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Review

Lung clearance index: Evidence for use in clinical trials in cystic fibrosis

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Abstract

The ECFS-CTN Standardisation Committee has undertaken this review of lung clearance index as part of the group's work on evaluation of clinical endpoints with regard to their use in multicentre clinical trials in CF.

The aims were 1) to review the literature on reliability, validity and responsiveness of LCI in patients with CF, 2) to gain consensus of the group on feasibility of LCI and 3) to gain consensus on answers to key questions regarding the promotion of LCI to surrogate endpoint status.

It was concluded that LCI has an attractive feasibility and clinimetric properties profile and is particularly indicated for multicentre trials in young children with CF and patients with early or mild CF lung disease. This is the first article to collate the literature in this manner and support the use of LCI in clinical trials in CF.

Keywords: Clinimetric properties; Multiple breath washout; Lung clearance index; Outcome measures; Surrogate endpoints

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1. Introduction

In the cystic fibrosis (CF) community, there is a need to focus on developing and evaluating endpoints for clinical trials in early disease. The European Cystic Fibrosis Society Clinical Trial Network (ECFS-CTN) has established a Standardisation Committee consisting of researchers with expertise in specific outcome measures. The Standardisation Committee is undertaking a rigorous evaluation of potential outcome measures for multicentre clinical trials in CF. This article summarises the group's work on lung clearance index (LCI).

A full description of the classification of outcome measures is provided in the first document in the series of articles from the ECFS-CTN Standardisation Committee (CFTR biomarkers group) [1]. Briefly, outcome measures fall into three classes: clinical endpoints, surrogate endpoints and biomarkers. Clinical endpoints reflect how a patient feels, functions or survives and detect a tangible benefit for the patient [2,3]. A surrogate endpoint is a laboratory measurement used to predict the efficacy of therapy when direct measurement of clinical effect is not feasible or practical. Ideally, surrogate endpoints should shorten the period of follow-up required. The link between the surrogate endpoint and long-term prognosis must be proven. Forced expiratory volume in one second (FEV_1) is still the only accepted surrogate outcome for the European Medicines Agency (EMA) and the North American Food and Drug Association (FDA). A biomarker is defined as "a

Table 1
Definitions and justification for clinimetric properties.

Clinimetric property	Definition	Justification of importance
Reliability	Degree to which a measurement is consistent and free from error	Important to quantify error (systematic and random) so that true changes can be discerned from changes due to normal fluctuations
Validity	Concurrent validity: Degree to which a test correlates with a "gold standard" criterion test which has been established as a valid test of the attribute of interest Convergent validity: Degree to which a test correlates with another test which measures the same attribute Discriminate validity: Degree to which a test differentiates between groups of individuals known to differ in the attribute of interest Predictive validity: Degree to which an attribute can be predicted using the result of a predictor test/or degree to which prognosis can be predicted	The gold standard outcome measures are often not feasible. Therefore it is important to know how an alternative outcome measure compares to the gold standard, and how different outcome measures compare. It is important to know the ability of outcome measures to discriminate between different groups
Responsiveness	Degree to which a test changes in response to an intervention known to alter the attribute of interest	Important attribute of tests used in clinical practice or research to assess treatment benefit (e.g. to identify improvements response to an intervention)

characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacologic response to a therapeutic intervention". Biomarkers are mainly used to explore proof-of-concept for a specific compound. Some are currently being considered for "promotion" to the status of surrogate endpoint.

Progression of lung disease in CF has slowed down [4], and therefore FEV_1 has become a less sensitive outcome measure. LCI has repeatedly been shown to be superior to FEV_1 to monitor early CF lung disease when FEV_1 is within normal ranges [5,6]. It thus appears a good candidate to become a new surrogate outcome measure in trials focusing on the early stages of disease. LCI may also be useful clinically to monitor patients with FEV_1 within normal ranges, however this article focuses on the use of LCI in clinical trials.

To gain acceptance of researchers and licensing bodies, an endpoint must however have a body of supporting evidence including acceptable clinimetric properties (Table 1) such as reliability, validity and responsiveness to treatment, and sufficient feasibility and safety. Clinimetric properties and feasibility are population and situation dependent, therefore data cannot readily be extrapolated to the CF population from other disease populations.

The aims of this project were 1) to review the literature on reliability, validity and responsiveness of LCI in patients with cystic fibrosis, 2) to gain consensus of the group on the feasibility of LCI and 3) to gain consensus on answers to key questions regarding the promotion of LCI to surrogate endpoint status.

2. Methods

An exhaustive literature search was conducted in MEDLINE, Allied and Complementary Medicine (AMED) and Embase using the following combination of keywords: ("lung clearance index" or "LCI" or "multiple breath washout" or "MBW" or "ventilation inhomogeneity" or "sulphur hexafluoride" or "SF6" or "nitrogen washout" or "helium washout" or "inert gas washout") and "cystic fibrosis". The search was limited to full text articles in the English language, with no limits on year of publication. A bibliography search was also conducted of all included articles and relevant reviews published until April 2013.

For clinimetric properties, data were extracted and tabulated for reliability, validity, correlation with other outcome measures, responsiveness and reference values. Definitions are given in Table 1.

To evaluate feasibility, data were extracted and tabulated on the proportion of attempts that were successful and reasons for excluding tests. An expert panel also discussed the following topics and reached consensus on each: risk involved, cost, ease of performance, ease of administration, time to administer, equipment and space needed and applicable age group. Specific advantages and limitations of infant pulmonary function were also discussed.

Narrative answers to 4 key questions were discussed by the expert panel during several face to face meetings

1) Does LCI have the potential to become a surrogate outcome?;
2) For what kind of therapeutic trial is LCI appropriate? (therapeutic aim, phase of trial, target population, number of patients involved, number of sites involved);
3) Within what timeline can change be expected and what treatment effect can be considered clinically significant?;
4) What are the most needed studies to further define LCI in patients with CF and to explore its potential as a surrogate marker? The consensus of the group is presented in the current article.

After preparatory work over a period of 6 months, participants with expertise in multiple breath washout met to discuss and develop consensus on the four key questions and feasibility (November 17 and 18, 2010, and June 9, 2011). The manuscript was developed which reports both the systematic review of clinimetric properties (performed by the core writing team (LK, KDB, IS, PR)) and the expert panel's discussions (four key questions and feasibility). This resulted in a draft manuscript which was circulated to the group for review and revision until group consensus was achieved.

3. Results

3.1. Use of LCI in clinical trials in CF

LCI derived from a multiple breath washout (MBW) provides a global measurement of ventilation inhomogeneity. It reflects abnormalities in ventilation in the respiratory tract compared to normal, including the small airways which are affected early in CF lung disease and where changes are not easily detected with traditional pulmonary function techniques such as spirometry [7]. The ability to identify early airway dysfunction in these "silent years", when FEV_1 is often within normal range, is of great importance for investigating new therapies in infants and young children and in those with mild disease [8]. LCI is beginning to be used as an efficacy endpoint in CF clinical trials. It was the primary outcome in a recent phase 2, multicentre trial of ivacaftor in patients with the G551D mutation and normal lung function [9]. It was used in single centre interventional studies of rhDNase and hypertonic saline in infants and children with CF [10–12]. It is one of the major secondary efficacy measures in the ongoing UK CF Gene Therapy Consortium's large, placebo controlled, multidose trial of non-viral gene therapy (http://clinicaltrials.gov NCT01621867).

LCI is derived from a MBW technique which can be performed either with inhalation of an inert tracer gas such as sulphur hexafluoride (SF_6) or helium, or by using 100% oxygen to wash out resident nitrogen. The latter technique has been available for several decades, takes slightly less time to perform and is gaining increasing attention [13]. In the case of an exogenous tracer, the gas is inspired until equilibrium is reached (i.e. concentration of tracer is equal in both inhaled and exhaled air). At this point the tracer gas source is removed and the individual breathes room air until the concentration of the tracer gas in exhaled air is 1/40th of the equilibrium concentration, an arbitrary concentration based on the lower limits of detection of the early nitrogen analysers. In the case of using nitrogen

Table 2
LCI validity.

N and subject type			Apparatus	Gas	Results for LCI	Results for FEV_1	Statistic	Author
LCI discriminates patients with CF from non-CF subjects								
71	CF	Infants	Mass spectrometer	SF_6	p = 0.002	p < 0.001*	Unpaired t-test	Hoo [21]
54	Non-CF							
14	CF	Infants	Exhalyzer D [a]	SF_6	p = 0.022	NR	NA	Belessis [22]
NR	Non-CF							
39	CF	Infants	Mass spectrometer	SF_6	p < 0.001	p < 0.001*	NR	Lum [23]
21	Non-CF	Infants			0.834 (0.05)	0.836 (0.05)*	Mean (SE) ROC;	
					N = 22 (56%)	N = 14 (36%)	N (%) individuals with abnormal test	
33	CF	Infants	Mass spectrometer	SF_6	Sensitivity (39.4%) Specificity (94.3%)	NR	Cross tabulation	Haidopoulou [24] (RPN)
35	Non-CF	Infants			AUC_{ROC} = 0.789 (0.68 to 0.90)		AUC_{ROC}	
47	CF	Infants and children	Exhalyzer D [a]	SF_6	p < 0.001	NR	NA	Belessis [22]
25	Non-CF							
30	Uninfected CF	Infants and children			p < 0.001			
25	Non-CF							
48	CF	Preschool	Mass spectrometer	SF_6	p < 0.001	p = 0.002	Unpaired t-test	Aurora [35]
45	Non-CF					p < 0.001*		
48	CF	Early school			p < 0.001	p < 0.001		
45	Non-CF					p < 0.001*		
73	CF	Children	Exhalyzer D [a]	N_2	p < 0.001	NR	Unpaired t-test	Singer [32] (Pediatr Pulmonol)
50	Non-CF							
17	CF	Children	Mass spectrometer	SF_6	p < 0.001	NR	NR	Amin [11]
28	Non-CF	Children						
45	CF	Children	Mass spectrometer	SF_6	p < 0.001	NS	MWUT	Keen [40]
35	Non-CF	Children						
22	CF	Children	Mass spectrometer	SF_6	p < 0.001	p < 0.001	Unpaired t-test	Aurora [30]
33	Non-CF	Children			Sensitivity = 95% Specificity = 97%	Sensitivity = 50% Specificity = 100%	Cross tabulation	
30	CF	Children	Mass spectrometer	SF_6	p < 0.001	p < 0.05*	Unpaired t-test	Aurora [33] (AJRCCM)
30	Non-CF	Children			Sensitivity = 77%	Sensitivity = 7%*		
56	CF	Children	Mass spectrometer	SF_6	p < 0.001	p < 0.001	NR	Owens [25]
52	Non-CF	Children						
43	CF	Children (<18 yrs)	Mass spectrometer	SF_6	p < 0.001	NS	Unpaired t-test	Gustafsson [26] (ERJ)
28	Non-CF							
60	CF	Children	Mass spectrometer	SF_6	Sensitivity (76.7%) Specificity (96.8%)	NR	Cross tabulation	Haidopoulou [24] (RPN)
62	Non-CF	Children			AUC_{ROC} = 0.94 (0.89 to 0.98)		AUC_{ROC}	
5	CF	Children	Modified Innocor [b]	SF_6	p = NS	NR	Wilcoxson	Pittman [41]
10	Non-CF							
68	CF	Children	EasyOne Pro [c]	SF_6	p < 0.001	NR	NR	Fuchs [42] (JCF)
38	Non-CF							
18	CF	Children	Modified Innocor [b]	SF_6	p = 0.022	NS	Unpaired t-test	Horsley [16] (RPN)
29	Non-CF	Children						
15	CF	Children	Exhalyzer D [a]	He	p < 0.001	NR	Unpaired t-test	Bakker [43]
15	Non-CF	Children						

26	CF	Children <18 yrs	Spiroson[d]	SF_6	$p < 0.001$	$p < 0.01$	Unpaired t-test	Fuchs [31]
22	Non-CF	Children <18 yrs						
10	CF	Children <10 yrs			$p = 0.009$	NS		
8	Non-CF	Children <10 yrs						
					Specificity = 100%	Specificity = 100%*		
					$AUC_{ROC} = 0.95$ (0.03)	$AUC_{ROC} = 0.66$ (0.07)		
					$p < 0.001$	$p < 0.05$*		
					$p < 0.001$	NS		
139	CF	Children and adults	EasyOne Pro[c]	SF_6	$p < 0.001$	$p < 0.001$	Unpaired t-tests	Fuchs [20]
102	Non-CF							(Pediatr Pulmonol)
11	CF	Children and adults	N_2 analyser	N_2	$p < 0.01$	$p < 0.001$	ANOVA	Gustafsson [19]
15	Asthma	Children						
18	Non-CF	Children						
25	CF	Adults	N_2 analyser	N_2	$p < 0.001$	$p < 0.001$	Unpaired t-test	Verbanck [17]
25	Non-CF							(ERJ)
22	CF	Adults	Modified Innocor[b]	SF_6	$p < 0.0001$	$p < 0.0001$	Unpaired t-test	Horsley [16]
17	Non-CF	Adults						(RPN)
33	CF	Adults	Modified Innocor[b]	SF_6	$p < 0.001$	$p < 0.001$	MWUT	Horsley [18]
48	Non-CF	Adults						(Thorax)

N and subject type			Apparatus	Gas	Comparison	Results for LCI	Results for FEV1	Statistic	Author
LCI differs between patients with CF who have different phenotypes									
47	CF	Infants and children	Exhalyzer D[a]	SF_6	With vs. without *P. aeruginosa*	$p = 0.038$	NA	NR	Belessis [22]
					With vs. without infection	NS	NA		
27	CF	Infants and children	Exhalyzer D[a]	SF_6	*P. aeruginosa* vs. other pathogen	$p < 0.01$	NA		
49	CF	Infants and children	Exhalyzer D[a]	SF_6	With vs without bronchiectasis	NS	NR	MWUT	Hall [27]
					With vs without air trapping	NS	NR		
30	CF	Children	Mass spectrometer	SF_6	With vs without *P. aeruginosa*	$p < 0.05$	NS	Unpaired t-test	Aurora [8] (AJRCCM)
22	CF	Children	Mass spectrometer	SF_6	With vs without *P. aeruginosa*	$p < 0.05$	NS	Unpaired t-test	Aurora [30]
43	CF	Children	Mass spectrometer	SF_6	CF with bacterial colonisation vs. CF without bacterial colonisation	$p < 0.01$	$p < 0.001$	Unpaired t-test	Gustafsson [26] (ERJ)
28	Non-CF	(<18 yrs)							
152	CF	Children	Pediatric Pulmonary Unit[e]	N_2	No infection vs. SA vs. PA vs. SA+PA	$p < 0.0001$	NR	Linear mixed effect model	Kraemer [44] (Resp Res)
18	CF	Children	Modified Innocor[b]	SF_6	Adults vs. children	$p < 0.0001$	NR	Unpaired t-test	Horsley [16]
22	CF	Adults							(RPN)
LCI is a more sensitive indicator of abnormalities than FEV_1									
47	CF	Infants and children	Exhalyzer D[a]	SF_6	Detection of *P. aeruginosa*	0.819 (0.686 to 0.951), $p = 0.004$	NA	AUC (95%CI)	Belessis [22]
						Sensitivity = 67% Specificity = 80% PPV = 47% NPV = 93%	NA	Sensitivity Specificity (%)	
49	CF	Infants and children	Exhalyzer D[a]	SF_6	Extent of bronchiectasis on HRCT	NS	NR	Multivariate regression coefficient	Hall [27]

(continued on next page)

Table 2 (*continued*)

N and subject type			Apparatus	Gas	Comparison	Results for LCI	Results for FEV1	Statistic	Author
LCI is a more sensitive indicator of abnormalities than FEV_1									
43	CF	Children	Mass spectrometer	SF_6	LCI(+)/FEV1(+) LCI(+)/FEV1(−) LCI(−)/FEV1(−) LCI(−)/FEV1(+)	n = 9 n = 18 n = 15 n = 1	NA	Number subjects (+=abnormal; −= normal)	Gustafsson [26] (ERJ)
28	Non-CF	Children			LCI(+)/FEV1(+) LCI(+)/FEV1(−) LCI(−)/FEV1(−) LCI(−)/FEV1(+)	n = 0 n = 0 n = 28 n = 0			
53	CF	Children	Mass spectrometer	SF_6	Concordance with abnormal Brody-II HRCT Total concordance with Brody-II HRCT result (both abnormal and normal)	39/53 (74%) 81%	18/57 (32%) 47%	Number (%) subjects	Owens [25]
44	CF	Children and adults	Mass spectrometer	SF_6	Abnormal when structural abnormalities on HRCT	Bronchiectasis Sensitivity = 85 (71 to 98)% Specificity = 50 (27 to 73)% HRCT Score Sensitivity = 93 (83 to 100)% Specificity = 65 (42 to 87)% Air trapping Sensitivity = 94 (82 to 100)% Specificity = 43 (25 to 61)%	Bronchiectasis Sensitivity = 19 (4 to 34)% Specificity = 89 (74 to 100)% HRCT Score Sensitivity = 26 (9 to 42)% Specificity = 100 (100 to 100)% Air trapping Sensitivity = 25 (4 to 46)% Specificity = 89 (78 to 100)%	Sensitivity and specificity % (95%CI)	Gustafsson [28]
34	CF	Children and adults	EasyOne Pro[f]	SF_6	Concordance with Bhalla CT Score Abnormal when structural abnormalities on HRCT	28/34 (82.3%) Sensitivity = 88 (69 to 97)% Specificity = 63 (26 to 90)% PPV = 88% NPV = 63%	NA (sample of patients with normal FEV_1) NA (sample of patients with normal FEV_1)	Number (%) patients Sensitivity and specificity % (95% CI)	Ellemunter [29]

* = $FEV_{0.5}$.

aLCI = alveolar lung clearance index, CF = cystic fibrosis, FEV_1 = forced expiratory volume in one second, LCI = lung clearance index, LCI(+) = abnormal LCI, LCI(−) = normal LCI; FEV_1(+) = abnormal FEV_1; FEV_1(−) = normal FEV_1, MES = modified emission spectro-photometer, NA = not applicable, NR = not reported, NS = not significant, SA = *Staphylococcus aureus*, PA = *Pseudomonas aeruginosa*; MS = mass spectrometer; USFS = ultrasonic flow sensor.

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

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

[c] EasyOne Pro, MBW Module (ndd Medizintechnik AG, Zurich, Switzerland) plus addition of CO_2 analyser (DUET ETCO2 Module, Welch Allyn OEM Technologies, Beaverton, OR, USA).

[d] Spiroson (ndd Medical Technologies) plus addition of CO2 analyser (DUET ETCO2 Module, Welch Allyn OEM Technologies, Beaverton, OR).

[e] Pediatric Pulmonary Unit (SensorMedics 220, Yorba Linda,CA, USA).

washout, which is a resident gas, 100% oxygen is delivered until mean expired nitrogen concentration falls below 1/40th of the original concentration. In both methods, LCI is calculated as the cumulative expired volume during the washout phase divided by the functional residual capacity (FRC) i.e. the number of FRC volume turnovers required to clear the tracer gas. FRC is derived from the cumulative exhaled marker gas concentration divided by the difference in end-tidal gas concentration at the start of the washout and the end-tidal concentration at the end of the washout. Individuals with greater ventilation inhomogeneity use a greater number of turnovers to clear the tracer gas and therefore will have a higher (more abnormal) LCI.

Many different systems have been or are being used to measure MBW in clinical trials in CF. For detailed guidelines about washout equipment specifications, test performance and data analysis we refer to a recent ERS/ATS consensus document [14]. Although the mass spectrometer is considered the gold standard gas analyser equipment, it is very expensive, custom built for MBW and therefore not suitable for widespread use [14]. The majority of published results to date are calculated by offline analysis using proprietary software. The use of the software requires training and there is an element of subjectivity in reading the results. For LCI to be used as an outcome measure in large-scale multicentre trials, it is necessary to implement a file transfer and central reading facility. Only with such measures can variability be reduced. Commercially available systems, compliant with the above ERS guidelines will provide the opportunity to standardise the procedure in future multicentre trials. The online Table E1 lists the currently commercially available apparatuses and some of their characteristics. Results from MBW tests using different gases are not interchangeable, e.g. on average, LCI determined by nitrogen washout is higher than LCI determined by washout of SF_6 [15]. Traditionally, the mean of 3 (or at least 2) valid LCI measurements with FRC not differing more than 10% have been reported. The recent ERS document describes acceptability criteria in great detail [14]. If all other criteria are met, the new advice is to only reject tests where FRC differs by >25% from the median values across the 3 tests. Most published studies pre-date this advice and have used a 10% criterion. Throughout the tables we will refer to the apparatus used to obtain the MBW measurements. Since most of the reported studies predate the ERS consensus, all necessary information is not always available.

3.2. Clinimetric properties of LCI

3.2.1. Reliability (Table E2 online)

The majority of studies on reliability were conducted in children, with fewer in infants and adults. In most reports, the mean coefficient of variation (CV) for LCI measurements within one session was low (between 3 and 7%) but the range was higher. A mean CV above 10% was reported in a study in children with CF using an Innocor with a closed circuit. Therefore this apparatus set-up is not recommended [14]. Both CV and ICC of measurements within one session were as acceptable in CF as in healthy controls. One study showed neither a significant nor systematic difference in LCI between repeated sessions of LCI measurements. A low variability between repeated sessions of LCI measurements has also been reported by others: mean CV of up to 9 % in the short and medium term and high intra-class correlation coefficients.

3.2.2. Validity (Table 2)

Overall, 22 out of 23 studies demonstrated the ability of LCI to discriminate between individuals with CF and healthy, non-CF subjects. Of these, 3 studies included adults only [16–18], the others included either children and adults (n = 2) [19,20], or children only (n = 18 studies including 4 studies also in infants [21–24]). Several studies demonstrated the ability of LCI to discriminate between groups of patients with CF and differing degrees of lung disease based on age, infection status or structural changes on high resolution computerized tomography (HRCT) of the chest. In this respect LCI is superior to FEV_1. In infants and children, six studies compared the sensitivity of LCI and FEV_1 as indicators of structural lung abnormalities demonstrating that for bronchiectasis and air trapping on HRCT, LCI is more sensitive but less specific than FEV_1 [22,25–29].

3.2.3. Correlation with other outcomes (Table 3)

Twenty one studies have examined the relationship between LCI and other outcome measures with the majority of studies focusing on FEV_1 and HRCT. In 10 studies in children and/or adults with CF, a significant but variable correlation between LCI and $FEV_1/FEV_{0.5}$ was demonstrated [16–18,20,21,29–33]. One study in preschool children reported a correlation with $FEV_{0.5}$, $FEF_{25–75}$ and sR_{aw}. These studies also pointed out that LCI is superior in detecting abnormalities. In infants with CF diagnosed via newborn screening (mean age 11 weeks) there was no correlation between LCI and $FEV_{0.5}$ [21]. In a mixed group of infants and toddlers (including two with CF), LCI correlated with the volume of trapped gas (expressed as percent of FRC) [34]. Abnormal LCI was shown to have a moderate to strong correlation with structural abnormalities evaluated separately or using global HRCT scores. Overall, correlation was good between LCI and bronchial wall thickening, mucus plugging and bronchiectasis, but weaker with air trapping. LCI was also shown to correlate with other outcome measures including, age, onset of infection, type of infection, inflammation measured in the bronchoalveolar lavage fluid, blood gas analysis, exhaled nitric oxide fraction, capnographic parameters, and symptom score.

3.2.4. Predictive validity (Table E3)

One study demonstrated the validity of LCI in preschool children as a predictive test of abnormal lung function at an early school age. Whilst positive predictive values for future abnormalities were also good for FEV_1, LCI had a stronger negative predictive value [35]. Further studies to investigate the relationship between LCI measurements and the long term course of CF (lung function, exacerbations etc.) are urgently required.

3.2.5. Responsiveness (Table 4)

Several studies provide information on responsiveness of LCI in small numbers of patients (range n = 11 to 38). In patients with CF, LCI was able to detect a treatment effect after four weeks of

Table 3
Cross sectional correlation between LCI and other measures.

N and subject type			Apparatus	Gas	Comparison	Result	Statistic	Author
In children and adults with CF, LCI correlates with specific spirometry parameters such as FEV_1 and MEF_{25}								
22	CF	Children	Mass spectrometer	SF_6	FEV_1	$r^2 = -0.62$, $p < 0.0005$	Linear regression	Aurora [30]
1					MEF_{25}	$r^2 = -0.46$, $p < 0.001$		
26	CF	Children	Spiroson [a]	SF_6	FEV_1	$r = -0.476$, $p = 0.014$	Spearman correlation coefficient	Fuchs [31]
2					MEF_{25}	$r = -0.523$, $p = 0.006$		
139	CF	Children and adults	EasyOne Pro [b]	SF_6	FEV_1 z-score	$p < 0.001$	NR	Fuchs [20] (Pediatr Pulmonol)
3					MEF_{25}	$p < 0.001$		
34	CF	Children and adults	EasyOne Pro [b]	SF_6	FEV_1	$r = 0.468$, $p = 0.005$	Pearson correlation coefficient	Ellemunter [29]
4								
33	CF	Adults	Modified Innocor [c]	SF_6	FEV_1	$r^2 = 0.69$, $p < 0.001$	Linear regression	Horsley [18] (Thorax)
5								
40	CF	Adults and children	Modified Innocor [c]	SF_6	FEV_1 z-score	$r = -0.86$, $p < 0.0001$	Spearman correlation coefficient	Horsley [16] (RPN)
6					Curvilinearity of washout tracing	$r = -0.88$, $p < 0.0001$		
22	CF	Adults			RV/TLC	$r = 0.73$, $p < 0.0002$		
25	CF	Adults	N_2 analyser	N_2	FEV_1	$r = -0.76$, $p < 0.001$	Spearman correlation coefficient	Verbanck [17] (ERJ)
7								
73	CF	Children	Exhalyzer D [e]	N_2	FEV_1 z-score	$r = -0.49$, $p < 0.001$	Pearson correlation coefficient	Singer [32] (Pediatr Pulmonol)
					FEV_1/FVC z-score	$R = -0.44$, $p = 0.003$		
					FEF_{25-75} z-score	$R = -0.51$, $p < 0.001$		
In preschool children with CF, LCI correlates with $FEV_{0.5}$, FEF_{25-75} and sR_{aw}								
30	CF	Children 2–5 yrs	Mass spectrometer	SF_6	sR_{aw}	$r^2 = -0.14$, $p = 0.04$	Linear regression	Aurora [8] (AJRCCM)
					$FEV_{0.5}$	$r^2 = 0.21$, $p = 0.01$		
					FEF_{25-75}	$r^2 = 0.28$, $p = 0.003$		
In infants with CF detected after newborn screening, LCI did not correlate with $FEV_{0.5}$								
71	CF	Infants after NBS Mean age 11 wks	Mass spectrometer	SF_6	$FEV_{0.5}$	NS	Pearson correlation coefficient	Hoo [21]
In a mixed group of infants and toddlers (including 2CF), LCI correlated with the proportion of trapped gas								
8	3 risk of atopy 3 ex-premie 2 CF With and without respiratory disease	Children	Mass spectrometer	SF_6	$V_{TG,\ SF6}$/FRC	$r^2 = 0.94$, $p < 0.001$	Linear regression	Gustafsson [26] (Pediatr Pulmonol 35:42–49)
LCI correlates well with parameters derived from imaging analysis.								
49	CF	Infants and children	Exhalyzer D [e]	SF_6	Extent of bronchiectasis on HRCT	NS	Spearman correlation coefficient	Hall [27]
					Extent of air trapping on HRCT	$r = 0.31$, $p = 0.03$		
57	CF	Children	Mass spectrometer	SF_6	Brody-II HRCT total score	$r = 0.77$	Spearman correlation coefficient	Owens [25]
					Brody-II bronchiectasis score	$r = 0.71$		
					Brody-II peribronchial thickening score	$r = 0.72$		
					Brody-II mucous plugging score	$r = 0.67$		
					Brody-II air trapping score	$r = 0.58$		
34	CF	Children and adults	EasyOne Pro [b]	SF_6	Bhalla HRCT score	$r = -0.54$, $p = 0.001$	Pearson correlation coefficient	Ellemunter [29]

44	CF	Children and adults	Mass spectrometer		HRCT scores	r = 0.65 to 0.85	Spearman correlation coefficient	Gustafsson [28]
26	CF	Children	Spiroson [a]	SF_6	Crispin-Norman X-ray score	r = 0.684, p = 0.001 No sig. correlation between CN score and FEV_1^*	Spearman correlation coefficient	Fuchs [31]
LCI correlates with some other parameters of disease severity								
71	CF	Infants	Mass spectrometer	SF_6	Homozygous F508del	NS	Linear regression	Hoo [21]
					Respiratory symptoms	NS		
					Positive growth (cough swab)	NS		
					Antibiotics	NS		
47	CF	Infants and children	Exhalyzer D [e]	SF_6	LCI vs. pathogen load CFU/mL)	R^2 = 0.10, p = 0.031	Linear regression	Belessis [22]
					LCI vs. IL-8	R^2 = 0.20, p = 0.004		
					LCI vs. neutrophil count	R^2 = 0.21, p = 0.001		
73	CF	Children	Exhalyzer D [e]	N_2	*P. aeruginosa* infection status	r = 0.75, p < 0.001	Pearson correlation coefficient	Singer [32] (Pediatr Pulmonol)
					PaO_2	r = −0.54		
142	CF	Children	Pediatric Pulmonary Unit [f]	N_2	Age	F = 22, p < 0.0001	Linear mixed effect model	Kraemer [45]
					Age at onset of chronic *PA* infection	F = 4.2, p = 0.02		
					CFTR genotype	NS		
178	CF	Children	Pediatric Pulmonary Unit [f]	N_2	PaO_2 <80 mm Hg	t-Statistic = −3.156, p = 0.002	Linear mixed model, adjusted by year at testing	Kraemer [46] (Respiratory Research)
					PaO_2 above or below 80 mm Hg	χ^2 = 9.644, p = 0.002	Chi square	
15	CF	Children	Exhalyzer D [e]	He	LCI vs. Mean nocturnal oxygen saturations	NS	Spearman correlation coefficient	Bakker [43]
					LCI vs. Mean cough (cough s/h)	NS		
15	Non-CF	Children			LCI vs. mean nocturnal oxygen saturations	NS		
					LCI vs. Mean cough (cough s/h)	NS		
68	CF	Children and adults	EasyOne Pro [b]	SF_6	Slope 2 of CO_2 expirogram	r = −0.198, p < 0.042	Pearson correlation coefficient	Fuchs [42] (JCF)
					Slope 3 of CO_2 expirogram	r = 0.376, p < 0.001		
					Capnographic index (KPI_v)	r = 0.610, p < 0.001		
45	CF	Children	Mass spectrometer	SF_6	$FENO_{50}$	r = −0.43, p = 0.003	Spearman correlation coefficient	Keen [40]
					Alveolar NO	r = −0.32, p = 0.037		
					$FENO_{50}$	β = −0.251 95%CI: −0.354 to −0.147, p < 0.001	Multiple regression model (dependent variable: log $FENO_{50}$)	
28	CF	Children	V_{max} 22D [d]	N_2	Change in CFCS in response to IVAB	r = 0.48, p = 0.01	NR	Robinson [36] (Pediatr Pulmonol)

CFU = colony forming units; $FEF_{25–75}$ = mean forced expiratory flow between 25 and 75% of exhaled vital capacity; $FENO_{50}$ = fractional exhaled nitric oxide, measured at a flow rate of 50 ml/s; FEVx = forced expiratory volume in x seconds; HRCT = high resolution computed tomography; IVAB = intravenous antibiotics; MEF_{25} = forced expiratory flow where 25% of the FVC remains to be expired; NS = not significant; USFS = ultrasonic flow sensor; NR = not reported; RV/TLC = ratio of residual volume to total lung capacity; sR_{aw} = specific airway resistance measured by body plethysmography; $V_{TG,\ SF6}$/FRC = volume of trapped gas as measured with sulphur hexafluoride as tracer gas.

[a] Spiroson (ndd Medical Technologies) plus addition of CO2 analyser (DUET ETCO2 Module, Welch Allyn OEM Technologies, Beaverton, OR, USA).

[b] EasyOne Pro, MBW Module (ndd Medizintechnik AG, Zurich, Switzerland) plus addition of CO2 analyser (DUET ETCO2 Module, Welch Allyn OEM Technologies, Beaverton, OR, USA).

[c] Modified Innocor (Innovision, Odense, Denmark).

[d] Vmax 22D spirometer and Spectra software (SensorMedics Corp., Yorba Linda, CA, USA).

[e] Exhalyzer D (Ecomedics AG, Duernten, Switzerland).

[f] Pediatric Pulmonary Unit (SensorMedics 220, Yorba Linda, CA, USA).

Table 4
Responsiveness of LCI in cystic fibrosis.

N	Subject type	Apparatus	Gas	Intervention	LCI results (mean SD)	Did other endpoints detect difference?	Statistic	Author
LCI decreases after 2 weeks treatment with IV antibiotics, and after 4 weeks treatment with hypertonic saline and rhDNase in patients with cystic fibrosis								
16	Children	Easyone Pro [a]	SF_6	Endurance training and flutter/PEP	p = NS pre-ACT: 7.76 (1.23), post-ACT: 7.96 (1.04)	NS	Paired t	Fuchs [47] (Pediatr Pulmonol)
11	Children and adults	MES [b]	N_2	Salbutamol, 5 mg once	p = NS	S_{acin} p < 0.01 FEV_1 p < 0.01	Paired t	Gustafsson [19]
20	Children	Mass spectrometer	SF_6	7% hypertonic saline, 4 ml BID 4 wk vs. Isotonic saline, 4 ml BID 4 wk	**p = 0.016** Rx effect: 1.16 (0.94), 95% CI [0.27 to 2.05] HTS: pre: 8.84 (1.95), post: 7.86 (1.71) ITS: pre: 8.71 (2.10), post: 8.89 (2.10)	No (spirometry NS)	Repeated measures ANOVA	Amin [10]
17	Children	Mass spectrometer	SF_6	rhDNase, 2.5 ml QD 4 wk vs. Placebo, 2.5 ml QD 4 wk	**p = 0.02** Rx effect: −0.90 (1.44) rhDNase: pre: 8.31 (1.48), post: 7.69 (1.65) Placebo: pre: 8.75 (1.72), post: 8.52 (1.19)	FEF_{25-75}%pred p = 0.03 FEF_{25-75} z-score p = 0.03	Mixed model	Amin [11]
28	Children	V_{max} 22D [c]	N_2	IV antibiotics	**p = 0.03** Rx effect: 3.8% decrease Admission: 10.10 range [6.87 to 14.83] Discharge: 9.62 range [7.37 to 13.45]	CFCS p < 0.01 FEV_1 p < 0.01 FVC p < 0.01 RV/TLC p < 0.05 VO_{2peak} p < 0.05	Paired t-test	Robinson [7]
38	Adults	Innocor [d]	SF_6	IV antibiotics	**p = 0.003** Rx effect: −0.8 (1.4) Start IVAB: 14.6 (2.7) End IVAB: 13.8 (2.4)	Yes [e]	Paired t-test	Horsley [37]

Abbreviations: CFCS = cystic fibrosis clinical score, FEV_1 = forced expiratory volume in 1 s, FVC = forced vital capacity, IQR = interquartile range, MES = modified emission spectrophotometer, NS = not significant; RV/TLC = residual volume to total lung capacity ratio, S_{acin} and S_{cond} additional LCI parameters (for more info see review, Robinson [7]), wk = weeks.

[a] EasyOne Pro, MBW Module (ndd Medizintechnik AG, Zurich, Switzerland) plus addition of CO_2 analyser (DUET ETCO2 Module, Welch Allyn OEM Technologies, Beaverton, OR, USA).

[b] Medscience 505 (Medscience Electronics, Inc., St. Louis, MO, USA).

[c] V_{max} 22D spirometer and Spectra software (SensorMedics Corp., Yorba Linda, CA, USA).

[d] Modified Innocor (Innovision, Odense, Denmark).

[e] Large number of endpoints explored: in general clinical observations, symptom scores, lung function, serum inflammatory markers and some structural endpoints improved.

inhalation of dornase alpha [10], four weeks of inhalation of hypertonic saline [11] and after a course of intravenous antibiotics for a respiratory exacerbation [10,11,36,37]. One short term study did not show a statistically significant treatment effect with 5 mg of inhaled salbutamol as measured by LCI in 11 children and adults with CF. Only S_{acin} improved, an index derived from MBW which reflects inhomogeneity in the airways close to or within the gas exchange zone [19]. It may not be surprising that LCI did not detect change; bronchodilators target larger airways whereas LCI is considered to be more reflective of ventilation homogeneity in smaller airways. There is also little information on the efficacy of inhaled bronchodilator therapy in CF using other outcome measures.

3.2.6. Reference values (Table 5)

Reported reference values predate the ERS guideline. We list the reported reference ranges according to gas used, set-up used and age category. It is important to note that reference values are dependent on age of participants, method of analysis (i.e. online vs. offline), software used, device and set-up and tracer gas used. Reference values are not interchangeable between different methods. In addition, we refer to an abstract containing reference values for commercially available equipment over a wide age range [38].

3.2.7. Feasibility of LCI (Table E4)

Feasibility data were collated from studies in CF, and are mainly from children; fewer studies have been conducted in adults or infants. In children, success rates ranged from 24% to 100%. The study with the lowest success rates was evaluating feasibility in the clinical setting in which strict time constraints were imposed (20 min for participant familiarisation and performance of measurement). This is not as relevant in clinical trials as there tends to be more time for participant familiarisation and performance of repeat measures [32]. In infants and preschool children, success rate can be lower. Common reasons for exclusion of tests include manoeuvres that are not technically acceptable (e.g. unstable breathing pattern) or lack of within-session reproducibility (i.e. no two curves within 10% for FRC measurement). The experience of several hundred LCI measurements in adults with CF in the UK CF Gene Therapy Consortium gene therapy studies indicates feasibility in this group of close to 100% (unpublished observations).

3.3. Group consensus on feasibility

MBW is a safe technique since it uses either oxygen for nitrogen washout or very low concentrations of inert tracer gases SF_6 and helium.

For young children, quiet breathing is performed using a face mask, whereas for older children and adults, a mouth piece is used. In neonates the test can be attempted during natural sleep. This is usually impossible beyond the neonatal period.

Few have embarked on LCI measurements in children under the age of three years, especially beyond the newborn period. From experience with other lung function tests, it is anticipated that the test duration and the need for regular and quiet breathing will imply sedation. As for any test done under sedation this requires close monitoring and is associated with a small risk. In infants with rapid breathing rates, the gas analyser must have a rapid response time. Commercial stand alone SF_6 analysers can be adapted to provide the rapid response times necessary to measure LCI in infants. Most studies in infants have used a mass spectrometer. The nitrogen washout technique has not yet been validated in infants in whom the impact of breathing 100% oxygen on ventilation pattern should be further explored.

In infants and preschoolers, MBW is simpler than forced expiratory techniques. MBW requires only quiet tidal breathing whereas the raised volume rapid thoraco-abdominal compression (RVRTC) technique requires high skill, long term and continuous training and numerous acceptability criteria. RVRTC feasibility in infants has a much lower feasibility than LCI when comparing the percent of successful measurements (albeit between studies). A large multicentre trial evaluating feasibility in RVRTC also showed that feasibility was much lower in naive centres compared to more experienced ones, demonstrating the dependence on training and experience [67].

MBW takes more time than routine spirometry. In general, three repeat measurements are performed to generate a single mean value. In healthy subjects, both phases take approximately less than 5 min. Both wash-in and wash-out require less time in healthy subjects than in people with obstructive airways disease. The time needed increases relative to the increase of LCI. The nitrogen wash-out technique has the advantage of being shorter, as a wash-in is not needed before the 1st washout. The time the patient is attached to the equipment is also reduced since all wash-in phases are done with room air. Time requirements also increase when off-line analysis is used, however automated calculation of LCI from the MBW tracer helps to reduce analysis time. The manpower required increases when testing infants and young children, as at least two people are needed.

The equipment (hardware and software) and consumables required depend on the technique used [14]. In general the following should be considered; a trolley-mounted analyser or mass spectrometer, space for the tracer gas cylinder, a seat for the individual, a TV/DVD for distraction and a computer with software for data storage and analysis. These can easily be accommodated in most lung function laboratories. Tracer gas build-up in confined spaces should be prevented by good ventilation of the test room. In multicentre studies, the tracer gas used must be approved by all national authorities, which may limit the use of SF_6.

Ongoing developments may further improve LCI feasibility; assessing whether results from partial washout (first breaths) predict the 'standard' LCI value. The additional value of other indices derived from MBW, such as S_{acin} and S_{cond}, that describe the site of ventilation inhomogeneity, are being explored.

3.4. The "four key questions"

3.4.1. Question 1: Does LCI have the potential to become a surrogate outcome parameter?

LCI is potentially very valuable as a surrogate outcome parameter. It reflects disease in the peripheral airways which

Table 5
Reference values for LCI in healthy controls according to inert gas, age and apparatus used.

N	Age group	Additional info	Apparatus	Mean LCI Median*	SD SE*	Range IQR*	95% CI	Upper limit of normality	Author
SF_6									
201	Infants		Exhalyzer D [a]	6.6*	NR	5.5 to 8.6	NR	NR	Kieninger [48]
29	Infants	Preterm Time 1	Exhalyzer D [a]	7.3	NR	6.0 to 10.3	NR	NR	Sinhal [49]
29	Infants	Preterm Time 2	Exhalyzer D [a]	7.5	NR	6.3 to 10.6	NR	NR	Sinhal [49]
64	Infants	Full term	Exhalyzer D [a]	7.17	0.54	NR	NR	NR	Hülskamp [50]
59	Infants	Preterm	Exhalyzer D [a]	7.14	0.88	NR	NR	NR	Hülskamp [50]
16	Infants	Full term	Exhalyzer D [a]	6.51	0.27	NR	NR	NR	Riedel [51]
14	Infants	Preterm	Exhalyzer D [a]	6.54	0.49	NR	NR	NR	Riedel [51]
20	Infants	Facemask	Exhalyzer D [a]	6.6	0.8	NR	NR	NR	Schulzke [52]
20	Infants	Nosemask	Exhalyzer D [a]	7.2	0.9	NR	NR	NR	Schulzke [52]
25	Infants and children		Exhalyzer D [a]	6.45	0.49	5.42 to 7.37		7.41	Belessis [22]
39	Children		Exhalyzer D [a]	5.5*	NR	4.2 to 6.8	NR	NR	Kieninger [48]
185	Infants	Full term	Spiroson [b]	7.0	0.8	5.5 to 10.1	NR	NR	Latzin [53]
239	Infants	Preterm	Spiroson [b]	6.9	0.7	5.2 to 8.5	NR	NR	Latzin [53]
22	Children		Spiroson [b]	6.7	0.5	5.8 to 7.6	NR	7.77	Fuchs [31]
9	Adults		Spiroson [b]	7.10	0.30	NR	NR	NR	Fuchs [54]
10	Adults	Supine	Spiroson [b]	5.63	0.43	NR	NR	NR	Riedel [55]
10	Adults	Prone	Spiroson [b]	7.13	0.64	NR	NR	NR	Riedel [55]
10	Adults	Left lateral lying	Spiroson [b]	6.27	0.44	NR	NR	NR	Riedel [55]
10	Adults	Right lateral lying	Spiroson [b]	6.65	0.52	NR	NR	NR	Riedel [55]
22	Children (<18y)	Hannover	EasyOne Pro [c]	6.13	0.3	5.57 to 6.64	NR	7.0	Fuchs [56]
22	Children (<18y)	Innsbruck	EasyOne Pro [c]	6.27	0.5	5.36 to 7.06			
102	Children and adults		EasyOne Pro [c]	6.3	0.19	NR	NR	NR	Fuchs [20] (Pediatr Pulmonol)
10	Children		Modified Innocor [d]	5.98	1.22	3.74 to 7.53	NR	NR	Pittman [41]
29	Children		Modified Innocor [d]	6.24	0.47	5.14 to 7.05	NR	NR	Macleod [57]
12	Children		Modified Innocor [d]	6.3	0.5	5.6 to 7.1	NR	7.3	Horsley [18] (Thorax)
29	Children		Modified Innocor [d]	6.2	0.5	5.1 to 7.1	NR	7.5*	Horsley [16] (RPN)
48	Adults		Modified Innocor [d]	6.7	0.4	6.0 to 7.8	NR	7.5	Horsley [18] (Thorax)
17	Adults		Modified Innocor [d]	6.7	0.6	5.9 to 7.9	NR	7.5*	Horsley [16] (RPN)
									*ULN calculated from combined sample of adults and children**
21	Infants		Mass spectrometer	7.2	0.3	NR	NR	7.8	Lum [23]
45	Preschool		Mass spectrometer	6.69	0.5	NR	NR		Aurora [35]
45	Early school		Mass spectrometer	6.67	0.5	NR	NR		Aurora [35]
28	Children		Mass spectrometer	6.13	0.41	NR	NR	6.95	Amin [11]
72	Children		Mass spectrometer	6.6*	NR	6.5 to 6.7*	NR	NR	Sonnappa [58]
35	Children		Mass spectrometer	5.9*	NR	5.1 to 7.8	NR	NR	Keen [40]

31	Children		Mass spectrometer	6.89	0.44	NR		7.77	Aurora [8] (AJRCCM)
33	Children		Mass spectrometer	6.45	0.49	NR	NR	7.41	Aurora [40]
28	Children (<18 yrs)		Mass spectrometer	6.33	0.43	NR	NR	7.17	Gustafsson [26] (ERJ)
52	Children		Mass spectrometer	6.6	0.5	NR	NR	7.5	Owens [25]
9	Adults		Mass spectrometer	7.21	0.26	NR	NR	NR	Fuchs [54]
11	Adults	Standing, V_T of 750 ml	Mass spectrometer	7.10	0.17*	NR	NR	NR	Grönkvist [59]
11	Adults	Standing, V_T of 1000 ml	Mass spectrometer	7.05	0.15*	NR	NR	NR	Grönkvist [59]
11	Adults	Standing, V_T of 1250 ml	Mass spectrometer	7.05	0.17*	NR	NR	NR	Grönkvist [59]
11	Adults	Supine, V_T of 750 ml	Mass spectrometer	6.95	0.16*	NR	NR	NR	Grönkvist [59]
11	Adults	Supine, V_T of 1000 ml	Mass spectrometer	7.07	0.16*	NR	NR	NR	Grönkvist [59]
11	Adults	Supine, V_T of 1250 ml	Mass spectrometer	7.23	0.18*	NR	NR	NR	Grönkvist [59]

N	Age group	Additional info	Apparatus	Mean LCI Median*	SD SE*	Range IQR*	95% CI	Limits of normality	Author
N_2									
50	Children	Healthy	Exhalyzer D [a]	6.1	0.9	NR	NR	7.9	Singer [32]
20	Pre-term infants	Healthy	N_2 analyser	10.8	1.4	NR	NR	NR	Shao [60]
32	Infants	Preterm	N_2 analyser	11.3	2.05	NR	NR	NR	Hjalmarson [61]
53	Infants	Full-term	N_2 analyser	10.2	1.82	NR	NR	NR	Hjalmarson [61]
60	Adults	Female	N_2 analyser	6.26	0.44	NR	NR	NR	Verbanck [62]
60	Adults	Male	N_2 analyser	6.28	0.39	NR	NR	NR	Verbanck [62]
30	Adults	Female	N_2 analyser	5.77	0.50	NR	NR	NR	Verbanck [62]
30	Adults	Male	N_2 analyser	5.65	0.49	NR	NR	NR	Verbanck [62]
17	Adults		N_2 analyser	7.02	0.6	NR	NR	NR	Downie [63]
10	Adult	Female	N_2 analyser	7.6	1.0	NR	NR	NR	Arborelius [64]
11	Adult	Male	N_2 analyser	7.5	0.9	NR	NR	NR	Arborelius [64]
12	Children	Sitting	MES [e]	6.39	0.36	NR	NR	NR	Gustafsson [26] (Pediatr Pulmonol 36:34–42)
12	Children	Supine (0 min)	MES [e]	6.31	0.56	NR	NR	NR	Gustafsson [26] (Pediatr Pulmonol 36:34–42)
12	Children	Supine (30 min)	MES [e]	6.29	0.47	NR	NR	NR	Gustafsson [26] (Pediatr Pulmonol 36:34–42)
12	Children	Supine (60 min)	MES [e]	6.39	0.43	NR	NR	NR	Gustafsson [26] (Pediatr Pulmonol 36:34–42)
He									
28	Infants (3 to 28 mo)	Full term	Mass spectrometer	9.3	NR	NR	9.1 to 9.6	NR	Chakr [65]
18	Children		Mass spectrometer	6.50	0.45	NR	NR	NR	Aljassim [66]
18	Children		Mass spectrometer	6.54	0.47	NR	NR	NR	Aljassim [66]

MES = modified emission spectrophotometer, NR = not reported, V_T = tidal volume. * signifies median or IQR.

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

[b] Spiroson(R), Ecomedics AG, Duernten, Switzerland.

[c] EasyOne Pro, MBW Module (ndd Medizintechnik AG, Zurich, Switzerland) plus addition of CO2 analyser (DUET ETCO2 Module, Welch Allyn OEM Technologies, Beaverton, OR, USA).

[d] Modified Innocor (Innovision, Odense, Denmark).

[e] Medscience 505 (Medscience Electronics, Inc., St. Louis, MO, USA).

occurs early in CF lung disease and is not detected with traditional spirometric measures such as FEV_1. LCI has a significant and growing evidence base which indicates that its clinimetric properties are positive and more useful than traditional spirometric parameters in early or mild disease. LCI has a well-established and acceptable safety and feasibility profile throughout the spectrum of ages and severities of CF lung disease. The test performance has been standardised in a recent ERS/ATS guideline [14]. The use of LCI in multicentre clinical trials will be facilitated in the near future by the standardisation efforts such as those by the ECFS-CTN Standardisation Committee: agreed standard operating procedures for performance of the measurement and for training and certification procedures, central quality control and the availability of central over-reading. The availability of commercial systems and systems that do not require specific gases such as SF_6 may also boost more general use and facilitate standardisation between centres in large scale trials.

3.4.2. Question 2: For what kind of therapeutic trial is LCI appropriate? (therapeutic aim; phase of trial, target population, number of patients involved, number of sites involved)

At present LCI has mainly been used in phase two trials evaluating therapeutic benefit. A recent phase two trial of ivacaftor in patients with mild lung disease showed that LCI was more responsive to treatment than FEV_1 [9]. A post-hoc power analysis demonstrated a much lower number of patients needed when using LCI rather than FEV_1 as primary outcome. Since this was a multicentre trial, it also demonstrates the feasibility of using LCI across centres in different countries. The accumulating evidence indicates that, in addition to phase two trials, LCI is becoming applicable to phase three trials. Given LCI's greater sensitivity than FEV_1, it is especially appropriate for use in phase three trials in small populations (e.g. rare mutations), young children, patients with mild lung disease, or to reduce the number of subjects needed.

3.4.3. Question 3: Within what timeline can change be expected and what treatment effect can be considered clinically significant?

Available studies have not addressed how quickly LCI changes after an intervention. The biological mechanisms underlying abnormally raised LCI are thought to be (a) regional airway endoluminal obstruction by retained secretions, (b) regional airway obstruction due to mucosal airway inflammation and (c) regional remodelling/fibrosis/destruction of airways. Mechanisms (a) and (b) are amenable to change over days and improvements in LCI following treatment of acute CF exacerbations have been documented. A raised LCI might also have an irreversible part related to structural abnormalities (c).

The treatment effect that can be considered clinically significant should be larger than the difference in LCI seen between repeat measurements without intervention or change in clinical status. In healthy children and using SF_6 as inert gas and mass spectrometer as analyser, the CoR was 0.74 or 11% of the baseline value [39]. When using nitrogen washout and a commercial set-up, CoR was 0.6 in healthy children and 0.96 in children with CF [32]. For more data on test repeatability we refer to Table E2.

3.4.4. Question 4: What studies are needed to further define LCI in CF patients and its potential as a surrogate marker?

1. Clinical relevance: variability of LCI in preschool children and infants. Correlation of LCI with clinical outcome parameters such as time to pulmonary exacerbation. Use of LCI in a multicentre setting to study treatment benefit in preschool children and infants. Longitudinal evolution from birth in a large cohort of CF patients.
2. Methodology: further comparisons of LCI measured according to the recent consensus but using the different possible set-ups; normative ranges and CoR across ages and for all techniques.
3. Additional information compared to other outcome parameters: correlation with regional ventilation abnormalities as defined by imaging (e.g. hyperpolarized helium). Ideally these studies should be interventional (e.g. before and after treatment). Further correlations with inflammatory markers in bronchoalveolar lavage or/and sera.

4. Conclusion

This document provides an overview of the work of the ECFS-CTN Standardisation Committee on LCI. A systematic review of the clinimetric properties of LCI demonstrates its reliability, validity and responsiveness. LCI also has an attractive feasibility profile. It is particularly useful for multicentre trials in young children with CF and in patients with early or mild CF lung disease when FEV_1 is within normal range. This is the first article to collate the literature on LCI and CF in this manner and provides a strong evidence base to support the use of LCI in clinical trials in CF.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jcf.2013.09.005.

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