Multiple Breath Washout outcome measures in adults with bronchiectasis

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Take home message

$LCI^{2.5}$ and $LCI^{5.0}$ have robust properties as outcome measures and have superior sensitivity compared with spirometry measures, in differentiating between health and bronchiectasis disease. $LCI^{5.0}$ is shorter and more feasible than $LCI^{2.5}$.

Abstract 245 / 350

Background: Lung Clearance index (LCI) has good intra-visit repeatability with better sensitivity in detecting lung disease on CT scan compared to Forced Expiratory Volume in 1 second ($FEV_{1)}$) in adults with bronchiectasis. Alternative multiple breath washout (MBW) parameters have not been systematically studied in bronchiectasis.

Aim: To determine the validity, repeatability, sensitivity, specificity and feasibility of standard LCI ($LCI^{2.5}$), shortened LCI ($LCI^{5.0}$), $S_{cond}VT$ and $S_{acin}VT$ in a cross-sectional observational cohort of adults with bronchiectasis.

Methods: Cross-sectional MBN_2W data (Exhalyzer® D) from 132 patients with bronchiectasis across 5 UK centres (Bronch-UK Clinimetrics study) and 88 healthy controls were analysed.

Results: Within test repeatability (mean CV%) was <5% for both $LCI^{2.5}$ and $LCI^{5.0}$ in patients with bronchiectasis and there was no difference in mean CV% in $LCI^{2.5}$ and $LCI^{5.0}$ in patients with bronchiectasis compared to healthy volunteers. Moderate strength correlations were seen between FEV_1 and $LCI^{2.5}$ z-scores (r=-0.54), $LCI^{5.0}$ (r=-0.53), $S_{cond}VT$ (r=-0.35) and $S_{acin}VT$ (r=-0.38). The proportion of subjects with abnormal MBW (>2 z-score) but normal FEV_1 % predicted (<-2 z-score) was 42% ($LCI^{2.5}$) and 36% ($LCI^{5.0}$). Overall results from the receiver operator characteristic curve (AUC^{ROC}) indicated that $LCI^{2.5}$ had greatest combined sensitivity and specificity to

discriminate between bronchiectasis and control subjects, followed by $LCI^{5.0}$, FEV_1 and $S_{cond}VT$. There was a 57% time saving with $LCI^{5.0}$.

Conclusions: $LCI^{2.5}$ and $LCI^{5.0}$ had good within test repeatability and superior sensitivity compared with spirometry measures, in differentiating between health and bronchiectasis disease. $LCI^{5.0}$ is shorter and more feasible than $LCI^{2.5}$.

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INTRODUCTION

Bronchiectasis is a chronic, debilitating and progressive pulmonary disease with symptoms of recurrent cough, daily sputum production, recurrent chest infections, and a poor health-related quality of life (HRQoL) (1). Currently, Forced Expiratory Volume in 1s (FEV_1) is used for monitoring lung function however, it can be insensitive in mild-moderate disease and unresponsive to antibiotic treatment (2-4). Therefore, there is a need for exploration of more sensitive outcome measures to monitor disease progression and measure treatment efficacy in clinical trials. Lung Clearance Index (LCI), derived from Multiple Breath Washout (MBW) testing is a measure of ventilation inhomogeneity (VI), or uneven gas mixing. Initial studies in bronchiectasis have also shown promise demonstrating the validity of LCI, with significant differences between health and disease (4-6) significant correlation with High-Resolution Computed Tomography (HRCT) scores and superior sensitivity to early changes in lung disease compared to FEV_1 (4, 5, 7). Verbanck and colleagues demonstrated that increased lung disease severity (number of bronchial segments) resulted in an associated LCI increase in bronchiectasis demonstrating the potential clinical application of LCI in this disease (8).

A large number of parameters reflecting VI are collected during MBW. LCI is one of the most commonly reported parameters. A drawback of LCI is that testing can be prolonged and therefore difficult for some patients to tolerate, compromising the utility of the test. These issues are particularly relevant in the bronchiectasis population who are elderly and frequently have multiple co-morbidities (9). The investigation of other MBW parameters and the flexibility of the current LCI end-point ($1/40^{th}$ of starting concentration of tracer gas) is an important area for

research as it could improve the feasibility of this test. As shown in CF, shortened LCI point (test endpoint $<1/40^{th}$ of starting concentration of tracer gas) is potentially a more feasible measure to perform with comparable repeatability and sensitivity to standard LCI (10). Furthermore, ventilation inhomogeneity within different zones of the lung i.e. phase III analysis including convection-dependent zones ($S_{cond}VT$) and diffusion-convection dependent zones ($S_{acin}VT$) can characterise regional ventilation inhomogeneity. Two studies have shown elevation in these measures in bronchiectasis compared to health (6, 8). However it is unclear whether $S_{cond}VT$ or $S_{acin}VT$ provide additional physiological information to FEV_1 or LCI, or if they are suitable outcome measures for interventional studies in bronchiectasis.

Further study is warranted to investigate alternate MBW parameters in order to determine if they have potential as surrogate endpoints for use in bronchiectasis clinical trials. To gain acceptance of researchers and licensing bodies, an endpoint must have a body of supporting evidence including acceptable clinimetric properties. The objectives of this study were to determine and compare the

- concurrent validity,
- intra-visit (within test) repeatability,
- sensitivity, specificity and
- feasibility (test duration)

of standard LCI ($LCI^{2.5}$), shortened LCI ($LCI^{5.0}$), $S_{cond}VT$ and $S_{acin}VT$ in adults with bronchiectasis. We hypothesized that the MBW outcomes of $LCI^{2.5}$ and $LCI^{5.0}$ would have comparable clinimetric properties to spirometry outcome measures. Some of the results have been reported previously in the form of an abstract (11).

METHODS

Participants

Data from patients recruited to the BronchUK Clinimetrics study across five UK centres between November 2015 - June 2019 were analysed. Inclusion criteria were a proven and documented diagnosis of idiopathic or post-infectious bronchiectasis by HRCT scan including 2 or more lobes (no defined timeline between diagnosis and enrolment) and aged $\geq$ 18 years old, ability to perform spirometry and MBW testing and written informed consent. Other aetiologies were excluded in accordance with BTS guidelines (12) for example CF genetic testing, Primary Ciliary Dyskinesia

(PCD). Exclusion criteria also included pregnancy, current participation or previous participation in a clinical trial of an investigational medicinal product in the last 4 weeks. Eligible participants were identified using leaflets advertising the study in patient waiting areas and by research co-ordinators screening clinic lists. Interested participants were screened against the inclusion/exclusion criteria. This study analysed data from the first clinically stable visit i.e. no pulmonary exacerbation within the previous 4 weeks (a cross-sectional observational cohort). Pulmonary exacerbation was defined as an acute respiratory infection requiring oral or IV antibiotics as outlined by O'Donnell and colleagues (13). This study was ethically approved and prospectively registered (reference MR/L011263/1, ClinicalTrials.gov Identifier: NCT02468271).

Healthy volunteers were recruited to collect reference MBW and spirometry data to enable comparison with disease data (sponsor Queen's University Belfast; reference 18.26v3). Volunteers were aged 18-80 years. Exclusion criteria included a current or previous history of a respiratory condition, on long term oxygen therapy, history of recent pneumothorax, history of recent eye surgery, history of recent sinus surgery, unstable cardiovascular status (i.e. myocardial infarction or worsening angina within 4 weeks), recent thoracic or abdominal surgery, tuberculosis, thoracic, abdominal or cerebral aneurysms, use of antibiotics in the previous 4 weeks or any other medical condition that could impair spirometry or MBW tests.

Multiple Breath Nitrogen Washout test

A valid MBW test was a requirement for enrolment in the BronchUK Clinimetrics study. A MBN$_2$W test using the Exhalyzer D® machine (Eco Medics AG, Duernten, Switzerland, Spiroware software (version 3.1.6) was used to derive all MBW indices. For MBW data accuracy and to facilitate up to date data analysis, MBN$_2$W data were re-calculated using Spiroware software version 3.3.1 and spx data files (14). All operators across sites completed a standardised training and certification process in MBW testing before study start (15) (online supplement). Subjects were required to complete a minimum of 2 valid and repeatable trials. In accordance with the inert gas consensus statement, tests where FRC differs by >25% from the median FRC value across the three trials were automatically rejected. FRC or LCI variability >10%, (difference between maximum and minimum values) triggered further investigation (16). Shortened LCI was determined from a

washout endpoint $1/20^{th}$ of starting concentration of tracer gas (i.e. 5% N_2 or $LCI^{5.0}$) and standard LCI was determined from a washout endpoint $1/40^{th}$ of starting concentration of tracer gas (i.e. 2.5% N_2 or $LCI^{2.5}$). Only those tests with ≥ 3 valid trials were used to calculate the CV% and the phase III parameters ($S_{cond}VT$ and $S_{acin}VT$) in accordance with the consensus statement for inert gas washout measurements (16). $S_{cond}VT$ reflects ventilation heterogeneity occurring in the conducting airways, where gas transport is driven by differences in pressure gradients and thus by convection. $S_{acin}VT$ is thought to reflect ventilation heterogeneity occurring at and beyond the acinar region of the lung, where gas transport is driven by differences in concentration and thus by diffusion. $S_{cond}VT$ and $S_{acin}VT$ relative to tidal volume were calculated by Spiroware (i.e. phase III slope was multiplied by the expired volume in litres to produce a volume-normalized phase III slope) as previously described (17, 18). Small breaths with $\geq 25\%$ deviation from the median of all breaths were excluded from the $S_{cond}VT$ or $S_{acin}VT$ calculation (automated by the software). All MBW tests were centrally over-read by trained personnel using pre-defined technical and quality criteria to ensure test validity and derivation of results (19, 20).

Test duration (minutes) for all trials performed was calculated retrospectively from the software for $LCI^{2.5}$ and $LCI^{5.0}$. Test time duration included both test time (including at least 2 trials) plus the waiting time before the next trial which ensured that $N_2\%$ returned to baseline (set by the equipment as 1.5 times the duration of the previous trial and calculated manually). Time duration did not include pre-phase or breaths taken after the defined cut off time.

Spirometry

Spirometry was conducted after MBW testing in accordance with American Thoracic Society/European Respiratory Society standards (21) and the measures of FEV_1, Forced Expiratory Flow at 25-75% (FEF_{25-75}), Forced Vital Capacity (FVC) and FEV_1/FVC ratio were recorded. Time of the participants last administered bronchodilator was recorded. GLI reference ranges were used (22).

Statistical analysis

Statistical analysis was conducted using SPSS (version 25), GraphPad Prism (version 8.3.1) and MedCalc (version 19.6) software. Subject characteristics in the disease and healthy control cohorts

were summarised and compared using Students t-test and Pearson Chi Square test. Within-test repeatability of MBW measures was assessed using the CV% of tests including at least three trials. In addition, Bland-Altman plots (i.e. the 95% limits of agreement between the first and third washout) were inspected. Paired sample t-tests were used to compare CV% and test time duration of MBW measures. P-values and confidence intervals are presented. Z-scores for all MBW parameters were calculated from the healthy study population. Z-scores for spirometry values were calculated from reference data (22). An $LCI^{2.5}$, $LCI^{5.0}$, $S_{cond}VT$ and $S_{acin}VT$ z-score of >2 was considered abnormal. A FEV_1, FEF_{25-75} and FVC z-score lower than −2 was considered abnormal. Independent samples t-tests were used to compare healthy and disease. The relationship between spirometry (as current gold standard measurement of lung function) and MBW parameters (z-scores) was assessed using scatter plots and Pearson's correlation coefficient.

Strength of correlations was graded as strong (≥0.7), moderate (≥0.5-0.69), weak (≥0.30 – 0.49) or no association (<0.29) (23). The sensitivity of $LCI^{2.5}$, $LCI^{5.0}$, $S_{cond}VT$, $S_{acin}VT$ was explored by summarising the proportion of individuals with an abnormal MBW result but a normal spirometry result. Sensitivity, specificity, and positive and negative predictive values were calculated. Sensitivity % was defined as the % of patients who had an abnormal test. Specificity was deinfed as the % of controls who had a normal result. The positive predictive value (the probability that an abnormal result means you have bronchiectasis) was defined as the number of true positives / (number of true positives + false positives) x 100. The negative predictive value (the probability that a normal result means that you do not have bronchiectasis) was defined as the true negative rate / (true negative rate + false negative rate) x 100 (a value from 0-100% with 100% indicating a perfect test). Furthermore, receiver operator characteristic (ROC) curves (area under the receiver operator characteristic curve [AUC^{ROC}] and SE) for all measures were used to assess and compare diagnostic accuracy (a value of 0 to 1, with 1 indicating a perfectly accurate test). The AUC^{ROC} curves were compared as described in (24).

Furthermore, the relationship between MBW parameters and age was assessed using scatter plots and Pearson's correlation coefficient. The sensitivity and specificity of MBW parameters were also explored using adjusted Z-scores representative of the data from healthy controls ≥60 years old only.

RESUTS

Participants

Table 1 presents the participant demographics. The majority of patients with bronchiectasis (64%) were female, the mean age was 66 years and patients had mild-moderately impaired lung function as measured by FEV_1% predicted. Patients had on average 2.5 (2.1) pulmonary exacerbations in the previous year.

One hundred and thirty-five healthy volunteers were enrolled and attempted MBW and spirometry. Eighty-eight had valid paired MBW and spirometry data and were used in this analysis; (14/135 (10%) had invalid spirometry, 6/135 (4.4%) had a FEV_1% predicted <80% and 27/135 (20%) had an invalid MBW test).

Table 1: Demographics, spirometry and MBW data for bronchiectasis and healthy control participants

	Bronchiectasis	Healthy control	Mean difference (95% CI) and p-values
N	132	88	
Mean (SD) age (years)	65.6 (11.3)	49.1 (17.4)	-16.5 (-20.3 to -12.7) p<0.001*
n (%) M:F	47 (36): 85 (64)	29 (33): 59 (67)	n/a p=0.69
Median (IQR) Body Mass Index (BMI)	n/a	26.2 (6.7)	n/a
n (%) patient chronically colonised with P. aeruginosa	22/132 (16.7)	n/a	n/a
Mean (SD) number of pulmonary exacerbations in the previous year	2.5 (2.1)	n/a	n/a

Multiple Breath Washout outcome measures in adults with bronchiectasis

Mean (SD) FEV$_1$ (% predicted)	70.6 (19.1)	99.3 (11.0)	-28.6 (-32.7 to -24.6) p<0.0001*
Mean (SD) FEV$_1$ z-score	-1.84 (1.2)	-0.06 (0.8)	-1.8 (-2.0 to -1.5) p<0.0001*
Mean (SD) FVC (% predicted)	84.8 (18.3)	104.9 (13.9)	-20.1 (-24.6 to -15.6) p=0.0001*
Mean (SD) FVC z-score	-1.01 (1.2)	0.29 (1.0)	-1.3 (-1.6 to -1.0) p<0.0001*
Mean (SD) FEF (% predicted)	52.6 (33.1)	84.1 (25.3)	-31.5 (-39.7 to -23.3) p<0.0001*
Mean (SD) FEF z-score	-1.68 (1.2)	-0.57 (0.8)	-1.1 (-1.4 to -0.8) p<0.0001*
Mean (SD) LCI$^{2.5}$ (no turnovers)	10.8 (2.6)	6.7 (0.9)	4.1 (3.6 to 4.6) p<0.0001*
Mean (SD) LCI$^{2.5}$ z-score	4.8 (3.1)	-0.007 (1.0)	4.8 (4.2 to 5.3) p<0.0001*
Mean (SD) LCI$^{2.5}$ CV%	3.1 (1.9)^	2.9 (1.3)#	0.2 (-0.3 to 0.7) p=0.43
Mean (SD) LCI$^{2.5}$ % difference between 2 trials	5.0 (4.1)^^	3.4 (2.9)##	1.5 (-0.3 to 3.4) p=0.12
Mean (SD) LCI$^{5.0}$ (no turnovers)	7.6 (1.5)	5.2 (0.6)	2.4 (2.1 to 2.7) p<0.0001*
Mean (SD) LCI$^{5.0}$ z-score	3.9 (2.6)	-0.002 (1.0)	3.9 (3.4 to 4.5) p<0.001*
Mean (SD) LCI$^{5.0}$ CV%	2.9 (1.9)^	3.2 (1.5)#	-0.3 (-0.8 to 0.3) p=0.35
Mean (SD) LCI$^{5.0}$ % difference between 2 trials	5.0 (3.7)^^	4.1 (2.6)##	0.9 (-1.0 to 2.8) p=0.35
Mean (SD) S$_{cond}$VT	0.05 (0.03) ^	0.02 (0.16) (#	0.03 (0.02 to 0.04)

9

Multiple Breath Washout outcome measures in adults with bronchiectasis

			p<0.0001*
Mean (SD) S_{cond}VT z-score	1.85 (1.63)^	0.01 (1.00)#	1.8 (1.4 to 2.3) p<0.0001*
Mean (SD) S_{cond}VT CV%	38.0 (32.9)^	70.8 (43.4)#	-32.7 (-44.6 to -20.4) p<0.0001*
Mean (SD) S_{acin}VT	0.35 (0.21)^	0.15 (0.12)#	0.20 (0.1 to 0.3) p=0.001*
Mean (SD) S_{acin}VT z-score	1.74 (1.79)^	0.002 (1.00)#	1.7 (1.2 to 2.3) p<0.0001*
Mean (SD) S_{acin}VT CV%	15.2 (10.7)^	30.0 (27.0)#	-14.5 (-23.3 to -5.6) p=0.002*

Bronchiectasis: ^n=91 ($\geq$ 3 trials) (except for S_{acin}VT n=57); ^^n=41 (2 trials)

HC: # n=70 (3 trials) (except for S_{acin}VT n=42); ##n=18 (2 trials)

CI: confidence intervals.

*p<0.05

Within-test repeatability

Compared to healthy controls the mean CV% and the mean % difference of LCI$^{2.5}$ and LCI$^{5.0}$ in patients with bronchiectasis was not different (Table 1). In patients with bronchiectasis with tests with $\geq$ 3 trials (n=91), there was no difference in the mean CV% of LCI$^{2.5}$ and mean CV% LCI$^{5.0}$ (mean difference [SD] =0.19 [2.4]), p=0.46.

The Bland-Altman plots for patients with bronchiectasis show a mean difference (95% limits of agreement) of 0.05 (-1.50 to 1.60) for LCI$^{2.5}$ compared with -0.03 (-0.94 to 0.88) for LCI$^{5.0}$. Importantly there was no clear pattern of greater variability in subjects with more advanced disease in either LCI$^{2.5}$ or LCI$^{5.0}$ (Figures E1a-b; supplementary material). Comparably, the mean difference (95% limits of agreement in healthy controls was -0.10 (-0.82 to 0.62) for LCI$^{2.5}$ and -0.07 (-0.79 to 0.64) for LCI$^{5.0}$ (Figures E2a-b; supplementary material).

Both S_{cond}VT and S_{acin}VT had high levels of within-test variability and higher levels of variability in patients with bronchiectasis compared to healthy controls (Table 1).

Multiple Breath Washout outcome measures in adults with bronchiectasis

Relationship between $LCI^{2.5}$, $LCI^{5.0}$, $S_{cond}VT$ and $S_{acin}VT$ and FEV_1 z-scores in disease

The relationship between $LCI^{2.5}$ and FEV_1 z-scores was of moderate strength (r=-0.54) (Figure 1a). $LCI^{2.5}$ had superior sensitivity with 55/132 (42%) of patients with an abnormal $LCI^{2.5}$ but a normal FEV_1 z-score. The relationship between $LCI^{5.0}$ and FEV_1 z-scores was also of moderate strength (r=-0.53) (Figure 1b). Results indicated that $LCI^{5.0}$ had superior sensitivity with 48/132 (36%) of patients with an abnormal $LCI^{5.0}$ but a normal FEV_1 z-score.

The relationship between $S_{cond}VT$ and FEV_1 (r=-0.35) and between $S_{acin}VT$ and FEV_1 **z-**scores (r=-0.38) was weak (Figure 2a and 2b). 19/91 (21%) of patients had an abnormal $S_{cond}VT$ but a normal FEV_1 z-score however, 17/91 (19%) had a normal $S_{cond}VT$ but an abnormal FEV_1 z-score.

9/58 (16%) of patients had an abnormal $S_{acin}VT$ but a normal FEV_1 z-score however, 14/58 (24%) had a normal $S_{acin}VT$ but an abnormal FEV_1 z-score.

Relationship between $LCI^{2.5}$, $LCI^{5.0}$, $S_{cond}VT$ and $S_{acin}VT$ and FEF_{25-75} z-scores in disease

The relationship between $LCI^{2.5}$ and FEF_{25-75} z-scores was weak (r=-0.44) (Figure 3a) however, $LCI^{2.5}$ had superior sensitivity with 59/132 (45%) of patients with an abnormal $LCI^{2.5}$ but a normal FEF_{25-75} z-score. The relationship between $LCI^{5.0}$ and FEF_{25-75} z-scores was also weak (r=-0.42) (Figure 3b). Results indicated that 52/132 (39%) of patients had an abnormal $LCI^{5.0}$ but a normal FEF_{25-75} z-score.

There was no relationship between $S_{cond}VT$ and FEF_{25-75} (r=-0.22) or between $S_{acin}VT$ and FEF_{25-75} z-scores (r=-0.13).

The relationship between $LCI^{2.5}$, $LCI^{5.0}$, $S_{cond}VT$ and $S_{acin}VT$ and FVC z-scores in bronchiectasis are presented in supplementary material Figures E3a-d.

Relationship between all MBW and spirometry parameters in health

Scatterplots presenting the relationship between all MBW and spirometry parameters in healthy volunteers is presented in supplementary material Figures E4a-d (MBW parameters and FEV_1 z-scores), Figures E5a-d (MBW parameters and FEF_{25-75} z-scores) and Figures E6a-d (MBW

parameters and FVC z-scores), showing the majority of data falling within the normal range (MBW parameter <2 and spirometry parameter > -2).

Senstivity and specificity

The agreement between each MBW parameter and a diagnosis of bronchiectasis is shown in Table 2. All measures had a high specificity to detect a normal result in healthy controls as well as high positive predictive values (the probability that an abnormal result means you have bronchiectasis). However, the sensitivity of the measure to detect patients who had an abnormal test and negative predictive values (the probability that a normal result means that you do not have bronchiectasis) were notably higher in $LCI^{2.5}$ and $LCI^{5.0}$ compared to all other measures studied including all spirometry measures.

Table 2: Agreement between MBW and spirometry parameter z-scores and the diagnosis of bronchiectasis

	$LCI^{2.5}$ z-score	$LCI^{5.0}$ z-score	FEV_1 z-score	FVC z-score	FEF z-score	$S_{cond}VT$ z-score	$S_{acin}VT$ z-score
Sensitivity %	80%	72%	43%	17%	37%	43%	35%
Specificity %	98%	96%	98%	98%	98%	97%	93%
Positive predictive value %	98%	96%	97%	92%	96%	95%	87%
Negative predictive value %	77%	69%	53%	44%	51%	57%	51%

As shown by the receiver operating curve, overall $LCI^{2.5}$ had greatest diagnostic accuracy to discriminate between subjects with bronchiectasis and healthy control subjects, followed by $LCI^{5.0}$, FEV_1, $S_{cond}VT$, FVC, $S_{acin}VT$, FEF_{25-75} (Figure 4, Table 3).

Table 3: Summary of Receiver – operator characteristic (ROC) statistics for MBW and spirometry z-scores (bronchiectasis versus. Healthy controls)

Variable	Area	SE	*P* Value	95% Confidence Intervals	
				Lower	Upper
$LCI^{2.5}$ z-score	0.98	0.01	<0.0001	0.96	1.00
$LCI^{5.0}$ z-score	0.97	0.02	<0.0001	0.93	1.00
FEV_1 z-score	0.91	0.03	<0.0001	0.86	0.97
$S_{cond}VT$ z-score	0.88	0.03	<0.0001	0.82	0.95
FVC z-score	0.84	0.03	<0.0001	0.76	0.92
$S_{acin}VT$ z-score	0.84	0.04	<0.0001	0.86	0.97
FEF_{25-75} z-score	0.80	0.04	<0.0001	0.72	0.89

On comparison of the ROC curves, statistically, $LCI^{2.5}$ was more sensitive and specific than all spirometry parameters (difference between the areas range 0.07-0.18; p<0.05). $LCI^{5.0}$ was more sensitive and specific than FEF_{25-75} and FVC (difference between the areas range 0.16 – 0.12; p<0.05), however there was no difference between AUC^{ROC} $LCI^{5.0}$ and AUC^{ROC} FEV_1 (difference between the area 0.05; p=0.09).

In addition, the wider CI of AUC^{ROC} $LCI^{5.0}$ meant that difference between AUC^{ROC} $LCI^{2.5}$ and AUC^{ROC} $LCI^{5.0}$ was significant (difference between the area 0.02; p=0.02).

Regarding potential confounding variables, there was no relationship between any MBW or spirometry outcomes and number of pulmonary exacerbation in the previous year (r=<2.0).

Relationship with age

There was a significant positive correlation between age and $LCI^{2.5}$ (Figure E7), $LCI^{5.0}$ (Figure E8), ScondVT (r=0.28; p=0.02) and SacinVT z-scores (r=0.34; p=0.03) (Figures not shown) in the healthy control cohort.

Results using adjusted z-scores representative of the data from healthy controls ≥60 years old only (n=28, mean (SD) $LCI^{2.5}$ = 7.5 (0.9), showed that there was agreement between $LCI^{2.5}$ and FEV_1 z-scores in 81/132 (61%) of patients (co-normal [35/132] or co-abnormal [46/132]) (Figure E9a). Results indicated that $LCI^{2.5}$ had superior sensitivity with 40/132 (30%) of patients with an abnormal $LCI^{2.5}$ but a normal FEV_1 z-score.

13

Regarding $LCI^{5.0}$ data from healthy controls ≥60 years old only (n=28, mean (SD) $LCI^{2.5}$ = 5.7 (0.6), there was agreement with FEV_1 z-scores in 77/132 (58%) of patients (co-normal [36/132] or co-abnormal [41/132]). Results indicated that $LCI^{5.0}$ had superior sensitivity with 39/132 (30%) of patients with an abnormal $LCI^{5.0}$ but a normal FEV_1 z-score (Figure E9b).

Using the adjusted z-scores, the proportion of patients with an abnormal LCI but a normal FEV_1, was reduced compared to the main analysis using z-scores generated from the total healthy control cohort.

Test duration

Median (IQR) total test time duration (test time plus waiting time) was 35.8 (25.8-52.4) for $LCI^{2.5}$ and 20.3 (14.2 – 28.3) minutes for $LCI^{5.0}$. The mean (SD) time saving with the $LCI^{5.0}$ test was 17.4 (11.0) minutes, equivalent to a 57% time saving. The positive correlation between $LCI^{2.5}$ (r=0.62), $LCI^{5.0}$ (r=0.58) and the time saving indicated that the larger the LCI value (i.e. more severe the VI), the larger the time saving. We have previously reported on other aspects of feasibility of MBW testing in this cohort including success rates of 79% (25).

DISCUSSION

This is the first study to explore the outcome measure properties of a range of MBW parameters in a large cohort of high quality bronchiectasis and healthy control data. Our results demonstrate that $LCI^{2.5}$ is a repeatable measure which is a more sensitive measure of bronchiectasis lung disease than spirometry measures, in support of the conclusions from our previous study (4). Furthermore, in this study we have shown that $LCI^{5.0}$ has comparable repeatability, sensitivity and specificity to $LCI^{2.5}$ in discriminating between bronchiectasis and healthy subjects and is also more sensitive than spirometry measures. Importantly, $LCI^{5.0}$ is shorter to perform, making it potentially more tolerable for patients and more feasible in both the clinical and research environment. Whilst both $S_{acin}VT$ and $S_{cond}VT$ had good agreement with FEV_1, high levels of variability within a test limit their use as an endpoint.

Within test repeatability of MBW measures was assessed using the CV% of tests including at least three trials and results are comparable with other studies in bronchiectasis (4). However, the Bland Altman limits of agreement and levels of intravisit repeatability in this study are larger

than those reported previously (5). As MBW data collected using different systems are not comparable, these data using MBN_2W and Exhalyzer D are important in informing whether this is a reliable measure in heterogeneous group such as bronchiectasis.

Assessment of the relationship between MBW measurements and FEV_1 is an important step in the validation of alternate MBW parameters as study endpoints, as FEV_1 represents the current gold standard measurement of lung function. In this study, $LCI^{2.5}$ and $LCI^{5.0}$ had comparable strength of correlation with FEV_1 indicating validity as a potential surrogate outcome measures for bronchiectasis clinical trials.

The sensitivity and specificity data support the notion that an abnormal $LCI^{2.5}$ / $LCI^{5.0}$ is highly predictive of the presence of bronchiectasis and highlights the limitation of spirometric measures where values within the normal range do not rule out bronchiectasis.

Furthermore, the proportion (up to 45%) of bronchiectasis patients with abnormal gas mixing as measured by $LCI^{2.5}$ or $LCI^{5.0}$ whilst having no measurable airway obstruction or restriction as measured by FEV_1, FVC or FEV_1/FVC, highlights the potential clinical value of MBW testing in this disease population. Specifically, the low sensitivity of FEF_{25-75} was highlighted in this study, with $LCI^{2.5}$ and $LCI^{5.0}$ detecting large proportions (45% and 39% respectively) of patients with abnormal gas mixing in the presence of a normal FEF_{25-75} z-score result. Registry data demonstrate that spirometry remains normal in a significant proportion of patients and other groups have demonstrated that spirometry offers a limited view of bronchiectasis pathophysiological complexity (26, 27). The superior outcome measure performance by $LCI^{2.5}$ and $LCI^{5.0}$ as shown in this study, justify closer study of these parameters as potential endpoints and clinical markers of disease.

The finding of comparable repeatability, sensitivity and specificity between $LCI^{2.5}$ and $LCI^{5.0}$ is in agreement with CF and PCD studies using the same MBN_2W system (28, 29, 30) and other MBW equipment (10). In addition, there was a meaningful time saving with $LCI^{5.0}$ compared to $LCI^{2.5}$, highlighting the increased feasibility of using $LCI^{5.0}$ in potential future trials and clinical practice. This data along with our previous report of a 79% success rate in this bronchiectasis cohort, where MBW operators completed a training and certification process, will help inform use of MBW testing in future studies in this patient population (25). Increased feasibility is particularly important

in the bronchiectasis population, which include older patients with more severe disease and where lengthy testing sessions may be prohibitive.

This is the largest study of MBN_2W data in bronchiectasis exploring phase III analysis indices. In this study, a larger proportion of patients had an elevated $S_{cond}VT$ in comparison to $S_{acin}VT$ (value > ULN) indicating that convection-dependent VI in the conducting airways may be more affected than diffusion-dependent VI in the acinar airways in patients in this study. This is in agreement with the study by Verbanck et al. which also demonstrated a predominant relationship with $S_{cond}VT$ over $S_{acin}VT$ in their study group (n=15) (indices modified to avoid artefact caused by increased severity of ventilation inhomogeneity) suggesting that ventilation heterogeneity occurred between relatively large lung units (31). Horsley et al reported on similar phase III analysis indices (using SF_6 MBW) in CF (18). The authors reported that $S_{cond}VT$ was predominately abnormal very early in disease. In this study, neither $S_{cond}VT$ or $S_{acin}VT$ had superior sensitivity compared to FEV_1 highlighting the limitation of these measures in bronchiectasis as well as CF. In agreement with other studies, higher intra-test variability of both $S_{acin}VT$ and $S_{cond}VT$, compared to $LCI^{2.5}$ in agreement with other studies, further constrains their current applicability as an endpoint to $LCI^{2.5}$ in bronchiectasis (6).

Overall results from this study highlight that clinically, more than one type of assessment may be required to characterise lung function in bronchiectasis. Functional sub-groups have been effectively characterised by other groups using a combination of plethysmography, spirometry and gas transfer assessment (26). Multiple breath washout parameters may be another important tool in the tool kit to further our understanding of pulmonary pathophysiology, characterise the lung function and identify treatable traits in bronchiectasis.

This study has a number of limitations. The BronchUK study enrolled only patients with idiopathic and post-infective disease limiting applicability of the results to some aetiologies. COPD and asthma are common co-morbidities in bronchiectasis and further study of MBW in these other aetiologies/ comorbidities will also be helpful. Importantly, we did not have imaging data alongside lung function data, constraining the specificity estimates of our study.

Whilst the healthy control data included older subjects; more closely age matched data would strengthen conclusions. Therefore, the diagnostic properties (sensitivity and specificity) of the MBW parameters may be confounded by the assumption of a constant upper limit of normal.

Our analysis of the age - MBW parameter relationship showed that the significant correlation between age, $LCI^{2.5}$ and $LCI^{5.0}$ was driven in large part by the higher values seen in subject's $\geq$ 60 years old. This finding, together with the results from our subgroup analysis (using ULN representative of the age group $\geq$ 60 years) highlight that larger studies to determine the ULN for age subgroups would better elucidate the added value of MBW parameters compared to spirometry, across the adult age range. Reference data including the older age range, for all parameters of interest (as presented in childhood age range (32)) is an important area for future study in order to aid the interpretation of MBW parameters in other adult respiratory populations. All MBW data is equipment and software specific (re-calculated using Spiroware 3.3.1) and reference ranges cannot be extrapolated to other equipment (e.g. SF_6 MBW, other MBN_2W devices) or software versions.

This study reported on cross-sectional data only and further analysis of MBW outcomes in longitudinal data including inter-visit variability is needed to fully demonstrate the utility of MBW as a potential endpoint in bronchiectasis. Interrogation of longitudinal MBW data during treatment periods will facilitate the exploration of treatment effects, minimum clinically important differences and the generation of sample size calculations.

CONCLUSION

In this study, we have provided new evidence that $LCI^{2.5}$ and $LCI^{5.0}$ have good within test repeatability and superior sensitivity compared with spirometry measures, in differentiating between health and bronchiectasis disease. $LCI^{5.0}$ is shorter and more feasible than $LCI^{2.5}$. Indices of $S_{cond}VT$ and $S_{acin}VT$ may provide additional physiological information but care is needed when interpreting these indices due to the high levels of intra-test variability. Further research to demonstrate inter-visit repeatability and responsiveness of $LCI^{2.5}$ and $LCI^{5.0}$ is needed.

Multiple Breath Washout outcome measures in adults with bronchiectasis

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