A pilot study to test the feasibility of histological characterisation of asthma–COPD overlap

To the Editor:

Asthma and chronic obstructive pulmonary disease (COPD) are chronic respiratory diseases that share some common characteristics. Asthma is associated with airway hyperresponsiveness, airway inflammation and airflow limitation that is reversible [1]. COPD is characterised by persistent and progressive airflow limitation and airway inflammation [2]. In recent years, there has been an ongoing discussion of whether asthma and COPD are different diseases, since a significant proportion of patients with symptoms of obstructive lung disease has features of both asthma and COPD [3–5]. In this respect, the most clinically significant phenotypes are COPD patients with asthmatic features and asthmatic patients that smoke. These patients may necessitate different therapeutic approaches and, therefore, there is a great need for diagnostic criteria that would allow proper diagnosis and treatment [6, 7]. Histological analysis has been previously suggested as a tool to identify and understand overlapping clinical and physiological phenotypes, thereby helping to better design treatments and plan long-term management [8]. However, there are few studies that have examined the histological patterns of patients who may be characterised as having asthma–COPD overlap.

The present study is a pilot to provide data to inform the design and size of a study to test whether there are consistent histological differences between COPD patients with asthmatic features and asthma patients who have smoked. We included patients drawn from a cohort of COPD patients (total n=147) who underwent diagnostic bronchoscopy for clinical indications such as coin lesions (27%), evaluation of bronchoscopical or surgical low volume reduction procedures (25%), infiltrates (16%) and haemoptysis (8%). COPD patients had an average forced expiratory volume in 1 s (FEV_1) to forced vital capacity (FVC) ratio post-bronchodilation of 0.4 (range 0.3–0.5) and a smoking history of 43 pack-years (range 30–60). We also included a cohort of asthma patients (total n=19), with severe to very severe disease, that underwent bronchial thermoplasty. Diagnosis of COPD and asthma was based on European Respiratory Society/American Thoracic Society guidelines, according to Global Initiative for Chronic Obstructive Lung Disease and Global Initiative for Asthma criteria.

In the absence of a diagnostic criterion (or group of criteria) that reliably identify COPD patients with asthmatic features, we determined that COPD patients with an asthmatic component should fulfil three or more of the following criteria according to the most recently published consensus of asthma–COPD overlap [9]: blood eosinophils above 300 per L, normal (above 80% pred) diffusing capacity of the lung for carbon monoxide (D_{LCO}), exhaled nitric oxide fraction (F_{eNO}) above 25 ppb, FEV_1 post-bronchodilator above 80% pred, post-bronchodilator reversibility of airway obstruction above 200 mL, no hyperinflation on radiography, personal or family history of allergy, positive prick test, previous diagnosis with asthma, symptoms triggered by exercise, emotions, laughing, allergens, worse symptoms at night or morning, and onset of symptoms at age younger than 20 years. A total of 18 patients met these criteria (mean 3.3 criteria). Within the asthma cohort, we identified patients (n=7) that smoked (>10 pack-years). All patients underwent diagnostic bronchoscopy. In COPD patients, endobronchial biopsies were obtained from the right upper lobe and the right lower lobe. From asthma patients, 10 biopsies were obtained, one from the right upper lobe, two from the middle lobe, three from the right lower lobe, one from the lingula

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This pilot study suggests that specific histopathological findings, such as thickening of reticular basement membrane in COPD patients, may reveal an overlapping COPD–asthma phenotype with higher response to ICS/LABA http://bit.ly/2Lb5sAW

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and three from left lower lobe. Five sequential sections were prepared from each biopsy, stained with haematoxylin and eosin and evaluated by a pathologist who was blind to the final diagnosis for: 1) inflammation in the stroma, tissue lymphocyte infiltration and tissue eosinophilic infiltration, using a 0–3 scale: 0 absence/normal, 1 mild-moderate, 2–3 severe; 2) granulocytes in the stroma, granulocytes in the epithelium and goblet cells, using a 0–3 scale: 0 absence, 1 a few, 2–3 many; 3) glands (%); 4) thickening of reticular basement membrane (BM), using a 0–3 scale: 0 absence/normal, 1 mild-moderate, 2–3 severe; 5) airway smooth muscle cell (ASMC) mass (%); 6) distance between BM and ASMC in µm. More than 90% of the selected slides allowed satisfactory analysis of epithelial cells, BM, smooth muscle and mucosal glands (table 1).

We observed significant differences in histological parameters between patients of the total COPD and total asthma cohorts. Asthma patients had significantly higher tissue eosinophilic infiltration (p=0.048). Granulocytes in the stroma were higher in COPD patients (p=0.026, Chi-square) and were detected in the epithelium only in COPD patients. The presence of goblet cells was higher in COPD (p<0.001). 73.7% of asthma patients exhibited mild–moderate thickening of the BM, compared to 40.1% of COPD patients (p=0.026), and 21.1% of asthma patients exhibited severe thickening of the BM, compared to 48.2% of COPD patients (p=0.024). It has been suggested that the use of inhaled corticosteroid (ICS) alters histopathological findings in asthma and COPD, since ICS reduces eosinophilia, a hallmark of asthma, and increases neutrophilia, a hallmark of COPD [10]. In our study, a high number of patients in the COPD cohort (64.6%) and all patients in the asthma cohort were under treatment with ICS or with systemic steroids and this may have modulated the histological findings for airway inflammation and remodelling. However, when we dichotomised the analysis between COPD patients who were using ICS (n=95) and COPD patients who were not using ICS (n=49), we did not detect any significant differences in the histological parameters between these two groups. Similarly, in the group of COPD patients with asthma characteristics (n=18), there were no significant differences in the histological parameters between patients using ICS (n=9) and patients not using ICS (n=9), although the number of patients was small. Furthermore, when we analysed histological parameters in COPD patients (n=147) dichotomised according to post-bronchodilator reversibility of FEV_1, ⩾200 mL (n=20) and <200 mL (n=127), we observed similar values for both groups of patients.

COPD patients with and without asthma features had similar lung function, as assessed by post-bronchodilator FEV_1 % pred, FEV_1/FVC % pred, residual volume (RV) % pred, total lung capacity (TLC) % pred and RV/TLC % pred. According to the classification criteria used, COPD patients with asthma characteristics had higher D_{LCO} % pred (p=0.023), higher F_{ENO} (p=0.008) and higher reversibility (p=0.027), as compared with COPD patients without asthma characteristics (table 1). Histological characteristics, such as inflammation in the stroma, tissue lymphocyte infiltration, tissue eosinophilic infiltration, number of granulocytes in the stroma and in the epithelium, and number of goblet cells, were similar between COPD patients with and without asthma characteristics (table 1). Furthermore, mucous glands and average ASMC mass, as well as the distance between ASMC and BM, were not significantly different between the two groups of patients. However, severe BM thickening was found in more COPD patients with asthma features (p=0.021) (table 1). These results are in good agreement with the study of Al-Kassimi *et al.* [11], showing that in non-emphysematous COPD patients, BM is thickened and this is associated with their responsiveness to ICS/long-acting beta-agonist (LABA) treatment.

Patients with asthma and smoking history >10 pack-years had similar lung function to asthma patients with a smoking history of <10 pack-years, as revealed by post-bronchodilator RV % pred, TLC % pred, RV/TLC % pred, D_{LCO} % pred, FEV_1 % pred and FEV_1/FVC % pred that were similar among the two groups (table 1). Histological characteristics, such as tissue lymphocyte infiltration, tissue eosinophilic infiltration, number of granulocytes in the stroma and in the epithelium, and number of goblet cells, were similar between both asthma groups. Furthermore, mucous glands, average ASMC mass, the distance between ASMC and BM, and the thickening of the BM were similar between the two groups of asthma patients, although asthma patients with <10 pack-years smoking history had more severe inflammation in the stroma (p=0.008). The absence of statistically significant differences may reflect the presence of a type II error, but on the other hand, significant results may represent robust differences between the groups.

COPD patients with asthma features had a more severe airflow limitation when compared with asthma patients with a smoking history of >10 pack-years, as revealed by significantly lower values of FEV_1 % pred (p=0.016) and FEV_1/FVC (p=0.017). Whilst average ASMC were similar between the two groups, however, the distance between BM and ASMC was significantly lower (p=0.022) in the asthma group.

In conclusion, this pilot study suggests the hypothesis that specific histopathological findings, such as thickening of BM in COPD patients, may reveal an overlapping COPD-asthma phenotype with higher response to ICS/LABA. However, larger studies are needed to detect statistically significant differences and

TABLE 1 Characteristics of patients with chronic obstructive pulmonary disease (COPD), asthma and asthma–COPD overlap

Parameter	All COPD patients	All asthma patients	COPD patients without asthma features	Asthma patients <10 pack-years	Asthma patients >10 pack-years	COPD patients with asthma features[#]
Subjects	147	19	129 (87.8)	12 (61.1)	7 (38.8)	18 (12.2)
Age years	70.6±9.9	59.6±14.2	71.0±9.7	60.4±4.5	58.3±4.6	68.2±11.6
Male	98 (66.6)	8 (42.1)	82 (63.6)	5 (41.6)	3 (42.8)	16 (88.8)
Current smokers	55 (37.4)	17 (89.5)	47 (36.4)	10 (90.1)	7 (100)	8 (44.4)
Therapy						
SABA	24 (16.3)	12 (63.2)	23 (17.8)	9 (75.0)	3 (42.8)	1 (5.5)
SABA+LAMA	26 (17.7)	4 (21.0)	25 (19.4)	4 (33.3)	0 (0.0)	1 (5.5)
ICS	4 (2.7)	4 (21.0)	4 (3.1)	0 (0.0)	4 (57.1)	0 (0)
LABA+ICS	85 (57.8)	12 (63.1)	76 (58.9)	9 (75.0)	3 (42.8)	9 (50.0)
LABA	31 (21.1)	5 (26.3)	28 (21.7)	1 (8.3)	4 (57.1)	3 (16.7)
LAMA	95 (64.6)	8 (42.1)	85 (65.9)	5 (41.6)	3 (42.8)	10 (55.5)
Oxygen	10 (6.8)	0 (0.0)	10 (7.7)	0 (0.0)	0 (0.0)	0 (0)
Mucolytics	13 (8.8)	0 (0.0)	10 (7.7)	0 (0.0)	0 (0.0)	3 (16.7)
Oral corticosteroids	19 (12.9)	14 (73.7)	18 (13.9)	8 (66.6)	6 (85.7)	1 (5.5)
Post-bronchodilator RV % pred	178.4±58.9	124.5±30.4	181.5±58.8	122.2±28.9	131.3±40.7	155.9±56.7
Post-bronchodilator TLC % pred	121.0±22.1	104.8±13.2	122.2±22.1	104.7±14.4	105.3±11.2	112.3±21.1
Post-bronchodilator RV/TLC % pred	139.1±27.7	116.1±20.0	140.4±27.1	115.5±20.4	118.5±26.2	129.4±30.8
Post-bronchodilator FEV_1 % pred	48.8±22.4	65.4±15.8	46.8±19.9	62.1±13.8	71.0±18.4	61.8±32.5
Post-bronchodilator FEV_1/FVC % pred	39.7±14.4	52.1±9.8	38.6±13.4	50.6±8.1	54.6±12.7	47.0±19.1
Post-bronchodilator D_{LCO} % pred	50.8±19.7	92.7±20.9	49.3±18.9	92.6±24.4	92.8±15.5	61.5±21.9
Reversibility >200 mL	21 (14.2)	6 (31.6)	12 (9.3)	4 (33.3)	2 (28.6)	7 (38.9)
Reversibility mL	97.8±115.1	158.9±130.7	83.7±91.1	166.7±140.2	145.7±122.0	191.7±194.8
F_{ENO} ppb (exhalation flow rate 50 mL·s^{-1})	19.9±13.8	37.1±30.0	17.8±11.1	41.9±35.9	29.6±17.0	32.8±20.6
Blood eosinophils ×1059 per L[¶]	0.21±0.2	0.21±0.12	0.20±0.2	0.25±0.1	0.17±0.1	0.34±0.3
Blood leukocytes ×1059 per L[¶]	8.5±3.2	9.4±3.9	8.7±3.2	8.3±4.0	11.3±3.1	7.9±3.1
Blood neutrophils n[+]	6.1±3.0	7.2±3.3	6.2±3.1	6.5±3.4	8.2±3.0	5.3±1.8
Inflammation in the stroma[+]						
Absence	49 (33.3)	3 (15.8)	42 (32.6)	0 (0.0)	3 (42.8)	7 (38.9)
Mild–moderate	76 (51.7)	12 (63.2)	68 (52.7)	8 (72.7)	4 (57.1)	8 (44.4)
Severe	22 (15.0)	4 (21.0)	19 (14.7)	4 (33.3)	0	3 (16.7)
Tissue lymphocyte infiltration[+]						
Absence	16 (10.9)	2 (10.5)	14 (10.9)	1 (9.1)	1 (14.3)	2 (11.1)
Mild–moderate	107 (72.8)	12 (63.2)	95 (73.6)	7 (63.6)	5 (71.4)	12 (66.7)
Severe	24 (16.3)	5 (26.3)	20 (15.5)	4 (33.3)	1 (42.8)	4 (22.2)
Tissue eosinophil infiltration[+]						
Absence	89 (60.5)	8 (45.1)	81 (62.8)	4 (36.4)	4 (57.1)	8 (44.4)
Mild–moderate	51 (34.7)	8 (45.1)	41 (31.8)	5 (45.4)	3 (42.8)	10 (55.6)
Severe	7 (4.8)	3 (15.8)	7 (5.4)	3 (25.0)	0	0
Granulocytes in the stroma[+]						
Absence	102 (69.4)	18 (94.7)	90 (69.8)	12 (100)	6 (85.7)	12 (66.7)
A few	45 (30.6)	1 (5.3)	39 (30.2)	0	1 (14.3)	6 (33.3)
Many	0	0	0	0	0	0

Continued

TABLE 1 Continued

Parameter	All COPD patients	All asthma patients	COPD patients without asthma features	Asthma patients <10 pack-years	Asthma patients >10 pack-years	COPD patients with asthma features[#]
Granulocytes in the epithelium[+]						
Absence	120 (81.6)	19 (100)	106 (82.2)	12 (100)	7 (100)	14 (77.8)
A few	27 (18.4)	0	23 (17.8)	0	0	4 (22.2)
Many	0	0	0	0	0	0
Goblet cells[+]						
Absence	45 (31.3)	2 (10.5)	38 (29.5)	1 (8.3)	1 (14.3)	7 (42.4)
A few	43 (24.5)	15 (73.7)	39 (24.8)	10 (83.3)	5 (71.4)	4 (22.2)
Many	47 (44.2)	2 (10.5)	41 (45.7)	1 (8.3)	1 (14.3)	6 (33.3)
Not detectable[§]	12 (8.2)		11 (8.5)			1 (5.5)
BM thicknening[+]						
Normal	14 (9.3)	1 (5.3)	11 (8.5)	1 (8.3)		3 (16.7)
Mild–moderate	59 (40.1)	14 (73.7)	57 (44.2)	9 (75.0)	5 (71.4)	2 (11.1)
Severe	71 (48.2)	4 (21.1)	58 (45.0)	2 (16.7)	2 (28.6)	13 (72.2)
ASMC %	21.5±9.4	21.5±16.7	21.0±16.6	22.0±7.9	20.7±12.1	24.3±17.7
Distance BM–ASMC μm	80.5±55.5	62.6±21.1	80.4±55.9	62.8±23.8	62.1±23.8	81.3±55.2
Glands %	8.5±13.4	4.8±5.5	8.2±12.9	5.1±6.2	4.3±4.8	10.4±16.3
Eosinophils in BAL[f]	0.9±5.7	1.5±2.8	0.9±6.1	1.2±2.8	2.1±3.1	0.4±0.9
Leukocytes in BAL						
None	33 (22.4)	6 (31.6)	29 (22.4)	2 (16.7)	4 (57.1)	4 (22.2)
A few	69 (46.9)	13 (68.4)	59 (45.7)	10 (83.3)	3 (42.8)	10 (55.5)
Many	30 (20.4)		28 (21.7)			2 (11.1)
Excessive	10 (13.6)		8 (6.2)			2 (11.1)
Missing values	5 (3.4)		5 (3.8)			

Data are presented as n, n (%) or mean±SD. SABA: short-acting beta-agonist; LAMA: long-acting muscarinic antagonist; ICS: inhaled corticosteroid; LABA: long-acting beta-agonist; RV: residual volume; % pred: % predicted; TLC: total lung capacity; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; D_{LCO}: diffusing capacity of the lung for carbon monoxide; F_{ENO}: exhaled nitric oxide fraction; BM: reticular basement membrane; ASMC: airway smooth muscle cell; BAL: bronchoalveolar lavage. [#]: COPD patients were categorised as patients with asthma–COPD overlap if they had three or more of the following criteria: blood eosinophils above 300 per L, normal D_{LCO} (above 80% pred), F_{ENO} above 25 ppm, FEV_1 post-bronchodilator above 80% pred, post-bronchodilator reversibility of airway obstruction above 200 mL, no hyperinflation on radiography, personal or family history of allergy, positive prick test, previous diagnosis with asthma, symptoms triggered by exercise, emotions, laughing, allergens, worse symptoms at night or morning, onset of symptoms in age younger than 20 years old. [¶]: blood cells numbers represent absolute numbers. [+]: qualitative evaluation, 0–3 scale: 0 absence/normal, 1 mild–moderate, 2–3 severe. [§]: goblet cells could not be detected in tissue sections when the epithelium was detached. [f]: BAL cell numbers represent % of total cells.

similarities at the histopathological level between COPD with asthma and "smoking asthma". For instance, assuming a mean ASMC count of 21.0% in the group of COPD without asthmatic features and a mean ASMC count of 24.3% in the group of COPD with asthmatic features, a total of 180 patients, 90 in each diagnostic group, would be needed to achieve a significance level of $p<0.05$ with a power of 0.8. This pilot study provides important information that could guide the design of such studies.

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