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ORIGINAL RESEARCH

Feasibility and challenges of using multiple breath washout in COPD

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Background: Lung clearance index (LCI), derived from multiple-breath washout (MBW), is a well-established assessment of ventilation inhomogeneity in cystic fibrosis but has not been widely applied in other conditions characterized by heterogeneous airways disease, such as COPD. The aim of this study was to evaluate the sensitivity, repeatability, and practicality of LCI in patients with COPD.

Methods: Fifty-four COPD patients completed MBW using nitrogen as the washout tracer gas (MBW_{N2}, measured using an Exhalyzer™ device), spirometry, and plethysmography. Twenty patients repeated MBW_{N2}, MBW_{SF6} (using a separate Innocor™ gas analyzer to measure washout of the exogenous trace sulphur hexafluoride), and spirometry at a second visit ≥24 hours later.

Results: Mean (SD) COPD LCI measured by nitrogen washout (LCI_{N2}) was 12.1 (2.2); mean (SD) LCI Z-score 5.8 (2.0). LCI_{N2} increased across Global Initiative for Obstructive Lung Disease stages 1 to 3 and was abnormal (Z-score >1.65) in all COPD patients, including those with forced expiratory volume in 1 second (FEV_1) ≥80% predicted. LCI was repeatable (median intra-test coefficient of variation 4.1%) and reproducible (limits of agreement −1.8 to 1.6) after mean of 16 days. Functional residual capacity (FRC) measurements were significantly greater using nitrogen than SF_6 or plethysmography: mean FRC measured by nitrogen washout (FRC_{N2}) 139% predicted versus FRC measured by plethysmography 125% predicted, $p<0.0001$.

Conclusion: LCI is most suitable as a measure of early airways disease in COPD in those with well-preserved FEV_1, with similar repeatability and limitations to that observed in cystic fibrosis. Using the Exhalyzer system to perform MBW_{N2}, however, appeared to substantially over-read FRC. This discrepancy needs addressing before FRC_{N2} measurements made using this device can be reliably deployed.

Keywords: COPD, multiple breath washout, lung volumes, lung physiology, functional residual capacity, lung clearance index

Introduction

Multiple-breath washout (MBW) testing is now well established in cystic fibrosis (CF) research as a practical and reproducible measure of airways physiology that is both more sensitive at detecting the presence of disease and at monitoring treatment responses compared with conventional spirometry.[1] MBW involves tidal breathing to wash an inert tracer gas from the lungs and generates measures of lung volume (functional residual capacity; FRC) as well as of gas mixing efficiency.[2] The most important and commonly reported outcome is the lung clearance index (LCI), a summary measure of ventilation heterogeneity. It has been known for some time that COPD results in abnormal gas mixing,[3] and also that ventilation heterogeneity is an early feature of smoking-related lung disease.[4] Ventilation heterogeneity has been demonstrated in COPD using ventilation-MRI, and seems to relate to other measures of disease severity.[5] However,

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the applicability of current MBW technologies for measuring ventilation heterogeneity in COPD has not been studied.

The European CF Society have recently published recommendations for use of MBW in clinical trials in CF[6] and there is a drive for clinical application of these measurements.[7] MBW is now available in several CF centers in the US and Europe, the majority using nitrogen washout systems, which derive nitrogen concentration from measurements of expired O_2 and CO_2. Nitrogen washout is performed by breathing 100% oxygen. This differs from the majority of recent formative MBW studies where SF_6 was used as the tracer gas, with washout by breathing room air. Different technologies seem to deliver different measures of LCI and FRC,[8] and these are not interchangeable even between nitrogen washout systems.[9]

The role of MBW in COPD may differ from that in CF, being potentially more relevant in disease phenotyping and assessing treatment response than in detecting and preventing early disease progression. For MBW to be adopted in this way, it must be sensitive to disease identification, practical to perform,[10] and reproducible.[11] The applicability of MBW in adults with COPD has not previously been assessed.

We hypothesized that MBW would be a practical, repeatable, and potentially informative test in adults with COPD. The aims of this study were, therefore, as follows:

1. To assess practical limitations of MBW in patients diagnosed with COPD, including test time and success rates.
2. To assess intra-visit repeatability and inter-visit reproducibility of LCI and FRC.
3. To compare MBW outcomes with other lung function measures in patients diagnosed with COPD.
4. To compare indices from nitrogen washout with those from SF_6 washout.

Materials and methods

This study was performed at the Medicines Evaluation Unit, University Hospital of South Manchester, UK. COPD patients were aged >40 years and had a diagnosis of COPD in accordance with the 2013 Global Initiative for Obstructive Lung Disease (GOLD) recommendations.[12] This included a smoking history ≥10 pack-years with typical symptoms (1 or more of productive cough, breathlessness, and wheeze) and evidence of airflow obstruction (forced expiratory volume in 1 second [FEV_1]/forced vital capacity <0.7). Additional exclusion criteria included any respiratory tract infection or COPD exacerbations within 6 weeks of assessment.

Patients diagnosed with COPD continued all regular pharmacological therapies but restricted short-acting inhaled bronchodilators for a minimum of 8 hours, and long-acting inhaled bronchodilators for a minimum of 24 hours prior to assessments. The study was approved by the Greater Manchester South research ethics committee (reference: 10/H1003/108) and written informed consent was obtained from each subject.

Study design

Fifty-four patients diagnosed with COPD performed impulse oscillometry, MBW, gas transfer, whole-body plethysmography, spirometry, and a 6-minute walk test (6MWT) of exercise capacity (in that order). Subjects were invited to return for repeat MBW testing >24 hours later.

Assessments

Detailed descriptions of each of the measurements can be found in the online supplement. Multiple-breath nitrogen washout (MBW_{N2}) was performed as previously described[8] using an open circuit, bias flow system (Exhalyzer D®, Eco-Medics AG, Duernten, Switzerland). Analysis was performed using the associated software (Spiroware 3.1 EcoMedics AG). MBW_{N2} was performed 3 times and reported values for FRC measured by nitrogen washout (FRC_{N2}) and LCI measured by nitrogen washout (LCI_{N2}) are the average of at least 2 reproducible repeats. Age-matched Z-scores for LCI_{N2} were taken from those provided by the manufacturers.

A subset of patients performed additional MBW of SF_6 (MBW_{SF6}). This was carried out using an open circuit washin of 0.2% SF_6 followed by washout breathing room air, using a modified Innocor analyzer as previously described.[13] Both MBW_{N2} and MBW_{SF6} were performed according to consensus recommendations.[14]

Spirometry was performed according to the American Thoracic Society/European Respiratory Society recommendations[15] using a Sensormedics Vmax spirometer (Sensormedics Corporation, Yorba Linda, CA, USA). Reference values used were those of the 2012 Global Lung Initiative.[16]

Impulse oscillometry was performed using a Masterscreen impulse oscillometer (IOS; Erich Jaeger, Hoechenberg, Germany) in accordance with consensus recommendations.[17] Plethysmographic lung volumes were measured in accordance with the American Thoracic Society/European Respiratory Society recommendations[16] using a constant volume whole-body plethysmograph (Autobox 6200 DL, Sensormedics Corporation). Carbon monoxide diffusing capacity (DL_{CO}) and carbon monoxide transfer coefficient (K_{CO}) were measured using the Vmax 22 instrument (Sensormedics Corporation), according to ATS/ERS recommendations.[18] The reported value is the mean of at least 2 successful

readings within 10% of the highest value. The 6MWT test was conducted to a standardized protocol in accordance with current guidelines using a 20-meter course.[19]

Statistical analysis

A sample size of 10 patients would give 99% power to detect a 20% difference in LCI over historical control data and a sample size of 22 would be required to detect a 10% difference in LCI. We were interested, however, in how LCI compared with lung physiology across a range of disease severity and estimated that 50 patients would be sufficient to make reasonable inferences about how airway markers compared, and would give us enough subjects to get repeat LCI measurements on at least 20.

Data were analyzed using Prism (GraphPad Software Inc., San Diego, CA, USA). Parametric data were expressed as mean (SD) and non-parametric data expressed as median with interquartile range (IQR). For comparison of physiology parameters with GOLD stage, a 1-way analysis of variance was conducted, followed by Tukey's multiple comparison test to compare pairs of data. Correlations were assessed using a Pearson's correlation coefficient or a Spearman's rank correlation coefficient. Intra-visit repeatability was determined by calculating the coefficient of variation (CV) of the 3 recordings for each subject. Inter-visit reproducibility was assessed using Bland–Altman analysis[20] and intra-class correlation coefficient (ICC). ICC was calculated using SPSS (version 22, IBM SPSS inc, Armonk, NY, USA) using a 2-way mixed analysis of absolute agreement. For the comparison of MBW methods, outcomes were compared using a paired *t*-test and the agreement between the 2 systems assessed using Bland–Altman analysis.[20] Significance level was set at p=0.05.

Results

Fifty-four patients diagnosed with COPD (37 male) performed assessment of lung function and MBW. Mean (SD) age of subjects was 66 (7) years, with a median smoking history of 42 pack-years (full demographics presented in Table 1). Seventeen (31%) of the COPD patients were frequent exacerbators, defined as having ≥2 exacerbations in the last 12 months.

Practicality of MBW_{N2}

Four out of the 54 patients diagnosed with COPD (7%) were unable to complete MBW_{N2} due to leak at the mouthpiece, dry throat, or a sensation of dyspnea when breathing high-flow O_2. Nine repeat measurements were discarded for failure to meet quality control criteria. Patients with high minute ventilation would sometimes re-inspire expired air or room air from the end of the flow-past circuit. Overall, success rate was similar to that achieved for the other physiological assessments (Table S1). Median (IQR) total test time in COPD patients was 36.3 (23.8–44.9) minutes. Test time increased in those with higher LCI, r^2=0.24, p=0.0006 (Figure S1).

Table 1 Clinical characteristics and lung function measurements

Clinical characteristics	COPD (n=54)	
	Absolute values	% predicted
Sex (male/female)	37/17	
Age (years)	66 (7)	
Height (m)	1.67 (0.10)	
Weight (kg)	78.8 (18.6)	
Body mass index (kg/m^2)	27.8 (4.9)	
Smoking history (pack years)[a]	42.1 (31.7–55.5)	
Spirometry		
FEV_1 (L)	1.68 (0.6)	63.1 (18.4)
$FEF_{25\%-75\%}$ (L/s)	0.66 (0.35)	22.0 (11.2)
FVC (L)	3.62 (1.0)	106.6 (17.8)
FEV_1/FVC	0.48 (0.13)	
Multiple breath nitrogen washout		
MBW_{N2} test time (mins)	38.4 (19.9)	
FRC_{N2} (L)	4.35 (1.27)	135.7 (36.8)
LCI_{N2}	12.1 (2.2)	158.9 (29.3)
Plethysmography		
TLC (L)	6.30 (1.27)	105.5 (15.4)
Inspiratory capacity (L)	2.15 (0.59)	78.4 (15.2)
FRC_{pleth} (L)	3.99 (1.06)	123.0 (29.8)
RV (L)	2.95 (0.97)	127.9 (40.7)
RV/TLC %	46.6 (10.5)	
Gas transfer		
DL_{CO} (mmol·min^{-1}·kPa^{-1})	5.23 (1.78)	63.1 (18.4)
K_{CO} (mmol·min^{-1}·kPa^{-1})	1.07 (0.28)	79.6 (23.2)
Forced oscillation		
R_5–R_{20}[a] (kPa/L/s)	0.17 (0.11–0.25)	
X_5 (kPa/L/s)	–0.30 (0.16)	
CAT score[a]	18.0 (9.0–25.3)	

Note: Parametric data are expressed as mean and SD except [a]median and interquartile range.

Abbreviations: CAT, COPD Assessment Test; DL_{co}, carbon monoxide diffusing capacity; FEF, forced expiratory flow; FEV_1, forced expiratory volume in 1 second; FRC, functional residual capacity; FRC_{N2}, FRC measured by nitrogen washout; FRC_{pleth}, FRC measured by plethysmography; FVC, forced vital capacity; K_{CO}, carbon monoxide transfer coefficient; LCI_{N2}, lung clearance index measured by nitrogen washout; MBW_{N2}, multiple-breath washout using nitrogen; RV, residual volume; TLC, total lung capacity.

Repeatability and reproducibility of MBW in COPD

The median (IQR) intra-test CV of triplicate repeat FRC_{N2} measurements on the same visit was 4.3 (1.7–6.3) for FRC and 4.1 (2.2–7.1) for LCI.

Twenty clinically stable patients diagnosed with COPD performed repeat measurements of MBW_{N2} in triplicate after a mean (SD) interval of 16 (13) days. There was a mean difference in FRC_{N2} between visits of –0.4% predicted (absolute),

95% limits of agreement −28.9% to 28.1% (Figure S2). ICC for repeat FRC was 0.90 (95% CI: 0.76–0.96). For LCI_{N2}, the mean difference between visits was −0.11 with limits of agreement −1.79 to 1.59, approximately equivalent to ±15% of initial LCI (Figure S3). ICC for LCI was 0.94 (95% CI: 0.86–0.98).

Comparison of LCI with other lung physiology assessments

LCI_{N2} was raised in all patients with COPD (Z-score >1.65), including 10 (20% of the COPD patients) with GOLD stage 1 (defined as FEV_1 >80% predicted).[21] LCI_{N2} showed significant inverse correlation with FEV_1 Z-score (r^2=0.36, p<0.0001; Figure 1) and forced expiratory flow $(FEF)_{25\%–75\%}$ (r^2=0.36, p<0.0001). LCI_{N2} also showed significant positive correlations with $R_5–R_{20}$ (r^2=0.17, p=0.009), residual volume/total lung capacity (RV/TLC [%]) (r^2=0.16, p=0.02), R_{aw} (r^2=0.30, p=0.0006), and significant negative correlations with X_5 (r^2=0.35, p=0.0006), sG_{aw} (r^2=0.18, p=0.012) and DL_{CO} (r^2=0.34, p=0.0001). There was no correlation between LCI_{N2} and 6MWT distance. Full details of these correlations are presented in Table S2 and Figures S4–S11.

LCI_{N2} also increased significantly with greater GOLD stage. Mean (SD) LCI_{N2} in GOLD stage 1 was 10.2 (1.2), versus GOLD stage 2 (FEV_1 50%–80%), 11.9 (1.8) p=0.048, or GOLD stage 3 (FEV_1 30%–50%), 14.19 (2.3) p<0.0001

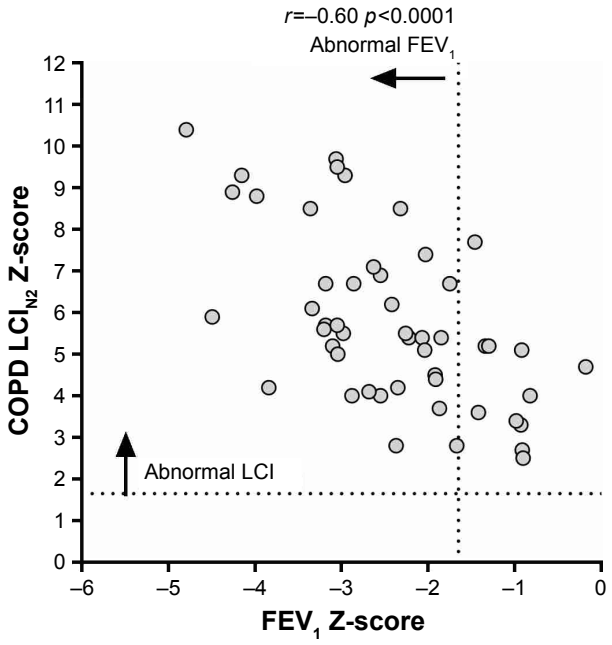

Figure 1 COPD LCI_{N2} Z-score versus FEV_1 Z-score.
Notes: Z-scores were calculated using the GLI 2012 reference equations for FEV_1[17] and those provided by Ecomedics for LCI. The horizontal dotted line represents the upper limit of normal for LCI_{N2}. The vertical dotted line represents the FEV_1 Z-score of −1.65 (lower limit of normal range).
Abbreviations: FEV_1, forced expiratory volume in 1 second; GLI, global lung initiative; LCI_{N2}, lung clearance index measured by nitrogen washout.

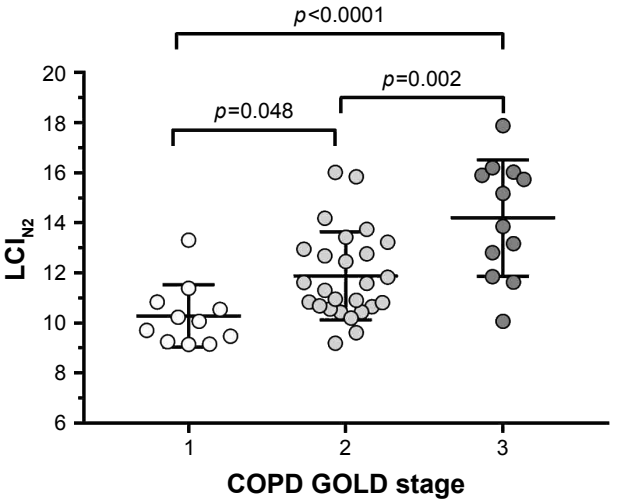

Figure 2 LCI_{N2} versus COPD GOLD stage.
Abbreviations: GOLD, Global Initiative for Obstructive Lung Disease; LCI_{N2}, lung clearance index measured by nitrogen washout

(Figure 2). There was only a single subject with GOLD stage 4 COPD, omitted from this analysis. Difference between lung physiology outcomes for the different GOLD stages (1–3) is presented in the OLS (Table S3). In addition to LCI, there was statistically significant separation between all 3 GOLD stages for sG_{aw} and $FEF_{25\%–75\%}$.

Comparison of FRC assessments

Seventeen patients diagnosed with COPD completed separate sequential assessments of FRC and LCI using both N_2 and SF_6 as the washout gases (Table 2). There was no significant difference in LCI measured by sulphur hexafluoride washout (LCI_{SF6}) and LCI_{N2} (11.9 [3.1] versus 12.3 [2.2], p=0.4).

Table 2 Clinical characteristics and lung function measurements of COPD patients with paired MBW assessments

Clinical parameter	COPD (n=17)	p-value
FRC (L)		
FRC_{N2}	4.24 (1.47)	0.002
FRC_{SF6}	3.49 (1.00)	
FRC (%) predicted		
FRC_{N2}	136.8 (42.8)	0.002
FRC_{SF6}	112.4 (28.0)	
Lung clearance index		
LCI_{N2}	12.3 (2.2)	0.4
LCI_{SF6}	11.9 (3.1)	
FEV_1 (%)	64.2 (21.1)	
FVC (%)	101.4 (13.6)	
FEV_1/FVC	0.5 (0.1)	

Note: Parametric data are expressed as mean and SD, and comparisons were performed using Student's *t*-test.
Abbreviations: FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; FRC, functional residual capacity; FRC_{N2}, FRC measured by nitrogen washout; FRC_{SF6}, FRC measured by sulphur hexafluoride washout; LCI_{N2}, lung clearance index measured by nitrogen washout; LCI_{SF6}, lung clearance index measured by sulphur hexafluoride washout; MBW, multiple-breath washout.

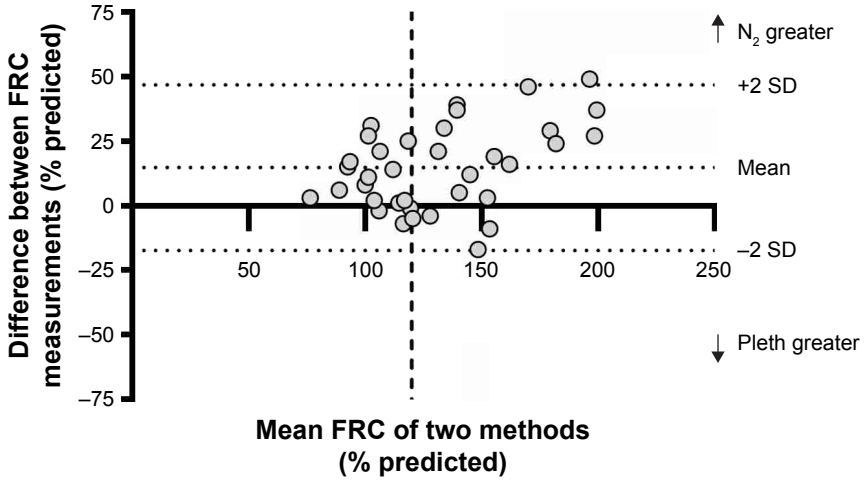

Figure 3 Bland–Altman comparison between FRC_{N2} and FRC_{pleth} in subjects with COPD.
Notes: The central dotted line represents the mean difference, and the upper and lower dotted lines represent the limits of agreement. The vertical line represents hyper-inflation, defined as FRC_{N2} >120% predicted. Data points plotted above the x-axis represent subjects in which FRC_{N2} was greater than FRC_{pleth}.
Abbreviations: FRC, functional residual capacity; FRC_{N2}, FRC measured by nitrogen washout; FRC_{pleth}, FRC measured by plethysmography.

FRC_{N2}, however, was significantly elevated compared with FRC measured by sulphur hexafluoride washout (FRC_{SF6}). Mean (SD) of FRC_{N2} was 136.8 (42.8) % predicted compared with 112.4 (28.0) % for FRC_{SF6}, p=0.002. The difference between FRC_{N2}% and FRC_{SF6}% increased disproportionally as FRC% increased. The mean difference was 24.4 (26.4) % predicted with limits of agreement –27.3 to 76.1 % predicted (Figure S12).

FRC values measured by plethysmography and nitrogen washout were also compared for the whole cohort at visit 1. Overall, FRC_{N2} was significantly greater than FRC measured by plethysmography (FRC_{pleth}) (139.3% versus 124.5%, $p<0.0001$). Mean difference between FRC_{N2} and FRC_{pleth} was 14.8% predicted (limits of agreement –17.3 to 46.8; Figure 3), with a disproportionate increase in FRC_{N2} in those with higher FRC % predicted.

Discussion

This is the first study to compare different MBW techniques, alongside detailed physiological phenotyping, in patients diagnosed with COPD. The specific objectives were to understand more about the repeatability and practical limitations of MBW in COPD. This study found LCI to be sensitive to detect mild COPD, being elevated even in those in GOLD stage 1. Intra-visit repeatability was similar to that reported in CF studies[8,13] and inter-visit reproducibility was similar to that of CF adults.[22] Finally, LCI has physiologically valid correlations with other small airways measurements (including X_5, R_5–R_{20}, RV/TLC, R_{aw}) as well as other markers of COPD severity, such as DL_{CO}. This accords with recent work showing that smokers with preserved FEV_1 had evidence of small airways damage, gas trapping, and impaired gas transfer.[23,24] Abnormality in single-breath nitrogen washout in symptomatic smokers and in MBW parameters in asymptomatic smokers (>10 pack years) has also been previously demonstrated.[24,25] The particular advantages of LCI as an outcome for assessing these early changes is that the methodology is standardized,[14] with good reproducibility, and with apparently good sensitivity. This last feature is harder to quantify, however, since there is no gold-standard definition of COPD that does not involve spirometry. It is nevertheless reassuring that LCI was abnormal (Z-score >1.65) in all the subjects with previously defined COPD and that it appeared to be more sensitive in this regard at detecting early airway change than the other lung physiology assessments. As with severe CF, however, the measurement appears less useful in those with more advanced COPD. In these patients, the test takes much longer to complete and the overall ventilation heterogeneity signal likely reflects a mixed pattern of disease. Thus, any signal from potentially reversible small airways disease will be overwhelmed by the signal from other lung regions where damage is more advanced and irreversible. These data and observations, therefore, support a role for MBW in measuring disease severity in patients with mild-to-moderate (GOLD stage 1–2) COPD, and possibly in detecting disease before it fulfills current spirometry-driven criteria.

An important caveat, however, exists for the specific nitrogen washout system used in this study. An unanticipated finding was that the measures of FRC_{N2} were far greater not only than those of FRC_{SF6} but also FRC_{pleth}. Plethysmography measures all gas within the thoracic cavity and should,

therefore, always measure a greater value for "FRC" than that which is ventilated during the quiet tidal breathing of a MBW test. FRC_{pleth} and FRC_{N2} are similar in healthy subjects,[26] but increasing FRC_{pleth} should be apparent in disease as gas trapping worsens, leading to an even greater discrepancy between tidally ventilated and total end-expiratory lung volumes.[27] In this study, however, we have observed the opposite. Although both FRC_{pleth} and FRC_{N2} were elevated in COPD, FRC_{N2} was higher than FRC_{pleth} with an offset of almost 20%. This difference also worsened in those with more severe disease, implying that tidally ventilated lung volume becomes increasingly greater than total lung volume as disease progresses. Clearly, this conclusion cannot be correct.

A recent study compared 2 similar indirect N_2 devices (Exhalyzer D versus EasyOne Pro, NDD Medical Technologies, Zurich, Switzerland). A difference of 30% in FRC values was found between these systems, with the Exhalyzer D generating higher mean values,[9] consistent with the observations in this study. This suggests an over-reading of FRC by the Exhalyzer system, which may be device-specific. Recent attempts to correct for excretion of body nitrogen did not fully account for this error,[28,29] and are not included in this analysis. The limitations of this same MBW_{N2} system for measuring lung volumes have also recently been recognized in patients with CF,[30] and seem to have occurred despite promising in vitro data for this method.[7] Importantly, accurate FRC measurement is essential for all MBW outcome measurements, including LCI. Reassuringly, however, the SF_6 washout gave similar values for LCI despite much lower values for FRC. This may be because both FRC and cumulative expired volume are overestimated to the same extent with the nitrogen washout, but importantly suggests no loss of sensitivity by using SF_6.

In this study, we have assessed patients diagnosed with COPD over a wide range of severity, including at repeat time points. The lack of a simple positive control for COPD, however, means that we cannot easily assess what the response to therapy should be. We also did not include a healthy control population, but have instead used the reference data from previous age-matched cohorts provided by the manufacturer to calculate Z-scores. LCI was assessed on site, using the on-board software of the Exhalyzer-D device. Measurements were not subject to external over-read as recently recommended in clinical trials,[6] although the research team has considerable experience with MBW as a technique.

Conclusion

We have shown that LCI offers substantial promise as a measurement in COPD. The key practical features of MBW assessment in COPD are similar to those in CF, and LCI appears to work best as a measure of early airways disease in those with well-preserved FEV_1. However, the specific nitrogen washout system used here exhibited major limitations in terms of the accuracy of the FRC measurements, something that has not been adequately accounted for and which has significant implications for clinical trials and trial networks already in progress. Considerable caution should, therefore, be employed in interpreting nitrogen washout outcomes until the reasons for this error are better understood.

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