) control biopsies showing submucosal eosinophils (H&E, original magnification, 40x).

respectively). Neither eNO nor BAL eosinophilia correlated with intraepithelial eosinophilia. Regarding neutrophils, intraepithelial and submucosal neutrophils were related ($p = .006$), but neither correlated with BAL neutrophils. Therefore, the compartment relationships of

TABLE 2.—Relationship of invasive and non-invasive markers of inflammation.

Clinical diagnosis	Severe persistent asthma	
eNO avg, ppb*	38 ± 49 ^a	
BAL % eosinophils*	12 ± 19 ^b	
Intraepithelial eosinophils, % grade 1	67	
Submucosal eosinophils, % of each grade	0	9%
	1	12%
	2	30%
	3	6%
	4	42%
BAL % neutrophils*	29 ± 28	
Intraepithelial neutrophils, % grade 1	42 ^c	
Submucosal neutrophils, % of each grade	0	45%
	1	36%
	2	15%
	3	3%
	4	0%
BAL % lymphocytes*	9 ± 11	
Intraepithelial lymphocytes, % grade 1	61	
Submucosal lymphocytes, % of each grade	0	3%
	1	15%
	2	15%
	3	42%
	4	24%

Note *Expressed as mean ± SD.

^aSignificantly correlated with BAL % eosinophils ($p = .049$, Spearman's $\rho = 0.36$) and with submucosal eosinophils ($p = .045$, Test for trend).

^bSignificantly correlated with submucosal eosinophils ($p < .0001$, Test for trend).

^cSignificantly correlated with submucosal neutrophils ($p = .006$, Fisher's exact test). BAL: bronchoalveolar lavage; eNO: exhaled nitric oxide.

eosinophilic inflammation in severe persistent asthma patients are opposite those for neutrophilic inflammation.

Pre- vs. Post-BT (Table 3 and Figure 3)

Three patients underwent EBBx with histologic grading before and after undergoing BT. Prior to BT, all three patients had prominent smooth muscle on biopsy. Only one patient had substantial submucosal eosinophilic inflammation, and none had neutrophilic inflammation. Following BT, smooth muscle was no longer prominent in all three patients. One patient continued to have severe submucosal eosinophilic infiltrates and additionally developed severe submucosal neutrophilic infiltrates. The other two patients did not have changes in submucosal inflammation.

DISCUSSION

EBBx is becoming an important component in the assessment of patients with severe persistent asthma, despite being an invasive procedure. These patients have failed conventional therapy, and there is a need to further

TABLE 3.—Endobronchial biopsy characteristics in three patients before and after bronchial thermoplasty.

Patient	Epithelial denudation	Goblet cell hyperplasia	Reserve cell hyperplasia	Basement membrane	Smooth muscle	Mucous glands	Submucosal eosinophils	Submucosal neutrophils	Submucosal lymphocytes
1	1→2	3→0	1→0	1→1	1→0	0→0	0→0	0→0	1→1
2	2→2	3→1	1→1	1→1	1→0	1→0	1→0	1→1	1→1
3	2→2	3→2	1→1	1→1	1→0	0→0	4→4	0→4	2→1

Note: Numbers expressed as grade pre-bronchial thermoplasty → post-bronchial thermoplasty (see Supplementary Material e-Figure 1b for grading details).

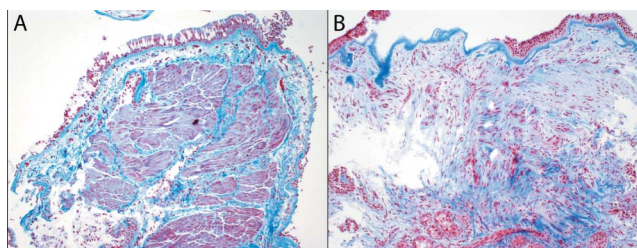


FIGURE 3.—Bronchial Thermoplasty. Endobronchial biopsy from right lower lobe shows (A) prominent smooth muscle (red) before bronchial thermoplasty but (B) not afterwards (3-week interval) in the same patient (trichrome stain, original magnification, 10x).

characterize their disease process (19). In this sense, the pathologist must systematically assess for inflammatory, structural, vascular, and infectious processes on biopsy. We have developed a novel standardized histologic grading system of EBBx for patients with severe persistent asthma that could serve as a guide for therapeutic decision making in this population. We have applied this system to assess the airway wall for changes in the same patients before and after undergoing BT. We have also shown that eNO, a non-invasive marker of airway inflammation, correlates with submucosal and BAL eosinophilia, but not intraepithelial eosinophilic inflammation.

To our knowledge, this is the first report of the histopathologic findings on EBBx in severe persistent asthma patients prior to and following BT. We found that there was prominent smooth muscle prior to BT, and that following the procedure, the smooth muscle was no longer prominent. These findings are consistent with early studies of the effects of radiofrequency ablation on canine airways (20). These findings are also consistent with an early feasibility study of BT in humans, which reported on the post-BT histologic resection findings in non-asthmatic patients (21). Our study differs from the feasibility study, since our patients had severe persistent asthma and have had histopathologic assessment of pre- and post-BT EBBx.

Prominent airway smooth muscle is a well-known histopathologic feature of asthma. However, studies of airway smooth muscle in the research setting have relied on using resection or surgical specimens with proper orientation and established techniques such as stereology (22, 23), which is not feasible for practicing pathologists. An important translational finding of our study is the utility of a semi-quantitative assessment of airway smooth muscle in human EBBx taken for diagnostic purposes and embedded without particular regard to orientation.

Without additional research techniques such as area profiles (22, 24), we found that the presence of both intraepithelial and submucosal eosinophils correlated with a diagnosis of severe persistent asthma. We found that basement membrane thickening, as assessed by light microscopy, was not a specific histologic feature of severe persistent asthma since it was present in both severe persistent asthma and control biopsies. Therefore, finding basement membrane thickening would not be a good substitute for determining sub-basement membrane fibrosis in

severe persistent asthma (25–29), and should not be used as a criterion for diagnosis of severe persistent asthma by light microscopy.

There has been increasing emphasis on the role of the airway epithelium in the pathogenesis of asthma, and epithelial cell injury has been studied in humans and mouse models (30, 31). We found epithelial denudation to be common in patients undergoing EBBx. Our findings agree with those reported by Ordóñez et al., who found epithelial desquamation to be frequent and non-specific in EBBx taken for any reason (32, 33). We did not assess markers of damage, such as CD44 (34) or EGFR (35) in the patients or in the control biopsies. However, our findings confirm that epithelial denudation should not be considered a surrogate marker of epithelial damage in severe persistent asthma (36). As there are currently no asthma therapies specifically targeting epithelial damage (37), this finding is useful only in the setting of asthma diagnosis on biopsy. Additionally, our finding of significant goblet cell hyperplasia in severe persistent asthma patients agrees with previous studies (27, 38–40) showing that goblet cell hyperplasia is a consistent and specific histologic finding in severe persistent asthma.

We studied the relationships of the neutrophils, eosinophils, and lymphocytes within the BAL fluid, the airway epithelium, and the airway submucosa in severe persistent asthma patients, and compared eosinophilic inflammation in the various compartments with eNO. Previous studies comparing BAL eosinophils with EBBx eosinophils in the submucosa showed them to be correlated in refractory asthma (14), as well as in mild and moderate asthma (41), but not in asthma in remission (42). We also found a correlation between BAL eosinophils and submucosal eosinophils in severe persistent asthma patients. Interestingly, while Maestrelli et al. (41) utilized the common asthma EBBx research technique of counting all inflammatory cells in a 100- μ m depth of sub-basement membrane covering the entire area of the biopsy, Foresi et al. (42) counted the number of eosinophils in ten high-power fields, and Good et al. (14) counted the peak number of cells per high-power field. Our methodology was most similar to that of Good et al. (14), however, we went one step further to group the cell numbers into grades of rare, mild, moderate, and severe, and showed that a distinction, at least for eosinophils, need only be made between rare and more than rare. Since there is reproducibility across

these studies utilizing different counting techniques, our methodology can be easily and confidently implemented by the practicing pathologist, thereby bridging the clinical-translational research gap and bringing the histologic diagnosis of asthma up to date with current research.

Significant eosinophilic inflammation in severe persistent asthma patients on EBBx combined with the correlation between submucosal eosinophils and BAL eosinophils, as well as with eNO, indicates that the less-invasive measures of airway eosinophilia may be suitable for detecting significant eosinophilic airway inflammation in patients clinically diagnosed as having severe persistent asthma.

However, the same cannot be said for neutrophilic airway inflammation. In our patient population, there was no significant neutrophilic airway inflammation, and no correlation between intraepithelial or submucosal neutrophils and BAL neutrophils. These findings are in contrast to studies implicating neutrophilic airway inflammation in the pathophysiology of asthma. For example, studies of induced sputum in persistent and other asthmatics have identified a phenotype of asthmatics with increased neutrophils (43–46). More recently, it has been suggested that asthmatic patients with increased sputum neutrophils have an airway factor which delays neutrophil apoptosis (47), and that these patients have a systemic inflammatory response different from other asthmatics (48). Wenzel et al. have also shown increased neutrophils in tissue on EBBx of severe asthmatics with or without eosinophilic inflammation as compared to mild asthmatics and normal controls (49). The lack of a significant neutrophilic inflammatory component in our severe persistent asthma patients may be due to sampling or due to differences in the pathophysiology of disease in this patient population. Therefore, further study of the pathophysiology of neutrophilic airway inflammation in asthma is warranted.

There are several limitations to our study. This study is intended to provide a framework for the clinical diagnostic assessment of EBBx in patients diagnosed as having severe persistent asthma. Biopsy assessment utilizing the standardized histologic grading system is not meant to diagnose severe persistent asthma *per se*. Assessment of clinical parameters, including detailed demographic data, clinical asthma phenotypes, and response to therapy is beyond the scope of this study. The control group of biopsies was chosen to represent diagnostic material from patients undergoing biopsy for some indication other than asthma, however, whether any of these biopsies were from patients with asthma is not known, and whether they had BAL collected is not known. Future studies should examine how the grading system performs in patients across the range of asthma severity, from mild to severe asthma. Regarding the inflammatory findings, although the presence of increased submucosal eosinophils was significantly more common in severe persistent asthma than in controls, there was overlap which could affect the specificity of the finding. Future studies should examine how the histologic findings based on our grading system compares

with induced sputum results. Regarding the structural findings, we did not compare our semi-quantitative grading system with appropriately oriented and traditionally measured parameters, as this was technically not feasible given that the biopsies were collected as diagnostic material. More grades of airway smooth muscle prominence (e.g., on a scale of 1 to 5) could further increase the utility of the histologic grading system, and could be tested in future studies. Studies quantifying airway smooth muscle directly comparing our approach with more traditional measurements may be useful. Also, although biopsies were obtained from the same location in each patient, there may be anatomic differences in the structures present at that location in each patient, which cannot be controlled for when assessing the structural features on biopsy. Interobserver variability was also not assessed in this study, and future studies should examine the reproducibility of the histologic grading system. Multiple comparisons between asthma and control biopsies as illustrated in Figure 1 may have led to overall inflation of type I error. However, the significant differences we describe have been previously reported and are biologically plausible, making it less likely that our findings are spurious.

CONCLUSIONS

We have developed a standardized histologic grading system for the assessment of structural and inflammatory components on EBBx in patients with severe persistent asthma. Compared to controls, more severe persistent asthma patients had goblet cell hyperplasia, intraepithelial eosinophils, intraepithelial lymphocytes, and prominent smooth muscle, and fewer severe persistent asthma patients had prominent mucous glands. We have also shown that eNO, a non-invasive marker of airway inflammation, correlates with submucosal and BAL eosinophilia, but not intraepithelial eosinophilic inflammation in this population of severe persistent asthmatics. We present histologic findings in three patients before and after undergoing BT and show decreased smooth muscle on post-BT biopsies.

Findings of eosinophilic inflammation or prominent smooth muscle can guide clinical management towards targeted anti-inflammatory medications (14, 16) or BT, respectively. This suggests the utility in knowing the histologic differences for applying the appropriate therapy, and our grading system will help to empower diagnostic pathologists to describe this meaningful phenotypic information. We propose that this grading system is useful for practicing pathologists assessing EBBx from severe persistent asthma patients for diagnostic and clinical research purposes and can lead to more personalized asthma treatment plans.

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DECLARATION OF INTEREST

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SUPPLEMENTARY MATERIAL

E-TABLE 1. Clinical definition of severe persistent asthma

-
- Referred by physician for further evaluation for severe persistent asthma
 - Symptoms compatible with lower airway disease
 - Acute bronchodilator response OR positive methacholine challenge
 - Never smoker OR smoker with <10 pack years
 - Any age of onset
 - No evidence of other recognized cause of lower airway disease: bronchopulmonary dysplasia, cystic fibrosis, sarcoidosis, obliterative bronchiolitis, bronchiectasis, lymphocytic bronchitis
-

Endobronchial Biopsy: A Guide for Asthma Therapy Selection in the Era of Bronchial Thermoplasty

METHODS SUPPLEMENT

Bronchoscopy, Bronchoalveolar lavage (BAL) fluid collection, and endobronchial biopsy (EBBx) collection

Diagnostic bronchoscopy was performed utilizing an Olympus therapeutic size video flexible bronchoscope (BF-1TQ180) with a 2.8 -mm channel. Five ml of 1% lidocaine were nebulized prior to sedation and 2-ml aliquots of 1% lidocaine were applied to the vocal cords (total of 6 to 12 ml). Another total of 8 to 14 ml of 1% lidocaine was used below the cords in the airways. Sedation was achieved using aliquots of Midazolam and Fentanyl, with average total doses ranging from 3 to 10 mg and 50 to 150 mcg, respectively, as well as 50 mg of intravenous diphenhydramine in most cases. Supplemental oxygen was applied as needed and standard sedation monitoring was used. Once sedated, standard airway inspection was done followed by BAL of the right middle lobe (RML, either RB4 or RB5) using 120 ml of room temperature normal saline with an in-line trap connected to a suction port via wall-based suction. Then, using Conmed Precisor (model # 100503) alligator cup forceps, 4 to 6 EBBx of the right lower lobe (RLL) were taken at the carinas separating RB8a and RB8b, RB9a and RB9b, and RB8 from RB9. Additional samples in some cases were taken from within RB10 or at the carina separating RB9 from RB10. Total “scope in” time ranged from 9 to 14 minutes. Estimated total blood loss was 1 to 3 ml.

Our study of the inflammatory component did not include an assessment of induced sputum. The exact site of origin of induced sputum material is unknown, and 10 to 20% of subjects are unable to produce an adequate sputum

specimen.⁸ We, therefore, focused our study on BAL and EBBx, which could safely and reliably be obtained in every patient.

BAL fluid processing and analysis

BAL fluid was analyzed in a diagnostic hematopathology laboratory manually using a hemocytometer, and the differential slides were prepared using a CytoSpin 4 Cytocentrifuge (Thermo Scientific, Waltham, MA).

EBBx histology

Ten tissue levels (L1 through L10 at 5 microns each) were cut using standard diagnostic histology technique and placed on glass slides. Standard hematoxylin and eosin (H&E) staining was performed on L1, L5, and L10. Additional unstained levels were utilized for special stains in some cases, including trichrome to highlight smooth muscle and fibrosis, VVG stain to highlight elastic fibers, Gomori methenamine silver stain for fungal elements, or CD21 immunostain to confirm bronchus-associated lymphoid tissue (BALT). All H&E-stained levels were examined at 40x power by two pulmonary pathologists (and) to determine the appropriate synoptic parameters (e-Figure 1). Airway smooth muscle and submucosal mucous glands were categorized as prominent if there were large smooth muscle bundles or submucosal mucous glands identified in each biopsy fragment. The “Comments” section included a statement indicating the presence or absence of granulomas or vasculitis, as well as the adequacy of the biopsy, for example, if no smooth muscle was sampled. Additionally, a clinical–pathologic correlation as to whether the findings were consistent with eosinophilic, neutrophilic, mixed granulocytic, or pauci-granulocytic asthma was made.

Bronchial Thermoplasty (BT) patient selection

Our selection criteria for undergoing BT included adults aged 18 to 65 years with severe persistent asthma despite adherence to high-dose inhaled corticosteroids and long-acting beta agonists, documented CT imaging excluding other diagnoses such as allergic bronchopulmonary aspergillosis or hypersensitivity pneumonitis, normal alpha-1-antitrypsin genotype, negative ANCA, and normal IgE levels. Exclusion criteria included unstable co-morbid conditions, sensitivity to required medications, internal pacemaker, defibrillator, or neurostimulator, prior BT treatment, and any other diagnosis that would explain an etiology to their severe persistent asthma.

A. ASTHMA and OBSTRUCTIVE LUNG DISEASE SYNOPTIC REPORT

EPITHELIUM:

Denudation: _____absent _____focal (one piece involved) _____extensive (>one piece involved)

Goblet cell hyperplasia: _____unable to assess _____normal _____one focus _____>one focus

Reserve cell hyperplasia: _____absent _____present

Squamous metaplasia: _____absent _____present

Dysplasia: _____absent _____present

Intraepithelial eosinophils: _____absent _____present

Intraepithelial neutrophils: _____absent _____present

Intraepithelial lymphocytes: _____absent _____present

BASEMENT MEMBRANE: _____not thickened _____thickened (at all)

SUBMUCOSA:

Smooth muscle: _____not prominent _____prominent

Mucous glands: _____not prominent _____prominent

Elastic fibers: _____normal _____abnormal

Submucosal eosinophils (in most inflamed 40× field):

_____absent

_____rare (1–2 cells) _____mild (3–10 cells) _____moderate (11–20 cells) _____severe (>20 cells)

Submucosal neutrophils (in most inflamed 40× field):

_____absent

_____rare (1–2 cells) _____mild (3–10 cells) _____moderate (11–20 cells) _____severe (>20 cells)

Submucosal lymphocytes (in most inflamed 40× field):

_____absent

_____rare (1–5 cells) _____mild (5–10 cells) _____moderate (11–40 cells) _____severe (>40 cells)

Submucosal plasma cells (in most inflamed 40× field):

_____absent

_____rare (1–2 cells) _____mild (3–5 cells) _____moderate (6–10 cells) _____severe (>10 cells)

Submucosal mast cells (in most inflamed 40× field):

_____absent

_____rare (1–2 cells) _____mild (3–5 cells) _____moderate (6–10 cells) _____severe (>10 cells)

COMMENTS:

E-FIGURE 1.—Standardized Histologic Grading Form for Endobronchial Biopsies. (A) This form can be included in the diagnostic pathology report. Parameters may be revised depending on diagnostic utility (e.g., we found no significant difference between asthma and controls for denudation). (B) For the purposes of statistical comparisons, each parameter was converted to a numerical grade. For some comparisons, “present” was defined as any grade greater than *0 or †1.

B. ASTHMA and OBSTRUCTIVE LUNG DISEASE SYNOPTIC REPORT

EPITHELIUM:

Denudation*: 0 absent 1 focal (one piece involved) 2 extensive (>one piece involved)

Goblet cell hyperplasia†: 0 unable to assess 1 normal 2 one focus 3 >one focus

Reserve cell hyperplasia: 0 absent 1 present

Squamous metaplasia: 0 absent 1 present

Dysplasia: 0 absent 1 present

Intraepithelial eosinophils: 0 absent 1 present

Intraepithelial neutrophils: 0 absent 1 present

Intraepithelial lymphocytes: 0 absent 1 present

BASEMENT MEMBRANE: 0 not thickened 1 thickened (at all)

SUBMUCOSA:

Smooth muscle: 0 not prominent 1 prominent

Mucous glands: 0 not prominent 1 prominent

Elastic fibers: 0 normal 1 abnormal

Submucosal eosinophils (in most inflamed 40× field):

0 absent

1 rare (1–2 cells) 2 mild (3–10 cells) 3 moderate (11–20 cells) 4 severe (>20 cells)

Submucosal neutrophils (in most inflamed 40× field):

0 absent

1 rare (1–2 cells) 2 mild (3–10 cells) 3 moderate (11–20 cells) 4 severe (>20 cells)

Submucosal lymphocytes (in most inflamed 40× field):

0 absent

1 rare (1–5 cells) 2 mild (5–10 cells) 3 moderate (11–40 cells) 4 severe (>40 cells)

Submucosal plasma cells (in most inflamed 40× field):

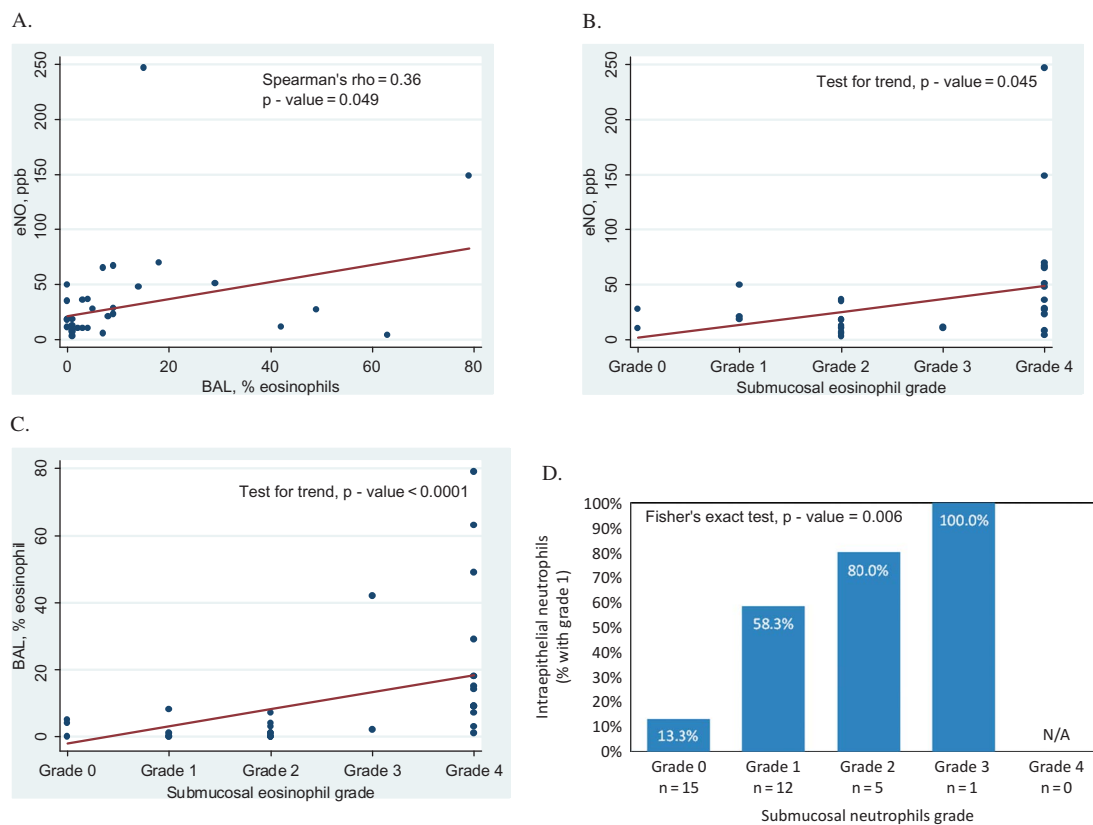
0 absent

1 rare (1–2 cells) 2 mild (3–5 cells) 3 moderate (6–10 cells) 4 severe (>10 cells)

Submucosal mast cells (in most inflamed 40× field):

0 absent

1 rare (1–2 cells) 2 mild (3–5 cells) 3 moderate (6–10 cells) 4 severe (>10 cells)



E-FIGURE 2.—Significant Inflammation Relationships. (A) Exhaled nitric oxide (eNO) is significantly associated with bronchoalveolar lavage (BAL) % eosinophils (Spearman's $\rho = 0.36$, p -value = .049). (B) eNO is significantly associated with submucosal eosinophils (Test for trend, p -value = .045). (C) BAL % eosinophils is significantly associated with submucosal eosinophils (Test for trend, p -value < .0001). (D) Presence of intraepithelial neutrophils (% with grade 1) is significantly associated with submucosal neutrophils (Fisher's exact test, p -value = .006).