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Lung clearance index in adults and children with cystic fibrosis

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Abstract

Background: Lung clearance index (LCI) has good clinimetric properties and an acceptable feasibility profile as a surrogate endpoint in Cystic Fibrosis (CF). Although most studies to date have been in children, increasing numbers of adults with CF also have normal spirometry. Further study of LCI as an endpoint in CF adults is required. Therefore, the purpose of this study was to determine the clinimetric properties of LCI over the complete age range of people with CF.

Methods: Clinically stable adults and children with CF and age matched healthy controls were recruited.

Results: LCI and spirometry data for 110 CF subjects and 61 controls were collected at a stable visit. CF Questionnaire-Revised (CFQ-R) was completed by 80/110 CF subjects. Fifty-six CF subjects completed a second stable visit. The LCI CV% was 4.1% in adults and 6.3% in children with CF. The coefficient of repeatability of LCI was 1.2 in adults and 1.3 in children. In both adults and children, LCI ($AUC^{ROC}=0.93$ and 0.84) had greater combined sensitivity and specificity to discriminate between people with CF and controls compared to FEV_1 ($AUC^{ROC}=0.88$ and 0.60) and FEF_{25-75} ($AUC^{ROC}=0.87$ and 0.68). LCI correlated significantly with the CFQ-R treatment burden in adults ($r=-0.37$; $p<0.01$) and children ($r=-0.50$; $p<0.01$). Washout tests were successful in 90% of CF subjects and were perceived as comfortable and easy to perform in both adults and children.

Conclusions: These data support the use of LCI as a surrogate outcome measure in CF clinical trials in adults as well as children.

Introduction

Lung clearance index (LCI), a measure of ventilation inhomogeneity derived from multiple breath washout (MBW), has good clinimetric properties and an acceptable feasibility profile as a surrogate endpoint in CF^{1, 2}. The majority of studies have been in children² where it has most potential as a useful marker to detect early changes in lung disease. These studies have shown good short- and medium-term repeatability of LCI in health and disease³⁻⁶ and correlation with parameters of chest Computed Tomography (CT)⁷⁻⁹, body plethysmography¹⁰, spirometry (forced expiratory volume in 1 second [FEV₁], forced expiratory flow₂₅₋₇₅ [FEF₂₅₋₇₅])^{11, 12} and markers of pulmonary inflammation and infection³. LCI has also been shown to be more sensitive than FEV₁ in predicting¹¹ and detecting^{4, 13, 14} early lung disease. In addition, LCI predicts pulmonary exacerbations and correlates with respiratory symptoms as measured by the CF Questionnaire-Revised (CFQ-R)¹⁵. The responsiveness of LCI in children with CF has also been demonstrated post intervention with hypertonic saline^{6, 16}, DNase¹⁷ and in children and adults receiving ivacaftor¹⁸. Furthermore, MBW testing is considered feasible in an outpatient setting⁵.

Studies of LCI involving adults with CF have also demonstrated encouraging results but are fewer in number^{8, 19-22}. As increasing numbers of adult patients with CF also have normal FEV₁, more sensitive endpoints are required to assess new therapies in this age group²³. Improved survival means that adults are beginning to outnumber children in most developed countries²⁴ and a large proportion of clinical trials are aimed at adults where efficacy must be demonstrated prior to testing treatments in younger patients. A recent workshop report has highlighted the potential use for LCI in older subjects with normal FEV₁²⁵ however gaps in the current evidence remain concerning inter-visit reliability, correlation with patient reported outcomes and patient acceptability. Furthermore, only one study has directly compared adults and children with CF²⁶. Further research is required to validate LCI as outcome measure across the age range in CF.

In this study we determined the intra-visit repeatability, sensitivity and specificity and inter-visit repeatability of LCI compared with FEV₁ and FEF₂₅₋₇₅. We also assessed the correlations between LCI, FEV₁, FEF₂₅₋₇₅ and CFQ-R domain scores and determined the feasibility and acceptability of LCI. Results were reported separately for adults and children with CF. Some results of this work have been presented in abstract format²⁷⁻²⁹.

Methods

Adults and children with CF were recruited as part of a larger study investigating the prevalence and potential pathogenicity of anaerobic bacteria in CF lung disease. CF subjects were recruited during routine outpatient appointments at the adult and paediatric CF Centres, Belfast Health and Social Care Trust (Belfast City Hospital and Royal Belfast Hospital for Sick Children). All subjects were enrolled for the first study visit when clinically stable (cross-sectional arm). Subjects were aged ≥ 6 years, had a confirmed diagnosis of CF³⁰ and were clinically stable (with no pulmonary exacerbation requiring intravenous antibiotics in the previous 4 weeks). A subgroup of subjects' (≥ 12 years) were recruited to a longitudinal arm in which repeat stable visits were performed at 3 monthly intervals. Due to the difficulties of collecting sputum samples from subjects < 12 years old, these subjects participated in the cross-sectional study only. The current study reports on MBW, spirometry and questionnaire data only.

An exclusion criterion for MBW testing specifically was infection with *Burkholderia cepacia* complex infection (due to equipment infection control issues). On obtaining written informed consent, subjects were enrolled for a study visit and completed a CFQ-R, a MBW test, 2 Visual Analogue Scales (VAS) for evaluating the ease and comfort with MBW testing and spirometry. For the second stable visit, procedures were completed in the same order.

Adult and child healthy control (HC) subjects were invited to attend a single study visit by means of email circulation amongst work colleagues at Queen's University Belfast and Belfast Health and Social Care Trust. Subjects were aged ≥ 6 years, had no history of a respiratory condition or use of antibiotics in the previous 4 weeks. On obtaining written informed consent, subjects completed spirometry and a MBW test.

All subjects were recruited during a 32-month period (October 2010-June 2013). This study was approved by the Office for Research Ethics Committees Northern Ireland (reference number: 10/NIR01/41).

Participants individually completed an age-appropriate version of the CFQ-R before any other procedure. Children aged 6-11 years old completed the questionnaire with the assistance of the researcher or a parent if necessary. Adolescent and adult data for subjects aged ≥ 14 years old was collected in 1 version of the questionnaire and child data for subjects aged 6-13 years old in another version.

The MBW test to measure LCI was performed using the modified InnocorTM device as previously validated¹⁹ (e-Appendix 1). The test was performed either before or at least 30 minutes after performing spirometry to try to avoid any effects of a forced manoeuvre on LCI. Analysis of MBW data was performed using the Simple Washout programme. Three tests were attempted and the mean LCI was calculated from a minimum of 2 technically valid and

repeatable tests (e-Appendix 1). The upper limit of normal (ULN) for LCI was calculated from the cohort of healthy controls (mean LCI + 1.96 SD). Z-scores were also calculated from HC group values. An LCI z-score of >2 was considered abnormal. Feasibility of MBW was assessed as the number of successful test sessions/attempted test sessions.

Two VAS were completed at the first stable visit to score perceived comfort and ease in carrying out the MBW test. The VAS consisted of a scale from 0 to 10 cm with zero representing not comfortable/not easy and 10 representing extremely comfortable/extremely easy (e-Appendix 2).

Spirometry was measured according to ATS/ERS guidelines³¹ using a MicroLab (ML3500 MK8) spirometer (CareFusion, Kent, UK). Predicted values were calculated from reference ranges for all ages³². The lower limit of normal of 80% for FEV₁ and FEF₂₅₋₇₅ was used, as this is the level that is historically used in clinical practice. Z-scores were also calculated from HC group values. A FEV₁ and FEF₂₅₋₇₅ z-score of <-2 was considered abnormal.

Statistical analysis

Data were analysed using PASW Statistics (version 18, IBM software, USA) and Prism (Version 5.01 GraphPad Software Inc.). Intra-visit repeatability was assessed using the coefficient of variation (CV %) (i.e. within subject SD/mean) of triplicate LCI readings. In addition, Bland-Altman plots (i.e. the 95% limits of agreement between the first and third washout tests) were inspected. A learned effect if present, would most likely be apparent between the 1st and 3rd test. Finally, the coefficient of repeatability (CoR) (i.e. $1.96 \times \text{SD of the differences}$) was calculated and compared to CoR and within subject differences (considered to represent a significant change) reported in the literature (0.96 to 1.30 in children and adults with CF^{5,33,34}). Subjects with only 2 washout repeats were excluded from intra-visit repeatability analysis. The sensitivity (proportion of true positives who are test positive) and specificity (the proportion of true negatives who are test positive) of LCI, FEV₁ and FEF₂₅₋₇₅ to the presence of CF, was assessed in the adult and child groups. Receiver-operator characteristic (ROC) curves (Area under the Curve [AUC^{ROC}] and Standard Error [SE]) for LCI, FEV₁ and FEF₂₅₋₇₅ were used to assess combined sensitivity and specificity. ROC curves were compared using a Venkatraman's test³⁵. In a subgroup, the long-term inter-visit repeatability across 2 stable visits was assessed using Bland-Altman plots and the interclass correlation coefficient (ICC) and 95% confidence intervals (CI). Correlations were assessed

using Pearson's correlation coefficient. Differences between means were assessed using paired t-tests. A p-value of <0.05 was considered statistically significant.

Results

Participants and data collected

e-Figure 1 describes study recruitment for subjects with CF. 122 subjects with CF were enrolled and completed the first stable visit. LCI results from 6 subjects were not repeatable and 6 subjects were unable to complete the MBW procedure; one experienced discomfort with the mouthpiece, 5 did not have time to complete a minimum of 2 tests. Therefore, data from 110 subjects with CF (n=43 children) was used for analysis. Fifty-six subjects (n=12 children) completed a second stable visit (≥ 3 months later).

e-Figure 2 describes study recruitment for healthy subjects. Data from 61 subjects (n=31 children) were included in the analysis. Adult and child results were analysed and presented separately. All subject characteristics are displayed in Table 1.

Intra-visit repeatability

In the adult group, the LCI CV% was not significantly different between CF and HC groups. However, the LCI CV% was significantly different between children with CF and HC and was higher in children with CF compared with adults with CF (Table 1). The Bland-Altman plots (Figures 1a and b) show that there was no evidence of greater variability in subjects with more advanced disease in the CF adult or child group. The CoR (1.2 in CF adults and 1.3 in CF children) was equivalent to 12% of the mean LCI value in CF adults and 17% of the mean LCI value in CF children (Table 2). The age, gender and spirometry of patients included in this analysis (3 washout repeats) did not differ significantly from those excluded from the analysis (2 washout repeats) (e-Table 1).

Sensitivity and specificity

The ULN of LCI (mean LCI plus 1.96 SD) for adults was 7.5 and 7.3 for children. All control subjects had an LCI < the ULN. Whilst LCI had a narrow range in healthy controls, LCI increased with declining FEV₁ % predicted in both CF adult ($r=-0.68$, $p<0.0001$) (e-Figure 3) and CF child ($r=-0.66$, $p<0.0001$) subjects (e-Figure 4). LCI also increased with declining FEF₂₅₋₇₅% predicted in both CF adult ($r=-0.59$, $p<0.0001$) (e-Figure 5) and CF child ($r=-0.48$, $p<0.0001$) subjects (e-Figure 6).

In adult subjects the sensitivity and specificity of LCI to CF was 81% and 100%, respectively compared to 63% and 100% for FEV₁ % predicted and 80% and 63% for FEF₂₅₋₇₅ % predicted. Considering CF subjects only, 14/67 (21%) CF adults had an abnormal LCI and a normal FEV₁ % predicted. Two CF adults (3%) had a normal LCI and an abnormal FEV₁ % predicted (e-Figure 3). Seven (12%) CF adults had an abnormal LCI and a normal FEF₂₅₋₇₅ % predicted. Four CF adults (7%) had a normal LCI and an abnormal FEF₂₅₋₇₅ % predicted (e-Figure 5). Overall, in adults LCI ($AUC^{ROC}=0.93$; $SE=0.03$; $p<0.0001$) had greater combined sensitivity and specificity to discriminate between CF and controls compared to FEV₁% predicted ($AUC^{ROC}=0.88$; $SE=0.03$; $p<0.0001$) and FEF₂₅₋₇₅ % predicted ($AUC^{ROC}=0.87$; $SE=0.04$; $p<0.0001$) (Figure 2a). Comparison of the ROC curves showed that LCI AUC^{ROC} was only significantly better than FEF₂₅₋₇₅ AUC^{ROC} ($p=0.02$) but not FEV₁ AUC^{ROC} ($p=0.12$). LCI had significant sensitivity and specificity to detect abnormal FEV₁ ($AUC^{ROC}=0.91$; $SE=0.03$; $p<0.0001$) and FEF₂₅₋₇₅ % predicted ($AUC^{ROC}=0.81$; $SE=0.05$; $p<0.0001$) in adults (e-Figure 7 and 8).

In the child subjects, the sensitivity and specificity of LCI to CF was 46% and 100% respectively compared to 31% and 81% for FEV₁ % predicted and 70% and 55% for FEF₂₅₋₇₅. Considering CF subjects only, 8/43 (19%) children had an abnormal LCI in the presence of a normal FEV₁ % predicted. Four (9%) children had a normal LCI and an abnormal FEV₁ % predicted (e-Figure 4). Three (7%) CF children had an abnormal LCI and a normal FEF₂₅₋₇₅ % predicted. Twelve CF children (28%) had a normal LCI and an abnormal FEF₂₅₋₇₅ % predicted (e-Figure 6). Overall, in children LCI ($AUC^{ROC}=0.84$; $SE=0.05$; $p<0.0001$) had greater combined sensitivity and specificity to discriminate between CF and controls compared to FEF₂₅₋₇₅ % predicted ($AUC^{ROC}=0.68$; $SE=0.07$; $p=0.02$). FEV₁ % predicted ($AUC^{ROC}=0.60$; $SE=0.07$; $p=0.15$) was not statistically significant in discriminating between CF and HC child subjects (Figure 2b). Comparison of the ROC curves showed that LCI AUC^{ROC} was significantly better than FEV₁ AUC^{ROC} ($p=0.0002$) and FEF₂₅₋₇₅ AUC^{ROC} ($p=0.03$). LCI also had significant sensitivity and specificity to detect abnormal FEV₁ ($AUC^{ROC}=0.77$; $SE=0.07$; $p<0.0001$) and FEF₂₅₋₇₅ % predicted ($AUC^{ROC}=0.69$; $SE=0.06$; $p<0.007$) (e-Figure 9 and 10) in children.

Inter-visit repeatability

Fifty-six CF subjects (n=12 children) completed a second stable visit that included MBW testing (Table 1). Due to smaller numbers completing 2 stable visits, data from adults and

children were combined for analysis. Visits were on average (SD) [range] 213 (114) [67-614] days apart.

There was no significant difference between repeat measurements across an average period of 8 months (e-Table 2). The ICC (95% CI) for LCI (0.96; 0.94 to 0.98; $p<0.0001$), FEV₁ % predicted (0.91; 0.84 to 0.94; $p<0.0001$) and FEF₂₅₋₇₅ % predicted (0.91; 0.85 to 0.95; $p<0.0001$) all demonstrated excellent agreement with statistical significance.

Correlations with CFQ-R domain scores

Of the 110 CF subjects, 80 completed the CFQ-R at their first stable visit consisting of 55 adolescents/adults (≥ 14 years old) and 25 children (6-13 years). Domain scores for CFQ-R adolescent/adult (≥ 14 years), CFQ-R child (6-13 years) are summarised in e-Table 3. Table 3 summarises the correlation coefficients between the CFQ-R domains scores, LCI, FEV₁ and FEF₂₅₋₇₅. Significant correlations were observed with CFQ-R treatment burden only (e-Figure 11 and e-Figure 12). The relationship between CFQ-R respiratory symptoms and LCI and between CFQ-R respiratory symptoms and spirometry were not significant.

Feasibility and acceptability

Of the 122 CF subjects recruited, 110 (90%) of MBW tests were successful with a minimum of 2 technically valid and repeatable tests obtained.

All but 1 of the 110 CF subjects completed both VAS (child aged 6 years unable to understand the instructions). Both adults and children reported high levels of comfort (mean VAS score [SD] 8.0 [1.5] Vs. 8.2 [1.8]; $p=0.39$) and ease (mean VAS score [SD] 9.0 [1.3] Vs. 9.0 [1.7]; $p=0.94$) with the MBW test and there was no significant difference between scores in the 2 age groups.

Discussion

This study demonstrates that LCI has good clinimetric properties over a wide age range in CF subjects from school age to older adulthood. Considering intra-visit repeatability of LCI, the within-visit CV% for CF subjects was better in the adult group (4.1%) compared with the child group (6.3%). Both results were comparable to within-visit CV% of 4-8% reported in recent studies^{3, 5, 12} and in a study using the same MBW device¹⁹. Adult and child CF subjects had similar intra-visit variability in LCI compared with their HC counterparts, with no significant difference in the mean differences between the first and third tests. The CoR (1.2 in CF adults and 1.3 in CF children) was similar to levels considered to represent significant change in recent

trials (0.96-1.30)^{5, 11, 34}. The CoR statistic is useful to indicate the degree of variability above which a treatment effect would be expected to be observed³⁶; however caution is required with respect to the direct interpretation of CoR as the level of treatment effect. This statistic confounds individual level changes, only measuring within-subject variation. In order to obtain a more meaningful threshold for treatment effect, an assessment of how much change is required for the patient to report benefit may be more informative. Importantly, the Bland-Altman plots demonstrate that there is no evidence of increased variation within washouts in subjects with more severely impaired lung function (i.e. a higher LCI) across both the adult and child CF groups. However, as the analysis protocol applied its own repeatability criteria (excluding those subjects who had an FRC > 10% and/or an LCI of >20% across ≥ 2 tests), this cohort of CF subjects represent the “best” subjects in terms of intra-visit repeatability. These criteria may inadvertently exclude subjects with more severe lung function and therefore a more variable LCI. Nevertheless, these data suggest that levels of variation in LCI are relatively stable in subjects with an LCI of up to 17.2 lung turnovers. A potential limitation of the study relates to the timing of MBW during the study visit which was performed before or 30 minutes after spirometry. This schedule was in place to minimise disruption for the patient and to ensure that data collection for the study could take place during a routine outpatient appointment. However, previous studies have shown little to no short term impact on LCI as a result of forced manoeuvres^{37, 38}.

This was the first study to investigate the longer term stability (> three months) of LCI in those with stable disease. Other studies assessing intra-visit repeatability in children performed repeat sessions up to a maximum of 12 weeks apart⁶. The results from this analysis indicated good long-term repeatability of LCI with stability of measurements across an average period of eight months. This data addresses an important question around the clinimetrics of LCI as a surrogate measure of lung function, providing evidence that LCI has low levels of variation in a combined adult and child group comparable with spirometry over a long-term period.

This study also demonstrates that LCI has excellent sensitivity and specificity (AUC^{ROC}) when discriminating between CF and HC subjects across the age range. LCI AUC^{ROC} was significantly better than FEF_{25-75} AUC^{ROC} in adults and both FEV_1 AUC^{ROC} and FEF_{25-75} AUC^{ROC} in children. A proportion of CF subjects in both age groups had an abnormal LCI in the presence of normal FEV_1 (21% in adults; 19% in children) or a normal FEF_{25-75} (12% in adults and 7% in children). FEF_{25-75} had equal (adults) or better (children) sensitivity

than LCI but was much less specific. In children, FEF₂₅₋₇₅ detected a proportion of subjects (30%) with an abnormal reading in the presence of a normal LCI. Given that LCI is reflective of ventilation distribution in both small and large airways, perhaps normal gas mixing in the large airways masked abnormality in the small airways in these patients. However, almost half of healthy subjects also had an abnormal FEF₂₅₋₇₅ which highlights that this measure was not specific to disease in this group and perhaps not reliable. The limitation of FEV₁ was highlighted specifically in the child group where the values were well preserved and therefore did not differentiate well between health and disease. The adult data specifically highlights that an abnormal LCI in the presence of a normal FEV₁ can range from mildly abnormal to grossly increased values, indicating that even advanced ventilation inhomogeneity may not be detected by FEV₁ or FEF₂₅₋₇₅.

The domain of treatment burden emerged as an important aspect of health related quality of life in this study, correlating with LCI and FEV₁ in both age groups and with FEF₂₅₋₇₅ in adults. The association between LCI, spirometry and CFQ-R respiratory symptoms were not significant in this study, unlike those reported in children by Vermeulen and colleagues¹⁵. In this study, patients with grossly elevated LCI (>10 z-scores) reported respiratory symptoms within the same range as those patients who had moderately elevated LCI z-scores, diminishing the association. A possible reason why treatment burden was important in this study may be due to the significant treatment demands placed on subjects with CF which may be increasing with the emergence of new and more aggressive therapies. Previous studies of patient perceived treatment burden have shown it to be independent of disease severity³⁹. In this study it significantly correlated with the respiratory symptoms domain in the adult group ($r=0.36$; $p=0.006$), indicating that a higher treatment burden was associated with more symptoms and likely, more severe disease. Overall, the significant correlations observed were of weak to moderate strength ($r= -0.37$ to -0.50) however, they are similar to those reported in the initial validation studies of the CFQ-R with FEV₁ ($r= 0.23$ to 0.45)⁴⁰.

Reported success rates for achieving valid and repeatable tests have ranged from 24 - 100%; however, the majority are >70%, given adequate time to ensure collection of valid data (2 to 3 tests)^{5, 41}. A drawback of the test is that sessions can often take > 30 minutes for each individual. Our finding that MBW tests were feasible to perform (90% success rate) and that child and adult subjects with CF found the MBW to be equally acceptable adds to the current feasibility profile of LCI.

Conclusions

This study shows that the clinimetric properties of LCI (validity reliability, sensitivity, specificity, feasibility and acceptability) in adults with CF are comparable to those in children with CF. These data support the use of LCI as a surrogate outcome measure in CF clinical trials in adults as well as children.

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KO'N and J.S.E. take responsibility for (is the guarantor of) the content of the manuscript, including the data and analysis.

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Tables

Table 1: CF and HC subject characteristics

	Visit 1			Visit 1			CF subjects completing 2 visits
	Adult CF (n=67)	Adult HC (n=30)	p-value	Child CF (n=43)	Child HC (n=31)	p-value	CF (n=56) ^f
Median (IQR) age in years	27.0 (16.0)	27.0 (7.0)	0.82	11.7 (3.4)	10.8 (3.4)	0.32	23.5 (15.8)
n (%) F508del homozygous	21/67 (31)	n/a	n/a	19/43 (44)	n/a	n/a	20/56 (36)
n (%) chronic <i>P. aeruginosa</i> infection ⁴²	33/67 (49)	n/a	n/a	7/43 (16)	n/a	n/a	19/56 (34)
Females: males (n)	28:39	16:14	0.70	19:24	14:17	<0.0001	31:25
Mean (SD) FEV ₁ % predicted	71.8 (20.3)	100.5 (11.0)	<0.0001	85.2 (16.6)	90.0 (12.2)	0.21	77.3 (18.9)
Mean (SD) FEV ₁ z-score	-2.3 (1.7)	0.1 (1.0)	<0.0001	-1.2 (1.4)	-0.8 (1.0)	0.15	-1.9 (1.6)
Median (IQR) FEF ₂₅₋₇₅ % predicted	40 (46.7) ^a	90.9 (33.32)	<0.0001	66.0 (27.6)	80.4 (23.2)	0.02	53.8 (52.0) ^g
Mean FEF ₂₅₋₇₅ z-score	-2.4 (1.6) ^a	-0.4 (0.8)	<0.0001	-1.7 (1.5)	-1.0 (1.2)	0.02	-2.0 (1.7) ^g
Mean (SD) LCI no. turnovers [range]	10.3 (3.0) [5.9-17.2]	6.5 (0.5) [5.6-7.3]	<0.0001	7.8 (1.8) [5.4-12.9]	6.3 (0.5) [5.5-7.3]	<0.0001	9.4 (2.6)
Mean (SD) LCI z-score	7.8 (5.8)	-0.1 (1.0)	<0.0001	2.9 (3.5)	-0.3 (1.0)	<0.0001	6.0 (5.2)
Mean (SD) LCI intra-visit CV %	4.1 (2.4) ^b	4.5 (2.9) ^c	0.57	6.3 (3.0) ^d	4.5 (2.3) ^e	0.01	4.3 (3.0)

^aFEF₂₅₋₇₅ data was recorded for 59/67 of adult subjects with CF.

^bLCI CV% calculated from 49/67 adult CF subject data i.e. those with 3 valid and repeatable LCI readings. LCI from n=18 were calculated from two washout readings, therefore CV% not calculated.

^cLCI CV% from calculated from 28/30 adult HC subject data. LCI from n=2 were calculated from two washout readings.

^dLCI CV% calculated from data from 37/43 child subjects with CF. LCI from n=6 was calculated from two washout readings.

^eLCI CV% from 28/31 child HC subjects. LCI from n=3 was calculated from two washout readings.

^f Subject demographics at visit 1.

^gFEF₂₅₋₇₅ data was recorded for 51/56 CF subjects completing 2 visits.

Table 2: LCI intra-visit repeatability data for CF adults and CF children

	Mean (SD) LCI 1st washout	Mean (SD) LCI 3rd washout	Mean difference (SD) 3 rd -1 st	CoR
CF adults (n=49)	9.8 (2.5)	10.0 (2.6)	0.2 (0.6)	1.2
CF children (n=37)	7.9 (1.9)	8.0 (1.9)	0.1 (0.7)	1.3

Table 3: Correlations between CFQ-R domain scores, LCI and spirometry in adolescent/adults (n=55) and children (n=25).

CFQ-R domains	LCI z-score		FEV ₁ z-score		FEF ₂₅₋₇₅ z-score	
	Adult	Child	Adult	Child	Adult	Child
Physical functioning	-0.18	-0.18	0.25	0.03	0.06	0.21
Role/School functioning [^]	-0.03	-	0.17	-	0.08	-
Vitality [^]	0.01	-	-0.06	-	-0.17	-
Emotion	-0.07	-0.23	0.02	0.00	-0.01	-0.08
Social functioning	-0.18	-0.33	0.15	0.16	-0.01	-0.01
Body image	-0.05	-0.26	-0.03	0.11	-0.01	0.04
Eating disturbances	0.01	-0.25	0.08	0.09	-0.02	0.11
Treatment burden	-0.37**	-0.50**	0.43**	0.44*	0.38**	0.19
Health perceptions [^]	-0.07	-	0.11	-	-0.05	-
Weight [^]	0.20	-	-0.12	-	-0.12	-
Respiratory symptoms	-0.15	-0.23	0.17	0.07	0.19	-0.01
Digestion symptoms	-0.10	-0.12	-0.07	0.15	-0.07	0.19

Correlation coefficient expressed as an r value

*p<0.05 **p<0.01

[^] Adolescent/adult CFQ-R domains only

Figure legends

Figure 1 (a) and (b): Bland-Altman plot for the first and third washout repeats for (a) adult (n=49) and (b) child (n=37) CF subjects.

Figure 2 (a): CF vs. HC: Adult ROC curve demonstrating the sensitivity and specificity of LCI, FEV₁% predicted and FEF₂₅₋₇₅% predicted to CF compared with HC subjects.

Figure 2 (b): CF vs. HC: Child ROC Curve demonstrating the sensitivity and specificity of LCI, FEV₁% predicted and FEF₂₅₋₇₅% predicted to CF compared with HC subjects.

e-Figure 1: Flowchart demonstrating recruitment of CF subjects

e-Figure 2: Flowchart demonstrating recruitment of HC subjects

e-Figure 3: LCI vs. FEV₁ % predicted in CF adults (triangles) and controls adults (circles). *The corresponding lines display the regression line for each group.*

e-Figure 4: LCI vs. FEV₁ % predicted in CF children (triangles) and child controls (circles). *The corresponding lines display the regression line for each group.*

e-Figure 5: LCI vs. FEF₂₅₋₇₅ % predicted in CF adults (triangles) and adult controls (circles). *The corresponding lines display the regression line for each group.*

e-Figure 6: LCI vs. FEF₂₅₋₇₅ % predicted in CF children (triangles) and child controls (circles). *The corresponding lines display the regression line for each group.*

e-Figure 7: Adult ROC curve demonstrating the sensitivity and specificity of LCI to Normal Vs. abnormal FEV₁ in CF subjects.

e-Figure 8: Child ROC curve demonstrating the sensitivity and specificity of LCI to Normal Vs. abnormal FEV₁ in CF subjects.

e-Figure 9: Adult ROC curve demonstrating the sensitivity and specificity of LCI to Normal Vs. abnormal FEF 25-75 in CF subjects.

e-Figure 10: Child ROC curve demonstrating the sensitivity and specificity of LCI to Normal Vs. abnormal FEF 25-75 in CF subjects.

e-Figure 11: Relationship between CFQ-R treatment burden and LCI z-score in adults (open circles) and children (closed circles). The vertical dashed line represents the ULN for LCI z-score.

e-Figure 12: Relationship between CFQ-R treatment burden and FEV₁ z-score in adults (open circles) and children (closed circles). The vertical dashed line represents the LLN for FEV₁ z-score.