



Early View

Research letter

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

E. Papakonstantinou, S. Savic, A. Siebeneichler, W. Strobel, P. W. Jones, M. Tamm, D. Stolz

Please cite this article as: Papakonstantinou E, Savic S, Siebeneichler A, *et al.* A pilot study to test the feasibility of histological characterisation of asthma-COPD overlap. *Eur Respir J* 2019; in press (<https://doi.org/10.1183/13993003.01941-2018>).

This manuscript has recently been accepted for publication in the *European Respiratory Journal*. It is published here in its accepted form prior to copyediting and typesetting by our production team. After these production processes are complete and the authors have approved the resulting proofs, the article will move to the latest issue of the ERJ online.

Copyright ©ERS 2019

A pilot study to test the feasibility of histological characterization of asthma-COPD overlap

E. Papakonstantinou¹, S. Savic², A. Siebeneichler¹, W. Strobel¹, P. W. Jones³, M. Tamm¹, D. Stolz¹

¹Clinic of Pulmonary Medicine and Respiratory Cell Research, University Hospital, Basel, Switzerland,

²Department of Pathology, University Hospital of Basel, Switzerland, ³Global Respiratory Franchise,
GlaxoSmithKline, Brentford, UK

Corresponding Author

Prof. Daiana Stolz, MD, MPH

Clinic of Pulmonary Medicine and Respiratory Cell Research,
University Hospital Basel,
Petersgraben 4, CH-4031 Basel, Switzerland

Email: daiana.stolz@usb.ch

Tel: 0041-61-328 7193

Funding: Clinic of Pneumology of the University Hospital Basel and the Department of Biomedicine,
University of Basel, Switzerland

Take-home message

This is a pilot study suggesting 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.

Asthma and 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 characterized by persistent and progressive airflow limitation and airway inflammation (2). During the past years, there is a long discussion whether asthma and COPD are different diseases since a significant proportion of patients with symptoms of obstructive lung diseases 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 bronchoscopic or surgical low volume reduction procedures (25%), infiltrates (16%) and hemoptysis (8%). COPD patients had an average FEV1/FVC post bronchodilation of 0.4 [0.3-0.5] and a smoking history of 43 pack-years [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 ERS/ATS guidelines, according to GOLD and GINA 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/l, normal DLCO% pred (above 80%), FeNO above 25 ppb, FEV1% pred post bronchodilator above 80%, post-bronchodilator reversibility of airway obstruction above 200 ml, no hyperinflation in X-Ray, 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. A total of 18 patients met these criteria (mean=3.3 criteria). Within the asthma cohort, we identified patients (n=7) that smoked (>10 PY). 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 ten biopsies were obtained, one from the right upper lobe, two from the middle lobe, three from the right lower lobe, one from the lingula and three from left lower lobe. Five sequential sections were prepared from each biopsy, stained with hematoxylin and eosin and evaluated by a pathologist who was blind to the final diagnosis for: (i) 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; (ii) Granulocytes in the stroma, granulocytes in the epithelium and goblet cells, using a 0-3 scale: 0=absence, 1=a few, 2-3=many; (iii) Glands (%); (iv) Thickening of reticular basement membrane (BM), using a 0-3 scale: 0=absence/normal, 1=mild-moderate, 2-3=severe; (v) ASMC mass (%); (vi) distance between BM and ASMC in μm . More than 90% of the selected slides allowed satisfactory analysis of epithelial cells, basement membrane, 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 ICS alters histopathological finding in asthma and COPD since ICS reduce eosinophilia, a hallmark of asthma and increase 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 remodeling. However, when we dichotomized 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 analyzed histological parameters in COPD patients ($n=147$) dichotomized according to post bronchodilator reversibility of FEV₁, equal/greater than 200 ml ($n=20$) and below 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 FEV₁% predicted, FEV₁/FVC% predicted, RV% predicted, TLC% predicted and RV/TLC% predicted. According to the classification criteria used, COPD patients with asthma

characteristics had higher DLCO% predicted ($p=0.023$), higher FeNO ($p=0.008$) 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, 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, showing that in non-emphysematous COPD patients BM is thickened and this is associated with their responsiveness to ICS/LABA treatment (11). Patients with asthma that smoked >10 PY had similar lung function to asthma patients with a smoking history of <10 PY, as revealed by post bronchodilator RV% predicted, TLC% predicted, RV/TLC% predicted, DLCO% predicted, FEV1% predicted and FEV1/FEV 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 PY 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 PY, as revealed by significantly lower values of FEV1% pred ($p=0.016$) and FEV/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 similarities at histopathological level between COPD with asthma and 'smoking asthma'. For instance, assuming a mean average ASMC of 21.0 in the group of COPD without asthmatic features and a mean average ASMC 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.

Table 1. Characteristics of patients with COPD, Asthma and COPD-Asthma overlap

Parameter	All COPD Patients N=147	All Asthma Patients N=19	COPD patients w/o asthma features N=129 (87.8%)	Asthma patients (<10 PY) N=12 (61.1%)	Asthma patients (>10 PY) N=7 (38.8%)	COPD patients with asthma features N=18 (12.2%)
Age (y, mean \pm SD)	70.6 \pm 9.9	59.6 \pm 14.2	71.0 \pm 9.7	60.4 \pm 4.5	58.3 \pm 4.6	68.2 \pm 11.6
Gender Male, n (%)	98 (66.6)	8 (42.1)	82 (63.6)	5 (41.6)	3 (42.8)	16 (88.8)
Current smokers, n (%)	55 (37.4)	17 (89.5)	47 (36.4)	10 (90.1)	7 (100)	8 (44.4)
Therapy, n (%)						
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)
RV % pred post (mean \pm SD)	178.4 \pm 58.9	124.5 \pm 30.4	181.5 \pm 58.8	122.2 \pm 28.9	131.3 \pm 40.7	155.9 \pm 56.7
TLC % pred post (mean \pm SD)	121.0 \pm 22.1	104.8 \pm 13.2	122.2 \pm 22.1	104.7 \pm 14.4	105.3 \pm 11.2	112.3 \pm 21.1
RV/TLC % pred post (mean \pm SD)	139.1 \pm 27.7	116.1 \pm 20.0	140.4 \pm 27.1	115.5 \pm 20.4	118.5 \pm 26.2	129.4 \pm 30.8
FEV1 % pred post (mean \pm SD)	48.8 \pm 22.4	65.4 \pm 15.8	46.8 \pm 19.9	62.1 \pm 13.8	71.0 \pm 18.4	61.8 \pm 32.5
FEV1/FVC post (mean \pm SD)	39.7 \pm 14.4	52.1 \pm 9.8	38.6 \pm 13.4	50.6 \pm 8.1	54.6 \pm 12.7	47.0 \pm 19.1
DLCO% pred post (mean \pm SD)	50.8 \pm 19.7	92.7 \pm 20.9	49.3 \pm 18.9	92.6 \pm 24.4	92.8 \pm 15.5	61.5 \pm 21.9
Reversibility (>200 ml), n (%)	21 (14.2)	6 (31.6)	12 (9.3)	4 (33.3)	2 (28.6)	7 (38.9)
Reversibility (ml, mean \pm SD)	97.8 \pm 115.1	158.9 \pm 130.7	83.7 \pm 91.1	166.7 \pm 140.2	145.7 \pm 122.0	191.7 \pm 194.8
FeNO (ppb, mean \pm SD), exhalation flow rate: 50 ml/sec	19.9 \pm 13.8	37.1 \pm 30.0	17.8 \pm 11.1	41.9 \pm 35.9	29.6 \pm 17.0	32.8 \pm 20.6

Blood eosinophils (nx1059/l, mean ± SD)*	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 leucocytes (nx1059/l, mean ± SD)*	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, mean ± SD)**	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, n (%)	49 (33.3)	3 (15.8)	42 (32.6)	0 (0.0)	3 (42.8)	7 (38.9)
Mild-Moderate, n (%)	76 (51.7)	12 (63.2)	68 (52.7)	8 (72.7)	4 (57.1)	8 (44.4)
Severe, n (%)	22 (15.0)	4 (21.0)	19 (14.7)	4 (33.3)	0	3 (16.7)
Tissue lymphocyte infiltration**						
Absence, n (%)	16 (10.9)	2 (10.5)	14 (10.9)	1 (9.1)	1 (14.3)	2 (11.1)
Mild-moderate, n (%)	107(72.8)	12 (63.2)	95 (73.6)	7 (63.6)	5 (71.4)	12 (66.7)
Severe, n (%)	24 (16.3)	5 (26.3)	20 (15.5)	4 (33.3)	1 (42.8)	4 (22.2)
Tissue eosinophil infiltration**						
Absence, n (%)	89 (60.5)	8 (45.1)	81 (62.8)	4 (36.4)	4 (57.1)	8 (44.4)
Mild-moderate, n (%)	51 (34.7)	8 (45.1)	41 (31.8)	5 (45.4)	3 (42.8)	10 (55.6)
Severe, n (%)	7 (4.8)	3 (15.8)	7 (5.4)	3 (25.0)	0	0
Granulocytes in the stroma**						
Absence, n (%)	102 (69.4)	18 (94.7)	90 (69.8)	12 (100)	6 (85.7)	12 (66.7)
A few, n (%)	45 (30.6)	1 (5.3)	39 (30.2)	0	1 (14.3)	6 (33.3)
Many, n (%)	0	0	0	0	0	0
Granulocytes in the epithelium**						
Absence, n (%)	120 (81.6)	19 (100)	106 (82.2)	12 (100)	7 (100)	14 (77.8)
A few, n (%)	27 (18.4)	0	23 (17.8)	0	0	4 (22.2)
Many, n (%)	0	0	0	0	0	0
Goblet cells**						
Absence, n (%)	45 (31.3)	2 (10.5)	38 (29.5)	1 (8.3)	1 (14.3)	7 (42.4)
A few, n (%)	43 (24.5)	15 (73.7)	39 (24.8)	10 (83.3)	5 (71.4)	4 (22.2)
Many, n (%)	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 thickening**						
Normal, n (%)	14 (9.3)	1 (5.3)	11 (8.5)	1 (8.3)		3 (16.7)
Mild-moderate, n (%)	59 (40.1)	14 (73.7)	57 (44.2)	9 (75.0)	5 (71.4)	2 (11.1)
Severe, n (%)	71 (48.2)	4 (21.1)	58 (45.0)	2 (16.7)	2 (28.6)	13 (72.2)
Average ASMC (%) (mean ± SD)	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, mean ± SD)	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 (%) (mean ± SD)	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**** (mean ± SD)	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 (n, %)	33 (22.4)	6 (31.6)	29 (22.4)	2 (16.7)	4 (57.1)	4 (22.2)
A few (n, %)	69 (46.9)	13 (68.4)	59 (45.7)	10 (83.3)	3 (42.8)	10 (55.5)
Many (n, %)	30 (20.4)		28 (21.7)			2 (11.1)
Excessive (n, %)	10 (13.6)		8 (6.2)			2 (11.1)
Missing values	5 (3.4)		5 (3.8)			

^a COPD patients were categorized as patients with ACO if they had 3 or more of the following criteria: blood eosinophils above 300, normal DLCO% pred (above 80%), FeNO above 25 ppm, FEV1% pred post bronchodilator above 80%, post-bronchodilator reversibility of airway obstruction above 200 ml, no hyperinflation in X-Ray, 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

****BAL cell numbers represent % of total cells

References

1. Chung KF, Wenzel SE, Brozek JL, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J* 2014;43(2):343–373.
2. Vestbo J, Anderson W, Coxson HO, et al. Evaluation of COPD longitudinally to identify predictive surrogate end-points (ECLIPSE). *Eur Respir J* 2008;31:869–873.
3. Gibson, P. G. & Simpson, J. L. The overlap syndrome of asthma and COPD: what are its features and how important is it? *Thorax* 2009;64:728–735.
4. (GINA) GIfA. Asthma, COPD and the asthma-COPD overlap syndrome (ACOS) (2014).
5. Barrecheguren, M., Esquinas, C. & Miravittles, M. The asthma-chronic obstructive pulmonary disease overlap syndrome (ACOS): opportunities and challenges. *Curr Opin Pulm Med* 2015;21:74–79 (2015).
6. Soler-Cataluna J J, Cosío B, Izquierdo JL, López-Campos JL, Marín JM, Agüero R, Balloira A, Carrizo S, Esteban C, Galdiz JB, González MC, Miravittles M, Monsó E, Montemayor T, Morera J, Ortega F, Peces-Barba G, Puente L, Rodríguez JM, Sala E, Sauleda J, Soriano JB, Viejo JL. Consensus document on the overlap phenotype COPD-asthma in COPD. *Arch Bronconeumol* 2012;48:331–337.
7. Kankaanranta H, Harju T, Kilpeläinen M, Mazur W, Lehto JT, Katajisto M, Peisa T, Meinander T, Lehtimäki L. Diagnosis and pharmacotherapy of stable chronic obstructive pulmonary disease: The Finnish Guidelines Guidelines of the Finnish Medical Society Duodecim and the Finnish Respiratory Society. *Basic Clin Pharmacol Toxicol* 2014; 116:291–307.
8. Maud T, Dolhnikoff M. Pathologic similarities and differences between asthma and chronic obstructive pulmonary disease. *Curr Opin Pulm Med* 2008;14:31-38.
9. Sin DD, Miravittles M, Mannino DM, et al. What is asthma–COPD overlap syndrome? Towards a consensus definition from a roundtable discussion. *Eur Respir J* 2016; 48: 664–673.
10. Fattahi F, Vonk JM, Bulkman N, Fleischeuer R, Gouw A, Grünberg K, Mauad T, Popper H, Felipe-Silva A, Vrugt B, Wright JL, Yang HM, Kocks JW, Hylkema MN, Postma DS, Timens W, Ten Hacken NH. Old dilemma, asthma with irreversible airway obstruction or COPD. *Virchows Arch* 2015;467:583-593.
11. Al-Kassimi AF, Alhamad HE, et al. Can computed tomography and carbon monoxide transfer coefficient diagnose an asthma-like phenotype in COPD? *Respirology* 2017;22:322-328.