

## RESEARCH LETTER

## Multiple-breath washout measurements can be significantly shortened in children

Multiple-breath washout (MBW)-derived lung clearance index (LCI) is a sensitive measure of ventilation inhomogeneity in patients with cystic fibrosis (CF), but LCI measurement is time consuming. We systematically assessed ways to shorten LCI measurements.

In 68 school-aged children (44 with mild CF lung disease) three standard nitrogen ( $N_2$ ) MBWs were applied. We assessed repeatability and diagnostic performance of (1) LCI measured earlier from three MBW runs and (2) LCI measured at complete MBW (1/40th of starting  $N_2$  concentration) from two runs only.

Compared with the standard LCI from three complete MBW runs, the new LCI based on three  $N_2$  MBW runs until 1/20th, or two complete runs until 1/40th, provided similar or better repeatability as well as sensitivity and specificity for CF lung disease. Alternative ways to measure LCI reduced test duration in children with CF by 30% and 41%, respectively.

LCI measurements can be reliably shortened in children. These new MBW protocols may advance the transition of LCI from research into clinical settings.

Multiple-breath washout (MBW)-derived lung clearance index (LCI) is a sensitive measure of increased ventilation inhomogeneity in cystic fibrosis (CF) lung disease.<sup>1 2</sup> However LCI is rarely

measured routinely due to time-consuming protocols, that is, at least three MBW runs with tracer gas washout until 1/40th of its starting concentration.<sup>3</sup> In this prospective study, we systematically assessed ways to shorten LCI measurements in school-aged children with CF, and in healthy controls.

We used a previously described nitrogen ( $N_2$ ) MBW equipment (Exhalyzer D, Eco Medics AG, Switzerland)<sup>4</sup> and measured standard LCI ( $LCI_{std}$ ) in 80 children (50 with CF) aged 5.7–16.9 years. Alternative MBW outcomes were (1) mean LCI derived from empirically chosen earlier time points based on three MBW runs and (2) mean LCI from two complete runs. Quality criteria for alternative LCI were defined as (1) predictive value ( $R^2$ ) for  $LCI_{std} > 90\%$ , (2) coefficient of variation (CV)  $\leq 7\%$  and (3) sensitivity and specificity for CF lung disease within  $\pm 3\%$  of  $LCI_{std}$  (detailed in the online supplementary (OLS)).

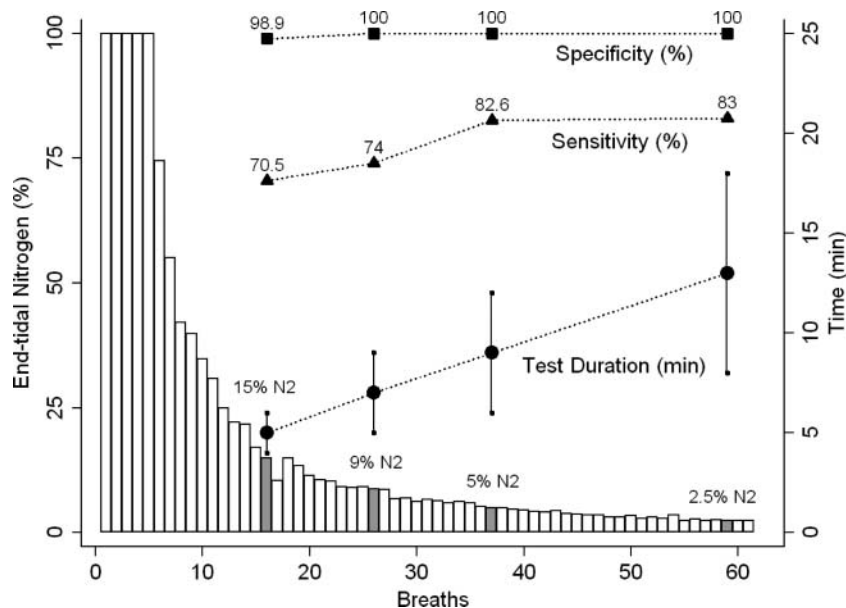
Technically acceptable triplicate  $N_2$  MBW ( $n=204$ ) were obtained in 68 (31 boys) children (44 with CF).  $N_2$  MBW until 5%  $N_2$  ( $LCI_5$ ), that is, 1/20th of  $N_2$  starting concentration, was the earliest time point to meet all quality criteria (figure 1).  $LCI_5$  predicted 93% of  $LCI_{std}$  (table 1 and figure 1 OLS). CV of  $LCI_5$  was 5.1%, and 5.4% for  $LCI_{std}$  (table 2 OLS).  $LCI_5$  and  $LCI_{std}$  were both highly specific (100%), and provided a sensitivity of 82.6% and 83.0%, respectively, for CF lung disease (figure 1). Time savings were on average (95% CI) 3.7 (3.1–4.4) min. (30%) in CF. LCI from two complete runs also met the quality criteria and provided

significant time savings (table 3 and figure 2 OLS). Detailed results and figures are given in the OLS.

By convention, at least three MBW runs until 1/40th were recommended to measure LCI due to  $N_2$  sensor characteristics, estimated  $N_2$  blood gas exchange, and possibly due to lack of online quality control. We show that LCI measurements can be reliably shortened. Both new LCI measures may enhance feasibility and acceptance in younger children and in patients with more advanced lung disease. In children with predominantly mild CF lung disease, LCI based on two MBW runs seems attractive, as complete washout is usually achieved fast. This was confirmed in a retrospective study by Robinson *et al*<sup>5</sup> where LCI of two complete runs adequately identified increased ventilation inhomogeneity in CF.  $LCI_5$  may be more applicable in advanced lung disease where complete  $N_2$  MBW is time consuming. Further studies are needed to establish the applicability of shortened MBW for different populations and settings. Long-term repeatability and response to intervention need to be compared between shortened MBW protocols. Detailed discussion on methodological and physiological aspects are provided in the OLS.

Both new LCI measures based on three  $N_2$  MBW runs until 1/20th, or two runs until 1/40th, significantly shorten MBW in children without compromising diagnostic performance for increased ventilation inhomogeneity in mild CF lung disease. These data support the transition of LCI measurement from research into clinical routine.

**Figure 1** Diagnostic performance and duration of nitrogen multiple-breath washout. A typical example of a nitrogen multiple-breath washout ( $N_2$  MBW) curve in a boy with cystic fibrosis schematically illustrates the course of diagnostic accuracy and test duration for the lung clearance index (LCI) measured at various time points (grey bars). End-tidal  $N_2$  concentration normalised to 100% (left y-axis) is plotted (white bars) against washout breaths (x-axis). By convention,  $N_2$  MBW is performed until 1/40th (2.5%  $N_2$ ) of the  $N_2$  starting concentration to measure LCI. We calculated LCI at arbitrary earlier time points: 15%, 9% and 5%  $N_2$  (grey bars). Specificity (rectangles) and sensitivity (triangles) for CF lung disease increase over the washout. Mean  $\pm$  SD of test duration (right y-axis) are displayed as circles.



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## Online supplement

### **Multiple-breath washout measurements can be significantly shortened in children**

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## Methods

### *Study subjects*

In this prospective cross-sectional study 50 children with cystic fibrosis (CF) and 30 healthy controls aged between six and seventeen years at the Children's University Hospital of Bern were enrolled between November 2011 and May 2012. Children with CF performed nitrogen multiple-breath washout (N<sub>2</sub>MBW) and spirometry in that order, controls performed N<sub>2</sub>MBW. Outcomes were lung clearance index (LCI), functional residual capacity (FRC), and forced expiratory volume in one second (FEV<sub>1</sub>) reported in z-scores (1). Data from these children have not been published before. We recruited children with confirmed CF attending the outpatient clinic and age-matched healthy controls from local schools and playgroups. Exclusion criteria for children with CF were fever or acute respiratory deterioration. Exclusion criteria for controls were doctor diagnosis of asthma or history of respiratory tract infection within the last four weeks. All children performed three N<sub>2</sub>MBW according to current guidelines (2).

### *Nitrogen multiple-breath washout*

Three complete N<sub>2</sub>MBW until 1/40<sup>th</sup> of the starting N<sub>2</sub> end-tidal concentration (cet), *i.e.* 2.5% N<sub>2</sub> cet from initial normalized N<sub>2</sub> cet, were performed as described previously (3;4). We used a recently validated setup (Exhalyzer D and Spiroware 3, Eco Medics AG, Duernten, Switzerland) (3-5). Children performed N<sub>2</sub>MBW while sitting upright, wearing a nose clip, and tidally breathing through a snorkel mouth piece. N<sub>2</sub>MBW was stopped after three breaths below 2.5% N<sub>2</sub> cet. Time for measurements and between tests (measurement time plus one minute) was recorded. End-tidal N<sub>2</sub> cet was controlled prior to the next N<sub>2</sub>MBW. N<sub>2</sub>MBW runs were accepted if FRC varied less than 20% (6).

### *Early stop in multiple-breath washout*

We calculated standard LCI ( $LCI_{std}$ ) at the classical time point, *i.e.*  $1/40^{th}$  of the starting  $N_2$  conc. = 2.5%  $N_2$  cet, and nine earlier alternative time-points of the three  $N_2$ MBW curves from each child. Using customized software (Matlab R2006a; The MathWorks, Inc.) we calculated the standard LCI ( $LCI_{std}$ ) at 2.5%  $N_2$  cet and nine LCI from empirically chosen  $N_2$  cet cut-offs: 18% ( $LCI_{18}$ ), 15% ( $LCI_{15}$ ), 12% ( $LCI_{12}$ ), 9% ( $LCI_9$ ), 7% ( $LCI_7$ ), 6% ( $LCI_6$ ), 5% ( $LCI_5$ ), 4% ( $LCI_4$ ), 3% ( $LCI_3$ ), and the standard 2.5% ( $LCI_{2.5} = LCI_{std}$ )  $N_2$  cet. All LCI were calculated as cumulative expired volume/FRC at the respective cut-offs. Standard and alternative LCI give the number of lung turn overs required to washout out the lungs until the respective target  $N_2$  cet is reached, thus respective LCI units are lung turnovers (TO).

### *Less than three multiple-breath washout tests*

From standard  $N_2$ MBW until 2.5%  $N_2$  cet, we assessed duration and diagnostic performance of (i) single LCI ( $LCI_{single}$ ) of the first  $N_2$ MBW run and of (ii) mean LCI ( $LCI_{double}$ ) of the first and second  $N_2$ MBW runs in comparison to the standard mean LCI ( $LCI_{std}$ ) of three runs.

### *Ethics statement*

The study was approved by the Ethics Committee of the Canton of Bern, Switzerland. The children`s assent was obtained and parents or caregivers provided written informed consent for this study.

### *Statistical Methods*

A recruitment goal of 80 children was chosen to match our sample size estimation ( $N = 60$ ) taking into account a failure rate of approximately 20% as described previously in school-aged children (7-9). Prior to analysis we defined quality criteria to be met by alternative LCI measurements: (i) Predictive value ( $R^2$ ) for  $LCI_{std} > 90\%$  or limits of agreement  $\leq 5\%$  (ii)

coefficient of variation (CV) or relative difference between two  $\text{LCI} \leq 7\%$  and (iii) sensitivity and specificity within  $\pm 3\%$  of  $\text{LCI}_{\text{std}}$ .  $R^2$  for  $\text{LCI}_{\text{std}}$  was calculated from uni-variable linear regression models. Intra-test repeatability of LCI from three  $\text{N}_2\text{MBW}$  runs ( $\text{LCI}_{\text{std}}$ ,  $\text{LCI}_{18-3}$ ) was calculated as intra-test CV. Intra-test repeatability of  $\text{LCI}_{\text{double}}$  was calculated as the relative difference (%) of the first minus the second LCI divided by the mean of both LCI ( $\text{LCI}_{\text{double}}$ ). Agreement between  $\text{LCI}_{\text{single}}$  and  $\text{LCI}_{\text{double}}$  with  $\text{LCI}_{\text{std}}$  was assessed by Bland Altman plots (10). By means of receiver operating characteristic curves (ROC) and LCI cross-plots, concordance between LCI, and sensitivity and specificity for CF lung disease were assessed. Upper limit of normal (ULN; mean LCI + 1.96 standard deviations [SD]) and LCI z-scores were defined from the healthy children. LCI z-score values were calculated from the corresponding LCI values in a child with CF minus the mean LCI of the healthy children divided by the LCI SD of healthy children. Thus normal ranges for standard and alternative LCI were based on the study's healthy cohort. Test duration was compared by paired t-test. P-values  $< 0.05$  were considered statistically significant. All analyses were done using Stata<sup>TM</sup> (Stata Statistical Software: Release 11. College Station, TX: StataCorp LP).

## Results

Technically acceptable triplicate N<sub>2</sub>MBW were obtained in 68 (31 boys) children (44 with CF). Reasons for exclusion were leaks in 7 and FRC variability > 20% in 5 children. Mean (range) age of children with CF was 12.0 (5.7 to 16.9) and 12.4 (7.3 to 15.9) years in controls. ULN for LCI<sub>std</sub> was 7.56 (6.44+[1.96\*0.57]) (Table 1). LCI<sub>std</sub> identified 36 (81.8%) CF children with abnormal lung function (LCI<sub>std</sub>> 7.56 TO and >2 z-scores). 27 (61.4%) children with CF had normal FEV<sub>1</sub> z-scores (< -2 z-scores) but abnormal LCI<sub>std</sub> (> 2 z-scores). Mean (SD) FRC intra-test CV in CF was 6.2% (4.2%) and 5.4% (3.7%) in controls. FRC variability was < 10% in 56 children (82%), better than 15% in 11 children (16%), and 17.5% in one child.

### *Early stop in multiple-breath washout*

Calculating standard and shortened LCI was feasible in all N<sub>2</sub>MBW measurements (n = 204, Table 1). LCI<sub>5</sub>, reflecting N<sub>2</sub> washout until 1/20<sup>th</sup>, was the earliest time point in N<sub>2</sub>MBW to meet the predefined quality criteria (i) R<sup>2</sup> > 90%, (ii) CV < 7%, and (iii) sensitivity and specificity within 3% of LCI<sub>std</sub> (Table 1 and 2). LCI<sub>5</sub> predicted 93% of LCI<sub>std</sub> (Figure 1). CV of LCI<sub>5%</sub> was 5.1%, compared to 5.4% of LCI<sub>std</sub> (Table 2). LCI<sub>5</sub> and LCI<sub>std</sub> were both equally specific (100%) and provided a sensitivity of 82.6% and 83.0% for CF lung disease. ULN for LCI<sub>5</sub> was 5.53 (4.86+[1.96\*0.34]). LCI<sub>5</sub> identified 35 (79.5%) CF children with abnormal lung function (LCI<sub>5</sub>> 5.53 TO and >2 z-scores). LCI<sub>5</sub> and LCI<sub>std</sub> concordantly classified 63 (92.6%) children (Figure 1). Mean (range) test duration for LCI<sub>5</sub> in CF was 8.8 (3.8-18.4) min. and in controls 8.5 (5.0-12.1) min. being significantly shorter than for LCI<sub>std</sub> in CF 12.6 (4.7-26) min. and controls 10.8 (5.9-15.6) min.. This represents an average time saving of 30% in CF (p < 0.001) and 21% in controls (p < 0.001, Table 1).

Every LCI calculated at cut-offs earlier than 5% N<sub>2</sub> cet was highly associated with LCI<sub>std</sub> and had low intra-test variability (CV) ranging from 4.8% to 5.4% in CF and from 5.0% to 6.8%

in controls. However, all time points for LCI earlier than 5% N<sub>2</sub> cet provided less sensitivity and specificity with more than 3% difference compared to LCI<sub>std</sub> (Table 1).

*Less than three multiple-breath washout tests*

LCI<sub>single</sub> and LCI<sub>double</sub> were very similar to and highly associated with LCI<sub>std</sub>, and both had similarly high diagnostic performance for CF lung disease (Table 3). Prediction of LCI<sub>std</sub> (R<sup>2</sup>) was 97% and 99%, respectively (Figures 2 and 3). Sensitivity and specificity were similar or even higher compared to LCI<sub>std</sub> (Table 3). LCI<sub>single</sub> and LCI<sub>double</sub> were significantly faster obtained than LCI<sub>std</sub>. For LCI<sub>double</sub>, relative mean (SD) intra-test difference between the first and second run was 6.8 (4.9)%. Mean (range) test duration for LCI<sub>single</sub> was 2.2 (0.4-5.2) min. in CF and 1.7 (0.9-2.8) min. in healthy controls, for LCI<sub>double</sub> 7.4 (2.4-16.0) min. in CF and 6.2 (3.5-9.4) min. in healthy controls (Table 3). Average time savings were 82% in CF and 84% in controls for LCI<sub>single</sub>, and 41% in CF and 42% in controls for LCI<sub>double</sub> (all p < 0.001). However, only LCI<sub>double</sub> was within 5% limits of agreement compared to LCI<sub>std</sub> (data not shown).

Based on these data, the shortest protocol for N<sub>2</sub>MBW would be LCI<sub>5</sub> from the first two N<sub>2</sub>MBW runs. However, LCI<sub>5</sub> based on two runs predicted only 88% of LCI<sub>std</sub>.



## Discussion

### *Comparison to previous studies*

N<sub>2</sub>MBW can be reliably and significantly abbreviated. LCI<sub>5</sub> and LCI<sub>double</sub> predicted 93-99% of LCI<sub>std</sub> and provided the same or better diagnostic performance for pediatric CF lung disease. These data may help implementing LCI measurement in clinical routine. Previous studies focused on classical tracer gas MBW until 1/40<sup>th</sup> showing reliability of LCI across age groups (7;11) or LCI sensitivity for *e.g.* structural changes in chest CT scans (12;13). None of these studies primarily aimed to increase practicability of LCI measurement for clinical routine. Recently one retrospective study in a large pediatric cohort (n = 198) elegantly showed that variability in FRC between two tests may approach 27% in children with CF and controls (6). Despite this variability, LCI derived from two sulfur hexafluoride (SF<sub>6</sub>)MBW runs classified 94% of children correctly. While these data confirm our findings (concordance of LCI<sub>double</sub> = 100%, Figure 3), they may be less applicable for clinical routine. Hard- and software used by Robinson *et al.* (6) are not available any longer and SF<sub>6</sub> use is restricted in many countries. The setup did not allow for real-time monitoring of FRC and LCI which certainly is important to prospectively assess whether two or three MBW are needed.

Abbreviated N<sub>2</sub>MBW protocols in our study significantly reduced test duration. These protocols potentially increase feasibility in routine care and acceptance in younger children. Previous studies have reported MBW success rates of 80% on average in school-age children for three valid MBW runs (7-9). Possible reasons for test failure were leaks or coughing and irregular breathing due to increasing discomfort. Earlier stop of MBW will *per se* increase success rates in any setting.

LCI<sub>double</sub> seems especially attractive in young children with predominantly mild CF lung disease. Complete N<sub>2</sub>MBW until 1/40<sup>th</sup> is usually achieved fast in mild CF lung disease. The LCI<sub>double</sub> provided the highest sensitivity in our study and thus provides sufficient confidence

to detect mild CF lung disease. The  $LCI_5$  may be more applicable *e.g.* in advanced lung disease where  $N_2$ MBW requires much time, and LCI measurement is more variable within test sessions. Above an  $LCI_{std}$  of 10 correlation with  $LCI_5$  gets poorer (Figure 1), mostly with a slight underestimation of  $LCI_{std}$  by  $LCI_5$ . The trend of higher  $LCI_{std}$  compared to  $LCI_5$  may be due to extremely slowly ventilated lung regions at the end of long washout and possible bias from tissue  $N_2$  for which no standard correction algorithm exists (14). Bias from tissue  $N_2$  may decrease with abbreviated  $N_2$ MBW test duration, thus  $LCI_5$  may be even more accurate in advanced lung disease than  $LCI_{std}$ . Which MBW protocols are best used in respective patient groups need to be established in future as well as comparison of long-term repeatability and response to intervention between MBW protocols.

#### *Physiological aspects*

We are the first to show that  $N_2$ MBW is not required until  $1/40^{th}$  of initial  $N_2$  cet to measure LCI. Our data clearly indicate that during the last third of  $N_2$ MBW, sensitivity and specificity of LCI only marginally increase while test duration significantly increases. Given the exponential decrease of  $N_2$  cet as also previously described (15), it is not surprising that LCI measured at  $N_2$  cet targets in the flatter tail of the  $N_2$ MBW curve contain very similar information. The shape of the  $N_2$ MBW curve also suggests at least two functional lung compartments being sequentially washed out with the second phase believed to reflect peripheral airways (16). This portioned washout behavior seems even more pronounced in advanced CF lung disease with prolonged washout in slowly ventilated lung regions. This probably explains lower agreement between  $LCI_5$  and  $LCI_{std}$  in those subjects with high  $LCI_{std}$  values (Figure 1). Nevertheless, we found that  $N_2$ MBW until  $1/20^{th}$  is sufficiently robust in the current study population consisting mainly of mild CF lung disease regarding the predefined quality measures. Despite lower agreement between  $LCI_5$  and  $LCI_{std}$  for higher

LCI<sub>std</sub> values, LCI<sub>5</sub> might be an even more robust marker of ventilation inhomogeneity in advanced CF lung disease, being less susceptible to bias from tissue N<sub>2</sub>.

We also show that performance of LCI<sub>double</sub> measured from two N<sub>2</sub>MBW is very similar to LCI<sub>std</sub> from three N<sub>2</sub>MBW. To our knowledge the only reason for three tests in previous studies is that tidal breathing online quality control is scarce thus requiring *post-hoc* quality control, *i.e.* calculating CV. Indeed, full FRC stability within tests cannot be achieved in children and even in adults (3;4). However, LCI is more robust than FRC. LCI, a volume ratio, partly cancels out variability of the denominator FRC. Our and previous data suggest that LCI from two MBW provide sufficient sensitivity and specificity for pediatric CF lung disease. This is even more true as modern MBW setups display flow-volume-loops, CEV, and FRC for online quality control. Considering potential bias on airway calibers due to prolonged dry tracer gas exposure, less than three MBW tests seem definitely preferable.

#### *Methodological considerations*

LCI<sub>5</sub> and LCI<sub>double</sub> reliably identified altered gas mixing efficiency in children with CF. The N<sub>2</sub> cut-offs and quality criteria were defined prior to analyses. However, cut-offs and quality criteria are arbitrary to some extent and further studies assessing these in different CF populations and other disease groups such as primary ciliary dyskinesia or sickle cell disease seem important (17). Whether our findings apply to customized equipment and other tracer gases needs to be determined.

Similar to most other MBW studies we used “CF diagnosis” as proxy for assessing sensitivity and specificity of LCI. Previous data suggest reasonable agreement between actual structural airway pathology detected in HRCT and LCI (12;13;18) . In our study, 36 (82%) children with CF had abnormal LCI<sub>std</sub>, which is in agreement with the prevalence in other pediatric CF cohorts (12). However, we did not apply HRCT to ascertain abnormal LCI findings. Further,

the impact of functional or radiological measures of small airway pathology on CF care and respiratory disease outcomes remains to be determined.

Additional analysis of N<sub>2</sub>MBW, *i.e.* phase III slopes for Scond and Sacin measurement, may be feasible from abbreviated N<sub>2</sub>MBW protocols. Due to the inherent variability of Scond and Sacin, two tests may be insufficient (3). However, Scond and Sacin were not the focus of this paper and may be less suitable for routine assessments (19;20).

## FIGURE LEGENDS

**Figure 1. Early stop in multiple-breath washout.** Concordance of lung clearance index (LCI) measured at 5% end-tidal nitrogen concentration ( $N_2$  cet) vs. standard LCI at 2.5%  $N_2$  cet from three  $N_2$ MBW. Healthy children ( $n = 24$ ) are displayed as closed circles and children with CF ( $n = 44$ ) as open circles. The dashed lines correspond to the upper limits of normal LCI (mean LCI + 1.96 standard deviations from healthy controls).

**Figure 2. Less than three multiple-breath washout tests - single LCI.** Concordance of lung clearance index (LCI) from the first nitrogen multiple-breath washout run vs. standard LCI (mean of three runs). Healthy children ( $n = 24$ ) are displayed as closed circles and children with CF ( $n = 44$ ) as open circles. The solid black line gives the line of identity. The dashed lines correspond to the upper limits of normal LCI (mean LCI + 1.96 standard deviations from healthy controls).

**Figure 3. Less than three multiple-breath washout tests - double LCI.** Concordance of lung clearance index (LCI) from the first and second nitrogen multiple-breath washout runs vs. standard LCI (mean of three runs). Healthy children ( $n = 24$ ) are displayed as closed circles and children with CF ( $n = 44$ ) as open circles. The solid black line gives the line of identity. The dashed lines correspond to the upper limits of normal LCI (mean LCI + 1.96 standard deviations from healthy controls).

**Table 1: The early stop - standard and alternative lung clearance index at different N<sub>2</sub> concentrations.**

N <sub>2</sub> cet (%)	ULN	Time (min)		Sens. (%)	Spec. (%)	Correct (%)	R <sup>2</sup>
		CF	Controls				
2.5*	7.56	12.6 ±4.5	10.8 ±2.9	83.0	100	88.2	n.a.
3	6.83	11.4 ±3.9	10.1 ±2.6	85.1	100	89.7	0.99
4	6.03	9.8 ±3.3	9.1 ±2.3	85.7	100	89.7	0.96
5	5.53	8.8 ±2.8	8.5 ±2.1	82.6	100	86.8	0.93
6	5.14	8.1 ±2.6	8.0 ±1.9	78.8	100	85.3	0.92
7	4.83	7.6 ±2.3	7.7 ±1.8	76.5	100	83.8	0.89
9	4.37	6.8 ±2.0	7.0 ±1.6	74.0	100	82.4	0.87
12	3.82	6.0 ±1.7	6.3 ±1.4	70.5	99.4	80.9	0.82
15	3.38	5.4 ±1.4	5.8 ±1.3	70.5	98.9	80.9	0.78
18	3.07	4.9 ±1.3	5.4 ±1.2	68.2	98.1	79.4	0.74

Lung clearance index (LCI) data from 68 children (44 with CF) with triplicate nitrogen multiple breath washout (N<sub>2</sub>MBW) tests. Test duration (time in minutes) required to clear the lungs until respective target end-tidal N<sub>2</sub> concentration (cet) is given as mean (±SD). \*2.5% N<sub>2</sub> cet of initial N<sub>2</sub> cet reflects the standard for LCI. Upper limits of normal LCI (ULN) is mean LCI + 1.96 SD from healthy controls. Sensitivity (Sens.), specificity (Spec.), and correctly classified (Correct) children are given in percent. R<sup>2</sup> gives the linear association of alternative LCI with standard LCI. n.a. = not applicable.

**Table 2: Variability of standard and alternative lung clearance index.**

	<b>Cystic Fibrosis</b>	<b>Healthy controls</b>
Standard LCI (TO)	10.1 ±2.5 (6.5-15.9)	6.4 ±0.6 (5.5-7.3)
Intra-test CV	5.4%	5.4%
LCI <sub>5</sub> (TO)	6.8 ±1.3 (4.8-9.4)	4.9 ±0.3 (4.2-5.4)
Intra-test CV	5.0%	5.1%

Standard lung clearance index (LCI) measured at 2.5% (1/40<sup>th</sup>) and at 5% (1/20<sup>th</sup>) of initial end-tidal nitrogen concentration differed significantly between 44 children with CF and 24 healthy controls ( $p < 0.001$  for both). Data are given as mean ±SD (range) and are compared by unpaired t-test. LCI units are lung turnovers (TO). LCI intra-test coefficient of variation (CV) is derived from three nitrogen multiple-breath washout runs.

**Table 3: Less than three - upper limit of normal, duration, and diagnostic accuracy of LCI.**

	ULN	Time (min)		Sens. (%)	Spec. (%)	Correct (%)	R <sup>2</sup>
		CF	Controls				
<b>LCI<sub>single</sub></b>	7.66	2.2 ±1.0	1.7 ±0.6	84	97.5	89	0.97
<b>LCI<sub>double</sub></b>	7.58	7.4 ±2.9	6.2 ±1.8	87	100	92	0.99
<b>LCI<sub>std</sub></b>	7.56	12.6 ±4.5	10.8 ±2.9	84	100	89	n.a.

Lung clearance index (LCI) data from 68 children (44 with CF) based on one (LCI<sub>single</sub>), two (LCI<sub>double</sub>), and three (standard - LCI<sub>std</sub>) nitrogen multiple breath washout (N<sub>2</sub>MBW) tests. Test duration (time in minutes) required to clear the lungs until 1/40<sup>th</sup> of initial end-tidal nitrogen concentration is given as mean (±SD). Upper limits of normal LCI (ULN) is mean LCI + 1.96 SD from healthy controls. Sensitivity (Sens.), specificity (Spec.), and correctly classified (Correct) children are given in percent. R<sup>2</sup> gives the linear association of alternative LCI with standard LCI. n.a. = not applicable.



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