Large airway wall vascularity in patients with asthma–COPD overlap: a bronchoscopy study

Received: 2 Jan 2024
Accepted: 2 March 2024

To the Editor:

Asthma COPD overlap (ACO) is a clinical term that describes patients with persistent airflow limitation, and clinical and physiological characteristics consistent with both asthma and COPD [1]. The hallmark pathological features of asthma and COPD are airway inflammation and remodelling. These have been considered as separate processes or perhaps as sequential, with early inflammation leading later to remodelling; however, little is known about these parameters in ACO. We have recently reported an increase in inflammatory cells such as macrophages and $CD8^+$ T-cells in the airway mucosa of patients with ACO [2]. Notably, our previous findings of airway remodelling in ACO airway suggested a thicker reticular basement membrane (RBM) and higher RBM cellularity [3]. Another crucial element of tissue remodelling is angiogenesis. In asthma, most studies reported an increase in tissue vascularity, while in COPD, there are conflicting reports on airway tissue vascularity contributing to disease [1, 4–8]. In this cross-sectional exploratory study, we hypothesised that tissue vascularity in the airway in ACO could be different from that in asthma, COPD and healthy controls. To evaluate this, we analysed large airway endobronchial biopsies from patients with ACO and compared them against healthy controls, patients with asthma, patients with COPD who were ex-smokers and current smokers, and normal lung function smokers (NLFSs).

We immunohistochemically stained 3-µm-thick bronchial biopsy sections following optimisation. The tissues were obtained from biobank and were collected from physician-diagnosed patients (diagnostic criteria previously published by our team [2, 3]) with ACO, asthma or COPD, and healthy controls and NLFSs. The patients with ACO in our study were ex-smokers; our biobank did not have any tissue available from current smokers with ACO. A representative tissue micrograph and counting strategy are provided in figure 1a, and the patient demography and baseline information are provided in figure 1b. Primary rabbit polyclonal anti-collagen IV (1:350) (ab6586; Abcam, Victoria, Australia) was used. Following staining, computer-assisted image analysis was performed as previously described [2, 3]. Stained tissues with visible epithelium, RBM and lamina propria were selected for image analysis. The images of the entire tissue area, including epithelium, RBM and lamina propria, were captured at 40× brightfield, avoiding the overlapping area between images. From five randomly selected images, collagen IV-positive vessels were counted in the epithelium, RBM and lamina propriate, *i.e.* 120 µm deep inside the tissue (figure 1a). The observer was blinded to the patient and diagnosis. The epithelium and RBM-associated vessels were presented as per mm of RBM length and the vessels in the lamina propria were presented as per mm^2 of the lamina propria area [9, 10]. Vessel count data distribution was evaluated using the D'Agostino and Pearson test, and intra- and intergroup variances were analysed using Kruskal–Wallis (nonparametric) with multiple comparisons using uncorrected Dunn's test.

Overall, the number of epithelial vessels in ACO (figure 1c) were similar to the healthy controls and asthma, and tended to be higher than the COPD ex-smokers and NLFSs. The number of epithelial vessels in COPD current smokers tended to be increased and in COPD ex-smokers, the vessel numbers tended to be decreased when compared with healthy controls; however, the differences were not statistically significant ($p=0.9891$ and $p=0.4412$, respectively).

The number of RBM vessels in patients with ACO (figure 1d) were significantly higher ($p<0.05$) than the healthy controls. Furthermore, the number of RBM vessels in ACO appeared to be higher than asthma

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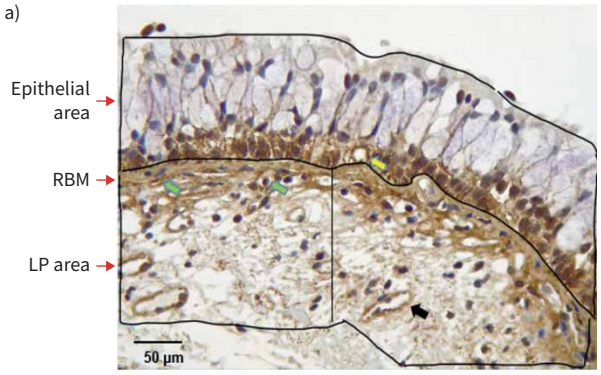
Large airway wall lamina propria in patients with asthma–COPD overlap is hypovascular with an increase in reticular basement membrane neoangiogenesis, reflecting smoking-related COPD-like pathology and potential epithelial-to-mesenchymal transition https://bit.ly/49DeoFX

Cite this article as: Dey S, Lu W, Weber HC, *et al*. Large airway wall vascularity in patients with asthma–COPD overlap: a bronchoscopy study. *ERJ Open Res* 2024; 10: 00002-2024 [DOI: 10.1183/23120541.00002-2024].

a)



b)

Parameter/group	HC	ACO	Asthma	COPD ES	COPD CS	NLFS
Subjects	10	12	14	10	12	12
Age, years	61 (25–68)	70 (52–77)	62 (26–81)	67 (46–78)	65.5 (51–78)	56.5 (41–72)
Smoking history, pack-years	0	22.5[#] (15–103)	0	36 (22–105)	34.8 (10–114)	31.5 (20–75)
ICS treatment, n	NA	6	6	NA	NA	NA
ICS dose, µg·day^{-1}	NA	800	750	NA	NA	NA
GINA diagnosis mild persistent/ moderate/severe, n	NA	0/0/4	5/1/8	NA	NA	NA
GOLD diagnosis stage I and II/stage III, n	NA	7/1	NA	10/0	12/0	NA
FEV_1, % pred	93 (75–114)	58 (35–96)	81.5 (48–108)	84.5 (54–113)	69 (49–92)	94 (79–113)
FEV_1/FVC, %	83 (73–86)	65.5 (31–84)	74 (52–90)	63.2 (55–69)	63.6 (50–75.3)	78 (70–85)

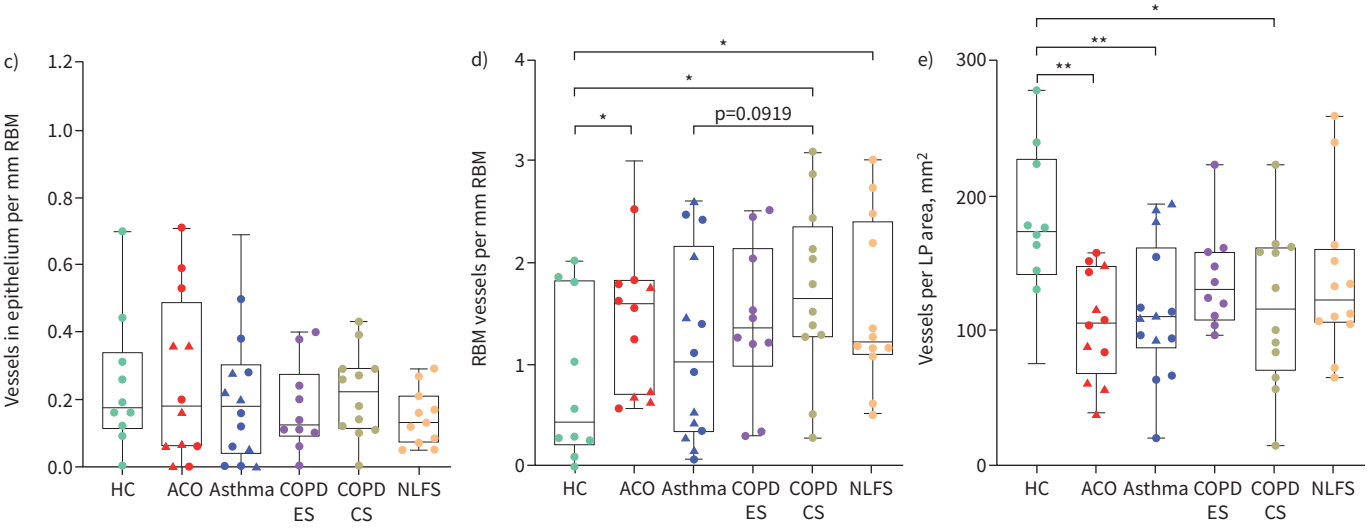


FIGURE 1 a) Tissue micrograph showing collagen IV-positive vessels in the epithelium (yellow arrow), reticular basement membrane (RBM) (green arrow) and lamina propria (LP) area (black arrow). b) Patient demographics (data presented as median (range)). 70 large airway endobronchial biopsy samples were collected from the participants, and tissue were obtained from the Tasmanian Respiratory Tissue Bank and Newcastle Biobank (Tasmanian Health and Medical Human Research Ethics Committee, ethics identifier H0013051; the Hunter New England Human Research Ethics Committee, reference number 05/08/10/3.09). Box plots showing c) epithelial vessels per mm of RBM, d) RBM vessels per mm of RBM and e) LP vessels per mm^2 area in healthy controls (HC), asthma–COPD overlap (ACO), asthma, chronic COPD ex-smokers (ES) and COPD current smokers (CS), and normal lung function smokers (NLFS). Triangles represent the vessel counts in patients treated with inhaled corticosteroids (ICS). The horizontal line inside each box represents the median; the top and bottom of each box represent the upper and lower quartiles, respectively; and the whiskers represent extreme values. GINA: Global Initiative for Asthma; GOLD: Global Initiative for Chronic Obstructive Lung Disease; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; NA: not applicable. [#]: all ex-smokers. *: p<0.05; **: p<0.01 for multiple comparisons.

(p=0.2316), COPD ex-smokers (p=0.7881) and NLFSs (p=0.9201); however, the difference was not statistically significant. A similar increase in the number of vessels was noted between the patients with ACO and COPD current smokers. We have also noted a statistically significant increase in the number of RBM vessels (p<0.05) in COPD current smokers compared to healthy controls. Although the asthma group appeared to have higher RBM vessels than the healthy controls, the difference was not statistically significant (p=0.3180). Among the asthma and COPD groups, the RBM vessels were greater in COPD current smokers, which was statistically significant (p<0.05) compared with asthma. Furthermore, RBM vessels in COPD current smokers also tended to be higher than in COPD ex-smokers, but was not statistically significant (p=0.4726).

In contrast to the RBM, we observed the lowest number of vessels in the lamina propria area of patients with ACO, which was statistically significant (p<0.01) compared with healthy controls (figure 1e). In addition, the

number of lamina propria vessels in ACO tended to be lower than in COPD ex-smokers and NLFSs; however, the difference was not statistically significant (p=0.1128 and p=0.1633, respectively). Furthermore, we noted similar RBM vessel numbers in ACO, asthma and COPD current smokers. The number of lamina propria vessels in asthma and COPD current smokers were also lower than in healthy controls ($p<0.05$ and $p<0.01$, respectively). Furthermore, the number of lamina propria vessels in COPD ex-smokers appeared to be lower than in healthy controls but the difference was not statistically significant (p=0.1351).

We checked the effect of inhaled corticosteroid (ICS) treatment in by dichotomising the vessel numbers in ACO patients with and without ICS treatment, and the results suggested a trend of lower numbers of vessels in the RBM (p=0.5049) and lamina propria (p=0.2132) of ICS-treated patients as compared with those not on ICS (median difference 0.71 per mm and 54.51 per mm^2, respectively) – although this was not statistically significant – suggesting an ICS treatment effect on the number of vessels. However, in COPD, we reported previously that inhaled fluticasone propionate normalises lamina propria vascularity and reduces RBM cellularity [10, 11]. Furthermore, the correlation analysis between the RBM vessels and RBM thickness was not significant in patients with ACO (Spearman's r= −0.0818, p=0.4090) or forced expiratory volume in 1 s/forced vital capacity ratio (Spearman's r=0.1888, p=0.2788).

Despite limited sample size, this cross-sectional study provides valuable insights into tissue vascularity in the mucosa of patients with ACO compared with the contributing diseases, smokers and healthy controls. We believe that these are the first observational findings of tissue vascularity in patients with ACO. Our findings suggest a prominent and contrasting change in RBM and lamina propria vascularity in ACO patients compared to healthy controls, with a similar observation for the contributing diseases asthma and COPD. Soltani and co-workers [4, 9] reported hypervascular RBM with higher vessel permeability and hypovascular lamina propria in smoking COPD patients. Kuwano *et al.* [12] reported a similar vascular area and vessel dimension in submucosa between COPD and healthy controls. Increased tissue vascularity was also reported in the submucosal area of patients with asthma [6, 12], possibly due to neovascularisation or angiogenesis in response to local inflammation and growth factor elaboration [13] causing more blood flow, perhaps to meet increased metabolic need. Although hypothetical at this stage, high RBM vascularity in ACO could be due to higher cellular activities with respect to epithelial–mesenchymal transition (EMT), which we previously reported in COPD [1, 3, 14]. ACO being the overlap of asthma and COPD disease, theoretically, it would not be wrong to state that some of the established pathology of tissue vascularity of either disease may also be active in ACO, as evident from our vascularity data on ACO. With respect to the effect of ICS on tissue vascularity in patients with ACO, it is well established that ICS has an effect on the reduction of tissue vascularity in asthma [15], our ICS data shows a similar trend in ACO, with six out of 12 subjects on ICS.

Our study has the limitation of not comparing the vascular area among the groups. In addition, one might think that age could be confounder; however, we have not noticed a consistent pattern of correlation between age and the vessel count among the groups. For some of our findings, we only noticed trends without statistically significant differences due to a small sample size. Future studies are needed with larger cohorts. In addition, our findings from this study were limited to patients with ACO who were ex-smokers. However, one can expect that current smoking will only lead to a more destructive pathology.

Taken together, our findings of high RBM vascularity in the large airway wall of ACO and decrease in LP vascularity are novel and indicate COPD-like pathology [9]. In COPD, increased vascularity is attributed to EMT changes leading to formation of pro-cancer stroma and airway fibrosis. EMT could also be an active process in patients with ACO but warrants further investigation. We believe our findings will enhance clinical understanding on ACO helping physicians with informed decision making.

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Provenance: Submitted article, peer reviewed.

Ethics statement: This study was approved by the Tasmanian Health and Medical Human Research Ethics Committee (ethics ID H0013051) and the Hunter New England Human Research Ethics Committee (ref. number 05/08/10/3.09).

Conflict of interest: S.S. Sohal reports an honorarium for lectures from Chiesi, travel support from Chiesi, AstraZeneca and GSK, and research grants from Boehringer Ingelheim and Lung Therapeutics, outside the submitted work; and has served on the Small Airway Advisory Board for Chiesi Australia for which an honorarium has been received, all disclosures made outside the submitted work. The remaining authors have nothing to disclose.

Support statement: This study was supported by the Clifford Craig Foundation, Launceston General Hospital. Funding information for this article has been deposited with the Crossref Funder Registry.

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