

Feasibility and Variability of Measuring the Lung Clearance Index in a Multi-Center Setting

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Summary. The Lung Clearance Index (LCI) is superior to spirometry in detecting early lung disease in cystic fibrosis (CF) and correlates with structural lung changes seen on CT scans. The LCI has the potential to become a novel outcome parameter for clinical and research purposes. However longitudinal studies are required to further prove its prognostic value. Multi-center design is likely to facilitate realization of such studies. Therefore the aim of the present study was to assess multi-center feasibility and inter-center variability of LCI measurements in healthy children and adolescents. Comparative measurements were performed in unselected patients with CF to confirm previous single-center results. LCI measurements were performed in eight centers using the EasyOne Pro, MBW Module (nDD Medical Technologies, Zurich, Switzerland). The overall success rate for LCI measurements was 75.5%, leaving 102/151 measurements in healthy volunteers and 139/183 measurements in patients with CF for final analysis. Age ranged between 4 and 24 years. Mean LCI (range of means among centers) was 6.3 (6.0–6.5) in healthy volunteers and thus normal. Inter-center variability of center means was 2.9%, ANOVA including Schffé procedure demonstrated no significant inter-center differences ($P > 0.05$). Mean LCI (range of means among centers) was 8.2 (7.4–8.9) in CF and thus abnormal. Our study demonstrates good multi-center feasibility and low inter-center variability of the LCI in healthy volunteers when measured with the EasyOne Pro MBW module. Our data confirm published LCI data in CF. However, central coordination, quality control, regular training, and supervision during the entire study appear essential for successfully performing multi-center trials. **Pediatr Pulmonol.** © 2011 Wiley Periodicals, Inc.

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INTRODUCTION

Although mortality has improved substantially over the last decades, pulmonary disease remains the major cause of morbidity and mortality in patients with cystic fibrosis (CF). Lung disease resulting from infection and inflammation already starts in the first years of life often without any apparent clinical symptoms or deterioration of conventional spirometry.^{1–3} The current challenge with regard to management of CF lung disease is to identify treatments that will halt pulmonary changes before irreversible damage has occurred.

A number of diagnostic tools have been applied to characterize lung disease in CF, including bronchoscopy and bronchoalveolar lavage and high resolution computed tomography (HRCT) for visualization of structural changes, both of which are currently being discussed as surrogate endpoints in CF clinical trials.^{4,5} However, bronchoscopy is an invasive procedure; the routine use of HRCT remains controversial because of the radiation exposure.⁶

In contrast, measurements of lung function are non-invasive. The only parameter of lung function that is currently recognized as a surrogate endpoint in CF trials is the forced expiratory volume in one second (FEV₁). However, FEV₁ mainly reflects large conducting airways⁷ and is relatively insensitive to changes in the small airways where CF lung disease starts. It has been demonstrated that significant structural lung damage can be present on HRCT despite normal spirometry.^{8–10} Furthermore, as a consequence of improved patient management, many children have FEV₁ well within the normal range and the rate of decline of FEV₁ has steadily declined over the last decade.^{11,12} Thus, power calculations show that many hundreds of patients would need to be treated for a long time to see any beneficial effect of a novel therapeutic approach in early lung disease when FEV₁ is used as the primary surrogate outcome parameter.^{4,5} Early CF lung disease is characterized by changes in the peripheral airways beyond the 8th bronchus generation resulting in inhomogeneous ventilation.⁷ Inert gas multiple breath wash-out (MBW) allows assessment of these peripheral airways. The Lung Clearance Index (LCI) is a marker of altered ventilation homogeneity that can be calculated from MBW curves.^{13,14} Previous cross-sectional studies carried out in single centers have demonstrated that the LCI identifies CF lung disease more often than FEV₁^{15–17} and that the LCI correlates with structural changes observed in CT scans.¹⁸ Almost 68% of a pediatric CF population with a normal FEV₁ presented both, structural changes in CT-scans and an increased LCI.^{8,9} In addition, compared to spirometry the LCI has the advantage that it is constant through childhood and adolescence eliminating the need to adjust for age,

height, or gender related differences.²² The LCI thus qualifies as a sensitive outcome parameter for both, clinical and research purposes.

However longitudinal studies are required before the discussion about the value of using the LCI as a potential new surrogate marker in clinical trials and for routine monitoring of CF lung disease can be concluded. As multi-center design is likely to facilitate realization of such studies, multi-center feasibility and inter-center variability needs to be assessed.

An MBW device using a sidestream ultrasonic flow sensor (*EasyOne Pro, MBW Module*, ndd Medical Technologies, Zurich, Switzerland) which has the potential of being used in clinical routine was validated in a stepwise single center validation program and the prototype has now reached a status allowing use in a multi-center study setting.^{15,19–23}

We hypothesize that

- assessment of the LCI in a multi-center network is feasible in healthy volunteers and patients with CF,
- observer and equipment related variability of the LCI is low between different centers, and
- previous results derived from single center studies are reproducible in a multi-center setting.

Therefore the aims of the present study were

- to establish a network of CF centers with an expertise in performing MBW,
- to assess multi-center feasibility of MBW using the *EasyOne Pro* in healthy volunteers and patients with CF,
- to assess inter-center variability in healthy volunteers, and
- to confirm previous, single-center results of the LCI in a multi-center setting.

METHODS

Study Sites and Ethics

For the present multi-center study six German CF centers (Hannover, Hamburg, Berlin, Munich, Essen, and Cologne) and the CF center in Innsbruck collaborated with the leading center in Wesel. The study was mainly conducted between the 1st of February and the 31st of July 2010.

The study coordinator visited each study site prior to the study in order to ensure correct and complete installation of software, hardware and further MBW related requirements, such as disinfection facilities, pulse oximetry, and television.

All staff assigned to and responsible for conducting the project was additionally introduced to the study and

trained in performing MBW during a workshop that was organized by the leading center Wesel in January 2010. Phone contact, supervision of measurements and additional site meetings with the study coordinator were performed on demand. A monitoring visit to the German study sites was performed after data collection was finished.

The present study was approved by the ethics committee of the Medizinische Hochschule Hannover, Germany, where the study was initiated originally.

According to present regulatory requirements each of the collaborating centers informed their respective local ethics committee about the project.

Informed written parental consent and—if applicable—children's assent were obtained prior to the measurements.

Study Population and Protocol

We aimed for a total sample size of $n = 160$ healthy controls and $n = 160$ patients with CF ($n = 20$ healthy volunteers and $n = 20$ patients with CF per center) collected in eight centers.

Healthy volunteers between 4 and 18 years of age were recruited from patient families and colleagues for a single test occasion including three single MBW measurements, spirometry, and a questionnaire.

Definition of a healthy individual excluded diagnosis of asthma, presence of other severe or chronic lung diseases, prematurity, and presence of other severe diseases.

For the CF population unselected patients between 4 and 18 years of age were recruited from the CF populations regularly attending CF clinics in each study site. Patients were asked to perform three single MBW measurements prior to spirometry during a routine visit at their CF clinic. Demographic data and the current status of *Pseudomonas aeruginosa* (PSA) infection were obtained from in- and out-patient records.

MBW

MBW measurements were performed using the Easy-One Pro, MBW Module (nDD Medical Technologies).²⁴ All participating centers purchased this equipment prior to the study.

The system consists of a side stream ultrasonic transducer for temperature and humidity independent sampling of the molar mass (MMss), a mainstream ultrasonic transducer for flow sampling and a side stream infrared CO₂ analyzer (DUET ETCO2 Module, Welch Allyn OEM Technologies, Beaverton, OR) to correct the MMss signal for exhaled CO₂. The gas bias flow system provides valve-controlled tracer gas delivery

containing 4% sulfur hexafluoride (SF₆), 21% oxygen and balanced nitrogen. WBreath[®] software (nDD, Switzerland) is used for data acquisition, storage, and analysis. Following visual quality control, LCI, and functional residual capacity (FRC) are automatically calculated according to published procedures^{15,25} that are implemented into the software.

LCI is defined as the number of lung volume turnovers needed until the lungs are cleared from the inert tracer gas that has been inhaled before. An increased LCI (>7.0) indicates ventilation inhomogeneity¹⁹ and thus, changes in peripheral airways.

MBW measurements are performed in a sitting position, breathing through a mouth piece, and wearing a nose clip. The patients watch a video during the test procedure to facilitate a regular breathing pattern. Pulse oximetry is used for monitoring oxygen saturation and heart rate during the measurement.

After 10 breaths of room air, the washin phase is started by switching the device to the tracer gas at end expiration. Having reached equilibrium, patients are switched back into room air to start the washout phase. The washout phase is defined to be complete when the tracer gas concentration has fallen below 2.5% (1/40) of the starting level.

Measurements were performed in triplicate and according to the protocol developed and practiced in the leading center.

The leading center in Wesel set up a central data base for all of the data generated in each of the study sites.

All MBW tests underwent central quality control in the leading center. Predefined quality control criteria for a technical acceptable test were the following:

- regular tidal breathing,
- stable plateau of the side stream MMss signal at the 4%-SF₆-level indicating end of washin phase,
- stable plateau of the side stream MMss signal at the room-air-level indicating end of wash-out phase, and
- absence of a leak.

Three technical acceptable MBW tests or at least two with an FRC difference of <10% were required for including the participant into final analysis. Analysis was performed centrally by a single experienced operator.

Spirometry

Measurements were performed according to ERS/ATS criteria using the local equipment available in each of the study sites.²⁶

Results of the best approach were documented in the test protocol and sent to the leading center for documentation.

Questionnaire

Healthy participants and their parents were asked to fill in a questionnaire regarding their medical history to identify exclusion criteria as defined above.

Statistics

Demographic data such as age, weight, and height are reported as mean, standard deviation (SD), and range. A one-way ANOVA analysis was used to assess center differences regarding age, sex weight, and height. An additional Scheffé procedure was used to account for unequal group sizes in the centers.

Unpaired *t* tests were used to assess differences between the patients with CF and the healthy population.

Individual data for LCI and FRC are reported as the mean of two to three technically acceptable washout maneuvers within one test occasion for each subject. According to published normal data assessed in healthy children and adolescents and with the same equipment used in this study, the upper limit of normal for the LCI was 7.0.¹⁹

Within-test repeatability is defined as the variability of LCI in an individual and is derived from the three or at least two single washout maneuvers within one test occasion with an FRC that differed by less than 10%. Within-test repeatability is expressed as the intra individual coefficient of variation (CV%) that is defined as $(SD/mean) \times 100$.

Spirometric parameters are expressed as Z-score (SD score = (measured value – predicted value)/SD within the reference population), where results < -2 SD are defined as abnormal.²⁷

Inter-center variability of the LCI in the healthy population was calculated from the mean LCI in either center and is expressed as CV%. A one-way ANOVA analysis including a Scheffé procedure was used for assessing inter-center differences of the LCI, Z-FEV₁, and Z-MEF₂₅ in this group.

For investigating the inter-center variability of the LCI in the healthy population it was estimated, that 10 individuals/center would be sufficient to detect a difference in LCI of >1.0 between the centers with a power of 94% at a two sided 5% significance level. This estimation was based on the known inter individual SD of the LCI (0.39) in healthy children and adolescents.¹⁹

The failure rate was estimated to be 10–25% depending on experience of the observer and age of the patient. This resulted in a minimum number of $n = 15$ of either healthy volunteers or patients with CF to include the group into subsequent analysis.

The statistical package used was SPSS version 16.0 for Windows.

RESULTS

Seven of eight MBW centers successfully performed MBW measurements. One of eight centers failed to recruit eligible subjects within the given time frame due to organizational reasons.

Six of seven centers provided a sufficient number of both measurements in patients and controls. One of seven centers only provided a sufficient number in patients. Measurements in healthy volunteers provided from this center ($n = 12$) were not included into any of the following calculations (but are provided in the discussion section to improve transparency of the data). Another three healthy volunteers were excluded due to presence of asthma or a history of prematurity that were defined as exclusion criteria prior to the study (Fig. 1).

One hundred eighty-three patients with CF and 136 healthy volunteers were recruited for this study. All MBW measurements underwent central quality control. Forty-four of 183 (24.0%) measurements in CF and 34/136 (25%) measurements in healthy volunteers were excluded due to lacking compliance related to irregular

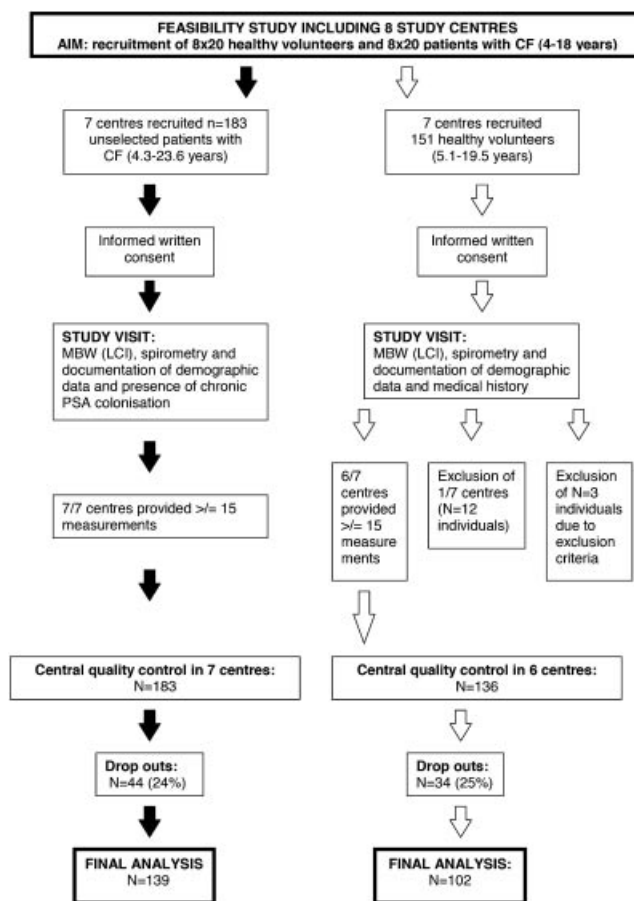


Fig. 1. Participant flow during the study.

tidal breathing, evidence of a leak or incomplete measurements. This led to a success rate for MBW within the entire study population of 75.5% that was not different between patients and healthy volunteers. However, the success rate between the centers ranged between 63% and 86% and was different between patients and healthy volunteers in some (Table 1).

Final analysis was therefore based on n = 102 measurements in healthy volunteers and n = 139 measurements in CF (Fig. 1).

Healthy Volunteers

Mean age (range) of the healthy volunteers was 12.5 (5.1–19.5) years. There were no statistical differences with regard to sex, height, and weight between the centers ($P \geq 0.05$); age was slightly different. Demographic data of each center are listed in Table 2.

Seventy-five of 102 (73.5%) healthy volunteers completed the questionnaire. Online Supplement Table S1 illustrates adherence to exclusion criteria defined for the healthy population. Lung function data derived from MBW (n = 102), spirometry (n = 75), and pulse oximetry (n = 101) in healthy volunteers are listed in Table 2. One of six centers included into final analysis did not perform spirometry in healthy controls (n = 19) at all. This was due to organizational reasons. N = 8 spirometries had to be excluded due to lacking compliance or insufficient technical quality.

Mean Z-FEV₁ was -0.12 and varied within ±1 SD between the centers ($P \leq 0.001$). Mean Z-MEF₂₅ was -0.76 and varied within 0.5 SDs between the centers ($P = 0.05$).

Mean LCI (SD) of six centers was 6.3 (0.19) with a variability of means among the centers of 2.9%. LCI was not different between the centers ($P \geq 0.05$) (Fig. 2).

There was no correlation between LCI and age, sex weight, or height ($P \geq 0.05$).

TABLE 1—Success Rate of MBW in Patients With CF (n = 183) and Healthy Controls (n = 136) Derived From Eight Centers

Center no.	Number of tests in healthy volunteers		Number of tests in CF	
	Provided	Valid(%)	Provided	Valid (%)
1	26	20 (77)	22	11 (50)
2	22	15 (68)	22	21 (96)
3	—	—	—	—
4	26	20 (77)	26	23 (89)
5	20	19 (95)	37	30 (81)
6	—	—	22	16 (73)
7	15	11 (73)	31	18 (59)
8	27	17 (63)	23	20 (87)

The LCI did not correlate with Z-FEV₁ ($P = 0.215$) or Z-MEF₂₅ ($P = 0.58$).

Patients With CF

Mean age (range) of the patients with CF was 11.8 (4.3–23.6) years. There were no statistical differences regarding age, gender, height, and weight between the centers ($P \geq 0.05$). Demographic data of each center are listed in Table 3.

Lung function data derived from MBW (n = 139), spirometry (n = 132), pulse oximetry (n = 99), and extent of chronic pseudomonas infection (n = 131) in patients with CF are listed in Table 3. LCI in patients with CF was increased in both any single-center and the entire study population. In contrast, mean Z-FEV₁ was within the normal range of ±2 SD in the entire study population.

However, mean Z-FEV₁ was normal in 5/7 centers, below -2 in the other two. These two centers were those with the highest mean LCI and the highest percentage of individuals with chronic PSA infection.

The lowest mean LCI among the participating centers was found in the center with the highest mean Z-FEV₁ and the lowest percentage of individuals with chronic PSA infection.

LCI in CF correlates with age ($P = 0.011$) (Fig. 3) and length ($P = 0.019$), but not with sex ($P = 0.546$) and weight ($P = 0.298$).

LCI in CF correlates with Z-FEV₁ ($P \leq 0.001$) and with Z-MEF₂₅ ($P \leq 0.001$).

CF and Healthy Volunteers

Demographic data in patients with CF and healthy volunteers were not different regarding age and sex, but different regarding height and weight.

For MBW measurements, the within subject repeatability expressed as intra-individual CV% was not different between patients and healthy volunteers ($P = 0.974$ for FRC and $P = 0.077$ for LCI) (Tables 2 and 3).

Lung function parameters were statistically different for LCI ($P \leq 0.001$, 95%CI: -2.22; -1.48), Z-FEV₁ ($P \leq 0.001$, 95%CI: 0.61; 1.88), Z-MEF₂₅ ($P \leq 0.001$, 95%CI: 1.69; 3.48), and oxygen saturation ($P \leq 0.001$, 95%CI: 0.79; 1.56). However, FRC did not differ between the groups ($P = 0.098$, 95%CI: -0.02; 0.29).

Individual results for the LCI in patients with CF compared to healthy volunteers are illustrated in Figure 3.

Online Supplement Figure S1a,b illustrate the relation between LCI and spirometry for individuals. The upper quadrant on the right hand side represents individuals with an abnormal LCI in the presence of a normal Z-FEV₁ and normal Z-MEF₂₅, respectively.

TABLE 2—Demographic and Lung Function Data of n = 102 Healthy Controls Derived From Six Study Centers

Center	No. 1 (n = 20)	No. 2 (n = 15)	No. 4 (N = 20)	No. 5 (n = 19)	No. 7 (n = 11)	No. 8 (n = 17)	No. 1–8 (n = 102)
Age (years)	10.8 (2.9)	12.1 (3.7)	14.3 (3.1)	12.5 (3.7)	11.0 (3.2)	13.7 (2.8)	12.5 ² (3.4)
Male (%)	12 (60.0)	8 (53.3)	8 (40.0)	4 (21.0)	5 (45.5)	10 (59.0)	47 (46.1)
Height (cm)	144.0 (15.0)	151.1 (14.2)	162.0 (12.4)	151.7 (20.4)	145.2 (18.6)	161.0 (15.8)	152.4 (19.0)
Weight (kg)	40.8 (15.8)	43.4 (14.8)	54.2 (14.8)	46.7 (17.8)	40.1 (14.4)	51.0 (19.3)	46.5 (16.8)
LCI	6.5 (0.52)	6.4 (0.51)	6.5 (0.42)	6.0 (0.32)	6.3 (0.55)	6.3 (0.53)	6.3 (0.49)
LCI _{CV} (%)	7.14 (4.04)	3.64 (1.81)	3.47 (2.64)	4.34 (3.11)	6.20 (2.71)	4.55 (2.50)	4.85
FRC (L)	1.23 (0.45)	1.45 (0.43)	2.12 (0.61)	1.58 (0.68)	1.37 (0.53)	1.71 (0.54)	1.60 (0.62)
FRC _{CV} (%)	8.86 (6.5)	7.12 (4.79)	5.10 (2.84)	5.1 (3.1)	6.00 (1.36)	5.06 (3.40)	6.22
Z-FEV ₁	0.92 (1.14)	−0.55 (1.11)	−0.16 (1.30)	nd ¹	−0.01 (0.79)	−0.93 (1.11)	−0.21 (1.28)
Z-MEF ₂₅	−0.61 (1.29)	−0.86 (1.3)	−0.34 (1.16)	nd	−0.69 (2.54)	−1.29 (1.62)	−0.76 (1.58)
O ₂ -Satur. (%)	99.1 (0.45)	98.5 (0.8)	98.6 (1.3)	97.5 (0.8)	98.8 (0.8)	97.8 (0.8)	98.4 (1.0)
Heart-rate/min	87.3 (8.9)	90.7 (12.4)	76.6 (10.4)	79.6 (10.7)	84.1 (5.6)	78.8 (8.9)	82.4 (10.9)

Results are reported as mean (SD) apart from sex. Sex is reported as number of individuals (%).

¹nd, not done.

²P ≤ 0.05 (using ANOVA-analysis with Scheffe procedure).

DISCUSSION

Results from the present study demonstrate good multi-center feasibility of MBW when using the EasyOne Pro, MBW Module and low inter-center variability of the LCI in healthy volunteers. Our LCI data both from healthy volunteers and patients with CF confirm published data obtained in single-center studies.^{16,17,19,22,28}

Measurement of the LCI using the EasyOne Pro, MBW Module in a multi-center network is feasible in both, healthy volunteers and patients with CF. Seven of eight of the participating centers successfully implemented MBW tests in their lung function laboratory.

Group training of all professionals prior to the study and continued individual training and supervision throughout the study resulted in an overall success rate for MBW measurements of 75.5%. The percentage of valid tests within the entire study population did not differ between patients and healthy volunteers. However, the success rate between the centers ranged between 63% and 86% and was different between patients and healthy volunteers in some instances (Table 1).

In our previous MBW studies, the success rate was 96% in healthy children and adolescents and 83% in children and adolescents with CF. Reasons for excluding a patient from final analysis were limited compliance and/or an irregular breathing pattern as equally observed in the present study. Measurements in both studies were performed by a single experienced observer and with the same equipment used for the present study.^{8,19} The lower success rate in the present multi-center study is most likely due to the fact that all but two centers had no prior specific expertise in performing MBW. Although difficult to demonstrate statistically, the success rate improved with increasing study duration and increasing experience with the test procedure in each of the centers. This experience should be considered when planning future multi-center trials. Analogous to studies using FEV₁ as outcome parameter, documentation of the feasibility to perform an acceptable LCI measurement at the screening visit should be eligibility criterion for future trials when using the LCI as outcome parameter.

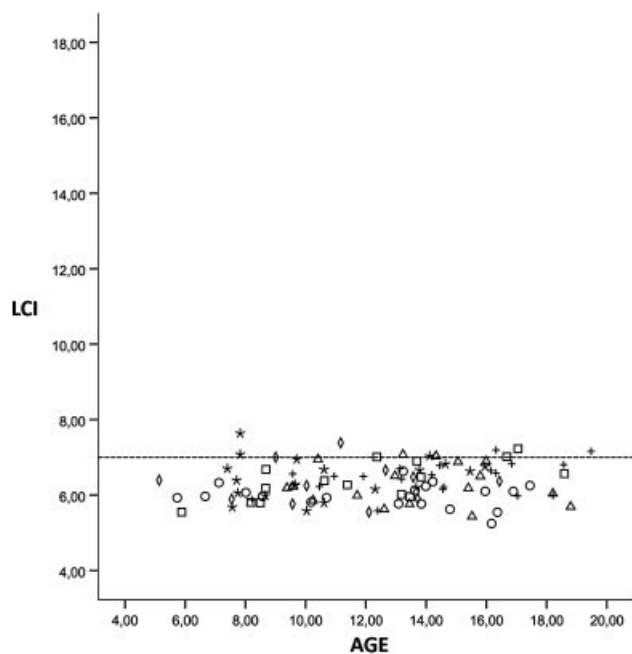


Fig. 2. LCI versus age in n = 102 healthy controls derived from six centers: Center 1, stars; center 2, squares; center 4, crosses; center 5, rings; center 7, rhombuses; center 8, triangles. Horizontal line: upper limit of normal (≤ 7) for the LCI using the EasyOne Pro, MBW Module.

TABLE 3—Demographic and Lung Function Data of n = 139 Patients With CF Derived From Seven Study Centers

Center	No. 1 (n = 11)	No. 2 (n = 21)	No. 4 (n = 23)	No. 5 (n = 30)	No. 6 (n = 16)	No. 7 (n = 18)	No. 8 (n = 20)	No. 1–8 (n = 139)
Age (years)	10.8 (2.5)	13.7 (4.2)	10.5 (3.7)	12.8 (3.0)	11.6 (3.2)	10.6 (3.0)	11.8 (3.4)	11.8 (3.5)
Male (%)	6 (54.0)	13 (61.9)	6 (26.1)	13 (43.3)	5 (31.3)	11 (61.1)	15 (75)	69 (49.6)
Height (cm)	138.3 (13.5)	153.7 (15.3)	141.1 (24.2)	150.8 (16.5)	145.6 (15.1)	139.3 (17.0)	147.6 (20.5)	146.1 (18.6)
Weight (kg)	29.6 (5.6)	44.4 (15.8)	36.9 (16.9)	41.7 (11.9)	37.4 (13.7)	34.3 (12.1)	37.4 (12.1)	38.3 (13.7)
LCI	8.57 (2.83)	8.83 (1.90)	8.07 (1.53)	7.45 (1.50)	8.86 (2.78)	8.03 (1.59)	8.01 (1.59)	8.17 (1.85)
LCI _{CV} (%)	6.21 (4.81)	6.13 (4.26)	6.31 (4.05)	5.94 (3.35)	4.30 (2.01)	5.48 (2.42)	4.62 (3.33)	5.64 (3.54)
FRC (L)	1.20 (0.62)	1.43 (0.57)	1.48 (0.68)	1.60 (0.59)	1.48 (0.48)	1.26 (0.42)	1.61 (0.74)	1.47 (0.60)
FRC _{CV} (%)	7.46 (3.18)	6.85 (4.10)	6.80 (6.27)	6.31 (4.08)	4.78 (3.82)	5.67 (2.42)	5.88 (3.78)	6.24 (4.22)
Z-FEV ₁	-1.79 (4.43)	-2.93 (3.75)	-1.03 (1.78)	-0.21 (1.69)	-2.58 (2.22)	-1.12 (1.93)	-0.79 (2.11)	-1.37 (2.63)
Z-MEF ₂₅	-3.71 (3.83)	-5.47 (4.54)	-2.78 (3.23)	-2.03 (3.67)	-4.90 (3.51)	-3.97 (3.10)	-1.68 (2.91)	-3.35 (3.76)
O ₂ -Satur. (%)	97.8 (1.0)	nd	97.1 (1.5)	96.9 (1.8)	nd ¹	97.4 (1.0)	97.0 (2.3)	97.2 (1.7)
Heart-rate/min	86.6 (9.1)	nd	94.4 (14.6)	85.1 (14.8)	nd	80.4 (8.3)	85.0 (12.4)	87.6 (13.7)
PSA (%)	2 (18.2)	7 (35.0)	4 (23.5)	4 (13.3)	6 (37.5)	3 (16.7)	3 (15.8)	29 (22.1)

Results are reported as mean (SD) apart from PSA infection and sex. PSA infection and sex are reported as number of individuals (%).
¹nd, not done.

Mean within-test repeatability was 5.6 in CF and 4.9 in healthy volunteers and was thus comparable to published data on LCI repeatability.^{16,28,29} This again proves reliability and validity of software, hardware, and test procedure in both, healthy volunteers and patients with CF, even in a multi-center setting with different observers and different local conditions.

Despite immense difficulties with recruiting particularly healthy volunteers in some of the centers, 6/8 centers finally provided a sufficient number of valid MBW

measurements in healthy volunteers for statistically detecting inter-center differences with a power of 94%. With regard to the extremely short study duration of 6 months the previously defined maximum age of 18 years was slightly extended (maximum age of 23.6 years) to facilitate recruitment within the short time frame given. This deviation is considered to be negligible for the outcome of the study.

Mean LCI and inter individual SD in each of the centers as well as for the entire healthy population was comparable to published data.^{16,17,22,28} Mean LCI in each of the centers as well as for the entire population was well below the defined upper limit of normal of <7.¹⁹ The inter-center variability of the LCI in healthy volunteers was 2.9% and was thus extremely low when compared to the variability of other lung function tests.³⁰ There was no significant difference in LCI between the centers.

To the best of our knowledge inter-center variability has only been reported from a study in infants which may therefore not be comparable. However, using an infant device for measuring MBW the authors reported an unexpected high inter-center variability that was suspected to be equipment related.³¹

One center provided a sufficient number of MBW measurements in patients with CF but recruited only 12 healthy controls. The problem in this center was that staff, responsible and trained for conducting the study, changed at study start. New staff had to be trained, thus leading to reduced recruitment of participants within the remaining study duration. MBW results calculated from this small control population were not included into final analysis but are briefly provided in this section to improve transparency of the entire data set: 8/12 (75%) measurements were technically acceptable. Age ranged between 4.3 and 14.4. Mean FRC was 1.4 L

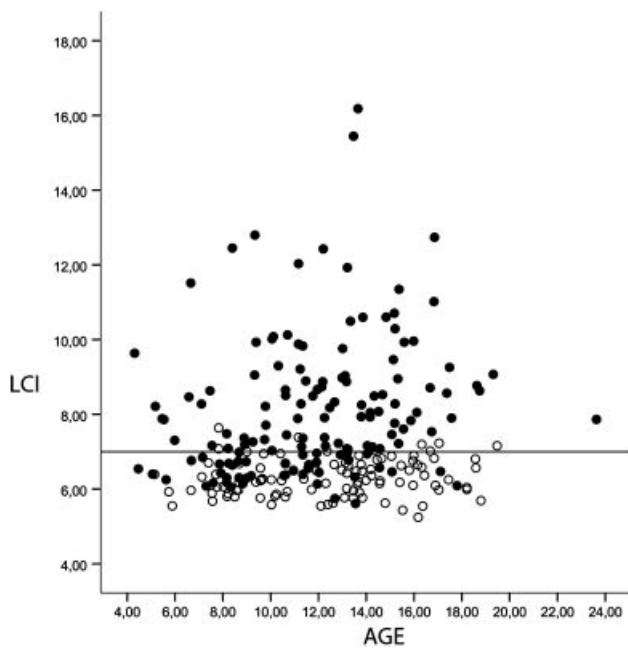


Fig. 3. LCI versus age in n = 102 healthy controls and n = 139 patients with CF. Horizontal line: upper limit of normal (≤ 7) for the LCI using the EasyOne Pro, MBW Module.

(0.4). Mean (SD) LCI was 6.7 (0.4), ranging between 6.3 and 7.58. One of eight subjects had an LCI of above the upper limit of normal of 7.0 (7.58). The intra individual coefficient of variation was 4.6. These results are similar to those obtained in the other centers and for the entire healthy study population. However as stated in the statistic section, the sample size of eight subjects was considered too low for calculating a representative mean LCI in healthy controls for this center aiming to assess inter-center variability.

FEV₁ differed significantly between the centers. On-line Supplement Figure S1a,b illustrates the wide inter individual range for FEV₁ and even more marked for MEF₂₅ in the healthy population. We did not perform central quality control for all spirometric measurements in the present study, as all spirometric measurements were performed by experienced technicians. It remains therefore open for discussion, whether these differences are due to different spirometry devices used in the centers as observed in previous multi-center studies.³² It appears likely, that variability could have been reduced with performing comparative spirometries prior to onset of the study in order to test reliability and accuracy measurements. Further improvement might have been achieved by central inspection of raw data and timely feedback regarding quality of the data obtained. However, for this study, spirometry was only used to characterize the healthy volunteers as healthy and the CF population as unselected and not as outcome parameter. Nevertheless, for future multi-center studies, central quality control should be implemented for techniques involved.

For the population of CF patients, our data confirm previous findings that MBW and the LCI are much more sensitive than spirometry and FEV₁^{16,17,22,28} re-emphasizing the potential clinical value of additional measurement of MBW in CF.

In summary, this is the first study to demonstrate good multi-center feasibility and low inter-center variability for MBW performed with the EasyOne Pro, MBW Module in children and adolescents.

The data generated in this project are important in the discussion of new outcome parameters for clinical and research purposes. However, central study coordination, training courses prior to the study and ongoing quality control appears mandatory for future multi-center studies to generate reliable results. Quality control should probably be extended even to routine tests like spirometry.

The MBW network as founded for this study presents a good opportunity to further evaluate the LCI as a better surrogate marker and for monitoring the clinical course and response to treatment in patients with CF.

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