original article

Endobronchial biopsy in the final diagnosis of chronic obstructive pulmonary disease and asthma: a clinicopathological study

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BACKGROUND: Asthma and chronic obstructive pulmonary disease (COPD) are chronic conditions with an increasing prevalence in developing countries. The evaluation of endobronchial biopsies has emerged as a tool to differentiate between both conditions via the measurement of the reticular basement membrane (RBM) thickness with various conclusions drawn from different studies.

OBJECTIVES: Compare the thickness of the RBM between asthma and COPD and evaluate other histomorphological features in both groups.

DESIGN: Prospective, descriptive and analytical.

SETTING: University teaching hospital.

PATIENTS AND METHODS: The study included patients with COPD and irreversible and reversible asthma with diagnosis based on clinical assessment, pulmonary function tests and high-resolution computed tomography scans. Endobronchial biopsies were obtained from all patients and, using a light microscope and a computerized image analyzer, the thickness of the reticular basement membrane was calculated in all patients. We also made a qualitative assessment of other histomorphological features.

MAIN OUTCOME MEASURES: Mean RBM thickness.

SAMPLE SIZE: Thirty male patients.

RESULTS: The mean RBM thickness in asthmatic patients was 8.9 (2.4) μm. The mean RBM thickness in COPD patients was 5.3 (1.1) μm. However, there was no thickening of the RBM in patients with reversible asthma. The RBM was significantly thicker in patients with irreversible asthma than in patients with COPD or reversible asthma. There were no significant differences in epithelial desquamation or metaplasia, mucosal or submucosal inflammation, the presence of eosinophils, submucosal glandular hyperplasia or submucosal smooth muscle hyperplasia between groups.

CONCLUSIONS: The thickness of the RBM is the only reproducible histopathological feature to differentiate COPD from irreversible asthma.

LIMITATIONS: The study included a limited number of patients. A qualitative approach was used to compare epithelial cell injury, inflammation, submucosal glandular and muscular hyperplasia.

CONFLICT OF INTEREST: None.

Asthma and chronic obstructive pulmonary disease (COPD) are the two most commonly encountered chronic respiratory diseases worldwide. In 2015, both conditions affected 18.4 million and 12.8 million people in the United States, respectively.[1] Asthma commonly causes reversible airway obstruction while COPD, by definition, is invariably irreversible by spirometry. Therefor the two conditions are distinguishable clinically and physiologically in the majority of cases. However, about 20% of asthmatics progress into airway remodeling associated with irreversible airways. Currently, differentiation between irreversible asthma in smokers and COPD patients is not always achievable. The 2017 version of the Global Initiative for Chronic Obstructive Pulmonary Disease (GOLD) states that "in some patients with chronic asthma, a clear distinction from COPD is not possible using current imaging and physiological testing techniques".[2] High resolution computed tomography (HRCT) has shown promising results in discriminating these two conditions, but it is still not recognized as a differentiating tool. Thus, the evaluation of endobronchial biopsies, in particular the qualitative and quantitative assessment of inflammatory cells and the measurement of the reticular basement membrane (RBM), has emerged as a potential tool for a proper diagnosis.

Several studies aimed to assess the structural abnormalities and airway remodeling in patients with asthma and COPD. James et al showed that, in autopsy samples, the thickness of the RBM in central airways, assessed by endobronchial biopsies, correlates with cartilaginous airway wall dimension.[3] The study concluded that the RBM thickness can be used as an index of airway remodeling in asthma.[3] The literature however is not unanimous as to whether the RBM is thickened in COPD with several studies reaching different conclusions. Some studies showed an increase in the RBM thickness though to a smaller extent than asthma.[4-9] A normal RBM thickness in COPD was reported by others.[10-12] Milanese et al studied a group of 33 patients including 11 patients with perennial allergic asthma, 8 patients with allergic rhinitis, 5 patients with seasonal allergic rhinitis and 9 patients with COPD.[8] The RBM thickness was significantly thicker in asthma (10.1 [3.7] μm) in comparison to COPD (5.2 [0.7] μm).[8] On the other hand, Liesker et al compared the RBM thickness between 24 asthmatic patients and 17 patients with COPD and found no significant difference between both groups, though both had a significantly thicker RBM when compared to healthy controls.[11] Some of the differences could be attributed to the variable degrees of airway obstruction within the studied groups,[4,5,11]

the inclusion of healthy individuals with no history of smoking[9] or the limited number of patients within the study.[10] The aim of this study was to compare the RBM thickness in clinically defined patients with asthma and COPD and to evaluate the inflammatory cellular infiltrate and epithelial changes in both groups.

PATIENTS AND METHODS

Patients were recruited by convenience from the outpatient respiratory care clinics. Inclusion criteria were that patients be ≥ 40 years of age with a previous or an active history of smoking of at least ten pack years for more than ten years. Other inclusion criteria were a history of chronic cough or dyspnea, forced expiratory volume in 1 second (FEV1) <80% predicted or emphysema diagnosed by CT scan involving five lobes of the lung regardless of spirometric values and FEV1/FVC <70%.

The study was approved by the Clinical Ethics Committee (No. E-12-667) and conducted at a teaching university hospital between 2011 and 2015 All subjects had signed an informed consent, agreeing to undergo the procedure.

Asthma was defined (as stated by the Global Initiative for Asthma) as "a chronic condition characterized by the presence of wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory flow limitation".[13] In addition the CT scan had to be free of bronchiectasis and emphysema. Similarly, the carbon monoxide diffusion coefficient had to be equal or greater than 80% predicted. Studies are unanimous that asthma, even when severe or irreversible, is associated with normal diffusion studies.[14-16] Irreversible asthma is defined as patients with asthma who may have an accelerated decline in lung function and who develop airway limitation that is not fully reversible.[13] The COPD definition was adopted from GOLD as "chronic cough or sputum production and dyspnea in the presence of a history of smoking >10 pack/year and a post bronchodilator FEV1/FVC <0.70".[2] In addition a CT scan had to show emphysema and Krogh factors (KCO) were below 80% predicted. Postmortem studies document emphysema to be uniformly present in COPD.[17] Similarly antemortem surveys document that patients free of emphysema by CT scan display features of asthma.[18-21] All patients were ≥40 years of age with no clinical history of atopy. All patients were either active smokers or had a previous history of smoking of at least ten pack years for more than 10 years. Inhaled corticosteroids (ICS) were discontinued for 4 weeks and tiotropium (Spiriva Handihaler; Boehringer Ingelheim; Ingelheim, Germany) 18 ug was prescribed once daily. Lung func-

tion tests comprised spirometry performed with reversibility, carbon monoxide transfer coefficient (K_{CO}) and arterial blood gases. A K_{CO} <80% predicted was considered indicative of emphysema and was followed by a HRCT scan of the lungs. The above described criteria have been used to differentiate between asthma and COPD in our hospital since 2009.[22]

All patients were started on budesonide 320 µg/formoterol 9 µg (Symbicort Turbuhaler; AstraZeneca, London, UK) twice daily and salbutamol 200–400 µg (Ventolin inhaler; GlaxoSmithKline, Brentford, UK) as required. Guided by the response to a 12-month trial of inhaled corticosteroids/ long-acting β2 agonists and the results of the above-described investigations, further therapy was aimed at an asthma or COPD phenotype.[22]

Fiberoptic bronchoscopy was performed under local anesthesia in all 30 patients. After visual inspection of the lower airways, 4-5 biopsies were taken from a lobar bronchus of the lower lobe. The specimens were fixed in formalin (4% buffered) and routinely processed to paraffin blocks. Four microsections were stained with a routine hematoxylin and eosin stain. Additional special stains for the basement membrane including Periodic Acid-Schiff (PAS) and Trichrome stain were obtained for all cases.

The slides were examined under a light microscope (Olympus, Tokyo, Japan) at ×400 magnification (×40 objective lens, ×10 eyepiece). Only sections perpendicular to the epithelial surface and the reticular basement membrane were evaluated. Inadequate specimens (small, squashed) were excluded. Point-to-point measurements of the reticular basement member at regular intervals of 20 µm, as described by Sullivan et al, were taken.[23] For each subject, at least 40 measurements were recorded by a pathologist and a clinician. A computerized image analyzer (NIS-Elements V.4, Nikon) was used to take the measurements and the mean RBM thickness in an individual subject was calculated as a mean of all measurements recorded by the observers.

HRCT images, using LightSpeed 16 or VCT XT; GE Medical Systems, Milwaukee, WI, USA, were scored by three observers (a radiologist and two clinicians) based on the Goddard's quantitative visual scoring of emphysema.[24] A score of 0 was assigned if there was no abnormality. A score of 1 was assigned if less than 25% of the parenchyma showed abnormalities suggestive of emphysema; if 25%–50% of the parenchyma had abnormalities suggestive of emphysema, a score of 2 was given; if however 51%–75% of the parenchyma had abnormalities suggestive of emphysema a score of 3 was assigned and if more than 75% of the parenchyma

had abnormalities suggestive of emphysema, a score of 4 was given. The images were evaluated at the level of the arch of the aorta, the bifurcation of the trachea and above the diaphragm. Emphysema was considered significant if the average scoring of the three cuts was ≥1 and a consensus score was reached in cases of interobserver variability.

Categorical variables were summarized as percentages and continuous variables as medians and interquartile ranges. The nonparametric Mann-Whitney U-test was used for testing mean differences in variables with non-normal distribution. The chi-square test was used for categorical data. Two-tailed P value <.05 was considered statistically significant.

RESULTS

All 30 patients were males. Twelve patients (40%) with COPD had a mean age of 64.3 years. Eight patients were active smokers at the time of the study while the remaining four were ex-smokers. The diagnosis of COPD was made on HRCT in 11 patients and K_{CO} in 12 patients, showing an agreement between both modalities in 96.7% of all patients. A total of eighteen patients (60%), 7 active smokers and 11 ex-smokers, had asthma and were significantly younger than the former group (mean age=55.8 years). Although their FEV1 was higher, 29% of patients had an FEV1 ≤50% predicted. K_{CO} levels were within normal limits in all asthmatic patients (≥80% predicted). They also had higher PaO2 and lower $PaCO_2$ levels. All COPD patients had lower FEV1 level and low K_{CO} except one. This patient had an emphysema score of 1.16 and a K_{CO} level of 90% predicted. A misclassified diagnosis by HRCT was suspected as the patient displayed a positive response to ICS. **Table 1** summarizes the comparative clinical characteristics of both groups.

The number of collected biopsies was 4-5 biopsies per patient. The mean number of RBM thickness measurements was similar in both groups of patients. The mean RBM thickness in asthma patients was 8.9 (2.4) µm compared with 5.3 (1.1) in COPD subjects. The difference in the RBM thickness was statistically significant between groups (P=.00032). The RBM was not thickened in any patient with COPD (**Figure 1**). It was, on the other hand, thickened in 15 out of 18 patients with asthma (**Figure 2**). Three patients within the asthma group failed to show a thickened RBM. These 3 patients had reversible asthma as their initial FEV1 was 75-78% predicted and normalized subsequently upon receiving ICS.

Though epithelial metaplastic changes were prominent in COPD patients, there were no statistically

significant differences in the extent of epithelial desquamation or metaplasia between groups (**Figure 3**). Eosinophils and other inflammatory cells including lymphocytes, plasma cells and less frequently neutrophils were noted in biopsies of both groups. Though more pronounced in asthmatic patients, there was no significant difference in the numbers or distribution of inflammatory cells between groups. Submucosal glandular hyperplasia was a common feature in COPD and asthmatic patients (40% and 50%, respectively). Smooth muscle hyperplasia was more dominant in asthmatics however did not reach a statistical significance (**Figure 4**). **Table 2** summarizes the comparative histopathological characteristics of both groups.

DISCUSSION

Structural changes, remodeling and airway flow limitation characterize both asthma and COPD with noticeable pathological differences. The most frequently studied marker of airway remodeling is the thickness of the RBM. Our study confirms the considerable thickening of the RBM in asthmatic patients in comparison to patients with COPD. This finding is consistent with the results of other published studies.[8,25,26] The mean RBM thickness in asthmatics was significantly higher than in COPD subjects (8.9 µm in asthmatics vs. 5.35 µm in COPD patients). The measurements of the RBM of the three patients with reversible asthma were 4.05 µm, 5.1 µm and 5.9 µm. Thus, upon excluding these 3 patients, there was no overlap between the ranges of RBM thickness in both groups (2.6-7 µm in COPD vs. 7.3-13.1 µm in asthma patients). These observations confirm that the thickness of the RBM cannot differentiate between reversible asthma and COPD, a finding that has been reported by previous studies.[11,27,28] Among 72 patients studied by Cohen et al, 31 patients had severe asthma, 9 had mild asthma, 11 were patients with chronic bronchitis and 21 were healthy controls.[28] It was observed that the RBM thickness was greater in subjects with severe asthma compared to the other groups, however it could not differentiate between patients with mild asthma, COPD or healthy individuals.[28] The absence of an overlap in the thickness of the RBM between asthmatic patients and patients with COPD has also been demonstrated previously.[7,22] Fabbri et al compared COPD patients with patients with irreversible asthma and found that the RBM thickness ranges between 6.6-7.4 µm in the latter group compared to 4.2-6.2 µm in the former.[7] Bourdin et al found that a threshold of 6.65 µm differentiated between patients with irreversible asthma and COPD with a sensitivity of 89% and specificity of 68%, though the RBM thickness could not distinguish mild

Table 1. Comparative clinical characteristics of patients in the COPD and asthma groups.

	Patients with COPD (n=12)	Patients with asthma (n=18)	*P* value
Age (years)	64 (10) (56-73)	58.5 (14) (41-69)	.0251
BMI (kg/m²)	23.76 (10.23) (15.7839.97)	29.71 (9.8) (21.1545.14)	.238
Baseline pulmonary function tests			
FEV1 (L) (%predicted)	49 (18.5) (28-80)	61 (19) (37-78)	.008
DLco (mmol/min/kPa) (%predicted)	37 (17) (16-74)	79 (19) (50-105)	<.0001
KCO (mmol/min/kPa) (% predicted)	56 (19.5) (23-90)	110.5 (30.3) (85-144)	<.001
Arterial blood gas			
PaO₂ (mm Hg)	65 (14) (49-79)	77.5 (7.8) (64-100)	<.001
PaCO₂ (mm Hg)	43 (9.3) (35-70)	40 (3.8) (31-56)	.049

Data are presented as medians (interquartile range) (range). Comparisons were done by Mann-Whitney U-test.

BMI: body mass index, DLco: carbon monoxide diffusing capacity, FEV1: forced expiratory volume in 1 second and KCO, carbon monoxide transfer coefficient.
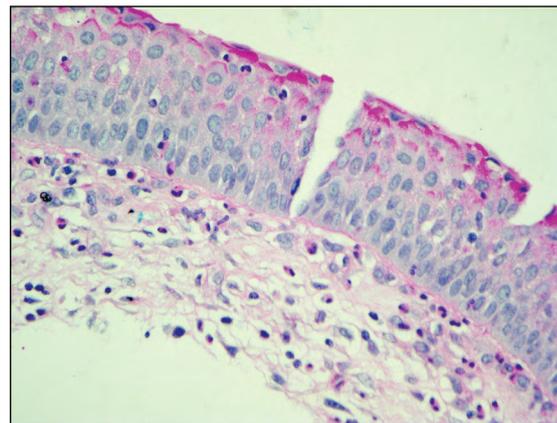
Figure 1. Endobronchial biopsy in a 56-year-old patient with chronic obstructive pulmonary disease. Note the thickness of the reticular basement membrane, which measured 2.6 µm in this case (PAS stain, ×40).

asthma from COPD, irrespective of the method used to measure it.[27] This observation is interesting because the only significant difference we found between patients with COPD and irreversible asthma was the thickness of RBM.

In this study, none of the other evaluated features including epithelial desquamation or metaplasia, mucosal or submucosal inflammation, the presence of eosinophils, submucosal glandular hyperplasia nor sub-
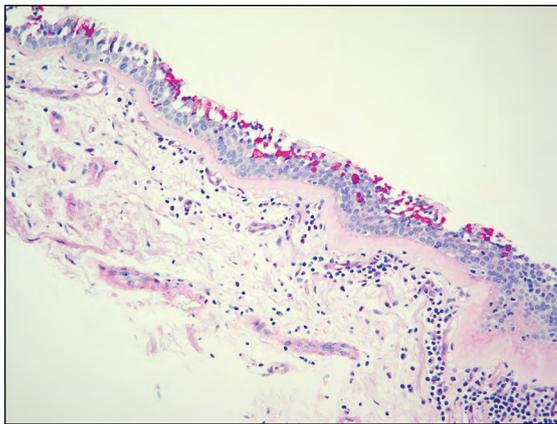
Figure 2. Endobronchial biopsy in a 62-year-old patient with irreversible asthma. In comparison to Figure 1, the reticular basement membrane is significantly thickened, measuring 13.1 μm (PAS stain, ×20).
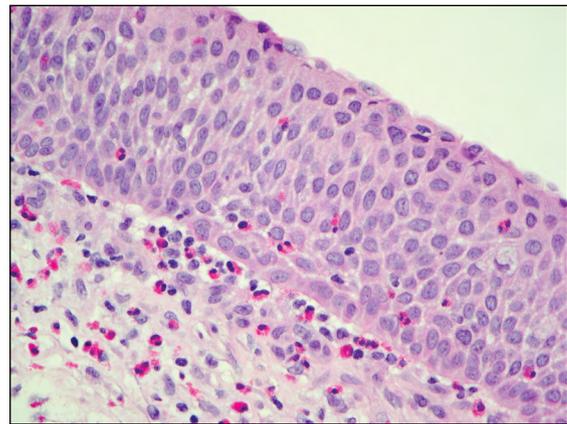
Figure 3. Epithelial metaplasia is apparent in this biopsy from the same patient in Figure 1. Note the presence of epithelial and submucosal eosinophils (Hematoxylin and eosin, ×40).

Table 2. Comparative histopathological characteristics of patients in the COPD and asthma groups.

	Patients with COPD	Patients with asthma	P value
RBM thickness (μm)	5.35 (1.47) (2.6-7)	8.8 (2.62) (4.0513.1)	.00032
Epithelial desquamation	3/12 (25%)	5/18 (27.78%)	.8683
Epithelial metaplasia	7/12 (58.33%)	7/18 (38.89%)	.3039
Mucosal inflammation	5/12 (41.67%)	10/18 (55.56%)	.4636
Mucosal eosinophils	2/12 (16.67%)	4/18 (22.22%)	.7143
Submucosal inflammation	5/12 (41.67%)	10/17 (58.82%)	.3711
Submucosal eosinophils	2/12 (16.67%)	6/17 (35.29%)	.2776
Submucosal glandular hyperplasia	2/5 (40%)	4/8 (50%)	.7353
Smooth muscle hyperplasia	3/6 (50%)	6/9 (66.67%)	.5554

Data are presented as numbers (percentages) and comparisons were made using chi-square test except for the RBM thickness for which the data are presented as medians (interquartile range) (range) and comparisons were done by Mann-Whitney U-test.

RBM: reticular basement membrane, n: number of patients.

The remaining samples were mostly mucosal and were devoid of submucosal glands and/or smooth muscle fibers: patients with submucosal glandular hyperplasia and smooth muscle hyperplasia, patients with asthma and submucosal inflammation, submucosal eosinophils, submucosal glandular hyperplasia, smooth muscle hyperplasia.
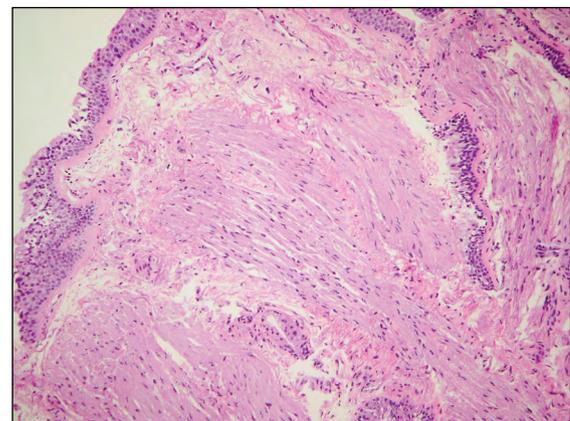
Figure 4. Submucosal smooth muscle hyperplasia in an endobronchial biopsy from a 65- year-old patient with irreversible asthma. The overlying thickened reticular basement membrane is also noted (Hematoxylin and eosin, ×40).

mucosal smooth muscle hyperplasia, was significantly different between the groups. Several studies have evaluated the extent of epithelial destruction and type of inflammation in asthma and COPD. Many of these studies were unable to find any differences in epithelial damage between healthy individuals, asthmatics, and COPD patients.[26-30] Kosciuch et al compared 20 patients with asthma to 12 with COPD and found no differences in the extent of epithelial damage, regardless of the extent or severity of the disease.[26] A total of 46 patients were included in the study by Fabbri et al. The group reported that, in comparison to patients with COPD (27 subjects), patients with asthma (19 sub-

jects) had significantly more eosinophils and fewer neutrophils in sputum, bronchoalveolar lavage and tissue biopsies. In addition to a higher CD4+/CD8+ ratio of infiltrating T-lymphocytes within the mucosa.[7] Epithelial metaplasia and goblet cell hyperplasia are, on the other hand, reported to be more frequently encountered in biopsies from COPD patients.[31,32] However, studies of submucosal smooth muscle hyperplasia in COPD have shown mixed results, with approximately half showing either an increase[33,34] or no significant change.[35,36]

Our study has numerous limitations. The study groups were relatively small. Valuable comparative data could have been obtained by adding a control group of healthy subjects. There were some clinical differences between both groups (e.g. mean age and duration of smoking) that could have affected our findings. In addition, the qualitative and relatively subjective way of evaluating the uneven distribution of inflammatory cells and submucosal glandular and muscular hyperplasia might have also limited our conclusions.

In summary, though the evaluation of surgically obtained endobronchial biopsies for differentiating asthma from COPD is not a common practice, the measurement of the RBM thickness is a reproducible tool that can serve this purpose. Larger scale studies, with a quantitative approach, are needed to support our results.

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