ORIGINAL RESEARCH LETTER

Influence of ventilation inhomogeneity on diffusing capacity of carbon monoxide in smokers without COPD

To the Editor:

Early detection of subclinical lung function impairment may enable a window of opportunity to slow down the progression of developing COPD [1, 2]. Single-breath carbon monoxide uptake in the lungs (D_{LCO}) can be used as a screening test for mild lung function impairment in smokers [3]. Yet despite being readily used in common practice, the physiology is complex and depends on gas ventilation in the airways, diffusion through the alveolar membrane and the volume of haemoglobin in the capillaries supplying ventilated alveoli [4]. As a result, mild changes in the peripheral airways often remain undetected and subsequent false normal D_{LCO} values limit the clinical utility of the test [5]. Increased ventilation inhomogeneity (VI), arising from uneven convective and diffusive gas transport, occurs early and may influence the D_{LCO} measurement method in smokers. The nitrogen multiple-breath washout (N_2MBW) method can reliably measure VI and is known to sensitively detect small airways disease in smoking adults with well-preserved forced expiratory volume in 1 s (FEV_1). Previous studies suggest that D_{LCO} correlates with VI; however, this association may have been mediated by airflow limitation from obstructed airways [6, 7]. Thus, until now the association of lung clearance index (LCI) with D_{LCO} remains unclear. The objective of this study was to investigate the influence of VI on D_{LCO} *in vivo* without inherent or induced airflow limitation. We hypothesised that D_{LCO} is associated with VI in smokers with preserved spirometry.

We performed a cross-sectional analysis in adult smokers enrolled from two prospective studies: a randomised controlled trial for tobacco cessation (ESTXENDS, www.clinicaltrials.gov identifier NCT03938298) and the Swiss Idiopathic Interstitial Pneumonia cohort (Swiss-IIP) [8]. For both cohorts, participants were enrolled consecutively in the pulmonary outpatient clinic and by advertisement between October 27, 2016 and November 30, 2019. Inclusion criteria were: age ⩾18 years and self-reported smoking of ⩾5 cigarettes per day for at least 12 months. Individuals with chronic lung disease, any inhaler medication or abnormal spirometry defined as FEV_1/forced vital capacity (FVC) <0.70 were excluded [9]. The study was approved by the local ethics committee (KEK BE 246/15, Basec PB 2016-01524; KEK BE 2017-02332) and written informed consent was obtained from all participants. The study setting was a pulmonary outpatient clinic, University Hospital, Bern, Switzerland.

Lung function testing was performed in accordance with current guidelines in the following order: N_2MBW (Exhalyzer D, Eco Medics AG, Duernten, Switzerland, Spiroware 3.1) during tidal breathing, D_{LCO} and spirometry (Jaeger MasterScreen™; CareFusion, Hochberg, Germany) [10, 11]. N_2MBW indices included LCI, a marker of global VI, S_{cond}, a marker of convection-dependent VI, and S_{acin}, a marker of diffusion-convection-dependent VI [12]. Breath-by-breath quality control was applied. Mean values of at least two acceptable MBW trials were reported. Additionally, demographics (age, sex, body mass index

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In smokers with preserved spirometry, D_{LCO} is associated with ventilation inhomogeneity arising from peripheral airways. Measurement of D_{LCO} to screen for early lung function abnormalities in smokers may be suboptimal and could be replaced by MBW.
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(BMI)) and smoking exposure in pack-years (PY) (packs of cigarettes per day×years of smoking) were assessed. To determine abnormal lung function, previously published upper and lower limits of normal (ULN) were applied (mean+1.96×SD) [12, 13].

Multivariable regression modelling was performed using D_{LCO} (%predicted) as the primary outcome. We included predictor and confounder variables selected *a priori* (LCI, S_{cond}, S_{acin}, FEV_1, sex, age, BMI, PY). Variables with p>0.2 were excluded stepwise in likelihood-ratio tests. Model coefficients and their precision were reported. Validity of regression assumptions were evaluated. For sensitivity testing, outliers identified using a leverage-*versus*-residual-squared plot were excluded and the analysis was repeated using only individuals with BMI⩽35 kg·m^{-2} to assess possible dependence of LCI and S_{acin} on BMI. A p-value <0.05 was considered statistically significant and analyses were performed using Stata 14.2 (StataCorp LP, College Station, TX, USA).

In total, 65 smokers were assessed for eligibility (n=36 ESTXENDS, n=29 Swiss-IIP). Reasons for exclusion were airflow limitation (FEV_1/FVC<0.70; n=6), failed quality control (N_2MBW, n=2; spirometry, n=8), and incomplete assessments (n=6). We analysed data from 42 individuals (45.2% females). Mean (SD) age and BMI were 39.3 (12.6) years and 26.4 (5.2) kg·m^{-2}. Median (interquartile range) smoke exposure was 18.5 (1.5–58.5) PY. FEV_1 and D_{LCO} were 96.5 (77.0–114.0) and 91.0 (60.0–128.0) % predicted. LCI, S_{cond} and S_{acin} were 7.6 (6.2–11.3), 0.025 (0.002–0.047) and 0.081 (0.046–0.256) units. In 14 (33.3%) individuals, LCI was above the ULN (8.25), whereas D_{LCO} was below 80% predicted in four (9.5%) (figure 1). Multivariable regression modelling showed that LCI and S_{acin} partially predicted D_{LCO}: 12.7% (adjusted regression R^2) of the variance in D_{LCO} was explained by LCI and S_{acin}, p=0.010. Predicted D_{LCO}=135.5–(6.1×LCI)+(63.4×S_{acin}). Per one unit increase in LCI, D_{LCO} decreased by −6.1% predicted. Sensitivity testing excluding outliers (predicted D_{LCO}=135.1–(6.1×LCI)+(63.7×S_{acin}); adjusted R^2: 0.142, p=0.024) confirmed the primary analysis.

In an additional sensitivity analysis in individuals with BMI ⩽35 kg·m^{-2} we found no influence of BMI on the association between LCI, S_{acin}, and D_{LCO} (data not shown).

In this study, results revealed that D_{LCO} may be influenced by VI in smokers with normal spirometry. LCI and S_{acin}, quantifying global and diffusion-convection-dependent VI, partly explained the variance in

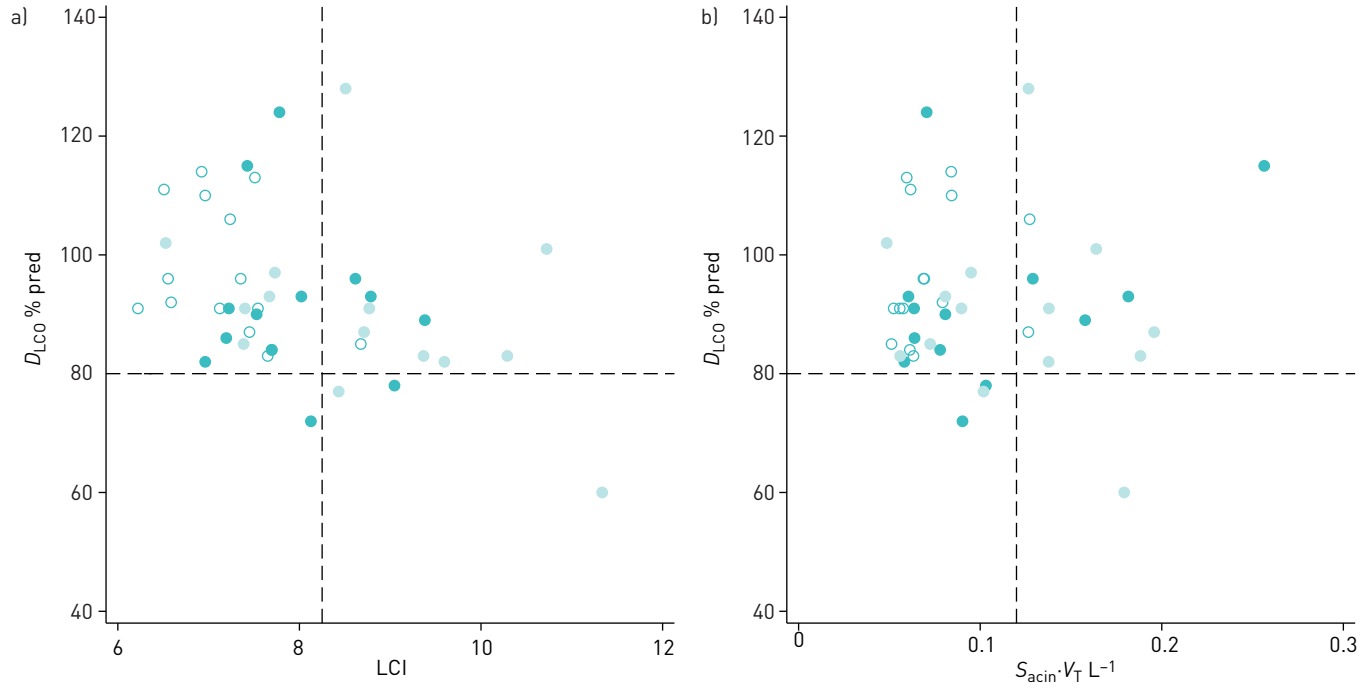

FIGURE 1 Association of carbon monoxide diffusion capacity and ventilation inhomogeneity. Diffusing capacity of carbon monoxide (D_{LCO}) in % predicted is plotted *versus* a) lung clearance index (LCI) and b) S_{acin} normalised for tidal volume (V_T) as recommended (S_{acin}·V_T). Dashed lines display upper and lower limits of normal. Individuals are displayed as circles with a colour scale indicating smoking exposure (tertiles): open circles, 1–10 pack-years; light filled circles, 11–29 pack-years; dark filled circles, 30–60 pack-years.

D_{LCO}, and furthermore were inversely related to D_{LCO}. These findings suggest that LCI and S_{acin} may be refined biomarkers in this population to quantify small airway dysfunction if airflow limitation is absent.

Given the relatively low R^2 value, the relationship of LCI and S_{acin} with D_{LCO} appears to be complex. Ventilation inhomogeneity may lead to CO maldistribution, affecting the D_{LCO} estimate. Several technical and physiological considerations should therefore be taken into account. More specifically, whilst both D_{LCO} and N_2MBW methods capture similar physiological aspects, they differ in measurement principles. CO and N_2 have almost identical molar masses (28.01 g·mol^{-1}) and susceptibility to VI should be comparable. D_{LCO} requires a maximal inspiration effort, whereas MBW is performed during relaxed tidal breathing. CO rapidly diffuses through the alveolar membrane during D_{LCO} measurement. Hardly any of the lungs' N_2 fraction passes the membrane during N_2MBW [11]. To isolate possible artefacts from VI on D_{LCO}, an airway model would be required.

Our findings are supported by previous studies suggesting that small airways dysfunction in current ex-smokers with COPD [14]. Importantly, LCI was more sensitive than D_{LCO} in capturing lung function abnormalities. Age and smoking history are known factors of lung function decline; however in our study, the association of VI with D_{LCO} was independent of BMI, age and smoking exposure. Diffusion-convection-dependent VI, global VI and D_{LCO} could be influenced by a common smoking-induced structural airway pathology. D_{LCO} measurement is considered sensitive to emphysema, which usually requires computed tomography scans for a definite diagnosis [5]. We hypothesise that VI may positively confound the association between D_{LCO} and structural airway pathology. In addition, D_{LCO} can also be confounded by other smoking-related changes, such as pulmonary vascular changes or increased CO-haemoglobin [15]. The utility of D_{LCO} to screen for early abnormalities in the lung function may be suboptimal and should be prospectively compared to MBW.

This study comes with a number of limitations which should be mentioned, such as the cross-sectional design, normative reference equations derived from diverse populations, and the lack of lung imaging to assess specificity of lung function abnormalities. Further evaluation of MBW breathing protocols requires additional studies.

In conclusion, our study suggests that the D_{LCO} measurement is influenced by VI. Ventilation inhomogeneity assessed by MBW may therefore become a refined biomarker in smokers with preserved spirometry to evaluate pre-COPD.

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