Association of lung clearance index with survival in individuals with cystic fibrosis

Johanna Manuela Kurz [1,2], Kathryn Angela Ramsey [1], Romy Rodriguez[1], Ben Spycher[3], Reta Fischer Biner[4], Philipp Latzin [1] and Florian Singer [1]

[1]Division of Respiratory Medicine, Dept of Paediatrics, Inselspital University Hospital Bern, University of Bern, Bern, Switzerland. [2]Graduate School for Health Sciences, University of Bern, Bern, Switzerland. [3]Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland. [4]Quartier Bleu, Lindenhofspital, Bern, Switzerland.

Corresponding author: Florian Singer (florian.singer@insel.ch)

Shareable abstract (@ERSpublications)
The lung clearance index (LCI) is a measure of global ventilation inhomogeneity that increases early during the course of cystic fibrosis (CF) lung disease. This study shows that LCI is associated with death or lung transplantation in individuals with CF. https://bit.ly/2T3ia9C

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Abstract

Background The lung clearance index (LCI) assesses global ventilation inhomogeneity and is a sensitive biomarker of airway function in cystic fibrosis (CF) lung disease. We examined the association of LCI with the risk of death or lung transplantation (LTx) in individuals with CF.

Methods We performed a retrospective analysis in a cohort of individuals with CF aged ⩾5 years with LCI and forced expired volume in 1 s (FEV_1) measurements performed between 1980 and 2006. The outcome was time until death or LTx. We used the earliest available LCI and FEV_1 values in a Cox proportional hazards regression adjusted for demographic and clinical variables. For sensitivity analyses, we used the mean of the first three LCI and FEV_1 measurements, stratified the cohort based on age, and investigated individuals with normal FEV_1.

Results In total, 237 individuals with CF with a mean (range) age of 13.9 (5.6–41.0) years were included. The time-to-event analysis accrued 3813 person-years and 94 (40%) individuals died or received LTx. Crude hazard ratios were 1.04 (95% CI 1.01–1.06) per 1.0 z-score increase in LCI and 1.25 (95% CI 1.11–1.41) per 1.0 z-score decrease in FEV_1. After adjusting LCI and FEV_1 mutually in addition to sex, age, body mass index and number of hospitalisations, hazard ratios were 1.04 (95% CI 1.01–1.07) for LCI and 1.12 (95% CI 0.95–1.33) for FEV_1. Sensitivity analyses yielded similar results and using the mean LCI strengthened the associations.

Conclusions Increased ventilation inhomogeneity is associated with greater risk of death or LTx. Our data support LCI as novel surrogate of survival in individuals with CF.

Introduction

Cystic fibrosis (CF) is one of the most prevalent inherited lethal multiorgan diseases [1]. Progressive chronic lung disease leads to lung function decline and respiratory failure, which remains the major cause of morbidity and mortality [2]. Spirometry-derived forced expired volume in 1 s (FEV_1) is used as a physiological surrogate to predict survival and referral for lung transplantation (LTx) [3]. Within the past 20 years, the progression of CF lung disease has slowed, and FEV_1 is often normal in early stages of the disease despite physiological and radiological signs of subclinical lung disease [4]. The lung clearance index (LCI) derived from multiple breath inert gas washout (MBW) quantifies global ventilation inhomogeneity and is a sensitive biomarker of central and peripheral airway function [5]. MBW is safe and requires minimal patient cooperation [6]. LCI is more strongly correlated with structural lung damage than FEV_1 [4]. In individuals with mild CF lung disease, FEV_1 is less responsive to treatment than LCI [7]. However, it is not clear whether an increased (abnormal) LCI is associated with death or LTx [8]. In particular, it is not known whether LCI can be considered as a surrogate end-point for survival.

The objective of this study was to determine the association of LCI with death or LTx. The secondary aim was to compare the strength of association of the two parameters LCI and FEV_1 with this outcome. We utilised an existing cohort of individuals with CF, who were followed longitudinally with MBW measurements performed during routine clinical visits since the 1980s [9]. We hypothesised that individuals with CF who have elevated LCI are at increased risk of death or LTx.

Methods

Study design

We performed a retrospective, observational analysis in a cohort of paediatric and adult individuals with CF at the paediatric CF outpatient clinic at the University Children's Hospital Bern (Bern, Switzerland). Source data were electronic patient records. We collated lung function and clinical data for the 3-year period following the first MBW measurement. Routine assessments at each clinical visit were: MBW measurements using nitrogen as tracer gas (N_2MBW) and spirometry, microbiological culture from cough swabs or sputum, body height and weight, records of medication, and number of exacerbations and hospitalisations [10]. Lung function indices from spirometry but not MBW were routinely disclosed to treating clinicians.

Ethical approval was obtained by the local ethics committee (KEK BE 2018-01642). Written informed consent from survivors and relatives of the individuals who died was not required from the ethics committee and therefore not obtained for this study. This study is registered at ClinicalTrials.gov with identifier number NCT04016194.

Inclusion and exclusion criteria

Individuals were screened by systematic review of electronic medical charts. Eligibility criteria were: confirmed CF diagnosis, age $\geqslant$5 years, available records on routine clinical care in the paediatric CF centre Bern. Inclusion criteria were: availability of at least one N_2MBW and FEV_1 measurement between 1 January 1986 and 31 December 2006. For patients born and diagnosed before 1989, diagnosis was based on clinical signs and sweat chloride results, and confirmed later by detection of two CF-causing mutations [11]. We excluded individuals if CF diagnosis was not confirmed or if lung function tests were available only after LTx.

Variables and definitions

Outcome was defined as time until death or LTx. The end of the study was 31 December 2018. We refer to survival as respiratory survival, where death and LTx are regarded as equivalent markers for terminal pulmonary disease, as previously described [12]. We included LCI as the primary and FEV_1 as the secondary predictor variable. We *a priori* selected the following variables to account for possible confounding [13]: year of birth, age at CF diagnosis, age at lung function measurement, sex and body mass index (BMI) (normalised to z-scores [14]). Birth year was used to account for temporal changes in medical care. In addition, the following clinical variables were investigated [15–17]: genotype, pancreas function, CF-related diabetes (CFRD), infection with *Staphylococcus aureus* and *Pseudomonas aeruginosa*, allergic bronchopulmonary aspergillosis (ABPA), antibiotic treatment, and number of pulmonary exacerbations and hospitalisations. All variables collected from the source data were entered into an online database (REDCap) [18]. Definitions of variables are summarised in supplementary table S1.

Lung function measurement

Trained lung function technicians performed N_2MBW according to in-house measurement standards using a customised open-bypass setup (SensorMedics 2200; SensorMedics, Yorba Linda, CA, USA) [19]. The N_2MBW setup and LCI analysis remained unmodified throughout the whole study period. N_2MBW analysis was performed offline; LCI was calculated according to the recommendations at that time (cumulative expired volume divided by function residual capacity) [10, 19]. LCI values are dimensionless "lung turnovers" and referred to as LCI units [6]. Generally applicable reference equations for MBW indices do not currently exist [20]. Therefore, LCI was standardised to z-scores using the distributional estimated mean±SD of LCI derived from the same N_2MBW setup in 54 healthy subjects aged between 7 and 16 years [19]: 7.64±0.86 units. z-scores were calculated as:

$$\frac{\text{observation} - \text{mean (healthy subjects)}}{\text{standard deviation (healthy subjects)}}$$

Spirometry was performed using a commercial setup (Jaeger, Würzburg, Germany) [10] and in accordance with standards of the European Respiratory Society (ERS)/American Thoracic Society (ATS) at that time

[21–23]. To assess lung function independent from sex, age and height, data were expressed as z-scores [24]. The Global Lung Initiative reference equations include data collected before 2006 and were therefore considered applicable.

We additionally expressed LCI and FEV_1 values as standard deviation score (SD-score) based on the current CF study population to account for unequal variances of LCI and FEV_1 in the CF population:

$$\frac{\text{observation} - \text{mean (subjects with CF)}}{\text{standard deviation (subjects with CF)}}$$

Statistical methods

In the main analysis, we fitted Cox proportional hazards regressions to investigate the association of baseline LCI with survival *versus* death or LTx. We used age (years) as the underlying time variable as this accounts for differences in mortality due to age and thus avoids the need to adjust for increasing age during follow-up [25]. Individuals entered the study at the age of the first MBW measurement and contributed time at risk until the event (death or LTx), loss to follow-up or the end of the study period, whichever occurred first. Time at risk of individuals who were lost to follow-up was right censored on the date of the last available visit. The baseline was defined as the first available LCI value (date of study entry) combined with the clinical information derived within the subsequent 3 years after study entry. In sensitivity analyses, we first included only individuals with at least three available LCI values, and used the mean of the first three available LCI and FEV_1 measurements within 3 years as baseline values. Average lung function values were expected to account for variability in lung function values between visits and to avoid possible confounding by indication [26]. As none of the included individuals received LTx or died within the first 3 years, follow-up started at the date of the third available LCI value and immortal time bias was avoided. Second, we stratified individuals based on age and repeated the analysis using the initial baseline definition (first available LCI and FEV_1 measurement) as in the main analysis 1) in children aged $\leqslant$16 years at baseline, 2) in individuals born within 30 years prior study end (excluding individuals born earlier than 1987) and 3) in adults aged >16 years at baseline. Third, we investigated the association of LCI with death or LTx including only individuals with normal FEV_1 ($FEV_1 \geqslant -1.96$ z-score). We report unadjusted and adjusted estimates (hazard ratios) and 95% confidence intervals. The Cox proportional hazards regression analysis was performed in five steps (supplementary figure S1): 1) crude model: unadjusted, including LCI and FEV_1 separately; 2) mutual model: including both LCI and FEV_1; 3) complete model: including LCI and FEV_1 separately, adjusted for all demographic and clinical variables; 4) reduced model: including LCI and FEV_1 separately, adjusted for selected demographic and clinical variables only; and 5) final model: including both LCI and FEV_1, adjusted for selected demographic and clinical variables. We selected all clinical variables *a priori*; variable reductions in the reduced and final models were based on examining correlations between clinical variables and stepwise removing variables with $p>0.2$ in likelihood ratio tests.

We computed Kaplan–Meier survival curves for the following two groups: 1) individuals with baseline LCI below the study population median and 2) individuals with baseline LCI above the study population median. We tested the proportional hazards assumption for LCI and FEV_1 [27]. Analyses were performed using Stata version 14.2 (StataCorp, College Station, TX, USA). p-values <0.05 were considered statistically significant.

Results

Study participants and descriptive data

In total, 263 individuals aged $\geqslant$5 years were treated in the CF centre at Bern between 1980 and 2006 and were assessed for eligibility (figure 1). Lung function data were available in 237 individuals with CF. These individuals (n=237, 47.7% females) were born between 1952 and 2000 and accrued 3813.3 person-years at risk during the study period. At baseline, mean±SD age was 13.9±8.2 years (range 5.6–41.0 years) (table 1). Mean±SD LCI and FEV_1 values were 8.7±7.3 z-score and −2.4±2.0 z-score, respectively. The majority of subjects (73.4%) were followed annually, 36.7% were followed biannually across the baseline period. Mean±SD time between baseline visits was 10.0±6.1 months and duration of follow-up was 16.1±6.6 years. 94 individuals (39.7%) received LTx or died by 31 December 2018. Mean±SD age at death or LTx was 30.0±10.0 years. 15 individuals (6.3%) were lost to follow-up within the study period and 143 individuals (60.3%) were alive at the end of the study (right censored). Reasons for loss to follow-up were clinical care elsewhere (n=10) and moving abroad (n=5). Compared with individuals who received LTx, individuals who died were older but had similar FEV_1, LCI and BMI at baseline. Follow-up

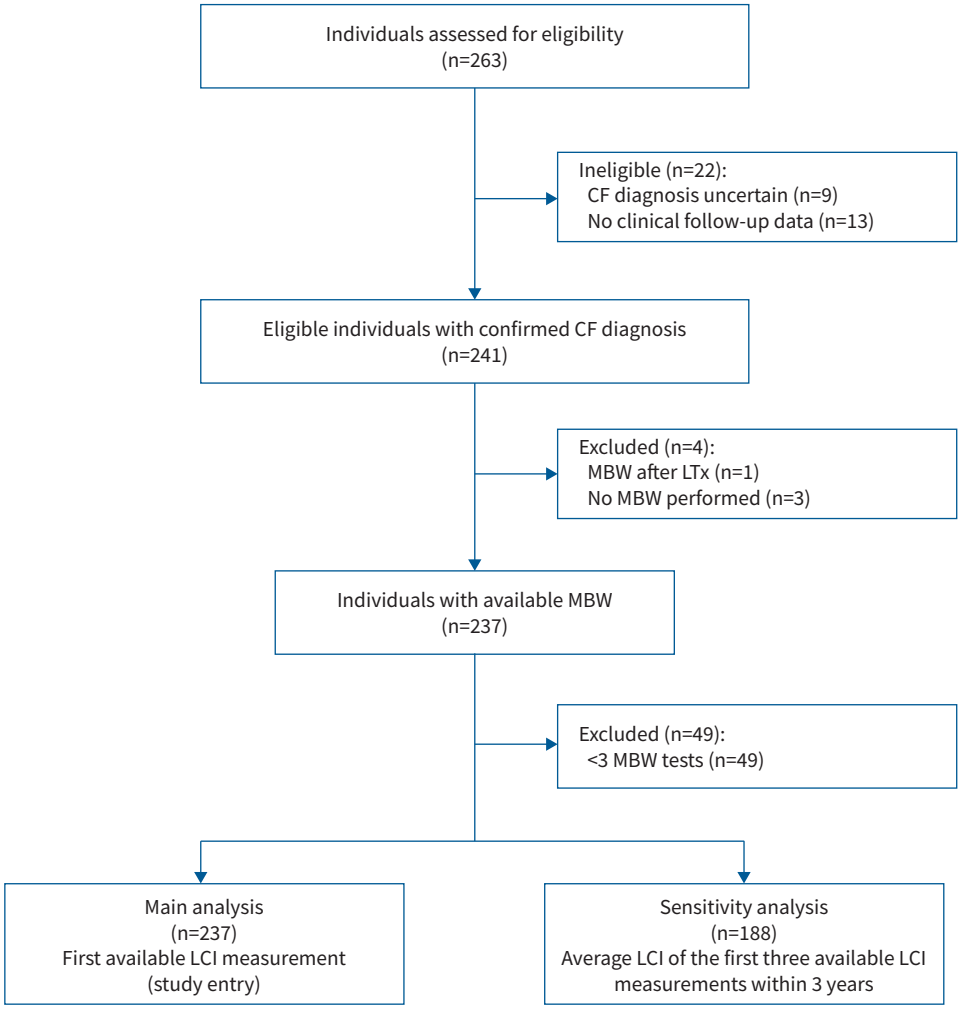

FIGURE 1 Participant flow diagram. CF: cystic fibrosis; MBW: multiple breath washout; LTx: lung transplantation; LCI: lung clearance index. Eligibility criteria: age ⩾5 years, confirmed CF diagnosis and routine clinical care in the CF centre at Bern between 1986 and 2006. Inclusion criteria: availability of at least one MBW test.

duration was comparable between individuals who died and those who received LTx (further details are provided in supplementary table S2). All included variables contained <5.0% of missing values.

Association of LCI with death or LTx

Higher baseline LCI and lower baseline FEV_1 were associated with increased risk (hazard ratio) of death or LTx in individuals with CF. In the main analysis, the estimated HR for death or LTx from the crude model was 1.04 (95% CI 1.01–1.06) per 1.0 z-score increase in LCI and 1.25 (95% CI 1.11–1.41) per 1.0 z-score increase in FEV_1 (table 2). After adjusting for selected demographic and clinical variables (sex, age, BMI, year of birth and number of hospitalisations) in the reduced model, the HR was 1.04 (95% CI 1.01–1.07) for LCI and 1.18 (95% CI 1.01–1.38) for FEV_1. Consequently, per 1.0 z-score increase in LCI, the risk of dying or receiving LTx increased by 4%. Per 1.0 z-score decrease in FEV_1, the risk of dying or receiving LTx increased by 18%. Therefore, an increase by 2.4 z-score in LCI or a 0.6 z-score decrease in FEV_1 was associated with the same risk increase of 10%. In the final model, we mutually adjusted LCI and FEV_1 in addition to the aforementioned variables. In this model, the HR for LCI was 1.04 (95% CI 1.01–1.07). The corresponding HR for FEV_1 was no longer statistically significant with the 95% CI including 1.00: 1.12 (95% CI 0.95–1.33). Results are displayed in figure 2. Estimates from the mutual and complete models can be found in supplementary table S3. Further details on the complete and final models are given in supplementary tables S6 and S7.

TABLE 1 Population characteristics (n=237)

Baseline	
Female	113 (47.7)
Year of birth	1983±10.7 (1952–2000)
Median (IQR) (range) age at CF diagnosis, years	0.0 (0.0–2.0) (0.0–28.0)
Age at study entry, years	13.9±8.2 (5.6–41.0)
BMI at study entry, z-score	−0.9±1.1 (−4.0–2.4)
CFTR mutation	
F508del homozygous	136 (57.4)
F508del heterozygous	74 (31.2)
Other	27 (11.4)
Pancreatic insufficiency	200 (84.4)
CF-related diabetes	49 (20.7)
LCI, units	17.5±7.3 (3.5–59.8)
LCI, z-score	8.7±7.3 (−5.4–50.9)
FEV_1, z-score	−2.4±2.0 (−6.4–1.7)
FEV_1, % pred	70.3±24.7 (19.8–120.8)
Pseudomonas aeruginosa	173 (73.0)
Staphylococcus aureus	137 (57.8)
ABPA	19 (8.0)
Individuals hospitalised at least once during baseline	107 (45.2)
Hospitalisations per individual during baseline, n	2.2±1.7 (1.0–9.0)
Antibiotic treatment (oral, inhaled, intravenous)	201 (84.1)
Inhaled medication (mucolytics, bronchodilators, steroids)	204 (86.1)
Follow-up duration until outcome event or end of study, years	16.1±6.6 (0.3–32.3)
Outcome (death or lung transplantation)	94 (39.7)
Death	41 (17.3)
Males	22 (53.7)
Females	19 (46.3)
Lung transplantation	53 (22.4)
Males	27 (50.9)
Females	26 (49.1)
Lost to follow-up	15 (6.3)
Person-years at risk	3813.3

Data presented as n (%) or mean±SD (range), unless otherwise stated. IQR: interquartile range; CF: cystic fibrosis; BMI: body mass index; CFTR: CF transmembrane conductance regulator; LCI: lung clearance index; FEV_1: forced expired volume in 1 s; ABPA: allergic bronchopulmonary aspergillosis. Baseline: first available LCI (=study entry) and corresponding FEV_1 values. Demographic and clinical data derived within the first 3 years after study entry. Study entry: date of third LCI value within the first 3 years after study entry; follow-up: duration (years) from study entry until outcome event or end of study (30 December 2018).

TABLE 2 Risk of death or lung transplantation (LTx) according to baseline lung function

	LCI	**FEV_1**
Per 1.0 z-score increase		
Crude model	1.04 (1.01–1.06), p=0.006	1.25 (1.11–1.41), p<0.001
Reduced model	1.04 (1.01–1.07), p=0.003	1.18 (1.01–1.38), p=0.043
Final model	1.04 (1.01–1.07), p=0.011	1.12 (0.95–1.33), p=0.164
Per 1.0 SD-score increase		
Crude model	1.30 (1.08–1.58), p=0.006	1.55 (1.22–1.96), p<0.001
Reduced model	1.34 (1.10–1.62), p=0.003	1.38 (1.01–1.88), p=0.043
Final model	1.30 (1.06–1.60), p=0.011	1.26 (0.91–1.74), p=0.164

Data are presented as crude and adjusted hazard ratios (95% CI) with p-values for the risk of death or LTx per 1.0 z-score increase in lung clearance index (LCI) or per 1.0 z-score decrease in forced expired volume in 1 s (FEV_1) and 1.0 SD-score increase in LCI or per 1.0 SD-score increase in FEV_1 in 237 individuals with cystic fibrosis using the first available LCI and corresponding FEV_1 value as baseline. Crude model: unadjusted HR per 1.0 z-score increase in LCI and 1.0 z-score decrease in FEV_1; reduced model: adjusted HR per 1.0 z-score increase in LCI and 1.0 z-score decrease in FEV_1, adjusted for the selected variables sex, age, body mass index, year of birth and number of hospitalisations; final model: HR per 1.0 z-score increase in LCI and per 1.0 z-score decrease in FEV_1, adjusted mutually in addition to the aforementioned variables. Results for the mutual and complete models are provided in supplementary table S3.

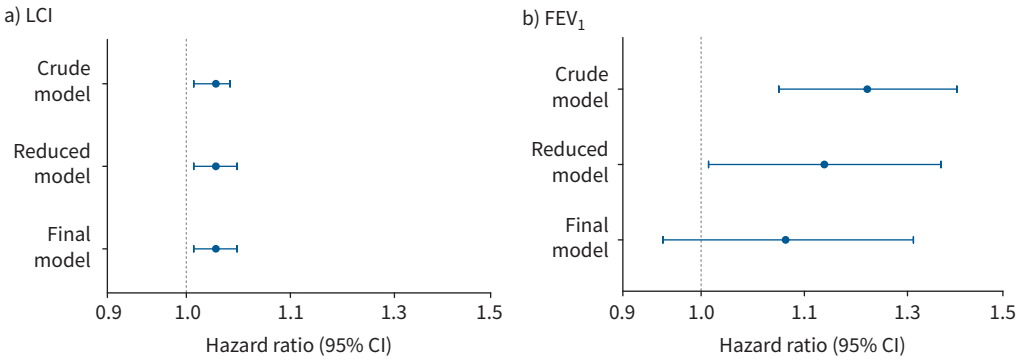

FIGURE 2 Risk of death or lung transplantation (LTx) according to baseline lung function: crude and adjusted hazard ratios (95% CI) for the risk of death or LTx a) per 1.0 z-score increase in lung clearance index (LCI) or b) per 1.0 z-score decrease in forced expired volume in 1 s (FEV_1) in 237 individuals with cystic fibrosis using the first available LCI and corresponding FEV_1 value as baseline. The *x*-axis shows log-transformed hazard ratio. The additive inverse of FEV_1 ($-FEV_1$) was used to allow better comparison of LCI with FEV_1. Crude model: unadjusted HR per 1.0 z-score increase in LCI and 1.0 z-score decrease in FEV_1; reduced model: adjusted HR per 1.0 z-score increase in LCI and 1.0 z-score decrease in FEV_1, adjusted for the selected variables sex, age, body mass index, year of birth and number of hospitalisations; final model: HR per 1.0 z-score increase in LCI and per 1.0 z-score decrease in FEV_1, adjusted mutually in addition to the aforementioned variables.

Effect sizes were influenced by the unequal variances of LCI and FEV_1 in this CF population (tables 1 and 2). To account for this, we expressed LCI and FEV_1 values as SD-scores based on the CF study population. In the crude model, the HR for death or LTx was 1.30 (95% CI 1.08–1.58) per 1.0 SD-score increase in LCI and 1.55 (95% CI 1.22–1.96) per 1.0 SD-score increase in FEV_1. In the final model, the HR for death or LTx was 1.30 (95% CI 1.06–1.60) per 1.0 SD-score increase in LCI and 1.26 (95% CI 0.91–1.74) per 1.0 SD-score decrease in FEV_1.

Kaplan–Meier survival curves show that individuals with LCI values above the population median LCI (7.3 z-score) had a higher risk of death or LTx compared with those with a LCI below the population median LCI at baseline (figure 3). Findings for FEV_1 were similar (supplementary figure S2). There was no evidence of a violation of the proportional hazards assumption (LCI: p=0.2; FEV_1: p=0.9).

Association of LCI with death or LTx in sensitivity analyses

Sensitivity analyses were performed in subgroups of individuals who 1) had three or more MBW tests within the first 3 years (n=188) (supplementary table S5), of which the average LCI of the first three visits was derived as an alternate baseline. Further sensitivity analyses were performed using the initial baseline (first available LCI or FEV_1) in subgroups of individuals who were 2) born after 1987 (n=102) (table 3) or 3) aged ⩽16 years (n=168) (table 3), or 4) had $FEV_1 \geqslant -1.96$ z-score (n=108) (supplementary table S5). Sensitivity analyses confirmed the primary analysis: LCI was similarly associated with death or LTx in children aged ⩽16 years and in younger individuals born after 1987 (figure 4); however, associations were weaker in individuals with normal FEV_1 and in adults (supplementary table S5). Using the mean over three LCI and FEV_1 measurements as baseline values resulted in higher estimates. The population characteristics in the sensitivity analyses are summarised in table 4 and supplementary table S4. Kaplan–Meier survival curves are shown in supplementary figure S3.

Discussion

Summary

This is the first study to show that LCI is associated with terminal pulmonary disease in individuals with CF. We found that per 1.0 z-score increase in LCI, the risk (hazard ratio) of death or LTx increased on average by 4%. After adjustment for heterogeneous variance of lung function values in the CF population, the risk of death or LTx increased on average by 30% per 1.0 SD-score increase in LCI. We verified this association in regression models adjusting for clinical and anthropometric variables, and in sensitivity analyses. Baseline lung function values averaged across three visits provided stronger association with death or LTx compared with baseline lung function including single LCI and FEV_1 values only. In children and younger individuals, LCI was stronger associated with death or LTx compared with older individuals born before 1987.

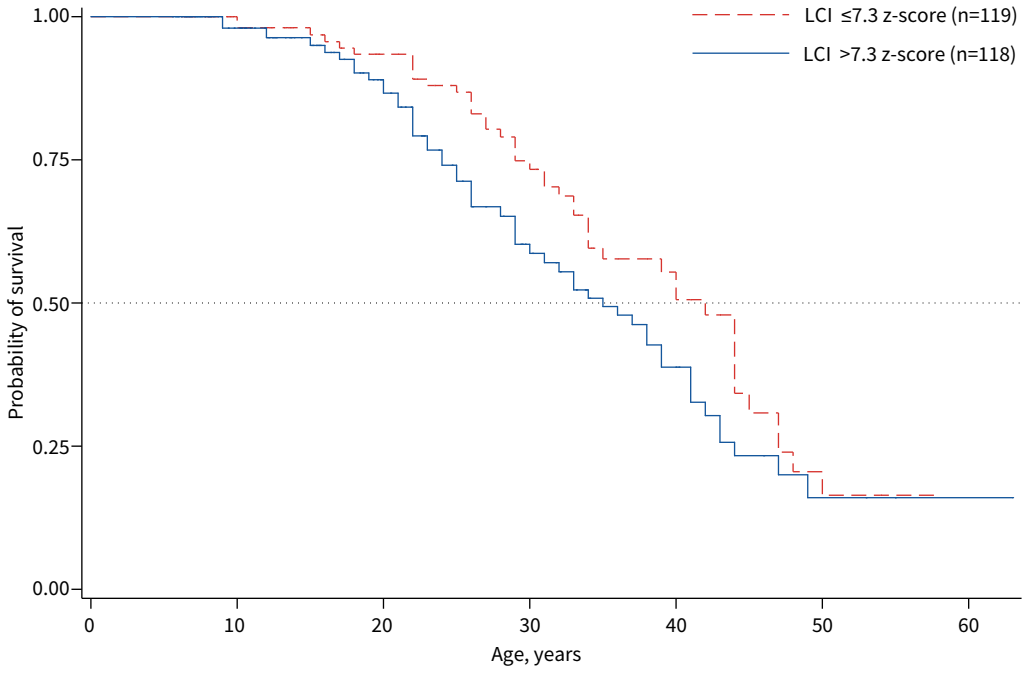

FIGURE 3 Respiratory survival in individuals with cystic fibrosis according to baseline lung clearance index (LCI): individuals with baseline LCI values below the study population median (≤7.3 z-score) or above the study population median (>7.3 z-score). Probability 0.50 (dashed line): 50% of the individuals in each group died or received lung transplantation.

Association of lung function with respiratory survival

This study links LCI with respiratory survival in individuals with CF. These data are essential for biomarkers to be recognised as surrogate end-points of long-term prognosis [8]. LCI was associated with death or LTx after adjusting for known risk factors and confounders (*e.g.* age) (supplementary figure S4) [28]. As expected, several variables were removed from the final model, as they either were strongly correlated with other variables or did not contribute to the association with death or LTx. In our population, the variables possibly influencing the association of LCI and FEV_1 with death or LTx may have been underestimated: pancreatic sufficiency was rare in the current population. Diagnosis and

TABLE 3 Risk of death or lung transplantation in the sensitivity analyses

	Individuals with ⩾3 LCI/FEV_1 measurements within 3 years (n=188)	Children ⩽16 years of age (n=168)	Individuals born after 1987 (n=102)
LCI			
Crude model	1.08 (1.04–1.13), p<0.001	1.05 (1.02–1.08), p=0.001	1.04 (1.00–1.08), p=0.042
Reduced model	1.08 (1.03–1.13), p=0.001	1.04 (1.01–1.08), p=0.007	1.04 (1.00–1.08), p=0.052
Final model	1.06 (1.01–1.12), p=0.025	1.04 (1.01–1.08), p=0.010	1.04 (1.00–1.08), p=0.054
FEV_1			
Crude model	1.37 (1.18–1.59), p<0.001	1.41 (1.19–1.67), p<0.001	1.62 (1.14–2.30), p=0.008
Reduced model	1.34 (1.06–1.69), p=0.006	1.17 (0.96–1.43), p=0.120	1.31 (0.85–2.02), p=0.219
Final model	1.24 (0.97–1.57), p=0.086	1.12 (0.91–1.38), p=0.294	1.29 (0.83–2.00), p=0.263

Data are presented as crude and adjusted hazard ratios (95% CI) with p-values for the sensitivity analyses using the average lung clearance index (LCI) and average forced expired volume in 1 s (FEV_1) of the first three available LCI and corresponding FEV_1 measurements as baseline in individuals with three or more multiple breath washout measurements within 3 years, and in age strata using the first available LCI and corresponding FEV_1 as baseline. Crude model: unadjusted HR per 1.0 z-score increase in LCI and 1.0 z-score decrease in FEV_1; reduced model: adjusted HR per 1.0 z-score increase in LCI and 1.0 z-score decrease in FEV_1, adjusted for the selected variables sex, age, body mass index, year of birth and number of hospitalisations; final model: HR per 1.0 z-score increase in LCI and per 1.0 z-score decrease in FEV_1, adjusted mutually in addition to the aforementioned variables.

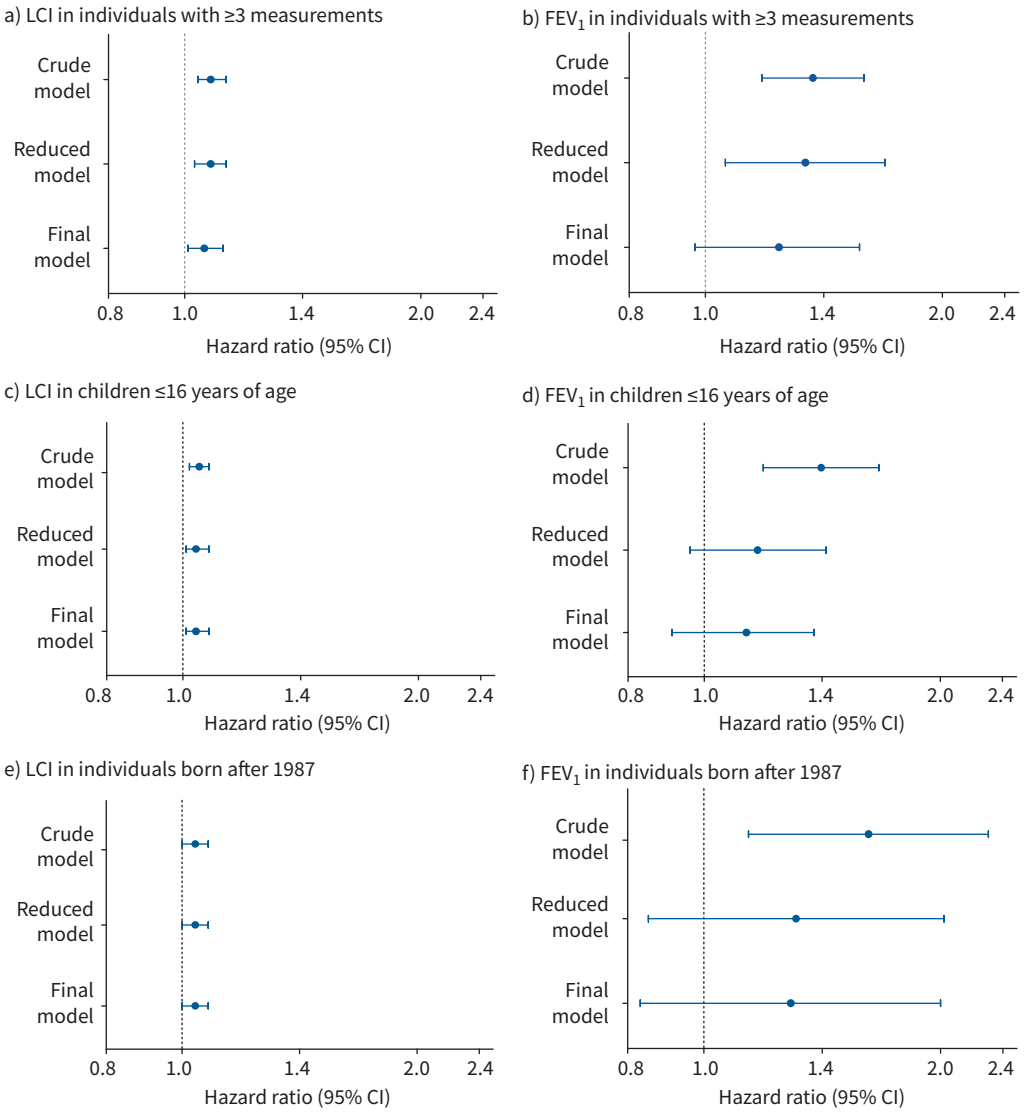

FIGURE 4 Risk of death of lung transplantation in the sensitivity analysis: crude and adjusted hazard ratios (95% CI) for the risk of death or lung transplantation in the sensitivity analyses. a) Per 1.0 z-score increase in lung clearance index (LCI) and b) per 1.0 z-score decrease in forced expired volume in 1 s (FEV_1) in individuals with at least three multiple breath washout measurements within 3 years after study entry using the average of the first three available LCI and corresponding FEV_1 values as baseline (n=188). c) Per 1.0 z-score increase in LCI and d) per 1.0 z-score decrease in FEV_1 in children ⩽16 years of age (n=168) using the first available LCI and corresponding FEV_1 value as baseline. e) Per 1.0 z-score increase in LCI and f) per 1.0 z-score increase in FEV_1 in individuals born after 1987 (n=102) using the first available LCI and corresponding FEV_1 value as baseline. The *x*-axis shows log-transformed HR. The additive inverse of FEV_1 ($-FEV_1$) was used to allow better comparison of LCI with FEV_1. Crude model: unadjusted HR per 1.0 z-score increase in LCI and 1.0 z-score decrease in FEV_1; reduced model: adjusted HR per 1.0 z-score increase in LCI and 1.0 z-score decrease in FEV_1, adjusted for the selected variables sex, age, body mass index, year of birth and number of hospitalisations; final model: HR per 1.0 z-score increase in LCI and per 1.0 z-score decrease in FEV_1, adjusted mutually in addition to the aforementioned variables.

management of CF-related complications such as CFRD evolved over time. Few other studies have assessed the degree to which LCI may predict the clinical course in CF. In school-aged children with CF, the risk of future pulmonary exacerbations increased by 12% after an LCI increase of ~0.5 units at baseline [29]. We have recently demonstrated that per 1.0 LCI unit increase, the risk of future pulmonary exacerbation increased by 13% in children and adults with primary ciliary dyskinesia [30].

TABLE 4 Population characteristics in the sensitivity analyses

	Individuals with ⩾3 LCI/FEV_1 measurements within 3 years (n=188)	Children ⩽16 years of age (n=168)	Individuals born after 1987 (n=102)
Females	96 (51.1)	78 (46.4)	49 (48.0)
Year of birth	1984±10.4 (1952–2000)	1988±6.7 (1971–2000)	1992±3.7 (1987–2000)
Median (IQR) (range) age at CF diagnosis, years	0.0 (0.0–2.0) (0.0–28.0)	0.0 (0.0–2.0) (0.0–14.0)	1.6 (0.0–2.0) (0.0–14.0)
Age, years	13.2±8.1 (5.6–41.0)	9.5±3.0 (5.6–16.0)	7.8±2.3 (5.6–17.7)
BMI, z-score	−0.9±1.0 (−4.0–1.8)	−0.8±1.0 (−4.1–1.8)	−0.3±0.8 (−2.6–1.8)
LCI, z-score	9.0±5.7 (−2.6–32.1)[#]	8.8±7.5 (−5.4–50.9)[¶]	9.5±8.0, −1.6–50.9)[¶]
FEV_1, z-score	−2.4±1.9 (−6.3–1.3)[#]	−1.8±1.8 (−6.1–1.7)[¶]	−1.1±1.47 (−4.5–1.7)[¶]
Outcome (death or LTx)	70 (37.2)	54 (32.1)	13 (13.7)
Lost to follow-up	9 (4.8)	8 (5.4)	4 (4.2)

Data presented as n (%) or mean±SD (range), unless otherwise stated. LCI: lung clearance index; FEV_1: forced expired volume in 1 s; IQR: interquartile range; CF: cystic fibrosis; BMI: body mass index; LTx: lung transplantation. [#]: in individuals with three or more multiple breath washout measurements within the first 3 years after study entry, we used the average LCI of the first three available LCI and corresponding FEV_1 values as baseline; [¶]: in individuals stratified by age, the first available LCI and corresponding FEV_1 were used as baseline.

FEV_1 and LCI measure two distinct features of lung physiology. We confirmed that FEV_1 continues to be an important surrogate end-point of survival in CF [3, 31]. However, after adjusting FEV_1 with LCI, the association between FEV_1 and survival was weakened substantially. In line with previous findings, sex, age, BMI and number of hospitalisations partially explained the association of FEV_1 with survival [28]. We assume that the ability of FEV_1 to predict survival may further decline in the current era of CF care including CF transmembrane conductance regulator (CFTR) modulators and evolving survival patterns [1, 28]. There are several reasons why LCI should be further considered. FEV_1 may remain normal in childhood and studies assessing treatment regimens targeted to improve lung function may be constrained by ceiling effects from normal FEV_1 [32]. In addition, the association between FEV_1 and structural lung disease is poor. LCI is a reliable measure of global ventilation inhomogeneity arising from central and mainly peripheral airways [33]. MBW is characterised by high feasibility, good repeatability and sensitivity to early lung disease [34, 35]. We have previously demonstrated the clinical utility of LCI to correlate with "gold standard" outcomes such as infection with *P. aeruginosa* and structural lung disease in individuals with CF [4, 5].

We show that both a single MBW measurement and averaged triplicate MBW measurements were associated with death or LTx. LCI averaged across three visits provided even stronger association with death or LTx compared with a single LCI value. Clinically important events such as ABPA or pulmonary exacerbations contribute to LCI variability and may therefore influence the predictive capacity of LCI [36, 37]. In our study, LCI, but not FEV_1, was associated with death or LTx in younger individuals and individuals with mild CF lung disease. Despite normal FEV_1, LCI was associated with shorter time to death or LTx. Longitudinal data indicate that LCI deteriorates more rapidly than FEV_1 in individuals with CF [10, 28]. We assume that clinical care improved during the study and possibly altered lung disease phenotypes from multiairway generation obstruction in older individuals to mainly peripheral airway generation obstruction in younger individuals [9]. The influence of birth year on the association of FEV_1 with survival was more pronounced compared with LCI, which indicated a temporal trend in our study. We considered birth year, age at CF diagnosis and age at baseline as proxies for improvements in clinical care and decreasing annual death rate in CF.

Strengths and limitations

Major strengths of our study were the wide spectrum of disease severity including individuals born over five decades, rolling enrolment over 20 years, follow-up duration of on average 16 years and the low number of missing values. While prevalence of *P. aeruginosa* was greater compared with most contemporary cohorts, spirometry indices were comparable. The observed rate of death and LTx was 39.7% in our study population, and agrees well with the expected event rate of ∼41.2% over a period of 33 years from 1986 until 2018 based on the data from the European Cystic Fibrosis Society Patient Registry [38]. It is important to note that baseline LCI and FEV_1 values used in our analysis represent snapshots in time when first measurements were taken in our population. It is probable that subsequent measurements of LCI and FEV_1 are more strongly associated with death or LTx. We defined age ⩾5 years at baseline as part of inclusion criteria as MBW was not applied in younger individuals. Individuals that

died or received LTx before the age of 5 years or generally before being able to perform MBW were not included in this analysis. As the clinical course usually differs between pre- and post-LTx, we did not include deaths post-LTx as study outcome [28]. Censoring subjects at the date of LTx did not underestimate the person-years at risk as we defined our outcome as death or LTx. Our study sample may not be entirely representative of present-day patient populations. We acknowledge the historical treatment regimens in our study. However, our sensitivity analyses showed that results remained closely similar in subsamples including younger individuals at baseline and individuals born in a more recent time period, subsamples that are arguably more representative of the present-day patients.

Further studies are required to externally validate our findings in large, contemporary populations. However, current CF cohorts investigating LCI started 15 years ago and therefore the association with survival can be studied in one or two decades at the earliest [39, 40].

The CF centre at Bern already had extensive experience in MBW at the time of our study and has collated one of the largest LCI datasets to our knowledge [10, 19]. N_2MBW was performed regularly during clinical visits but did not influence clinical decisions, reducing the risk of selection bias or confounding by indication. The N_2MBW setup was standard at that time and remained unchanged during the study period, but is no longer available. It appears unlikely that measurement error would have positively confounded the association of LCI with survival. Our findings appear relatively independent of evolving MBW technologies as suggested by the consistent risk estimates derived from normalised LCI values calculated from an external healthy population and the current study population. We acknowledge differences in age distribution between our CF study population and the only existing, younger, healthy reference population. We also accounted for the unequal variance of lung function indices in the study population using parametric (SD-score based on the study population) and nonparametric (median LCI based on the study population) approaches. However, absolute LCI values in our study should be interpreted cautiously. The upper limit of normal LCI was 9.5 units, which is higher compared with current N_2MBW setups [19, 41].

Clinical considerations

Our data support the use of LCI in routine clinical surveillance of individuals with CF. Future clinical decision algorithms for CF care may need to include LCI. We acknowledge that integrating LCI in clinical decision making warrants further study. LCI is increasingly being used as a study end-point in clinical trials assessing the efficacy of CFTR modulators in children [42]. Recent evidence suggests that LCI correlates with the extent of naïve CFTR function, suggesting that LCI can be considered as a candidate treatable trait [43]. An increase in LCI could be used to initiate CFTR modulator treatment in otherwise asymptomatic individuals with CF and normal FEV_1.

Conclusions

Our study showed that increased LCI is associated with a shorter time to death or LTx in individuals with CF. This finding suggests that LCI may be a promising surrogate end-point of death or LTx in individuals with CF. These data allow healthcare providers and individuals with CF to understand the potential future implications of elevated LCI values. Further research should investigate the association of LCI with death or LTx in individuals with CF in more detail, in particular regarding temporal relationships between LCI measurement and outcomes and the predictive value of LCI in different patient populations.

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