Diffusing capacity and alveolar attachments to small airways in smokers with and without COPD

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To the Editor:

COPD is a major smoking-related disease characterised by airflow limitation, defined as forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC)<0.7 on spirometry, and pathologically by alveolar destruction and airway disease [1]. Its high socioeconomic burden requires early diagnosis and intervention to prevent the development of COPD. In smokers, lung damage such as inflammation and wall remodelling of small airways and loss of alveolar attachments to the outer wall of these airways has occurred preceding developing COPD [2]. Thus, more sensitive measurements to detect these pathological changes than spirometry are warranted to detect early stage COPD.

The diffusing capacity of the lungs for carbon monoxide (D_{LCO}) is a physiological index that reflects smoking-related lung pathology. Impaired D_{LCO} increases the risk of developing COPD in smokers with normal spirometry [3]. Therefore, this study tested the hypothesis that small airway diseases and loss of alveolar attachments would be associated with lower D_{LCO} even in smokers without COPD by pathologically examining the small airways and neighbouring alveolar tissues in smokers both with and without COPD.

This retrospective analysis included ever smokers who underwent lobectomy for lung tumour resection at Kyoto University Hospital between 2006 and 2010. The patients with a history of asthma were excluded. Post-bronchodilator spirometry and D_{LCO} measurement with single-breath method were performed according to American Thoracic Society/European Respiratory Society guidelines, and full-inspiratory chest computed tomography (CT) scans were performed prior to the surgery. The diagnosis of COPD was based on FEV_1/FVC<0.7 [1]. FEV_1, FVC, the mean forced expiratory flow between 25% and 75% of the FVC ($FEF_{25–75\%}$) and D_{LCO} adjusted by haemoglobin concentration were normalised by reference values that were calculated using Japanese equations (FEV_1, FVC, and D_{LCO}) [4, 5] or Global Lung Initiative methods ($FEF_{25–75\%}$) [6]. Emphysema subtypes (centrilobular emphysema (CLE) and paraseptal emphysema (PSE)) on CT were visually assessed according to the Fleischner Society's statement [7, 8]. Pre-COPD was defined as no airflow limitation with at least one of the following conditions: 1) D_{LCO}<80% predicted (% pred); 2) FEV_1<80% pred; or 3) the presence of emphysema on chest CT scan [9]. This study was conducted in accordance with the Declaration of Helsinki statement and approved by the ethics committee of Kyoto University Hospital (G155, G0620, and R1852). Written informed consents were obtained from all the patients.

A piece of lung tissue distant from the tumour was obtained from resected lung, filled with Tissue Tek O.C.T Compound (Sakura Finetek, Osaka, Japan) through the bronchiole and immediately frozen in liquid nitrogen. Each 8 µm thickness section cut from frozen tissue was fixed with 4% paraformaldehyde, then stained with haematoxylin and eosin [10]. Small airways <2 mm in lumen diameter were identified in the microscopic images of lung sections (figure 1a). The number of alveolar attachments to the small airways (AAs) was manually counted and normalised by dividing the basement membrane perimeter (figure 1b) [11]. Wall thickness (WT) was also calculated by wall area divided by basement membrane perimeter (figure 1c).

Comparisons were performed by using a t-test or the chi-squared test depending on variables. Spearman's rank correlation coefficients were used to assess the relationship between histological indices and pulmonary function. All statistical analysis were performed using R statistical software version 4.3.0.

Shareable abstract (@ERSpublications)
The number of alveolar attachments to the small airways correlates with D_{LCO} in smokers, both with and without COPD. Early smoking-related lung pathology is reflected in impaired D_{LCO}. https://bit.ly/4fY3QUr

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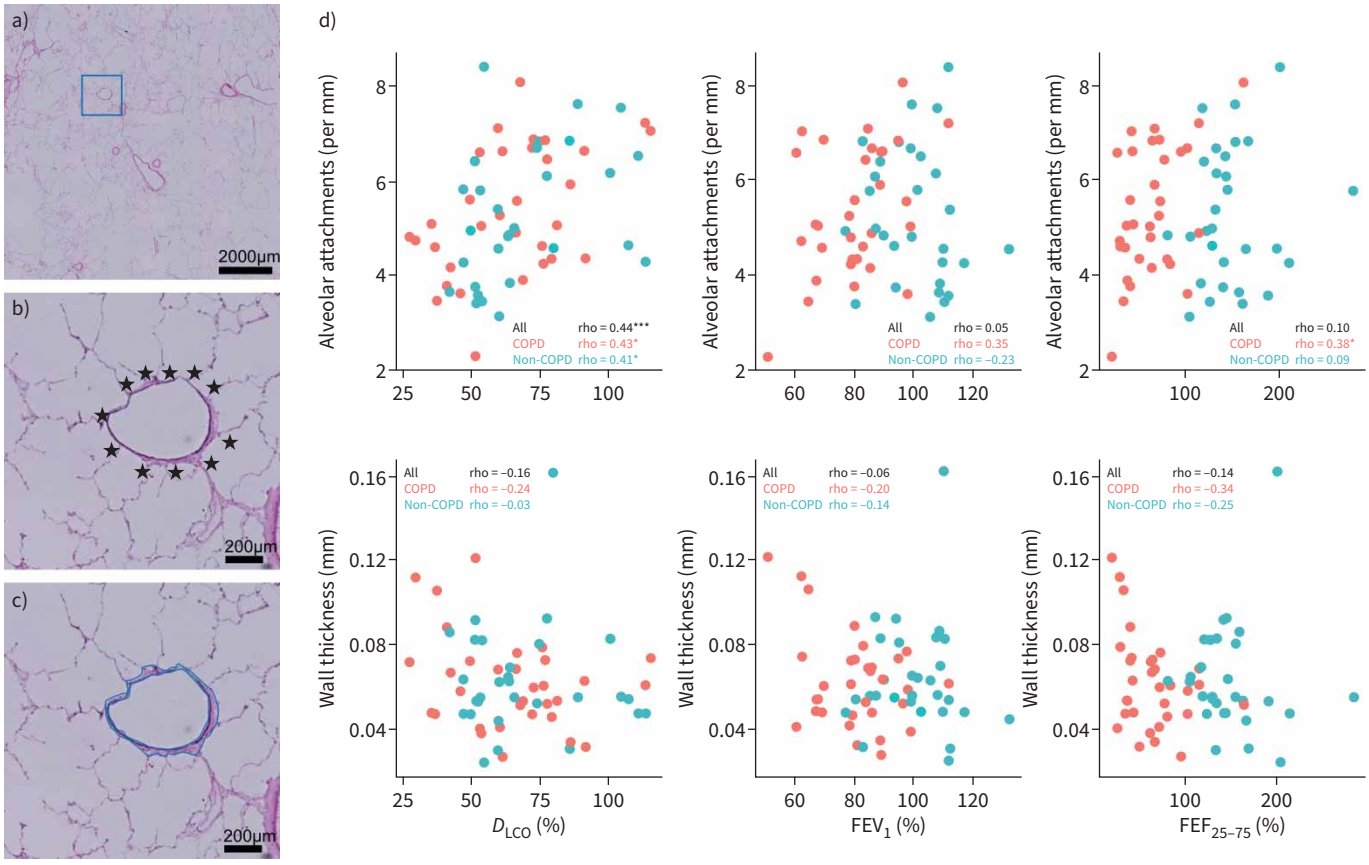

FIGURE 1 Representative histological image of haematoxylin and eosin staining, and the associations between the number of alveolar attachments, wall thickness, D_{LCO}, FEV_1 and $FEF_{25–75\%}$. a) A representative histological image of haematoxylin and eosin staining. b) and c) Magnified images of the blue box in (a). b) Alveolar attachment (stars). c) basement membrane perimeter (inner blue circle), and wall area (surrounded area between inner and outer blue lines) were measured manually. d) The associations between the number of alveolar attachments, wall thickness, D_{LCO}, FEV_1 and $FEF_{25–75\%}$. Each variable is expressed as % predicted. The red points represent patients with COPD and the blue points represent non-COPD patients. Spearman correlation coefficients were performed to assess the association between two variables (***: p<0.001, *: p<0.05). D_{LCO}: diffusing capacity of the lungs for carbon monoxide; FEV_1: forced expiratory volume in 1 s; $FEF_{25–75\%}$: mean forced expiratory flow at 25–75% of forced vital capacity.

The pathological lung tissue section was analysed in 32 patients with COPD and 29 ever smokers without COPD (non-COPD). Age, sex, body mass index (BMI) and smoking exposure were comparable between COPD and non-COPD (age: 69.2±7.8 *versus* 68.0±8.7, p=0.60; male: 81.2% *versus* 86.2%, p=0.86; BMI: 22.8±2.6 $kg \cdot m^{-2}$ *versus* 22.7±2.5 $kg \cdot m^{-2}$, p=0.85; current smoker: 43.8% *versus* 31.0%, p=0.45; smoking burden: 57.6±30.8 pack-year *versus* 54.7±37.9 pack-year, p=0.74). The existence of emphysema on CT was also similar (CLE: 93.8% *versus* 79.3%, p=0.20; PSE: 62.5% *versus* 62.1%, p=1.0). COPD had lower FEV_1 (80.5±13.6% pred *versus* 101.3±12.3% pred, p<0.001) and FEV_1/FVC (0.62±0.09 *versus* 0.78±0.05, p<0.001) than non-COPD, while FVC (101.3±13.7% pred *versus* 100.2±12.6% pred, p=0.73), D_{LCO} (63.8±22.3% pred *versus* 68.6±21.5% pred, p=0.74), AAs (5.35±1.4·mm^{-1} *versus* 5.26±1.4·mm^{-1}, p=0.81) and WT (0.06±0.02 mm *versus* 0.06±0.03 mm, p=0.86) were not different between COPD and non-COPD. Of 29 non-COPD, one met all pre-COPD criteria, 19 met both the D_{LCO} and emphysema criteria, five met only the emphysema criteria and two met only the D_{LCO} criteria.

As shown in figure 1d, AAs had a positive correlation with D_{LCO} in all subjects (rho=0.44, p<0.001), and in COPD and non-COPD (rho=0.43, p=0.01, and rho=0.41, p=0.03, respectively), but not with FEV_1. AA was also associated with $FEF_{25–75\%}$ in COPD (rho=0.38, p=0.03). Neither D_{LCO}, FEV_1 nor $FEF_{25–75\%}$ had any correlation with WT.

This histopathological study showed that lower number of alveolar attachments were associated with lower D_{LCO} but not with FEV_1, not only in smokers with COPD but also in those without COPD. Together with

previous studies showing that loss of alveolar attachments is an early pathology of lungs with COPD, our findings suggest that measurement of D_{LCO} in smokers may help identify smokers with the early stage of the disease before a definite COPD diagnosis is made on spirometry.

D_{LCO} and AAs were comparable among smokers with and without COPD, whereas FEV_1 and FEV_1/FVC were more deteriorated in those with COPD. VERLEDEN *et al*. [2] showed that the terminal bronchiole pathology including loss of AAs and lower D_{LCO} were observed in emphysematous pre-COPD. Furthermore, our finding shows the associations of a reduction in the number of AAs and an increase of WT with impaired D_{LCO} in smokers with and without COPD. Given that CLE originates from inflammation in terminal- and respiratory bronchiole and loss of AAs to the outer wall of these airways, our finding and VERLEDEN *et al*. [2] suggest that these primary changes of CLE might reduce the surface area of alveolar membrane and impair its function, leading to reduced D_{LCO}. These are also significant for suggesting that D_{LCO} reflects the pathological lung destruction more sensitively than airflow limitation and allows for diagnosing pre-COPD.

In this study, two-dimensional histological assessments were conducted. While histopathological analysis is an established method, recent technological advancements enable microscopic three-dimensional investigation using microCT or scanning electron microscopy (SEM). The number of terminal bronchiole and surface area density in microCT and interalveolar pores assessed by SEM are associated with COPD severity [2, 12]. These technologies may deepen understanding of the mechanisms underlying smoking-related pathology and physiological changes.

$FEF_{25–75\%}$, a functional small airway parameter, was associated with AAs only in COPD. Our findings were consistent with airflow limitation in COPD being mainly due to small airways less than 2 mm [13], and that the main determinant of airflow limitation was AAs [14], while it suggests that spirometric small airway parameters are not able to detect smoking-related pathology in smokers without COPD. Further studies including other functional airway parameters such as oscillometry are warranted.

There are some limitations. First, this study was retrospective nature and included a relatively small number of smokers. Second, a selection bias may exist, as the majority of non-COPD patients were pre-COPD. Third, morphological parameters other than AAs and WT such as alveolar surface area and lumen area could not be measured. Fourth, blood vessels were not assessed despite the potential involvement of pulmonary vascular abnormality in a reduction in D_{LCO}.

Smokers with COPD and even those without COPD showed a loss of alveolar attachments in small airways, that associated to D_{LCO}. Impaired D_{LCO} may serve as an early marker for pathological lung destruction in smokers.

Yusuke Shiraishi [1], Naoya Tanabe [1], Atsuyasu Sato[1], Tomoki Maetani [1], Ryo Sakamoto [2], Susumu Sato [1,3], Shigeo Muro [4], Hiroshi Date [5] and Toyohiro Hirai [1]

[1]Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan. [2]Department of Diagnostic Imaging and Nuclear Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan. [3]Department of Respiratory Care and Sleep Control Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan. [4]Department of Respiratory Medicine, Nara Medical University, Kashihara, Japan. [5]Department of Thoracic Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan.

Corresponding author: Naoya Tanabe (ntana@kuhp.kyoto-u.ac.jp)

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