Title: New directions on lung clearance index variability and feasibility

Authors: Katherine O'Neill[1], Clare Saunders[2].

[1]Centre for Experimental Medicine, Queen's University Belfast, [2]Royal Brompton Hospital and Imperial College London.

Correspondence email: k.oneill@qub.ac.uk

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The lung clearance index (LCI) measured by multiple breath washout (MBW) is accepted as a surrogate endpoint for clinical trials (1) and may be a useful test to clinically monitor lung function in children and adults with cystic fibrosis (CF). The review of evidence for the use of LCI in CF clinical trials published in 2014 concluded that LCI has good clinimetric properties including strong intra-test repeatability, strong correlation with validated measure of lung function (FEV_1), quality of life and pulmonary exacerbation in both child and adult groups (2). LCI is responsive to modulator therapy and is predictive of future lung function (1,3). In health, the longitudinal evolution of LCI declines from infancy to early childhood, stabilises in early childhood to adulthood and slowly increases thereafter with age (4). The 2014 review concluded that LCI has a low variability between repeated sessions (i.e. stable coefficient of variation [CV%]) with one study reporting a coefficient of reproducibility (COR) of 0.96 for measurements 24 hours apart (using MBN_2W and an Exhalyzer D[a]) (5). There have been more limited data on the longer term inter-test repeatability within the CF population, thus the interpretation of a clinically meaningful change is more difficult. Determination of the inter-test repeatability is very important to inform the treatment effect that can be considered clinically significant (i.e. larger than the difference in LCI seen between repeat measurements without intervention or change in clinical status). Recent published data may go some way to address these gaps in the literature (Table 1).

Using $MBWSF_6$ methodology, the COR of repeated measurements approximately 8 months apart was 1.40 (6,7). Using MBN_2W the % difference between repeated measurements ranging from 3 to 12 months apart, ranged from 17-25% with an average COR of 2.00, with good agreement reported between the 2 most recent publications in the current issue of JCF (Green et al and Svedberg et al) (3,8-10). Significantly, some studies (using MBN_2W) indicate that LCI variability is driven by disease state (8,10) with increasing LCI variability as the LCI value increases (both within and between session). This evidence supports expressing LCI variability as a relative and not an actual change and has implications for powering a study to detect a change in LCI, i.e. base the sample size on the meaningful change expected for that disease severity.

It is also now established that the differences between MBW methodologies must be considered when interpreting the study results. *In vivo* and *in vitro* studies have demonstrated significant differences between results derived from different MBN_2W devices and when different tracer gases are applied. Differences in results from different MBN_2W devices are driven by the measurement algorithms used in the software results. Therefore, results are not interchangeable until there is harmonisation of measurement algorithms (11). On comparing the different tracer gas methods, MBN_2W overestimates FRC and there is clear bias towards disproportionately higher LCI at higher mean values of LCI (i.e. more severe disease) compared with $MBWSF_6$ (12,13). Importantly, there is also evidence to show that tissue N_2 contribution impacts on washout, causing error in indirect measurement of N_2 which is more pronounced in longer washouts (i.e. patients with higher LCI values) (13,14). This evidence may provide some explanation for the greater inter-test variability seen in MBN_2W tests compared with SF_6.

Whilst there is greater inter-test variability seen in LCI using MBN_2W, the relative change in LCI was comparable to that seen in FEV_1 % predicted (10). Furthermore, inter-visit repeatability data for $MBWSF_6$ is limited to one study and further research is needed, particularly where MBN_2W with 100% O_2 is not a suitable approach in the infant age range. Decreased permeation in poorly ventilated lungs may also be a potential limitation of $MBWSF_6$ methodology which also requires further study (12). Relating to MBN_2W, further study of the impact of the application of N_2 tissue excretion corrections, the use of earlier test end-points and the use of using end-tidal concentrations versus volume averaged phase III expired concentrations to identify the LCI end-point, may reduce variability and improve agreement between results from different devices using different inert gases.

To facilitate such comparisons, future studies could describe inter-visit repeatability as a percentage change, in addition to a statistic (e.g. %CV, the intra-class correlation coefficient (ICC), the limits of agreement (LOA) from Bland–Altman plots, or COR); with caution using those where it is assumed that the measurement variability is independent of its magnitude (LOA and COR).

The differences between results from MBW technologies complicate the transition of MBW from the research setting to clinical care and necessitate standardisation of specific devices and protocols for multicentre use. Feasibility data summarised in 2014 review indicated variable success rates with MBW testing ranging from 24% to 100%. Not surprisingly, most challenges have been encountered in the infant and pre-school age range due to issues relating to patient co-operation, the requirement for patient interface adjustment, a sedation protocol and the associated logistics (2). Assessment of the most recent feasibility data in CF (Table 1) indicates improvements in successful application of MBW testing in the research and clinical setting, with success rates ranging from 73-99%

across a wide age range. Notably, in the current issue of JCF, Stahl et al demonstrate high success rates in the application of $MBWSF_6$ in sedated infants and preschool children with CF across 3 centres without prior MBW experience (15). Moreover, Downing et al assessed the feasibility of performing MBW in the routine outpatient setting in a group of children with respiratory disease (including patients with CF), reporting success rates comparable to those in the research setting (73%) (16). Reflecting on reasons for test exclusion, results from all studies indicate that patient factors (e.g. lack of cooperation) can influence success rates as much as technical or quality issues, especially in the younger age groups. To optimise feasibility, there is a need for continued adherence to the developed standardised training protocols (led by the North American central over reading centre; http://lab.research.sickkids.ca/ratjen/mbw-centre/) for operator training, certification and optimal testing approach, specific to the age group of interest (infants, pre-school, school age and adults). Ongoing consideration of operator role, knowledge and experience as well as external links to central over reading centres for support and quality control, will facilitate continuous learning and adherence to the standardisation document for MBW testing (17).

The most recent MBN_2W data indicates more variability in LCI than previously appreciated and support the reporting of percent change and the consideration of age and severity of target populations when powering a study based on LCI. Further longitudinal data on $MBWSF_6$ is required, especially in infants and young children. LCI is currently adopted in commercial interventional clinical trials. These will provide further important data from treatment and placebo groups on treatment response and inter-test repeatability. Improvements in testing success rates are likely the result of the emergence of key consensus guidance and work from clinical trial committees to standardise testing methods, training, data analysis and quality control (17-19), efforts which have strengthened the feasibility of LCI. Time constraints may continue to be a limiting factor in the clinical setting, particularly with MBN_2W where washout times are longer. Exploration of end of test cut-off concentrations earlier in the washout may improve feasibility of this test in the clinical as well as the research setting.

Some key questions that remain concern a clear definition of the patient subgroup where MBW would be indicated (i.e. where LCI would offer additional information to routine lung function testing) and how clinical decisions are made on the basis of LCI. A longitudinal multicentre, randomised blinded controlled trial to assess the impact of LCI on treatment decisions in CF patients would determine the utility of LCI and would be a major step in the transition of LCI from the research into the clinical setting.

Table 1: Inter-test variability and feasibility of LCI in CF

Inter-test variability						
N and subject type	**Apparatus**	**Gas**	**N measurements (time between)**	**Results**	**Statistic**	**Author**
77 CF Children aged 2.5-6 years	Exhalyzer D[a]	N_2	2 (24 hours apart) 2-5 (at 1, 3, 6 ,9 & 12 months)	+/- 17% +/- 25% 2.00	Percentage change Average Percentage change Average COR	(10)
25 CF Children aged 6-17 years	Exhalyzer D[a]	N_2	At least 2 visit (3 months apart)	7.4% +/- 17%	Mean CV% Upper limit of normal percentage change	(8)
78 CF Children aged 2.5-6 years	Exhalyzer D[a]	N_2	6 (3 months apart)	9.4% (0.8 to 33.7) 0.57	Median (range) CV% ICC	(3)
14 CF Children aged 5-18 years	Exhalyzer D[a] using Testpoint software	N_2	4 (1, 3, 6 and 12 months apart)	0.82 -21% to 25%	ICC Limits of agreement percentage difference	(9)

56	CF	Children and adults aged 6-67 years	Modified Innocor[b]	SF_6	2 (average 8 months apart [range 67-614 days])	4.3 (visit 1 vs 4.7 (visit 2) 1.4 0.96	Mean CV% CoR ICC	(6,7)

Feasibility							
Subject type		**Device and Gas**	**N measurements required for each session**	**Number of tests attempted**	**Number (%) of tests successful**	**Reasons for exclusion**	**Author**
CF	Children aged 6-17 years	Exhalyzer D[a] N_2	At least 2 within 1 session	109	106/107 (99)	NR	(8)
CF	Children aged 5-18 years	Exhalyzer D[a] N_2	At least 2 within 1 session	171	168/171 (98)	N=1 invalid N=1 only 1 valid trial	(9)
CF	Children aged 2.5-6 years	Exhalyzer D[a] N_2	At least 2 within 1 session	412	343/412 (83)	N=2 Equipment malfunction N=67 Did not meet quality control review criteria (19)	(3)
CF	Infants and children aged 2.3-12 years	Exhalyzer D[a] SF_6 and sedation protocol	At least 2 within 1 session	73	67/73 (91.8) (range 78.9-100%)	N=3 recurrent sighs N=3 technical problems	(15)
Respiratory disease including CF	Infants and children aged 2.1-5.9 years	Innocor SF_6[b]	At least 2 within 1 session	116	83/116 (73)	N= 9 noncooperation N=6 inability to tolerate the noseclips n=8 lack of understanding	(16)

						n=5 inability to maintain a proper seal around the mouth piece n=4 afraid to attempt to the test	
CF	Children and adults aged 6-67 years	Modified Innocor SF_6 [b]	At least 2 within 1 session	122	110 (90)	N=6 LCI not repeatable N=6 patient unable to complete 2 trials due to time limitations or discomfort with test.	(6)

[a]Exhalyzer D (Ecomedics AG, Duernten, Switzerland)

[b]Modified Innocor (Innovision, Odense, Denmark)

COR: Coefficient of repeatability

ICC: Intraclass correlation co-efficient

CV: Coefficient of variation

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