ORIGINAL RESEARCH

Assessment of Ventilatory Heterogeneity in Chronic Obstructive Pulmonary Disease Using the Inspired Sinewave Test

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Background: There is marked variability in the symptoms and outcomes of patients with chronic obstructive pulmonary disease (COPD) which are poorly predicted by spirometry/$FEV_{1\%pred}$. Furthermore, as spirometry requires the performance of potentially distressing respiratory manoeuvres which are to some extent user-effort dependent, there is need for non-invasive and simple-to-perform techniques to identify subtypes of COPD which are more closely related to clinically relevant outcomes.

Materials and Methods: The inspired sinewave test (IST) sinusoidally modulates the inspired concentration of a tracer gas (N_2O) over successive tidal breaths. A single-compartment tidal-ventilation lung model processes the amplitude/phase of the expired N_2O sinewave and estimates cardiopulmonary variables including: effective lung volume and indices of ventilatory heterogeneity (VH; ELV_{180}/FRC_{pleth} and ELV_{180}/ELV_{pred}). 83 COPD patients and 53 healthy controls performed the IST test, standard pulmonary function tests (Spirometry, body plethysmography and the single breath test of carbon monoxide uptake), and symptom severity questionnaires (COPD assessment test, CAT; mMRC dyspnoea-scale, mMRC-DS; Cough+Mucus score; C+M score).

Results: ELV_{180}/FRC_{pleth} and ELV_{180}/ELV_{pred} were significantly lower in patients with COPD vs healthy participants (0.34 ± 0.11 vs 0.68 ± 0.14 and 0.7 ± 0.27 vs 0.98 ± 0.15, respectively; $P<0.05$). Multivariable regression analysis demonstrated that ELV_{180}/FRC_{pleth} was a stronger and independent predictor of CAT, mMRC-DS and C+M score vs $FEV_{1\%pred}$. ELV_{180}/ELV_{pred} was a stronger and independent and better predictor of C+M score vs $FEV_{1\%pred}$. Phenotyping patients, based upon ELV_{180}/ELV_{pred} and $FRC_{\%pred}$, uncovered significant symptomatic differences between groups.

Conclusion: The IST indices of VH were superior and independent predictors of symptom severity vs $FEV_{1\%pred}$ and has potential as a non-invasive and simple-to-perform method to stratify patients into subgroups related to clinically relevant features of COPD.

Keywords: COPD, lung function test, nitrous oxide, medical device, ventilatory heterogeneity

Plain Language Summary

There is great need for novel techniques to identify subtypes of chronic obstructive pulmonary disease (COPD) which are closely related with patient outcomes. The inspired sinewave test (IST) can non-invasively measure lung volume, pulmonary blood flow, and estimate how evenly gas is distributed around the lung (ventilatory heterogeneity, VH). We examined whether measures of VH using the IST are associated with specific clinical features of COPD. 83 COPD patients and 53 healthy controls performed the IST test, as well as standard

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lung function tests and symptom severity questionnaires. The IST test had a very high sensitivity for detecting COPD, and its measurements of VH were a superior and independent predictor of symptom severity vs current standard tests (spirometry). It was also able to stratify patients into phenotypes related to clinically relevant features. The IST has potential as a non-invasive and simple-to-perform method to stratify patients into subgroups related to clinically important outcomes for COPD.

Introduction

In the UK, chronic obstructive pulmonary disease (COPD) accounts for over 1 million bed days and approximately 30,000 deaths each year, and it is the second highest cause of emergency admission.[1,2] However, the manifestation of COPD is very variable, where some patients have a faster decline in lung function and are at greater risk of symptom exacerbation than others.[3] The definition, diagnosis and management of COPD is fundamentally based upon airflow limitation, as assessed via spirometry/FEV_1. However, FEV_1 has only a weak relationship with symptom severity, the risk of exacerbation, and patient outcomes.[4] Furthermore, as spirometry requires precise and potentially distressing respiratory manoeuvres, whose reproducibility can be poor in elderly and paediatric populations,[5–7] there is clear need for simple-to-perform and non-invasive techniques to diagnose and identify subtypes of COPD which are more closely related to clinically relevant outcomes.[8]

The principal sites of pathophysiology in COPD are the small airways and lung parenchyma.[9–11] As the small airways form a vast array of parallel resistors each contributes only a small fraction to total airways resistance, so a significant degree of small airways disease can occur with little effect on FEV_1.[12] However, the obstruction of small airways and the enlargement of airspaces in emphysema has a greater impact on the distribution of ventilation than it does on total airways resistance, and thus techniques which can measure ventilatory heterogeneity (VH) may be sensitive to the early structural changes which occur in obstructive lung disease.[12,13]

The inspired sinewave technique (IST) is a non-invasive method to monitor cardio-pulmonary function which only requires passive patient cooperation.[14–17] The technique can measure effective lung volume (ELV), dead space (V_D), pulmonary blood flow (Q_P), and indices of VH can be calculated. The aim of the current investigation was to: 1) compare the IST indices of VH with measures obtained from standard pulmonary function tests (PFTs)

in patients with COPD and healthy never-smoker participants, and 2) examine whether IST measures are associated with specific clinical features of COPD independent of FEV_1.

Materials and Methods
Participants and Ethical Approval

In the study, 83 patients with COPD volunteered for participation (Table 1). Following clinical screening, patients were included if they met the following criteria: >40 years of age, no symptom exacerbation in the previous 8 weeks, >10 pack year smoking history and FEV_1/FVC <0.7. Patients with a history of asthma or pulmonary disease other than COPD were excluded. 53 never-smoker healthy

Table 1 Participant Characteristics and Pulmonary Function Test Measurements

	COPD		Healthy	
N	83		53	
Male/Female	48/35		35/18	
	Mean	**SD**	**Mean**	**SD**
Age (yrs)	67.35	9.91	64.69	11.64
Height (m)	1.69	0.09	1.70	0.12
Weight (kg)	78.35*	16.90	71.23	11.67
BMI	27.39*	5.30	23.77	4.11
FEV1 (L)	1.52*	0.64	3.28	0.99
FEV1% pred	55.08*	19.63	101.90	16.68
FVC (L)	3.12*	0.98	4.21	1.27
FVC % pred	84.53*	20.87	105.03	18.09
FEV1%FVC	48.46*	13.26	77.57	6.76
FEV1%FVC % pred	63.12*	18.26	96.56	7.25
TLC (L)	6.91*	1.61	6.16	1.38
TLC % pred	115.44*	20.41	100.68	20.85
RV (L)	3.59*	1.39	2.12	0.74
RV % pred	154.57*	51.87	101.70	21.52
FRC (L)	4.41*	1.47	3.39	0.84
FRC % pred	136.94*	40.21	106.86	17.37
T_{LCO}	4.90*	1.72	7.90	2.52
T_{LCO} % pred	60.54*	18.28	89.24	14.77
K_{CO}	1.02*	0.29	1.49	0.30
K_{CO} % pred	73.98*	22.09	96.22	16.10
Pack years	32.64	20.69	–	–
mMRC-DS	2.13	1.00	–	–
CAT	16.27	6.37	–	–

Note: *Statistically significant difference with healthy participants ($P<0.05$).
Abbreviations: SD, standard deviation; BMI, body mass index; PEF, peak expiratory flow; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; TLC, total lung capacity; RV, residual volume; FRC, functional residual capacity; T_{LCO}, transfer factor for carbon monoxide; K_{CO}, transfer coefficient for carbon monoxide; mMRC-DS, modified Medical Research Council dyspnoea scale, CAT; COPD assessment test; % predicted values obtained via reference values.[48,49]

participants also volunteered for the study (Table 1). All were defined as healthy by the following criteria: no history of respiratory disease or respiratory symptoms suggestive of disease, and no upper respiratory tract infections in the previous 8 weeks. All participants received written and verbal information regarding the study before providing their written informed consent. The study's protocol was approved by an NHS ethical committee (16/SC/0057; South Central - Hampshire B Research Ethics Committee) and conforms to the Declaration of Helsinki, 2013.

Protocol

All procedures were completed within the Respiratory Medicine Department, Oxford University Hospitals NHS Foundation Trust. The study visit day included: a medical history assessment, completion of the COPD assessment test (CAT;[18] and modified Medical Research Council dyspnoea scale,[19] standard pulmonary function tests (PFTs; spirometry, body plethysmography and the single breath test of carbon monoxide uptake; Jaeger MasterScope Body, Carefusion), and the IST. Using the first two questions of the CAT questionnaire an additional symptom score was produced, aiming to specify cough frequency and phlegm/mucus production (Cough + Mucus (C+M) score). In 21 COPD patients and 13 healthy participants (ie, 25% of each group), chest CT scans were obtained within 3 months of the study visit day. Unenhanced low dose CT scans were performed with a 64-slice scanner (Revolution GSI, GE Healthcare) and the following parameters: 0.625mm slice thickness, 60 mA, 100 kV, 40mm tube collimation, 0.984 beam peach, 0.5sec rotational time. Scans were analysed with Pulmonary Toolkit[20] to quantify the whole-lung emphysema extent. This was reported as percentage low attenuation area (%LAA), the percentage area of the whole-lung with attenuation values inferior to −950 Hounsfield units.

IST Test

Details of the IST and device have been detailed elsewhere,[16] but in brief: participants breathe quietly through a face mask, which is sealed around the mouth with no leaks. At the start of each inhalation a small quantity of N_2O is injected into the inspired air. Over successive breaths, the concentration of inspired N_2O oscillates in a sinewave pattern around a set mean (4%) with a predetermined amplitude and period. This results in end-tidal (ie, alveolar) N_2O concentrations to also oscillate in a sinewave pattern. All participants performed IST tests consisting of 4 minutes of forcing an inspired N_2O sinewave at periods of 180 seconds and 60 seconds.

Single-Compartment Lung Model

The amplitude/phase of the expired sinewave is processed by a single-compartment tidal ventilation lung model which can estimate cardiorespiratory variables such as V_A and $\dot{Q}_P$. ELV is the sum of V_A and airways dead space (V_D), as measured via a modified Bohr technique.[21] The period of the inspired sinewave used to estimate ELV is signified by the subscript (ie, ELV_{180} or ELV_{60}). Both modelling and empirical data have demonstrated that ELV_{180} has the closest agreement with FRC_{pleth}. The IST indices of VH are: 1) ELV_{180}/FRC_{pleth}, 2) ELV_{180}/ELV_{pred}, and 3) ELV_{60}/ELV_{180}; where lower values suggest greater VH and higher values suggest homogeneity. The first two indices rely on the nature of single compartment lung models. ELV is a perfectly predictable function of ventilation, sinewave period, and expired sinewave attenuation, and underestimations in ELV would suggest a degree of VH.[22] This is equivalent to multi-breath washout tests, where deviation from a perfectly exponential washout curve suggests a degree of VH. Therefore, greater VH results in larger disparities between ELV_{180} and: 1) FRC_{pleth} and 2) ELV_{pred} (a prediction of ELV_{180} based upon healthy participants.[16] The third index relies on ELV measurements becoming more dependent on sinewave period with increasing VH. The ELV of a single compartment lung will be the same regardless of the applied period, but with increasing VH measured ELV become increasingly dependent on the period used, appearing to decrease as the period shortens.[22] The lung model, estimation steps, and further details of the VH indices including the tests' repeatability are described elsewhere.[14–16]

Statistical Analysis

Analysis was conducted using, GraphPad Prism (GraphPad Software inc.), Matlab v2018b (Mathworks, Natick, MA, USA) and a standard statistical package (SPSS, Chicago, IL, USA). Values are means ± standard deviation unless otherwise stated. Comparisons between variables were assessed via Pearson coefficient for correlation tests, Student's t-test for continuous variables, and one-way ANOVA for categorical variables and, where appropriate, multiple comparison post hoc analysis with Bonferroni correction. Univariable and multivariable

linear regression analysis was performed on PFT data, CT scan analysis, and symptom scores with three independent variables: $FEV_{1\%pred}$, ELV_{180}/FRC_{pleth} and ELV_{180}/ELV_{pred}. The statistical significance level was set at $p<0.05$. R^2 and standardised regression coefficients were taken as a measure of association.

Receiver operating characteristic (ROC) curve analysis was performed to assess the diagnostic accuracy of IST indices of VH for the detection of spirometrically defined COPD ($FEV_1/FVC<0.7$; GOLD).[23] The area under the curve was calculated, and the cut-off point for each VH index was determined by using Youden's index (maximal sensitivity + specificity −1). When it became apparent that specificity could be improved by normalising ELV_{180}/FRC_{pleth} for age, a ROC curve for the additional parameter ELV_{180}/FRC_{pleth} %pred was constructed.

Results

Patients with COPD had a significantly lower mean $FEV_{1\%pred}$ (55.1 ± 19.6 vs 101.9 ± 16.7), $FEV_1\%FVC$ (48.5 ± 13.2 vs 77.6 ± 6.7), $T_{LCO\%pred}$ (60.5 ± 18.3 vs 89.2 ± 14.77), and ELV_{pred} (1.45 ± 0.53 vs 2.31 ± 0.78); and a significantly higher mean $FRC_{\%pred}$ (137 ± 40.2 vs 106.8 ± 17.4) in comparison to healthy participants (Table 1). There was no difference in age and height, but COPD patients had a higher mean BMI (27.4 ± 5.3 vs 23.77 ± 4.11).

Comparisons with Physiological Measures

Mean ELV_{180}/FRC_{pleth} and ELV_{180}/ELV_{pred} were significantly lower in COPD patients vs healthy participants (0.34 ± 0.11 vs 0.68 ± 0.14 and 0.7 ± 0.27 vs 0.98 ± 0.15, respectively), $FRC_{pleth}\%pred$ was significantly higher in COPD (1.37 ± 40.2 vs 106.9 ± 17.4), but there was no difference in ELV_{60}/ELV_{180} (Figure 1A–D). In COPD patients, there was a significant correlation between ELV_{180}/FRC_{pleth} and $FEV_{1\%pred}$ ($r = +0.58$, $P<0.05$) and $FRC_{pleth}\%pred$ and $FEV_{1\%pred}$ ($r = -0.47$, $P<0.05$), but no relationship was observed with ELV_{180}/ELV_{pred} and ELV_{60}/ELV_{180} (Figure 1E–H). Linear regression analysis between ELV_{180} vs FRC_{pleth} revealed a strong relationship for healthy participants ($y=0.74x - 0.19$, $R^2 = 0.64$) but a weaker relationship and lower regression coefficient in COPD patients ($y=0.19x + 0.59$, $R^2 = 0.28$; Figure 3).

ROC analysis of the predictive value of ELV_{180}/FRC_{pleth} and ELV_{180}/ELV_{pred} for COPD diagnosis is shown in Figure 2. ELV_{180}/FRC_{pleth} (cut-off value <0.51) had as a sensitivity of 0.97 and specificity of 0.81 for COPD diagnosis. However, an ELV_{180}/FRC_{pleth} %pred

value, which was based upon previous data from healthy participants[16] and normalises values for a given age, increased the specificity (0.98 vs 0.81) and AUC (0.98 vs 0.96) in comparison to ELV_{180}/FRC_{pleth}.

There were significant correlations between ELV_{180}/FRC_{pleth} and $T_{LCO\%pred}$, and %LAA ($r = +0.51$ and -0.50, respectively, $P<0.05$), and also between ELV_{180}/ELV_{pred} and $T_{LCO\%pred}$, and %LAA ($r = +0.41$ and -0.37, $P<0.05$; Figure 4). $FRC_{pleth}\%pred$ was also significantly correlated with %LAA ($r = -0.33$, $P<0.05$) but not with $T_{LCO\%pred}$ (Figure 4).

Comparisons with Symptoms/Clinical Outcomes

Figure 5 shows the relationships between ELV_{180}/FRC_{pleth} and ELV_{180}/ELV_{pred} and the 3 symptom scores. There was a significant correlation between CAT and ELV_{180}/FRC_{pleth}, ELV_{180}/ELV_{pred} and $FRC_{pleth}\%pred$ ($r = -0.52$, -0.36 and $+0.36$ $P<0.05$). ELV_{180}/FRC_{pleth} was significantly lower in COPD patients with a mMRC-DS of 2, 3, and 4 vs 0/1, and in those with a score of 4 vs 2/3 ($P<0.05$). In contrast, ELV_{180}/ELV_{pred} was only significantly lower in patients with a score of 4 vs 0/1, 2, or 3 ($P<0.05$). $FRC_{pleth}\%pred$ was significantly higher in patients with a mMRC-DS of 4 vs 0/1. There was no significant difference in ELV_{180}/FRC_{pleth} or $FRC_{pleth}\%pred$ between those with a C+M score of 0–3, 4/5, or ≥6. However, ELV_{180}/ELV_{pred} was significantly lower in those with a score of 4/5 or ≥6 vs 0–3, and in those with a score of ≥6 vs 4/5 ($P<0.05$).

Classifying patients based upon their ELV_{180}/ELV_{pred} and $FRC_{\%pred}$ values (phenotypes I–IV, Figure 6A) uncovered symptomatic differences between groups. Phenotype I displayed significantly lower severity of all symptoms (Figure 6B–D), and phenotype III had a significantly lower CAT score vs phenotype IV ($P<0.05$). Phenotype II had a significantly lower mean mMRC-DS vs phenotype IV ($P<0.05$) whereas there was no difference between groups III and IV. However, phenotype III did have a significantly lower C+M score in comparison to groups II and IV. Table 2 shows that ELV_{180}/FRC_{pleth} was an independent and better predictor of the three symptom scores, as well as $T_{LCO\%pred}$, in comparison to $FEV_{1\%pred}$. ELV_{180}/ELV_{pred} was an independent and better predictor of C+M score and $T_{LCO\%pred}$, in comparison to $FEV_{1\%pred}$.

Discussion

The current study presents a non-invasive and simple-to-perform technique to measure pulmonary function and has
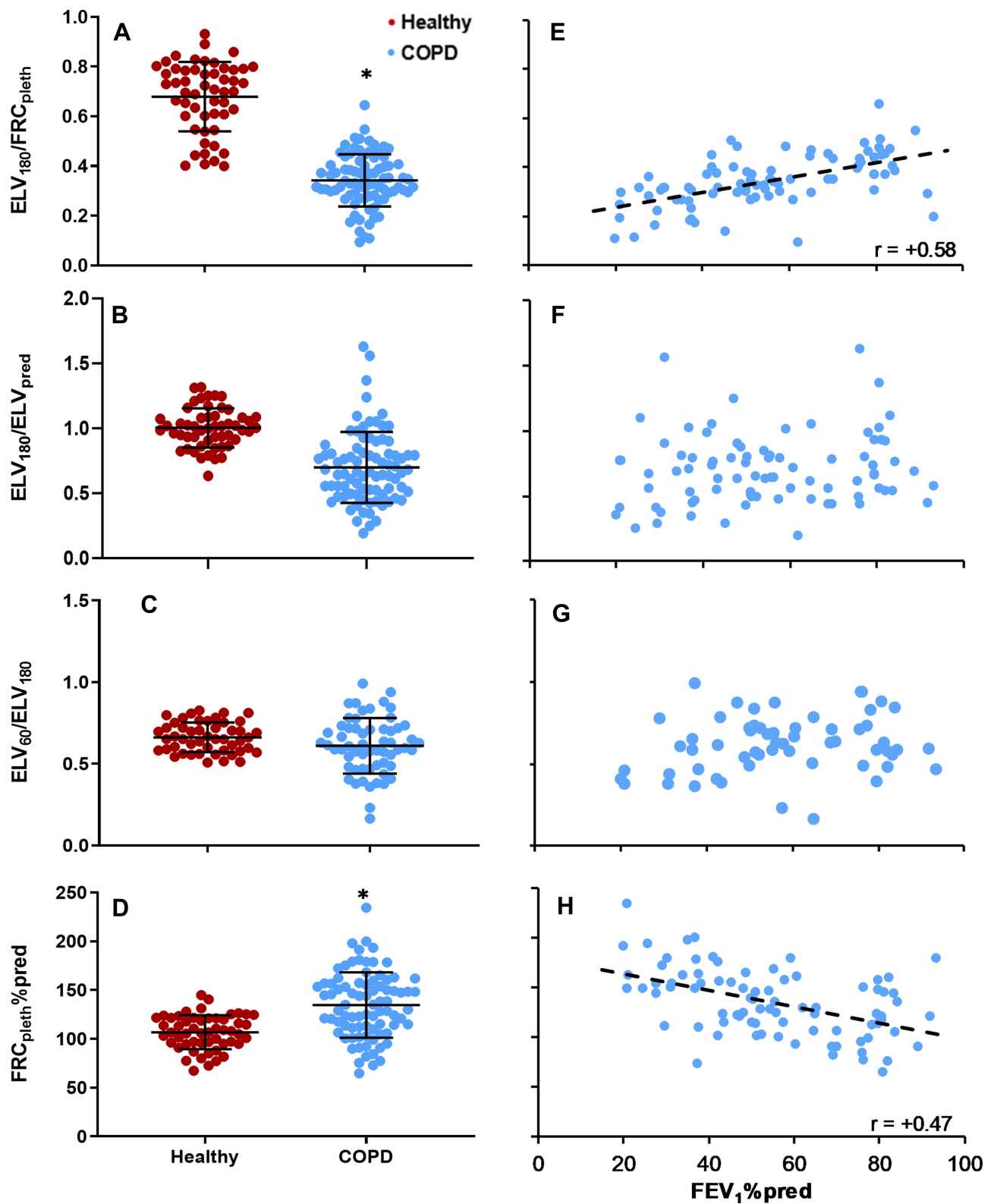
Figure 1 (**A–D**) Comparisons of (**A**) ELV_{180}/FRC_{pleth}, (**B**) ELV_{180}/ELV_{pred}, (**C**) ELV_{60}/ELV_{180} and (**D**) $FRC_{pleth}\%pred$ in COPD patients (blue) and healthy participants (red). Long horizontal bar = mean; short horizontal bar = standard deviation; *Statistically significant difference ($P<0.05$). (**E–H**) scatterplots of (**E**) ELV_{180}/FRC_{pleth}, (**F**) ELV_{180}/ELV_{pred}, (**G**) ELV_{60}/ELV_{180} and (**H**) $FRC_{pleth}\%pred$ vs $FEV_1\%pred$ in COPD patients with a linear regression line in D; r = +0.58 ($P<0.05$). For definitions see Table 1.
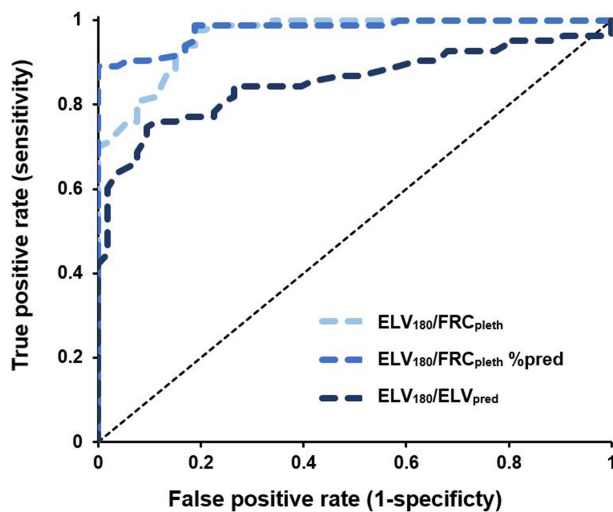
ROC measure	AUC	SE	YI	Cut-off	TPR	FPR
ELV_{180}/FRC_{pleth}	0.96	0.01	0.79	<0.51	0.97	0.81
ELV_{180}/FRC_{pleth} %pred	0.98	0.01	0.89	<80	0.91	0.98
ELV_{180}/ELV_{pred}	0.85	0.03	0.66	<0.81	0.76	0.91

Figure 2 Receiver operator characteristic (ROC) curves for ELV_{180}/FRC_{pleth}, ELV_{180}/FRC_{pleth} % pred and ELV_{180}/ELV_{pred}. When it became apparent that specificity could be improved by normalising ELV_{180}/FRC_{pleth} for age, a ROC curve for the additional parameter ELV_{180}/FRC_{pleth} %pred was constructed.
Abbreviations: AUC, area under curve; SE, standard Error; YI, Youden's index (maximal sensitivity+specifity-1).
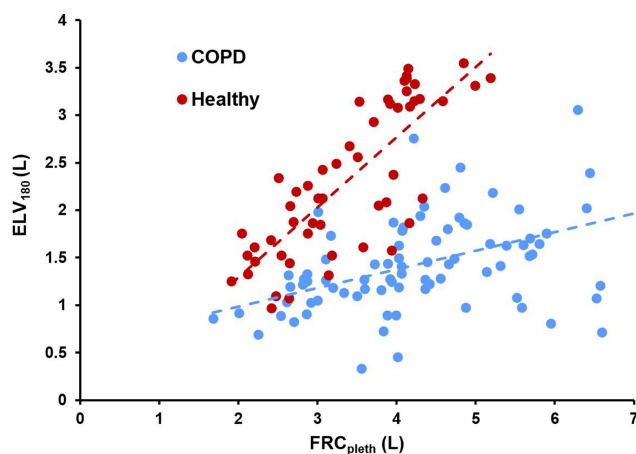

Figure 3 Scatterplot of ELV_{180} (L) vs FRC_{pleth} (L) with linear regression lines. Blue = COPD, Red = COPD. COPD, y = 0.19x + 0.59 (R^2 = 0.28); Healthy, y = 0.74x − 0.19 (R^2 = 0.64).

demonstrated several novel and clinically relevant findings. We have firstly shown that ELV_{180}/FRC_{pleth}, is significantly different between healthy participants and patients with COPD. This index (with a cut-off of ≤0.51) had a very high sensitivity (0.97) and moderately high specificity (0.81) for identifying spirometrically defined COPD. The relatively low specificity is largely

a consequence of the reduction in ELV_{180}/FRC_{pleth} with healthy ageing. Indeed, ≈50% of those >75 years of age had an ELV_{180}/FRC_{pleth} value below the 0.51 cut-off value. This is unsurprising as increases in VH with ageing is well established[24] and so must be accounted for. This was achieved by calculating ELV_{180}/FRC_{pleth}%pred, which improved the specificity (0.98 vs 0.81) and AUC (0.98 vs 0.96) in comparison to ELV_{180}/FRC_{pleth}.

ELV_{60}/ELV_{180} failed to discriminate between health and COPD. This is similar to our previous findings with young and elderly participants, where a detailed discussion of the index has been provided.[16] ELV_{180}/ELV_{pred} was significantly different between healthy participants and patients with COPD but displayed a lower AUC (0.85 vs 0.96) and sensitivity (0.76 vs 0.97) in comparison to ELV_{180}/FRC_{pleth}. The poorer sensitivity is likely related to the confounding influence of hyperinflation on measured ELV_{180}. Interestingly however, $FRC_{\%pred}$ and ELV_{180}/ELV_{pred} were only poorly related (Figure 6A) suggesting they may be sensitive to different underlying pathophysiology.

We have previously demonstrated that despite observing an increase in FRC_{pleth} by ~0.01L per year of healthy ageing, ELV_{180} reduces by ~0.01L per year – a likely consequence of increasing VH.[16,24] This contrasts with other gas dilution techniques which similarly demonstrate increases in FRC with age.[25] This suggests that ELV_{180} is sensitive to factors other than 'actual' lung volume (eg, VH) and so its value may have some independence form FRC_{pleth}. Indeed, it would explain the substantially weaker association between ELV_{180} and FRC_{pleth} in COPD patients vs healthy participants (Figure 3), as it likely reflects significantly greater patient VH.[26] This finding is important as it implies that the relationships in ELV_{180}/FRC_{pleth} (or ELV_{180}/ELV_{pred}) with disease/symptom severity are not simply a reflection of changes in lung volume (eg, hyperinflation) but are dependent on other aspects of lung pathology. Indeed, other evidence from the current study supports this. There was a stronger relationship between ELV_{180}/FRC_{pleth} and (i) FEV_1%pred, (ii) $T_{LCO\%pred}$, (iii) %LAA, (iv) CAT score and (v) mMRC dyspnoea score, in comparison to FRC_{pleth}%pred or ELV_{180}/EVL_{pred} alone (Figures 1, 4 and 5). This suggests the measure of ELV_{180}, and its presentation as a ratio of FRC_{pleth}, might provide added useful information.

For any new clinical tool to be adopted, it must either demonstrate an advantage/s over existing practice or show that it can provide important additional information. In the
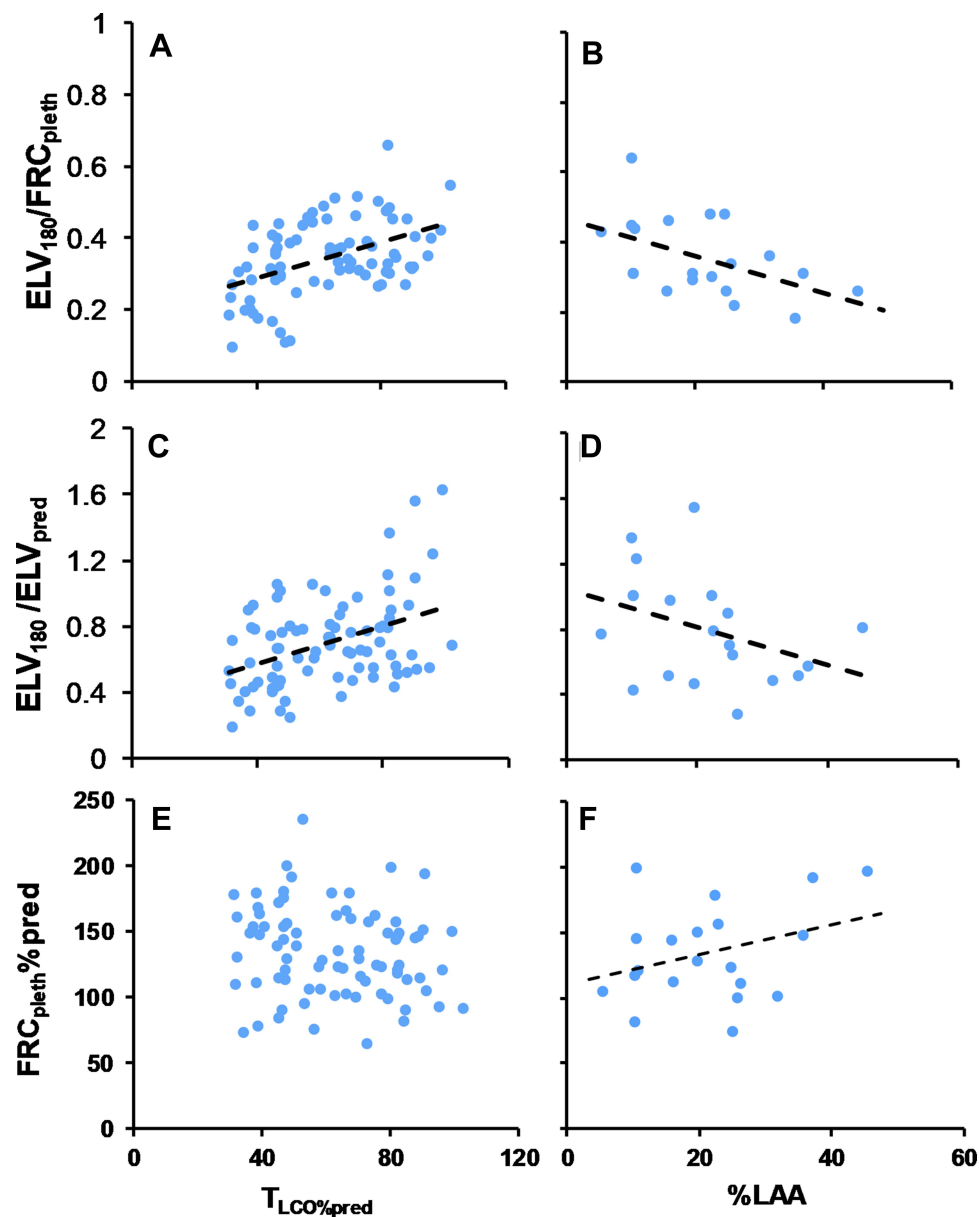
Figure 4 Scatterplots of ELV_{180}/FRC_{pleth} (**A** and **B**), ELV_{180}/ELV_{pred} (**C** and **D**) and $FRC_{pleth}\%pred$ (**E** and **F**) with $T_{LCO}\%pred$ and CT-derived morphological degree of emphysema (%LAA; low-attenuation area less than −950 Hounsfield units). See Table 1 for other definitions. Black linear regression lines are shown in A (r = +0.51), B (r = −0.5), C (r = +0.41), D (r = −0.37), and F (r = +0.33) respectively (*P*<0.05).

current study, we have shown that the IST's diagnostic ability goes beyond simply correlating with the spirometric/FEV_1 definition of COPD, as the indices of VH are independent and stronger predictors of several clinically relevant features (Table 2). The closer relationship of ELV_{180}/FRC_{pleth} with $T_{LCO}\%pred$ is expected, as reductions in effective lung volume (ELV) will likely be associated with a lower capacity for gas diffusion.[27] In addition, the single compartment lung model used to estimate cardio-respiratory variables[14,15] will be sensitive to abnormalities in gas exchange.

We have also demonstrated that ELV_{180}/FRC_{pleth} and/ or ELV_{180}/ELV_{pred} were independent and superior predictors of symptom severity in comparison to $FEV1_{\%pred}$. Interestingly however, ELV_{180}/FRC_{pleth} and ELV_{180}/ELV_{pred} show varying symptomatic relationships (Figure 5) which may suggest they reflect different phenotypes within the spectrum of COPD.[8] As stated above, ELV_{180} seems to have some independence from FRC_{pleth} (Figure 6A). If patients are categorised by the conventionally accepted threshold of lung hyperinflation ($FRC_{pleth}\%pred$ = 120%),[28] and also by the lower boundary of normality
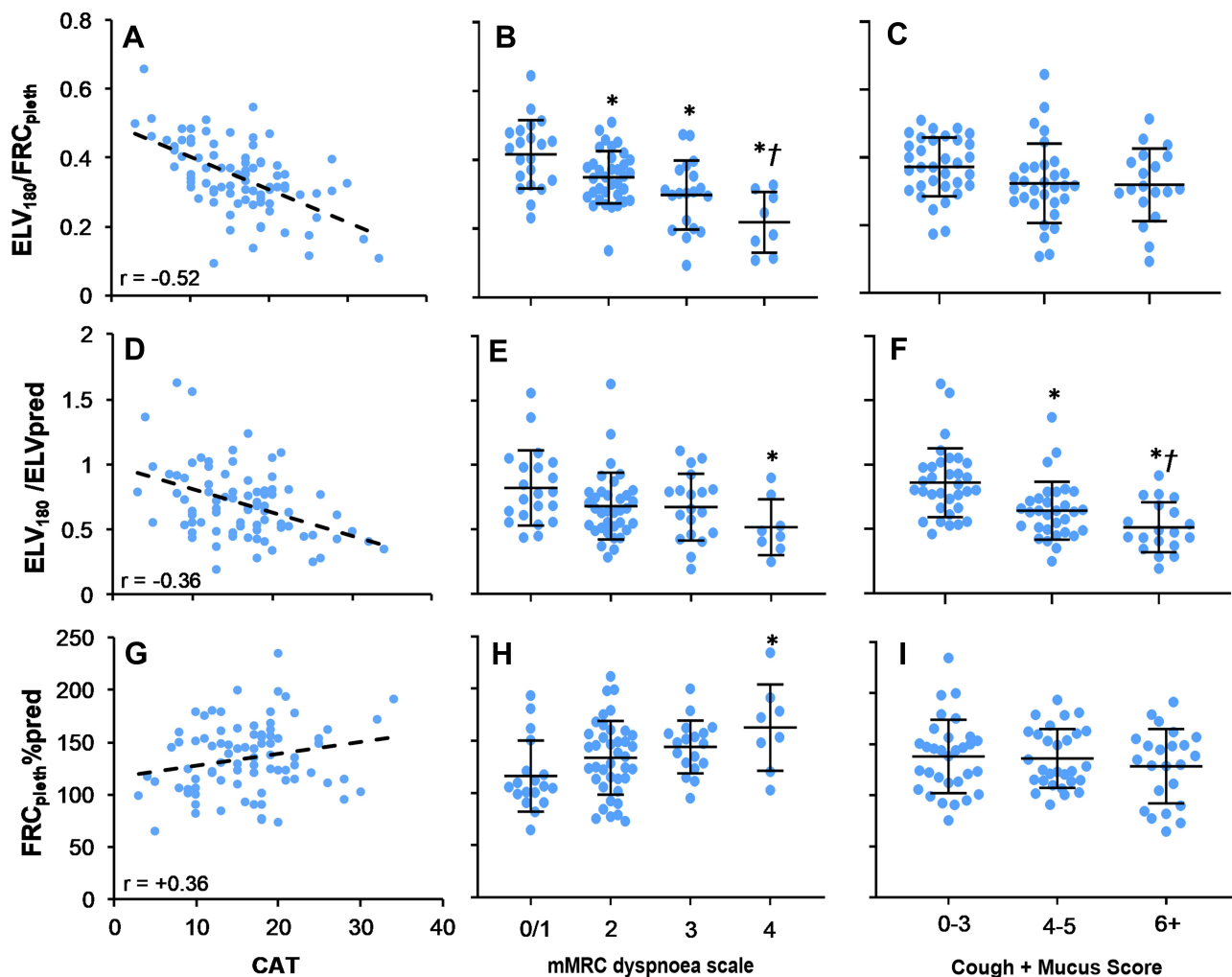
Figure 5 Scatterplots of ELV_{180}/FRC_{pleth}, ELV_{180}/ELV_{pred} and $FRC_{pleth}\%pred$ with the COPD assessment test (CAT), and dot plots of ELV_{180}/FRC_{pleth}, ELV_{180}/ELV_{pred} and $FRC_{pleth}\%pred$ and the modified Medical Research Council (mMRC) dyspnoea scale and a cough + mucus score derived from the first two question of the CAT (see methods for further details). Scatterplots (**A, D** and **G**) include a linear regression line, r = −0.52, r = −0.36 and r = +0.36, respectively ($P<0.05$). *Statistically significant difference ($P<0.05$) from mMRC Dyspnoea score = 0/1 (**B, E** and **H**) or Cough + Mucus score = 0–3 (**C, F** and **I**). †Statistical significant difference (P<0.05) from mMRC Dyspnoea score = 2 (**B** and **E**) or Cough + Mucus score = 4–5 (**C** and **F**).

for ELV_{180}/ELV_{pred} (ie, 2 Z-scores below mean ELV_{180} /ELV_{pred} in healthy participants ≈ 0.7), there is a spread of patients between each of these classifications ('phenotypes' I–IV, Figure 6A). Phenotype III and IV are characterised by hyperinflated lungs but have a "normal" or "abnormally low" effective lung volume (ELV_{180}) respectively. In contrast, phenotype II has non-hyperinflated lungs with a low ELV_{180}. Clearly, far fewer patients are classified as phenotype I (non-hyperinflated and "normal" ELV_{180}), and this is where the substantial majority of healthy participants lie (data not shown for clarity).

Figure 6B–D shows the variation in symptom type/ severity between these purported phenotypes. Patients within phenotype I had significantly lower symptom severities, whereas phenotype IV displayed the highest severities. Interestingly however, although there was no significant difference in dyspnoea severity between groups III and IV, phenotype II displayed significantly lower dyspnoea values (mMRC-DS). Conversely, phenotype III had a significantly lower cough frequency and/or mucus production in comparison to groups II and IV (C +M scale). However, as this latter finding was produced via an unvalidated approach it should be viewed with caution and, clearly, further work using validated methods is needed.[29,30] Interestingly though there was a strong linear association between question 4 of the CAT, which refers to sensations of breathlessness, and an independent and validated measure of dyspnoea (mMRC-DS; r = 0.81, P<0.05), suggesting that analysing CAT questions individually may hold some validity.
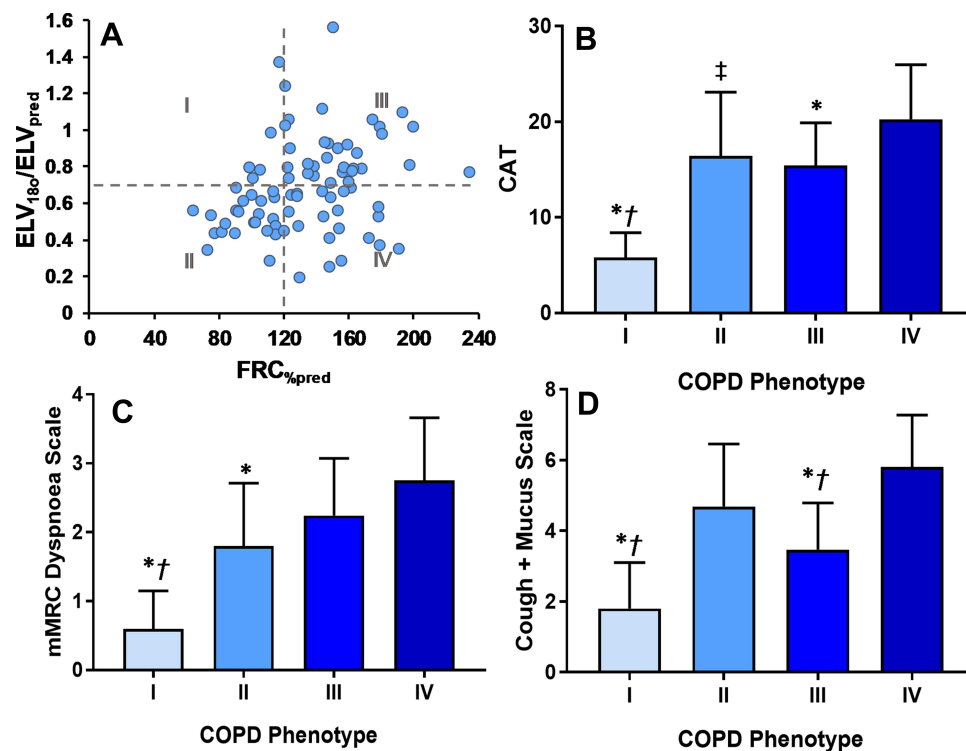
Figure 6 (**A**) Scatterplot of ELV_{180}/ELV_{pred} vs $FRC_{\%pred}$ for COPD patients. Patient data has been additionally categorised into quadrants ("COPD phenotype" I–IV) using the conventionally accepted threshold of lung hyperinflation ($FRC_{pleth}\%pred = 120\%$),[28] and the lower boundary of normality for ELV_{180}/ELV_{pred} (ie, 2 Z-scores below mean ELV_{180}/ELV_{pred} in healthy participants ≈ 0.7)/Quadrant I, $FRC_{\%pleth} <120\%$ and $ELV_{180}/ELV_{pred} ≥ 0.7$; II, $FRC_{\%pleth} <120\%$ and $ELV_{180}/ELV_{pred} < 0.7$; III, $FRC_{\%pleth} ≥120\%$ and $ELV_{180}/ELV_{pred} ≥ 0.7$; IV, $FRC_{\%pleth} ≥120\%$ and $ELV_{180}/ELV_{pred} < 0.7$. See Figure 5 and text for further details. (**B–D**) Bar charts of the mean (±SD) CAT, mMRC Dyspnoea, and Cough + Mucus scores for each phenotype (I–IV). *Statistical significant difference ($P<0.05$) from COPD phenotype III. †Statistical significant difference ($P<0.05$) from COPD phenotype II. ‡Near statistical difference ($P=0.09$) with COPD phenotype IV.

The physiological mechanisms underlying these findings require further examination, but there are considerable distinctions in the pathophysiology and molecular processes underlying chronic airways disease vs parenchymal disease which can be reflected in symptom manifestation.[31–33] The inflammation, goblet cell metaplasia, and structural remodelling in airways diseases can result in chronic cough, mucus production and inhomogeneous ventilation of lung compartments.[12,26,31,34] Under these conditions, the N_2O sinewave signal will be inhibited or prevented from entering poorly/non-ventilated regions, and so reduce the measured ELV. In contrast, the N_2O signal will likely be able to enter distal regions of emphysematous lungs, but the degradation of elastic parenchymal tissue is a principal cause of lung hyperinflation, and the alterations in pulmonary mechanics are often associated with severe dyspnoea.[28]

Taken together, this data suggests that in ELV_{180} may be sensitive to airways disease and provides a good predictor of cough frequency and mucus production. When normalised to FRC_{pleth} however (ELV_{180}/FRC_{pleth}), the measure appears to be a sensitive diagnostic tool for COPD, and is a good predictor of dyspnoea and the general burden of COPD symptoms (as quantified by CAT). Furthermore, patients with an abnormally low ELV_{180} and high FRC_{pleth} tend to have the most severe symptoms. Parenchymal and airways disease both occur to varying degrees across the spectrum of COPD patients, yet current management guidelines essentially disregard this heterogeneity.[33,35] The IST has potential as a simple and non-invasive method to stratify patients into subgroups based upon their "effective" lung volume and hence direct more individualised treatment strategies.[36]

ELV_{180}/FRC_{pleth} was significantly correlated with the CT-derived morphological degree of emphysema (% LAA), and the strength of association was similar to that of $FEV_{1\%pred}$. Emphysema detected by CT has been previously correlated with gross pathology[37] and is associated with important clinical outcomes, including mortality.[38] Lung parenchyma destruction has the potential to increase the disparity between ELV_{180} and FRC_{pleth}. FRC_{pleth} will be necessarily increased by hyperinflation, but ELV_{180}

Table 2 Univariable and Multivariable Linear Regression Analysis of ELV_{180}/FRC_{pleth} and ELV_{180}/ELV_{pred} as Predictors of Several Clinical Features (Dependent Variables) of COPD, Independent of $FEV_{1\%pred}$

Dependent Variable	Independent Variable/s	Univariable Analysis		Multivariable Analysis					
				ELV_{180}/FRC_{pleth} & $FEV_{1\%pred}$			ELV_{180}/ELV_{pred} & $FEV_{1\%pred}$		
		R^2	P	R^2	Std Coeff	P	R^2	Std Coeff	P
CAT	$FEV_{1\%pred}$	0.12	0.001	0.28	−0.47	0.48	0.22	−0.314	0.002
	ELV_{180}/FRC_{pleth}	0.26	<0.001		−0.83	<0.001			
	ELV_{180}/ELV_{pred}	0.13	0.001					−0.312	0.002
mMRC Dyspnoea Scale	$FEV_{1\%pred}$	0.06	0.028	0.27	0.08	0.5	0.12	−0.21	0.049
	ELV_{180}/FRC_{pleth}	0.26	<0.001		−0.56	<0.001			
	ELV_{180}/ELV_{pred}	0.07	0.017					−0.23	0.03
Cough + Mucus Score	$FEV_{1\%pred}$	0.02	0.657	0.16	0.19	0.15	0.33	0.23	0.8
	ELV_{180}/FRC_{pleth}	0.13	0.05		−0.41	0.002			
	ELV_{180}/ELV_{pred}	0.33	<0.001					−0.58	<0.001
$T_{LCO\ \%pred}$	$FEV_{1\%pred}$	0.13	<0.001	0.23	0.19	0.125	0.23	0.28	0.002
	ELV_{180}/FRC_{pleth}	0.25	<0.001		0.35	0.004			
	ELV_{180}/ELV_{pred}	0.16	<0.001					0.35	0.001
%LAA	$FEV_{1\%pred}$	0.24	0.027	0.33	−0.31	0.227	0.32	−0.47	0.042
	ELV_{180}/FRC_{pleth}	0.25	0.024		−0.32	0.224			
	ELV_{180}/ELV_{pred}	0.14	0.10					−0.33	0.091

Notes: Std Coeff, standardised regression coefficient (beta weight). This refers to the number of SDs the dependent variable will change per SD change of the independent variable. See Table 1, Figure 4 and/or methods for additional definitions.

may also become reduced by inhomogeneous enlargement of airspaces[9] and the increase of VH of the most distal acinar lung units – indeed emphysema is known to cause an increase in S_{acin}[39] (see below). Although ELV_{180}/FRC_{pleth} only performed similarly to $FEV_{1\%pred}$, it is worth noting that our current study is restricted to patients with a spirometric definition of COPD. It has long been argued that quantifying VH via washout techniques may be sensitive to early airway changes in smokers, before any measurable proximal airflow limitation.[39] As such, techniques such as IST have the potential to provide an earlier diagnosis of COPD ('GOLD 0'),[40] which warrants further work with smokers and assessing the association of IST measures with long-term outcomes.

Single and multiple-breath nitrogen washout (SBNW and MBNW) tests are becoming established clinical methods of assessing VH. These tests involve expelling alveolar nitrogen by inhaling 100% oxygen, and assessing the characterises of the single breath nitrogen expirogram (eg, Phase III slope; SBNW), or the clearance of nitrogen over multiple breaths (MBNW).[13] It has been shown that both

tests are sensitive to small airways disease in COPD patients and non-obstructed smokers, and have been associated with the severity of respiratory symptoms.[26,39,41,42] Given the parallels between techniques, future work should compare the performance of the IST with SBNW/MBNW in health and obstructive disease. Unlike SBNW, which assesses the entire airway, MBNW can probe VH at the acinar (S_{acin}) and conducting airways (S_{cond}) levels.[43] We also aim to develop more complex multicompartmental models that can provide additional information about different regions of the cardiopulmonary system. Unlike MBNW which is limited to assessing airways, the IST has the potential to analyse abnormalities in $\dot{Q}_P$. Currently, we are developing a simulation-based optimisation model, using Bayesian optimisation methods,[44] to provide further indices of lung heterogeneity using IST data. The concepts of the multi-compartmental lung model have been discussed elsewhere[45–47] and we aim for it to examine lung heterogeneity by recovering lognormal distributions of ventilation ($\dot{V}$) and $\dot{Q}_P$ fractions and assessing $\dot{V}-\dot{Q}_P$ mismatching. Initial analysis, using data from the current

study, shows the $\dot{V}$ and $\dot{Q}_P$ distributions are wider in COPD patients in comparison to healthy controls, and are similar to those demonstrated using other experimental techniques[46] (see [supplemental material](#) and [Figure 1S](#)). This clearly shows the potential for using more complex multi-compartmental models in the assessment of COPD, but further work is required.

Conclusions

The IST is a non-invasive method to monitor cardiopulmonary function which can produce clinically useful indices of VH: ELV_{180}/FRC_{pleth} and ELV_{180}/ELV_{pleth}. ELV_{180}/FRC_{pleth}, has a very high sensitivity for spirometrically defined COPD and its specificity improves if normalised for age. However, the IST's diagnostic ability goes beyond simply agreeing with FEV_1, as the indices of VH are independent and better predictors of several clinically relevant features of COPD including the type/severity of symptoms. Interestingly, ELV_{180}/FRC_{pleth} and ELV_{180}/ELV_{pleth} show varying symptomatic relationships which may suggest they reflect different phenotypes within the spectrum of COPD. The IST has potential as a simple-to-perform method to stratify patients into clinically relevant subgroups based upon their effective lung volume.

Abbreviations

BMI, body mass index; CAT, COPD assessment test; ELV, effective lung volume; FEV_1, forced expiratory volume in one second; FRC, functional residual capacity; FVC, forced vital capacity; IST, inspired sinewave test; K_{CO}, transfer coefficient for carbon monoxide; LAA, low attenuation area; mMRC-DS, modified Medical Research Council dyspnoea scale; PEF, peak expiratory flow; $\dot{Q}_P$, pulmonary blood flow; RV, residual volume; TLC, total lung capacity; T_{LCO}, transfer factor for carbon monoxide; $\dot{V}$, ventilation; V_A, alveolar volume; V_D, anatomical dead space; VH, ventilatory heterogeneity.

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Author Contributions

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Disclosure

Professor Andrew D Farmery reports a patent EP3122249 issued and Dr Phi Anh Phan reports a patent EP3122249A1 issued. The authors report no other conflicts of interest in this work.

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