Endothelial to mesenchymal transition is an active process in smokers and patients with early COPD contributing to pulmonary arterial pathology

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Shareable abstract (@ERSpublications)
Endothelial to mesenchymal transition is an active process in smokers and patients with early COPD, contributing to arterial remodelling and lung fibrosis in general. Endothelial cells in COPD are more than bystanders and should not be overlooked. https://bit.ly/49TtZBZ

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Abstract

Background We have previously reported pulmonary arterial remodelling in smokers and patients with early COPD, which can be attributed to endothelial to mesenchymal transition (EndMT). In this study, we aimed to evaluate if EndMT is an active mechanism in smokers and COPD.

Methods Immunohistochemical staining for the EndMT biomarkers CD31, N-cadherin, vimentin and S100A4 was done on lung resection tissue from 49 subjects. These comprised 15 nonsmoker controls (NC), six normal lung function smokers (NLFS), nine patients with small airway disease (SAD), nine current smokers with mild-moderate COPD (COPD-CS) and 10 ex-smokers with COPD (COPD-ES). Pulmonary arteries were analysed using Image ProPlus software v7.0.

Results We noted reduced junctional $CD31^+$ endothelial cells ($p<0.05$) in the intimal layer of all smoking groups compared to NC. We also observed increased abundance of the mesenchymal markers N-cadherin ($p<0.05$) and vimentin ($p<0.001$) in all smoking groups and across all arterial sizes *versus* NC, except for N-cadherin in large arteries in COPD-CS. The abundance of S100A4 correlated with arterial thickness (small: r=0.29, p=0.05; medium: r=0.33, p=0.03; large: r=0.35, p=0.02). Vimentin in the small arterial wall negatively correlated with forced expiratory volume in 1 s/forced vital capacity (r= −0.35, p=0.02) and forced expiratory flow rate at 25–75% of forced vital capacity (r= −0.34, p=0.03), while increased cytoplasmic CD31 abundance in the intimal layer of medium and large arteries negatively correlated with predicted diffusing capacity of the lung for carbon monoxide (medium: r= −0.35, p=0.04; large: r= −0.39, p=0.03).

Conclusion This is the first study showing the acquisition of mesenchymal traits by pulmonary endothelial cells from NLFS, SAD and mild-moderate COPD patients through EndMT. This informs on the potential early origins of pulmonary hypertension in smokers and patients with early COPD.

Introduction

COPD is characterised by progressive airflow limitation with variable chronic inflammatory response to cigarette smoking and environmental pollutants, especially gases emanating from fossil fuel burning [1, 2]. As a result, COPD patients develop several structural changes in the lung, including small airway wall

thickening and narrowing, large airway squamous cell metaplasia, mucus hypersecretion and smooth muscle hyperplasia [3–5]. Small conducting airways (<2 mm internal diameter) become the early and major site of airflow obstruction in smokers, representing a "silent zone" within the lung where obstructive airway disease can accumulate without being noticed, known as small airway disease (SAD) [6, 7]. Along with alveolar abnormalities, vascular modifications are also common in COPD, which can lead to pulmonary hypertension (PH), a common comorbidity associated with COPD [8, 9].

Vascular remodelling is not only restricted to patients with established COPD but is also seen in patients with SAD and in normal lung function smokers (NLFS) [10–13]. The most prominent feature of vascular remodelling in these groups of patients is intimal hyperplasia, primarily due to endothelial and smooth muscle cell proliferation [14]. Endothelial dysfunction is primarily responsible for the vascular changes observed in patients with COPD, including narrowing of arteries caused by the encroachment of the lumen and deposition of elastin and collagen [15–17]. Our recent study found that smoking, SAD and mild-moderate COPD were associated with pruning of and a decrease in the number of pulmonary vessels, with increased wall thickness and variable elastin deposition [8]. In addition, the proliferation of intima with aggressive encroachment into luminal space can be attributed to the ability of the endothelial cell to transform into a more proliferative phenotype, *i.e.* endothelial to mesenchymal transition (EndMT); however, its extent and molecular mechanism need further exploration [18–20]. EndMT is indicated by the loss or shift of junctional proteins such as platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31) from the endothelial cells, transforming them to migratory mesenchymal cells. An abundance of mesenchymal proteins such as N-cadherin, S100A4 and vimentin in the endothelial layer suggests EndMT activities [10, 21].

In this study, we hypothesise that the vascular remodelling in smokers and SAD and COPD patients could be attributed to the dynamic process of EndMT. Therefore, to evaluate the role and extent of EndMT in vascular remodelling, we differentially quantified the mesenchymal markers in each layer of pulmonary arteries across sizes in different patient cohorts and compared them with a normal control (NC) group. In addition, we descriptively analysed the localisation of endothelial markers in the junction and cytoplasm of endothelial cells. Furthermore, we evaluated whether the localisation of endothelial markers and abundance of mesenchymal markers were associated with the presence of vascular remodelling, and further correlated EndMT markers with lung function and other physiological parameters.

Materials and methods

Participants

Surgically resected human lung tissues were available from our biobank, with use approved by the Tasmania Health & Medical Human Research Ethics Committee (ethics no. H0012374). Tissue was used from participants with primary non-small cell lung cancer who consented to research procedures. The resected material was taken far from the primary tumour and contained non-cancer-affected pulmonary blood vessels. A single block and tissue section were used for each participant. Participants included six NLFS, nine SAD patients, nine current smokers with COPD (COPD-CS) and 10 ex-smokers with COPD (COPD-ES). COPD was categorised as Global Initiative for Chronic Obstructive Lung Disease 1 or 2 (forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC) <70% and FEV_1 >50% predicted). Participants classified as COPD-ES had at least 6 months of smoking-free history. In addition, tissue from 15 nonsmoker subjects who died of causes other than respiratory diseases were obtained from the James Hogg Lung Registry, University of British Columbia (ethical approval from the Providence Health Care Research Ethics Board H00–50110) to use as the NC group. Subject demographic and lung function measures, obtained from participants as part of standard clinical practice timed closely to the resection of lung tissue, are provided in table 1.

Immunohistochemical staining for EndMT markers

The resected lung tissue was deparaffinised using xylene and ethanol and rehydrated in distilled water as we have previously reported [22–24]. The antigen retrieval procedure was carried out using a Decloaking Chamber (Biocare Medical, Queensland, Australia) at 110°C for 15 min with target retrieval citrate buffer (pH 6.0; Dako S2369, Agilent Technologies, Mulgrave, VIC, Australia). Endogenous peroxidase blocking was processed using 3% hydrogen peroxide (v/v) in distilled water for 15 min. A protein block (serum-free; Dako X0909, Agilent Technologies) was used before applying primary antibody S100A4 for 30 min. The tissues were stained with EndMT primary antibodies, monoclonal mouse anti-human CD31 (1:40; Dako M0823, Agilent Technologies), rabbit polyclonal S100A4 (1:1000; Dako A5114, Agilent Technologies), mouse monoclonal vimentin (1:200; Dako M7020, Agilent Technologies) and mouse monoclonal anti-N-cadherin (1:100; Ab98952, Abcam, Melbourne, VIC, Australia) for 60 min at ambient temperature, followed by secondary horseradish peroxidase rabbit/mouse antibodies (EnVision Detection

TABLE 1 Participant demographics

Groups	NC	NLFS	SAD	COPD-CS	COPD-ES
Participants	15	6	9	9	10
Sex					
Female	8	4	7	5	3
Male	7	2	2	4	7
GOLD stage					
1	NA	NA	NA	3	6
2	NA	NA	NA	6	4
Age (years)	44 (19–63)	68.5 (52–79)	59 (42–84)	63 (59–78)	68 (56–85)
Smoking (pack-years)	0	24.75 (0.3–40)	40 (0–72)	30 (20–67)	30 (18–60)
FEV_1/FVC (%)		81.5 (70–90)	73.4 (69–78)	66 (60–70)	62.8 (55–69)
$FEF_{25–75\%}$ ($L{\cdot}s^{-1}$)		81.5 (71–116)	46.5 (31–69)	37 (28–47)	38 (20–55)
D_{LCO} (% predicted)		84.45 (50–95)	78.1 (54–91)	71 (34–84)	78 (51–105)

Data are expressed as n or median (range). NC: nonsmoker control; NLFS: normal lung function smoker; SAD: patients with small airway disease; COPD-CS: current smoker with COPD; COPD-ES: ex-smoker with COPD; GOLD: Global Initiative for Chronic Obstructive Lung Disease; NA: not available; FEV_1: forced expiration volume in 1 s; FVC: forced vital capacity; $FEF_{25–75\%}$: forced expiratory flow at 25–75% of FVC; D_{LCO}: diffusing capacity of the lung for carbon monoxide.

System, Dako K5007, Agilent Technologies) for 30 min. The protein markers were visualised as brown with the addition of DAB solution (EnVision Detection Systems, Dako K5007, Agilent Technologies). The nucleus was counterstained with haematoxylin (Australian Biostain P/L, Gillman, SA, Australia).

Measurement strategies for pulmonary arterial sizes and endothelial and mesenchymal markers

Measurements were made as previously described [8]; further details are given in the online supplementary material.

Correlations of mesenchymal markers with vascular remodelling markers and physiological parameters

Our previous study reported the morphometric assessment of arterial remodelling including increased thickness and variable elastin deposition in NLFS, SAD, COPD-CS and COPD-ES patients [8]. Here, we performed the correlation between vascular remodelling data and the abundance of EndMT markers to explore the association of EndMT with arterial remodelling. We also correlated the EndMT markers with physiological measures obtained from participants timed closely to the resection of lung tissue.

Statistical analysis

Cross-sectional data were tested for a normal distribution using the D'Agostino–Pearson omnibus normality test. Shapiro–Wilk normality test was used where the sample size was insufficient for the previous test. Based on the normality distribution, two-way ANOVA was performed with mean intergroup comparisons using Fisher's least significant difference test. Based on the normality distribution, univariate Spearman or Pearson r was used for correlation analysis. All analyses were performed using GraphPad Prism 9.3 (GraphPad, San Diego, CA, USA), with $p \leqslant 0.05$ considered significant.

Results

EndMT markers in the arterial wall

The pulmonary arteries of all the smoking groups, including NLFS, SAD, COPD-CS and COPD-ES, showed structural modification, such as luminal encroachment by the proliferative intimal cells and muscular hypertrophy, compared to NC (figure 1). Endothelial marker CD31 was localised at junctions in NC, while it was more diffuse across the cytosol in smoking groups. Greater expression of the mesenchymal markers vimentin, S100A4 and N-cadherin was seen in all layers, predominantly in the intima of smoking groups, compared to NC across all the arterial sizes. Comparatively, there was less N-cadherin staining than vimentin and S100A4 (figure 1).

Endothelial CD31 in the intima

We differentially counted cytoplasmic and junctional $CD31^+$ endothelial cells lining the intimal layer of the arterial wall. We found a reduced percentage of junctional $CD31^+$ endothelial cells in smoking groups compared to NC across all arterial sizes. NC had an average of 75–80% endothelial cells showing

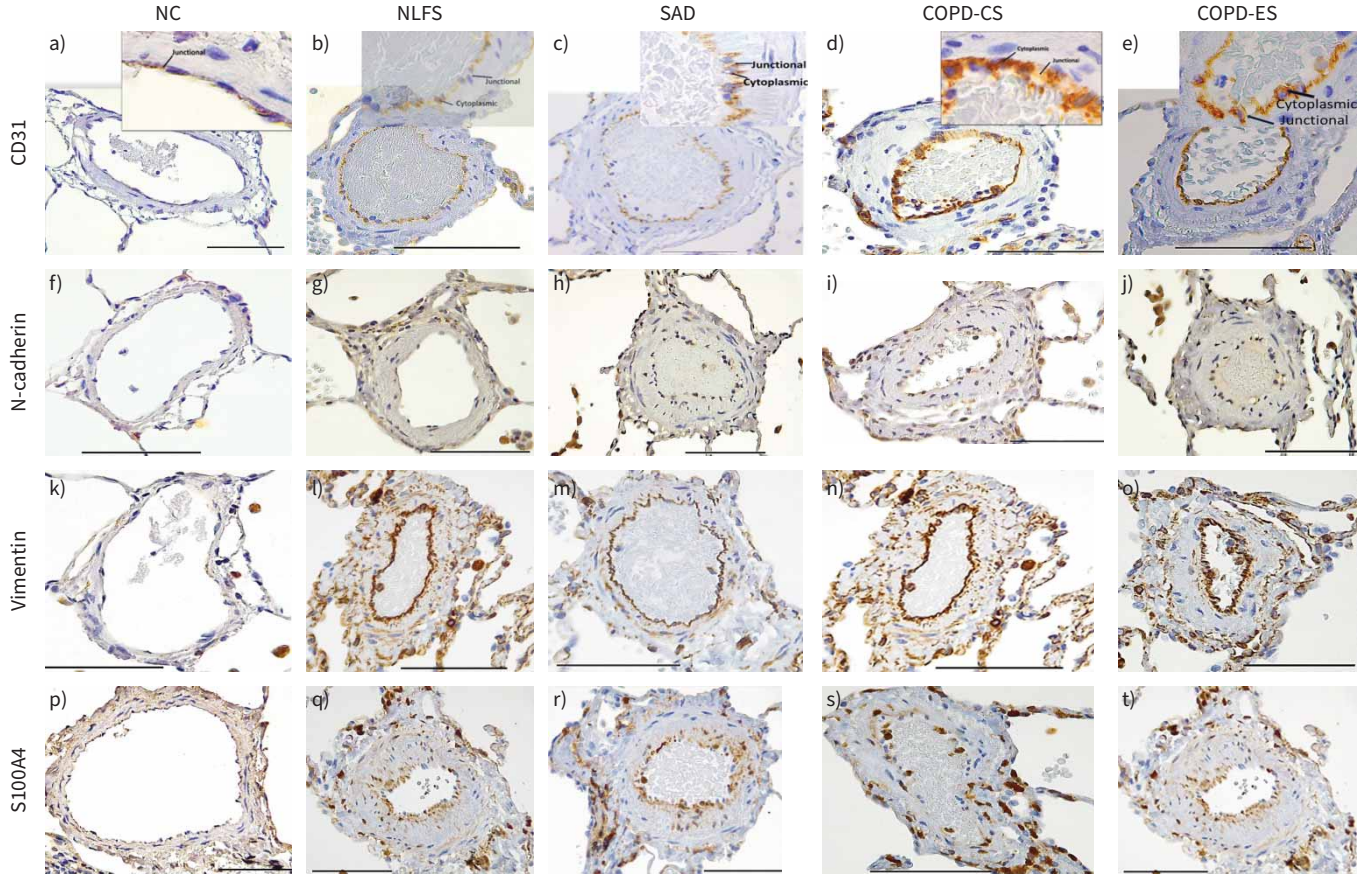

FIGURE 1 Representative images of immunohistology stained slides for pulmonary arteries from nonsmoker control (NC), normal lung function smoker (NLFS), small airway disease (SAD), current smoker with COPD (COPD-CS) and ex-smoker with COPD (COPD-ES) groups. Images were taken at ×40 magnification and ×100 magnification for the insert images at bright fields. Scale bars: 100 µm.

junctional CD31 marker, significantly reducing to 50–60% in all smoking groups in all arterial sizes ($p<0.05$) (figure 2a). In contrast, the percentage of cytoplasmic $CD31^+$ endothelial cells almost doubled in all smoking groups compared to NC. The percentage of cytoplasmic $CD31^+$ cells in the small arterial range was higher in SAD and COPD-CS than in NC ($p<0.05$), while in the medium and large arterial ranges, the percentage of cytoplasmic $CD31^+$ cells was significantly higher in all smoking groups compared to NC ($p<0.05$) (figure 2b).

Mesenchymal marker N-cadherin

The abundance of total N-cadherin in the arterial wall was found to be higher (2–3-fold) in all smoking groups compared to the NC across arterial sizes ($p<0.05$), except for large arteries in COPD-CS, which showed more abundant N-cadherin compared to NC but the difference was not statistically significant (figure 3a). The intimal N-cadherin was higher in medium arteries of NLFS, SAD and COPD-ES groups and as well as in large arteries of COPD-ES compared to NC ($p<0.05$), although a nonsignificant increase was seen across the pathological groups (figure 3c, d). In the media layer there was higher N-cadherin for smoking groups compared to NC in the medium arteries only ($p<0.05$) (figure 3c). Interestingly, the adventitial layer exhibited significantly more N-cadherin across all the arterial sizes across the various patient cohorts than NC (figure 3b–d). Notably, SAD patients had the most abundant N-cadherin in the adventitial layer of small arteries, which was statistically significant compared to NLFS, COPD-CS and COPD-ES ($p<0.05$) (figure 3b).

Mesenchymal marker vimentin

The abundance of the mesenchymal marker vimentin in the pulmonary arterial wall was measured in total and individual layers. Across all arterial sizes, the total vimentin was found to be significantly more abundant (4–6-fold) in all smoking groups compared to NC ($p<0.001$) (figure 4a). In small arteries, total

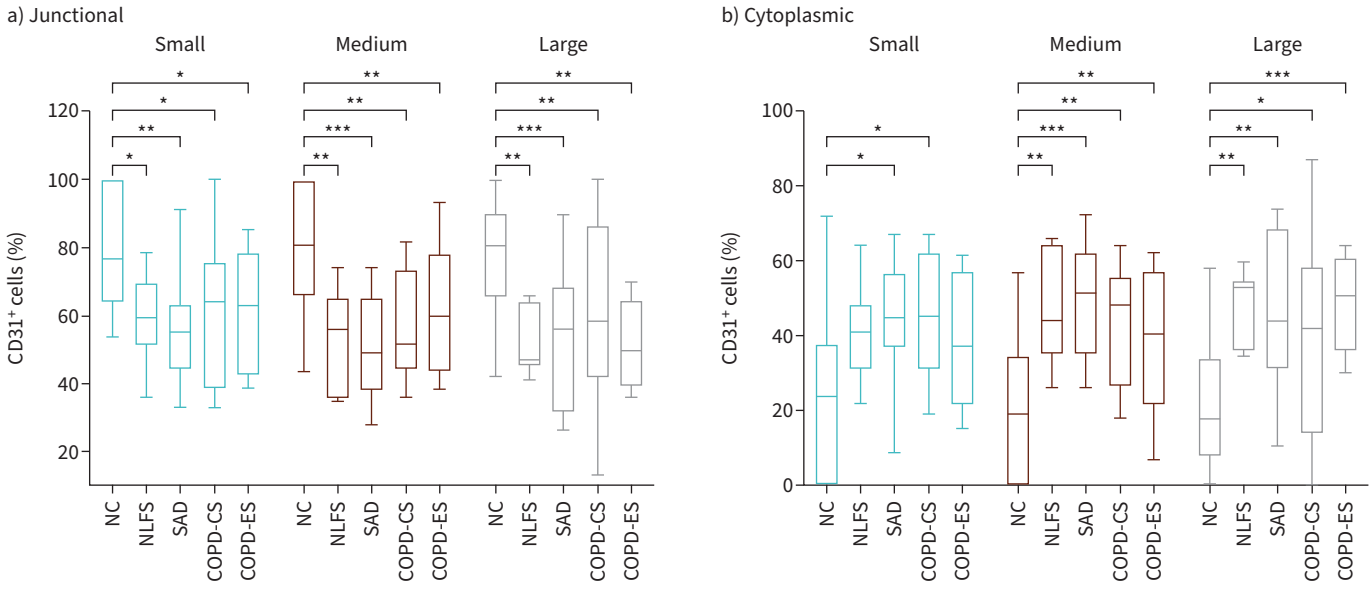

FIGURE 2 Graphical representation comparing the percentage of a) junctional CD31$^+$ cells and b) cytoplasmic CD31$^+$ cells in small (100–299 µm), medium (300–499 µm) and large (500–999 µm) arteries. NC: nonsmoker controls; NLFS: normal lung function smokers; SAD: patients with small airway disease; COPD-CS: current smokers with COPD; COPD-ES: ex-smokers with COPD. *: p≤0.05; **: p≤0.01; ***: p≤0.001.

vimentin was most abundant in COPD-CS among the pathological groups, statistically more so than in the SAD group (p<0.05) but not COPD-ES and NLFS (figure 4a). Vimentin was also significantly higher in each layer of NLFS, SAD, COPD-CS and COPD-ES patients compared to NC across arterial sizes (p<0.05) (figure 4b–d). COPD-CS had the most abundant vimentin in the intimal layer of small arteries, significant more than NLFS and SAD patients (p<0.05) but not COPD-ES (figure 4b). SAD patients had significantly higher vimentin than NLFS in the intimal layer of medium and large arteries (p<0.05) (figure 4c, d).

Mesenchymal marker S100A4

In contrast to vimentin, our results did not indicate any significant difference in total S100A4 abundance between the groups across the arterial sizes (figure 5a). However, we observed a higher abundance of S100A4 in the intimal layer of smoking individuals than in NC, which reached statistical significance in the smaller arteries of SAD and COPD-ES (p<0.05) (figure 5b) and in larger arteries of SAD and COPD-CS (figure 5d). Similarly, S100A4 was significantly higher in NLFS compared to NC and COPD-CS in the adventitial layer of larger arteries (p<0.05) (figure 5d). By contrast, the abundance of S100A4 in the media layer across the arterial sizes and in the adventitia layer of small and medium arteries did not show any significant difference between the groups (figure 5b–d).

Correlation of mesenchymal markers with physiological parameters and arterial thickness

We performed a correlation on the abundance of mesenchymal markers with physiological parameters and arterial thickness using the pathological groups including NLFS, SAD, COPD-CS and COPD-ES. The increased abundance of total vimentin in the small arterial wall was negatively correlated with FEV_1/FVC (r= −0.35, p=0.02) and forced expiratory flow rate at 25–75% of forced vital capacity ($FEF_{25–75\%}$) (r= −0.34, p=0.03) (figure 6a, b). Likewise, intimal S100A4 was negatively correlated with FEV_1/FVC across all the arterial sizes (small: r= −0.31, p=0.04; medium: r= −0.28, p=0.06; large: r= −0.36, p=0.02) (figure 6c). The abundance of intimal S1004 in larger arteries was also found to negatively correlate with $FEF_{25–75\%}$ (r= −0.33, p=0.03) across the pathological groups (figure 6d).

We also observed interesting correlations between the percentage of cytoplasmic CD31$^+$ cells in the intimal layer of medium and large arteries with diffusing capacity of the lung for carbon monoxide (D_{LCO}), which was significantly negative (medium: r= −0.35, p=0.04; large: r= −0.39, p=0.03) (figure 7a). Similar increases in vimentin abundance in the intimal layer of the small and medium arteries were negatively correlated with D_{LCO} (small: r= −0.47, p=0.008; medium: r= −0.38, p=0.02) (figure 7b). Furthermore, we found significant positive correlation between the abundance of total S100A4 and total arterial thickness

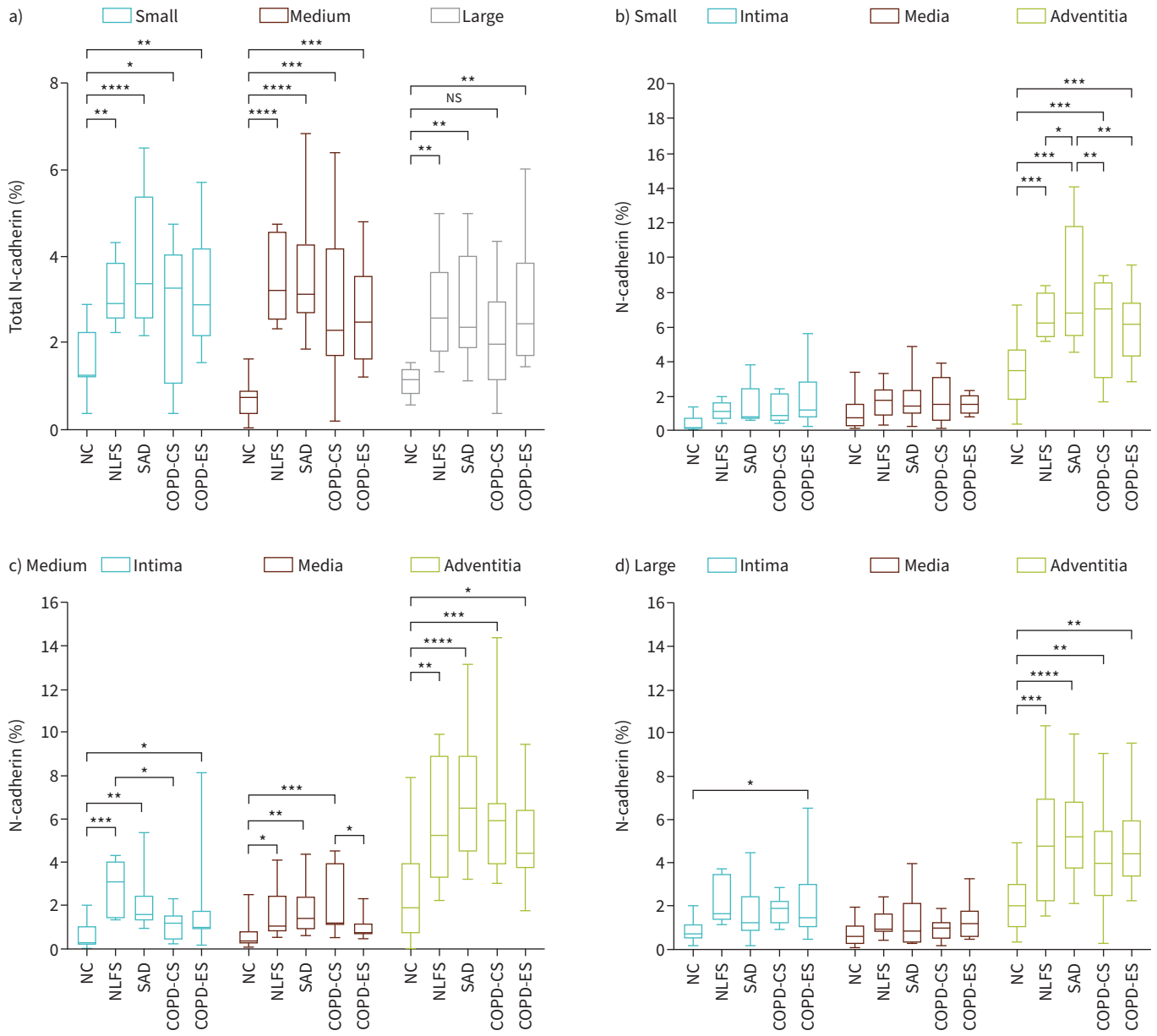

FIGURE 3 Abundance of total N-cadherin in a) the arterial wall in small, medium and large arteries; b) different arterial layers of small arteries (100–299 µm); c) different arterial layers of medium arteries (300–499 µm); and d) different arterial layers of large arteries (500–999 µm). NC: nonsmoker controls; NLFS: normal lung function smokers; SAD: patients with small airway disease; COPD-CS: current smokers with COPD; COPD-ES: ex-smokers with COPD; NS: nonsignificant. *: p⩽0.05; **: p⩽0.01; ***: p⩽0.001; ****: p⩽0.0001.

across the arterial sizes (small: r=0.29, p=0.05; medium: r=0.33, p=0.03; large: r=0.35, p=0.02) (figure 7c). Similarly, the abundance of cytoplasmic CD31 in the intimal layer of small arteries was positively correlated with the intimal thickness (r=0.45, p=0.005) (figure 7d). The abundance of vimentin and N-cadherin did not show any significant correlation with arterial thickness.

Discussion

This study, to the best of our knowledge, is the first descriptive study on EndMT in pulmonary arterial vasculature of NLFS, patients with SAD and current and ex-smokers with mild-moderate COPD. We have provided quantitative data on the abundance of EndMT markers across the arterial layers in different arterial sizes. This study shows that smoking, SAD and COPD are associated with increased cytoplasmic CD31 abundance in endothelial cells and increased mesenchymal proteins N-cadherin, vimentin and

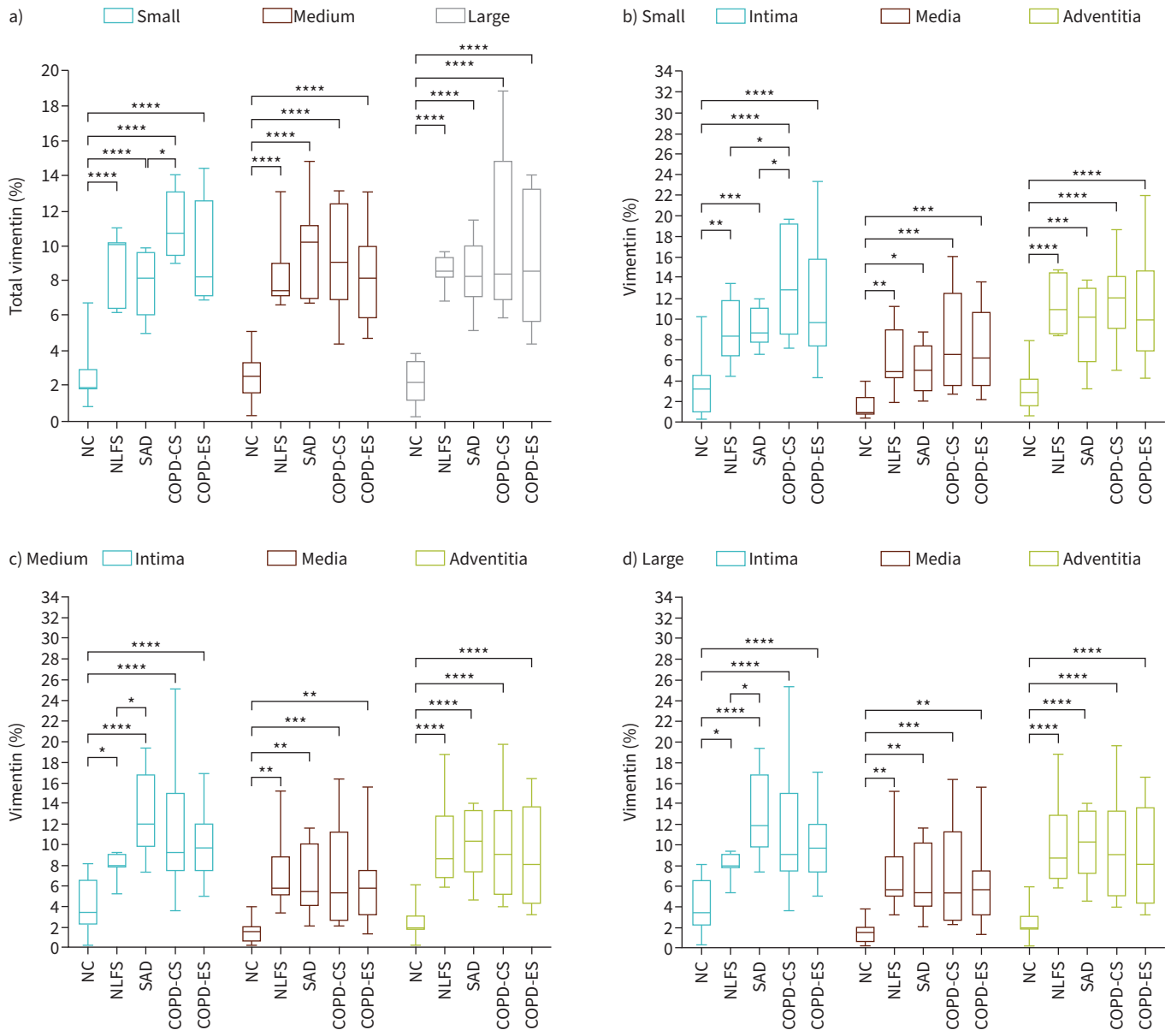

FIGURE 4 Abundance of total vimentin in a) the arterial wall in small, medium and large arteries; b) different arterial layers of small arteries (100–299 µm); c) different arterial layers of medium arteries (300–499 µm); and d) different arterial layers of large arteries (500–999 µm). NC: nonsmoker controls; NLFS: normal lung function smokers; SAD: patients with small airway disease; COPD-CS: current smokers with COPD; COPD-ES: ex-smokers with COPD. *: p⩽0.05; **: p⩽0.01; ***: p⩽0.001; ****: p⩽0.0001.

S100A4, suggesting active EndMT in the pulmonary arteries of these patients. We also observed a positive correlation between EndMT markers and arterial remodelling indicated by increased arterial thickness. Moreover, we found that EndMT in pulmonary arteries was associated with a decline in lung function as measured by FEV_1/FVC and D_{LCO}; however, mechanistic work is needed to further confirm such associations.

We observed significantly localised CD31 in endothelial cell junctions in NC, which was more diffuse and shifted towards the cytoplasm in the intimal endothelial cells of smokers and SAD and COPD patients. The diffusion or possible engulfment of this junctional protein is suggestive of compromised endothelial cell integrity, which results in loss of cell-to-cell contact and marked invagination of transitioning endothelial cells into subendothelial space. These changes partially show the potential migratory phenotype observed in endothelial cells undergoing EndMT [25].

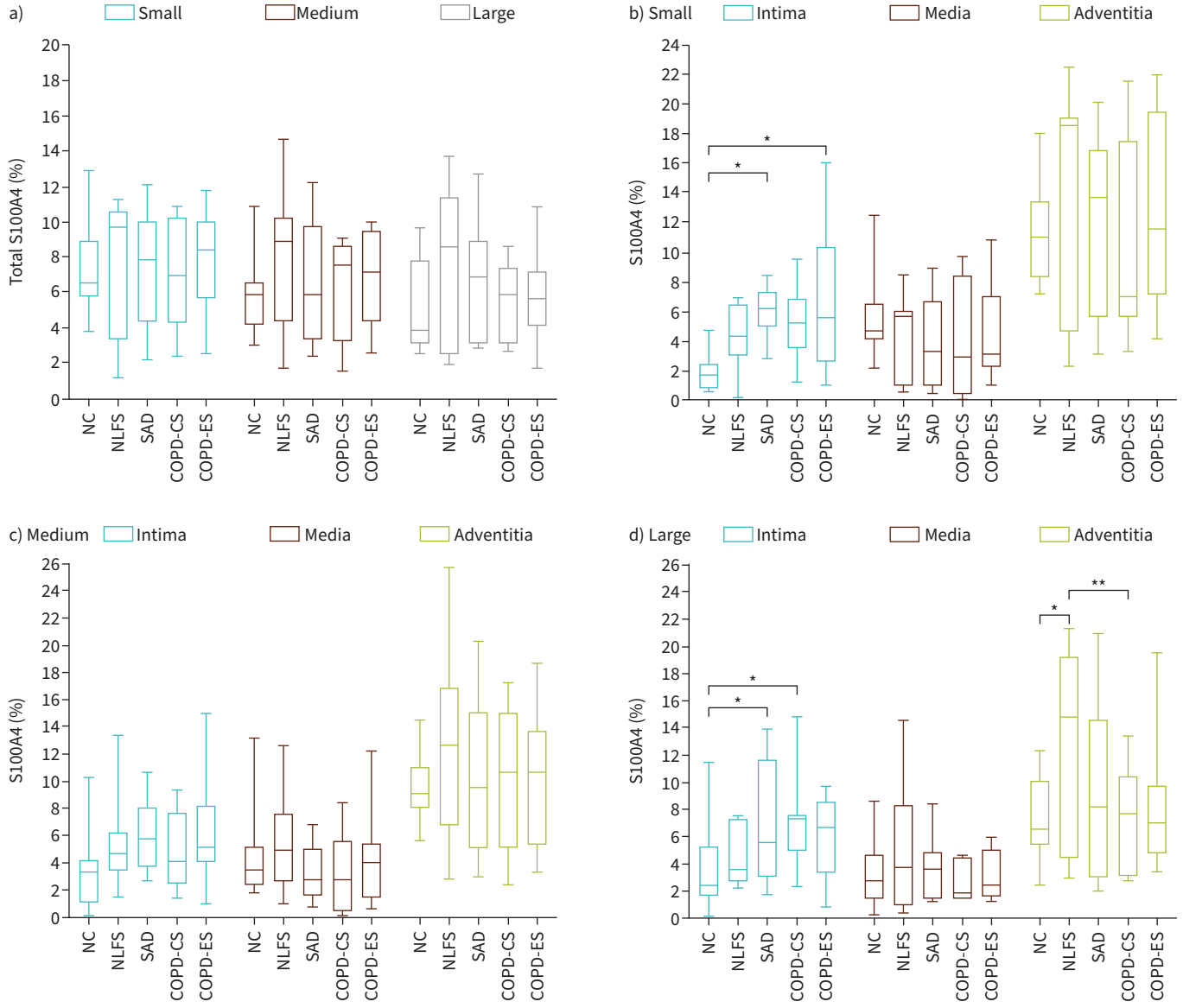

FIGURE 5 Abundance of total S100A4 in a) the arterial wall in small, medium and large arteries; b) different arterial layers of small arteries (100–299 µm); c) different arterial layers of medium arteries (300–499 µm); and d) different arterial layers of large arteries (500–999 µm). NC: nonsmoker controls; NLFS: normal lung function smokers; SAD: patients with small airway disease; COPD-CS: current smokers with COPD; COPD-ES: ex-smokers with COPD. *: p⩽0.05; **: p⩽0.01.

Furthermore, parallel to the shift of endothelial marker CD31 to the cytosol, we observed a simultaneous abundance of junctional mesenchymal marker N-cadherin. Endothelial cells express two cadherins: VE-cadherin, which is localised in junctions and serves to organise the junctional complex in these cells, and N-cadherin, which is extra-junctional and has an unclear role in normal endothelium [26]. However, switching VE-cadherin to N-cadherin and isolated upregulation of N-cadherin in endothelial cell junctions can promote motility and invasion [27]. In this study, we have shown a 2–3-fold higher total N-cadherin abundance in the arterial wall of smoking groups compared to NC. This elevated abundance was seen in all the individual layers and was most prominent in the adventitial layer. The emerging evidence suggests that the adventitia can serve as a staging ground for some of the earliest changes in the vessel wall [28, 29]. Available studies and our results of N-cadherin abundance indicate the likely initiation of vascular remodelling, which could be driven by the phenotypic transformation of cells in the adventitial layer [30].

Vimentin was the most significantly differentially expressed marker among all the mesenchymal markers. Total vimentin was significantly higher in all smoking groups compared to NC across the arterial sizes,

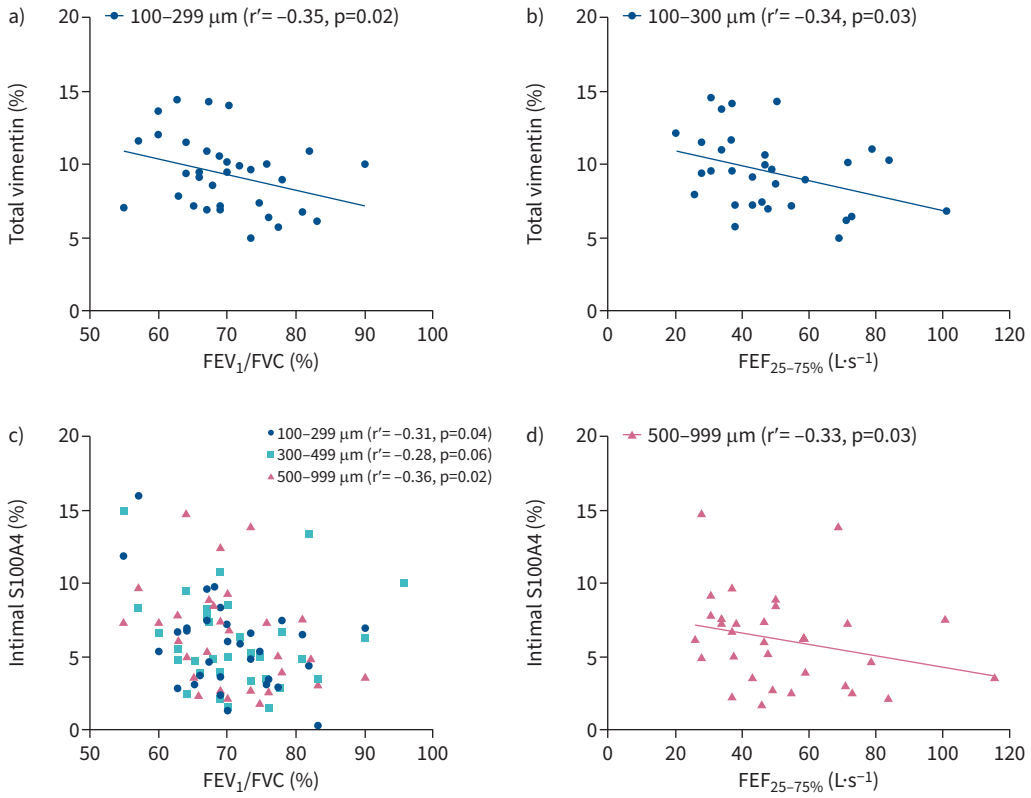

FIGURE 6 Correlation between a) total vimentin and forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC), b) total vimentin and forced expiratory fraction at 25–75% of FVC ($FEF_{25–75\%}$), c) intimal S100A4 and FEV_1/FVC and d) intimal S100A4 and $FEF_{25–75\%}$ across arterial sizes.

and was also increased across the arterial layers. Vimentin, a type III intermediate filament protein expressed in mesenchymal cells, is considered a marker of mesenchymal transition [31, 32].

S100A4 is a member of the S100 calcium-binding protein group involved in cell motility, angiogenesis, smooth muscle cell migration and proliferation [33]. Our results showed a greater abundance of S100A4, especially in the intimal layer of SAD and COPD patients, compared to NC. However, the difference in total and layer abundance of S100A4 across the groups in all arterial sizes was of a lower magnitude compared to other mesenchymal markers in this study. The smaller difference in S100A4 abundance in smoking and COPD patients compared to NC could be explained by the fact that S100A4 is a marker of early transition and at late stages it is overtaken by vimentin, as we observed in our study.

A positive correlation between EndMT markers and arterial wall thickness in smoking groups highlights a potential role for EndMT in arterial remodelling. We have shown that total S100A4 abundance in the smoker groups positively correlates with arterial wall thickness. Our finding follows Ambartsumian *et al.* [34], who reported that approximately 5% of mice ubiquitously overexpressing S100A4 (*Mts1*) develop pulmonary artery changes resembling plexogenic lesions. Similarly, Greenway *et al.* [35] found a positive correlation between S100A4 levels and the severity of vascular lesions in human plexogenic arteriopathy. Likewise, the shift of junctional to cytoplasmic CD31 in smoking groups of our study was directly proportional to the intimal thickness in small arteries. The connection between the shift of CD31 and arterial remodelling through the neo-expression of α-smooth muscle actin in the arterial wall in PH patients has been shown by Ranchoux *et al.* [25].

In assessing the impact of EndMT on lung physiology, we performed correlation analysis of mesenchymal marker abundance and lung function parameters. We identified that the abundance of vimentin and S100A4 in the pathological groups was associated with lower airflow parameters such as FEV_1/FVC and $FEF_{25–75\%}$, a measure of small airway calibre. Additionally, the higher intimal vimentin and cytoplasmic abundance of CD31 corelated with lower oxygenation capacity in smokers and COPD patients. Previous

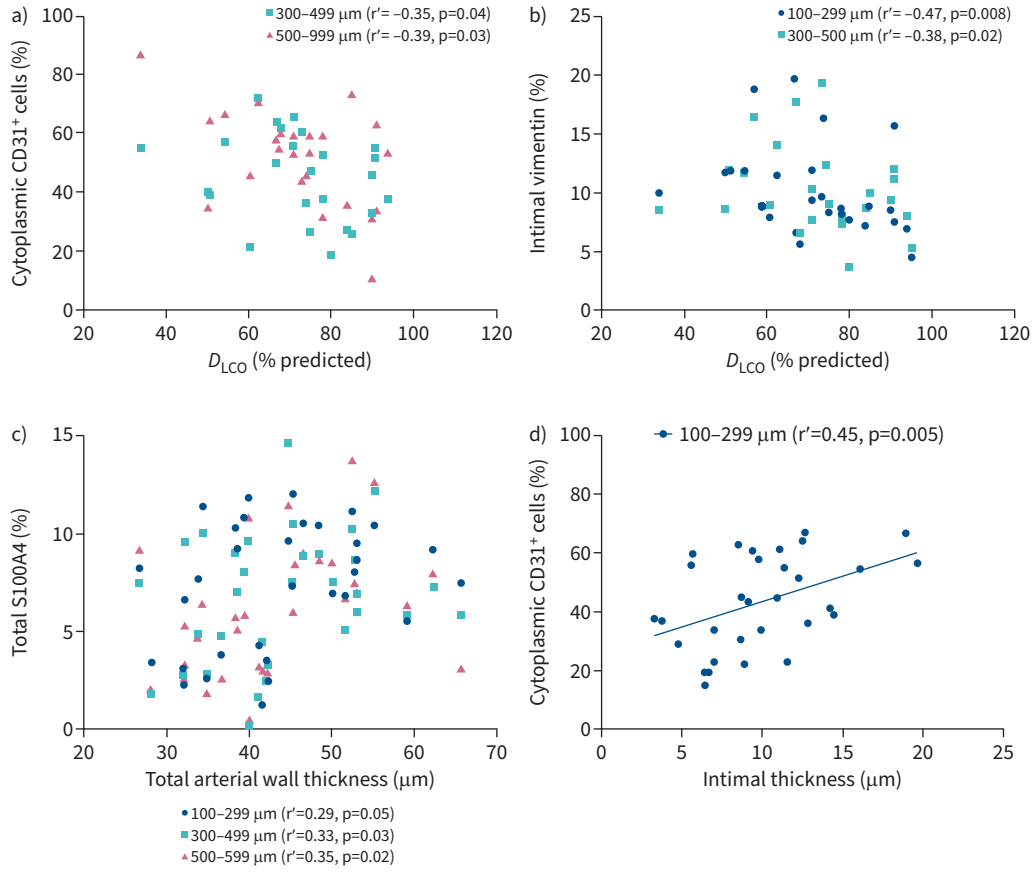

FIGURE 7 Correlation between a) cytoplasmic CD31$^+$ cells (%) and diffusing capacity of the lung for carbon monoxide (D_{LCO}), b) intimal vimentin abundance and D_{LCO}, c) total S100A4 abundance and total arterial wall thickness and d) cytoplasmic CD31$^+$ cells (%) and intimal thickness across small (100–299 µm), medium (300–499 µm) and large (500–999 µm) arteries.

research on idiopathic pulmonary fibrosis with endothelial dysfunction and active EndMT has shown a negative correlation between mesenchymal markers and D_{LCO} [36, 37]. The correlations of EndMT markers with vascular remodelling and lung gas exchange capacity (D_{LCO}) could indicate PH concomitant to the underlying pulmonary condition. EndMT is increasingly recognised as a source of vascular remodelling, which can be implicated in the development and progression of PH in smokers and SAD and COPD patients [38]. Our findings fit with the suggested pathology of PH.

There are limitations in the study. First, we had a small sample size for each group. However, our findings showed statistical significance in pathological groups compared to NC. Second, our pathological samples were obtained from COPD patients diagnosed with lung cancer. Although all tissue samples in this study were taken far away from cancer-affected areas, we are unable to rule out this effect completely. Third, due to the unavailability of cardiac function data in patients with COPD, we could not establish an association between EndMT and PH in this study. However, the positive correlation of EndMT with vascular remodelling and the negative correlation with D_{LCO} indicate the potential for early origin of PH in our patient cohort.

In summary, the shift of endothelial markers from the junction to the cytoplasm and the increased abundance of mesenchymal markers in NLFS and SAD and COPD patients indicate the acquisition of mesenchymal traits by endothelial cells through the EndMT process. Our work demonstrates that EndMT is associated with pulmonary arterial remodelling, further impacting the lung gas exchange capacities, and indicating the early signs of PH. Thus, the identification of this crucial role of EndMT in vascular remodelling and PH pathology in smokers and SAD and COPD patients can be used to identify novel therapeutic strategies and biomarkers targeting PH and fibrosis in these patients [39, 40].

Provenance: Submitted article, peer reviewed.

Ethics statement: This study was approved by the Tasmania Health and Medical Human Research Ethics Committee (ethics no. H0012374) and the James Hogg Lung Registry, University of British Columbia (ethical approval from the Providence Health Care Research Ethics Board H00–50110).

Conflict of interest: S.S. Sohal reports honoraria for lectures from Chiesi; travel support from Chiesi, AstraZeneca and GSK; and research grants from Boehringer Ingelheim and Lung Therapeutics, outside the submitted work. S.S. Sohal has served on the small airway advisory board for Chiesi Australia, for which an honorarium has been received. All the other authors do not have any conflict of interest to declare.

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