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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], Florian Singer[1]

1. Division of Respiratory Medicine, Department of Paediatrics, Inselspital University Hospital Bern, University of Bern, Bern, Freiburgstrasse 15, 3010 Bern, Switzerland
2. Graduate School for Health Sciences, University of Bern, Bern, Switzerland
3. Institute of Social and Preventive Medicine, University of Bern, Mittelstrasse 43, 3012 Bern, Switzerland
4. Quartier Bleu, Lindenhofspital, Bremgartenstrasse 117, 3012 Bern, Switzerland

CORRESPONDING AUTHOR

Florian Singer MD PhD

Division of Respiratory Medicine, Department of Paediatrics,

Inselspital University Hospital Bern, Freiburgstrasse 15, 3010 Bern, Switzerland;

Email: florian.singer@insel.ch; Phone : +4131632 2111; Fax: +41316325528

TAKE HOME MESSAGE:

Lung clearance index (LCI) is a measure of global ventilation inhomogeneity which 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.

Study registration number: NCT04016194

KEYWORDS (MeSH): Respiratory function tests; Cystic fibrosis, Analysis, Survival; Mortality; Lung transplantaion

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.

Objectives: 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 ≥ five years with LCI and 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 hazard 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 [95% CI] were 1.04 [1.01–1.06] per one z-score increase in LCI and 1.25 [1.11–1.41] per one z-score decrease in FEV_1. After adjusting LCI and FEV_1 mutually in addition to sex, age, BMI and the number of hospitalisations, hazard ratios were 1.04 [1.01-1.07] for LCI, and 1.12 [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 multi-organ 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 the first second (FEV_1) is used as physiological surrogate to predict survival and referral to 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 endpoint 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 FEV1 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 hypothesized 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 (NCT04016194) in a cohort of paediatric and adult individuals with CF at the paediatric CF outpatient clinic at the

University Children's Hospital Bern, Switzerland. Source data were electronic patient records. We collated lung function and clinical data from the three year period following the first MBW measurement. Routine assessments at each clinical visit were: MBW measurements using nitrogen (N_2) as tracer gas (N_2MBW) and spirometry, microbiological culture from cough swabs or sputum, body height and weight, records of medication, 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.

Inclusion- and exclusion criteria

Individuals were screened by systematic review of electronic medical charts. Eligibility criteria were: Confirmed CF diagnosis, age ≥ five 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 1986/01/01-2006/12/31. 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 on 2018/12/31. 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, normalized 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 the number of pulmonary exacerbations, and hospitalisations. All variables collected from the source data were entered into an online database (RedCap database)[18]. Variable definitions are summarized in Table S1, online supplement (OLS).

Lung function measurement

Trained lung function technicians performed N_2MBW according to in-house measurement standards using a customized open-bypass setup (SensorMedics 2200, Yorba Linda, CA, USA)[19]. The N_2MBW setup and LCI analysis remained unmodified throughout the whole study period. N_2MBW analysis was performed off-line; 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 LCI units[6]. Generally applicable reference equations for MBW indices do not currently exist[20]. Therefore, LCI was standardized to z-scores using the distributional estimates mean (standard deviation, 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) and 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 (GLI) 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 hazard regressions to investigate the association of baseline LCI with survival *vs.* 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 three subsequent 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 three 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 three 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 (i) in children aged $\leq$ 16.0 years at baseline, (ii) in individuals born within 30 years prior study end (excluding individuals born earlier than 1987) and (iii) in adults aged > 16.0 years at baseline. Third, we investigated the association of LCI with death or LTX including only individuals with normal FEV_1 ($FEV_1 \geq$ -1.96 z-

score). We report unadjusted and adjusted estimates (Hazard Ratios, HR), and 95% confidence intervals (CI). The Cox proportional hazard regression analysis was performed in five steps (Figure S1, OLS): *(i) Crude model:* unadjusted, including LCI and FEV_1 separately; *(ii) Mutual model:* including both, LCI and FEV_1; *(iii) Complete model:* including LCI and FEV_1 separately, adjusted for all demographic and clinical variables; *(iv) Reduced model:* including LCI and FEV_1 separately, adjusted for selected demographic and clinical variables only; *(v) Final model:* including both, LCI and FEV_1, adjusted for selected demographic and clinical variables. We selected all clinical variables *a priori*, variable reduction 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: *(i)* Individuals with baseline LCI below the study population median, and *(ii)* individuals above the study population median. We tested the proportional hazards assumption for LCI and FEV_1[27]. Analyses were performed using Stata 14.2 software package (StataCorp LP, College Station, TX, USA). P-values of <0.05 were considered statistically significant.

Results

Study participants and descriptive data

In total, 263 individuals aged five years and older were treated in the CF-centre Bern between 1980-2006 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 1952-2000 and accrued 3813.3 person-years at risk during the study period. At baseline, mean (SD) age was 13.9 (8.2) years with a range from 5.6 to 41.0 years (Table 1). Mean (SD) LCI and FEV_1 values were 8.7 (7.3) z-score and -2.5 (2.0) z-score, respectively. The majority of subjects (73.4%) were followed annually, 36.7% were followed bi-annually across the baseline period Average time between baseline visits was 10.0 (6.1) months and the mean duration of follow-up was 16.1 (6.6) years. Ninety-four individuals (39.7%) received LTX or died by 2018/12/31. Mean (SD) age at death or LTX was 30.0 (10.0) years. Fifteen 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 to individuals who received LTX, individuals who died were older but had similar FEV_1, LCI and BMI at baseline. Follow-up duration was comparable between individuals who died and those who received LTX, further details are provided in Table S2, OLS. All included variables contained less than 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 (HR) of death or LTX in individuals with CF. In the main analysis, estimated HR [95% CI] for death or LTX from the *Crude model* were 1.04 [1.01 – 1.06] per unit (1.0) z-score increase in LCI and 1.25 [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 the number of

hospitalisations) in the *Reduced model,* the HR was 1.04 [1.01 – 1.07] for LCI and 1.18 [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, were 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 [1.01 – 1.07]. The corresponding HR for FEV_1 was no longer statistically significant with the 95% CI including one: 1.12 [0.95 – 1.33]. Results are displayed in Figure 2. Estimates from the *Mutual* and *Complete models* can be found in Table S3, OLS. Further details on the *Complete* and *Final* models are given in Tables S6 and S7, OLS.

Effect sizes were influenced by the unequal variances of LCI and FEV_1 in this CF population (Table 1 and 2). To account for this, we expressed LCI and FEV_1 values as standard deviation scores (SD-score) based on the CF study population. In the *Crude model* the HR for death or LTX was 1.30 [1.08 – 1.58] per unit (1.0) SD-score increase in LCI, and 1.55 [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 [1.06 – 1.60] per 1.0 SD-score increase in LCI, and 1.26 [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 to those with a LCI below the population median LCI at baseline (Figure 3). Findings for FEV_1 were similar (Figure S2, OLS). 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 (i) had three or more MBW tests within the first three years (n = 188, Table S5, OLS) of which the average LCI of the first three visits was derived as alternate baseline. Further sensitivity analyses were performed using the initial baseline (first available LCI or FEV_1) in subgroups of individuals who were (ii) born after 1987 (n = 102, Table 4), or (iii) aged ≤ 16.0 years (n = 168, Table 4), or (iv) had $FEV_1 \geq$ -1.96 z-score (n = 108, Table S5, OLS). Sensitivity analyses confirmed the primary analysis: LCI was similarly associated with death or LTX in children aged ≤ 16.0 years and in younger individuals born after 1987. But associations were weaker in individuals with normal FEV_1 and in adults (table S5, OLS). 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 summarized in Table 3 and Table S4, OLS. Kaplan Meier survival curves are shown in Figure S3, OLS.

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 one z-score increase in LCI, the risk (HR) 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 one 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 to 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 to older individuals born before 1987.

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 recognized as surrogate endpoints of long-term prognosis[8]. LCI was associated with death or LTX after adjusting for known risk factors and confounders (e.g. age), see OLS 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 FEV1 with death or LTX may have been underestimated: Pancreatic sufficiency was rare in the current population. Diagnosis and 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 one LCI unit increase, the risk of future

pulmonary exacerbation increased by 13% in children and adults with Primary Ciliary Dyskinesia[30].

FEV_1 and LCI measure two distinct features of lung physiology. We confirmed that FEV_1 continues to be an important surrogate endpoint 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 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 characterized by a 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 *Pseudomonas 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 to 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 indicates 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 multi-airway generations obstruction in older individuals to mainly peripheral airway generations obstruction in younger individuals[9]. The influence of birth year on the association of FEV_1 with survival was more pronounced compared to 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 *Pseudomonas aeruginosa* was greater compared to 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 around 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 FEV1 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 FEV1 are more strongly associated with death or LTX. We defined age $\geq$ 5.0 years of age at baseline as inclusion criteria as MBW was not applied in younger individuals. Individuals that died or received LTX before the age of five 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. Yet, current CF cohorts investigating LCI started 15 years ago and therefore the association with survival can be studied earliest in one or two decades[39, 40].

The CF-centre 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 normalized 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 non-parametric (median LCI based on the study population) approaches. Yet, absolute LCI values in our study should be interpreted cautiously. The upper limit of normal LCI was 9.5 units which is higher compared to 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 endpoint 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.

Conclusion

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 endpoint 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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[1]Division of Pulmonary Medicine, University Hospital of Zurich, Zurich, Switzerland

[2]Division of Respiratory Medicine, Department of Paediatrics, Inselspital University Hospital Bern, University of Bern, Bern, Freiburgstrasse 15, 3010 Bern, Switzerland

[3]Studio Medico, Corso Pestalozzi 11, 6900 Lugano , Switzerland

[4]Department of Pneumology, University Hospital Basel, Switzerland

[5]Service de pneumologie, centre hospitalier universitaire Vaudois, 1011 Lausanne, Suisse

[6]Department of Nuclear Medicine and Radiology, Cantonal Hospital Lucerne, Lucerne, Switzerland

[7]Pneumologist, Service de Pneumologie, Hôpital Neuchâtelois, Neuchâtel, Switzerland

[8]Dept of Internal Medicine, University Hospital, Basel, Switzerland

[9]Dept of Paediatrics, Hospital of Bellinzona, Bellinzona, Switzerland

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Figure legends

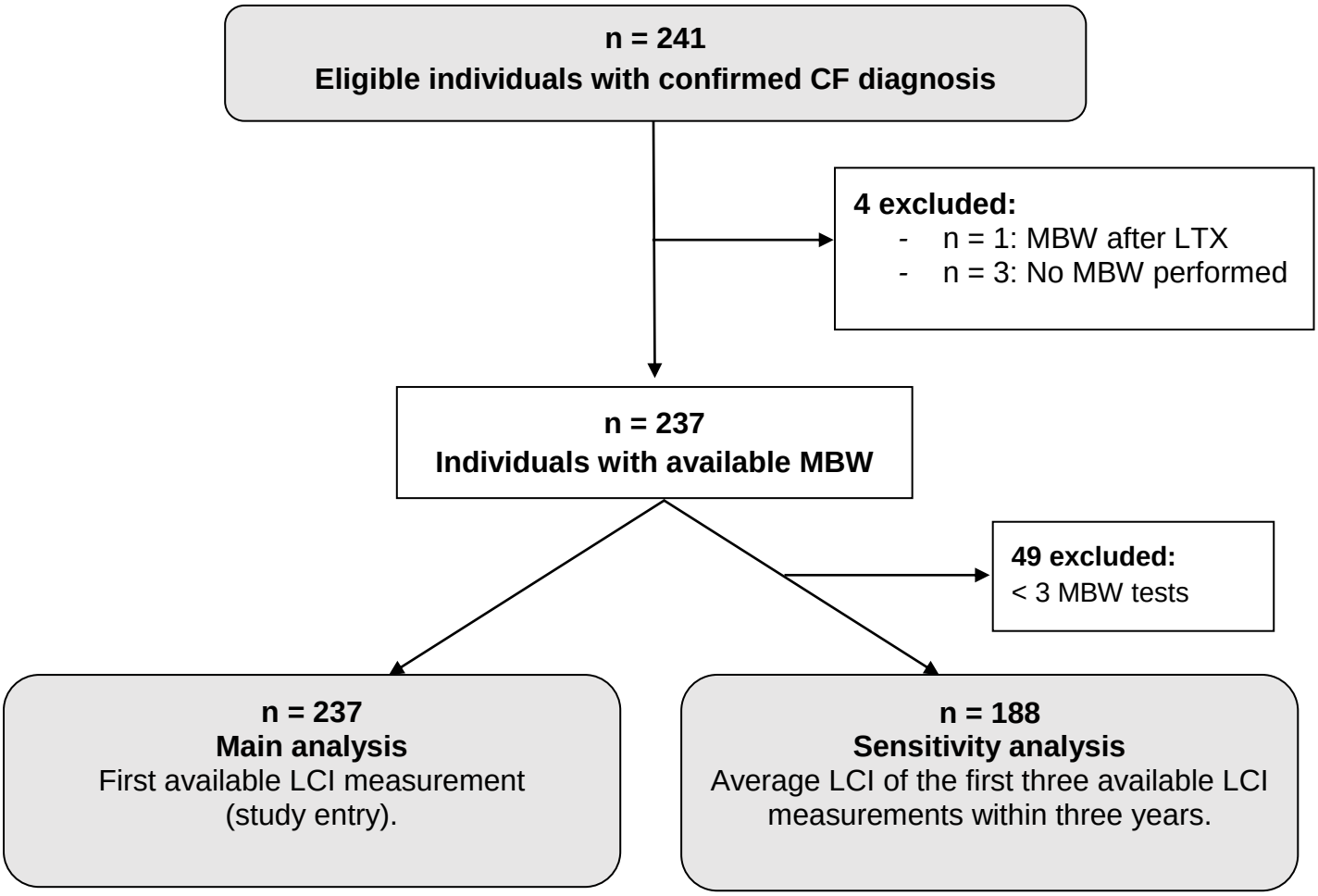
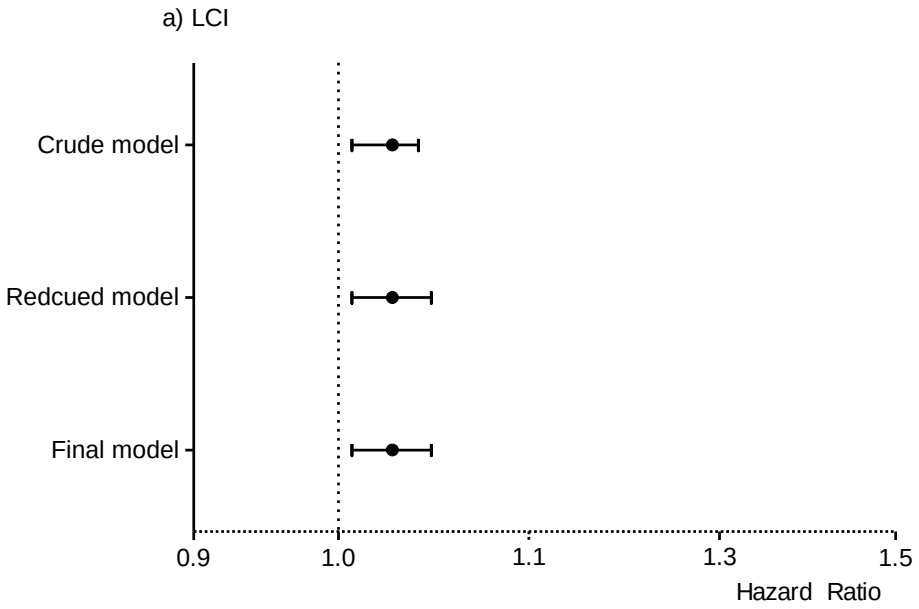


Figure 1. Participant flow diagram. *Eligibility criteria:* Age ≥ 5 years, confirmed CF diagnosis, routine clinical care in CF-centre Bern between 1986–2006. *Inclusion criteria:* Availability of at least one MBW test. CF = Cystic fibrosis, LCI = Lung clearance index, MBW = Multiple breath washout, LTX = Lung transplantation.



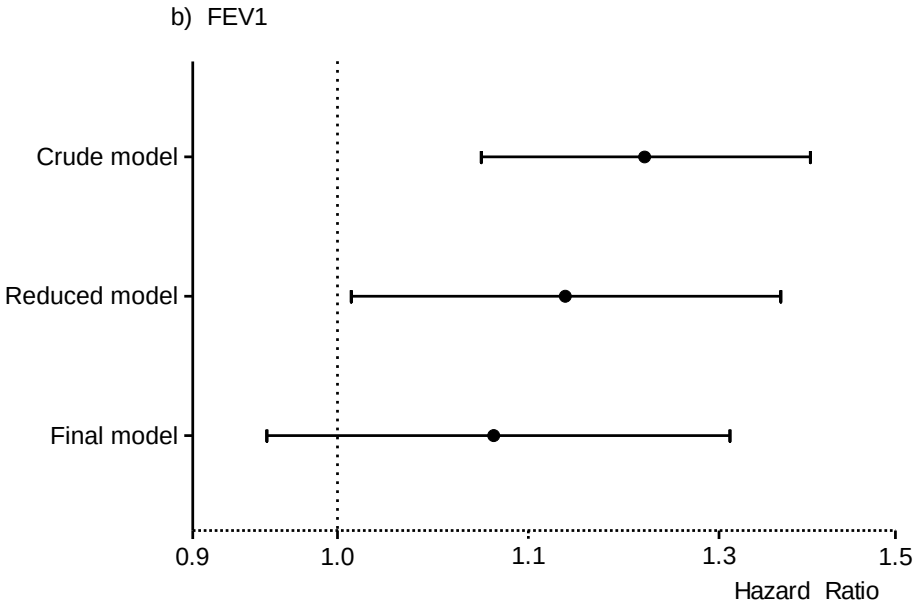


Figure 2. Risk of death or lung transplantation according to baseline lung function. Crude and adjusted Hazard Ratios [95% CI] for the risk of death or lung transplantation in a) per one z-score increase in LCI or b) per one z-score decrease in FEV_1 in 237 individuals with CF using the first available LCI and corresponding FEV_1 value as baseline. The closed circles

display the estimate (HR) and the horizontal lines indicate 95% CI; X-axis shows log-transformed HR. Additive inverse of FEV_1 (FEV_1*-1) was used to allow better comparison of LCI with FEV_1. *Definitions*: Crude model: unadjusted HR per one z-score increase in LCI and one z-score decrease in FEV_1; Reduced model: Adjusted HR per one z-score increase in LCI and one z-score decrease in FEV_1, adjusted for the selected variables sex, age, BMI, year of birth, number of hospitalisations; Final model: HR per one z-score increase in LCI and per one z-score decrease in FEV_1, adjusted mutually in addition to the aforementioned variables. BMI = Body mass index, CF = Cystic fibrosis, HR = Hazard Ratio, LCI = Lung clearance index, LTX = Lung transplantation.

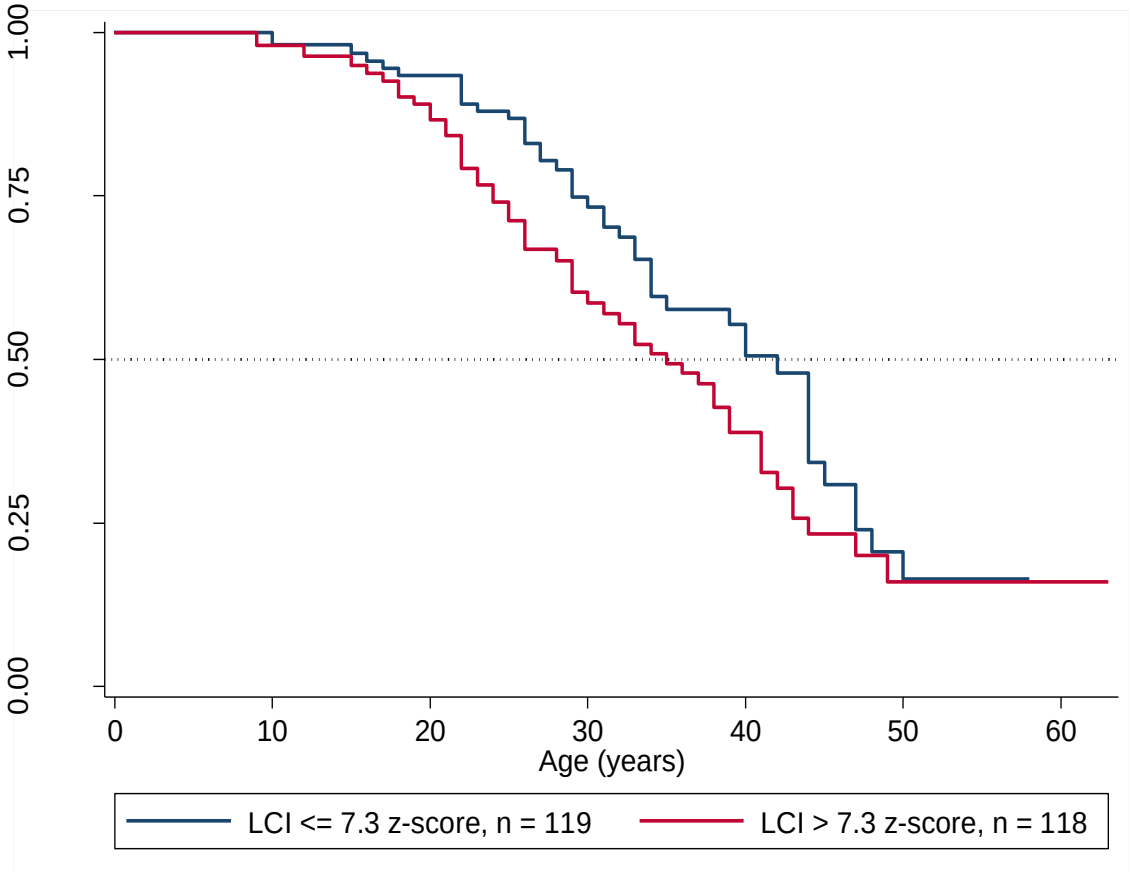
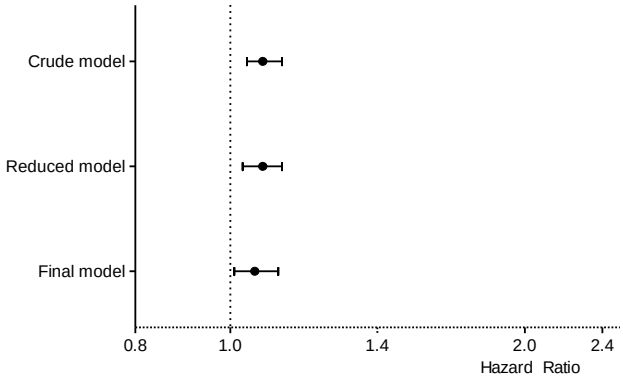
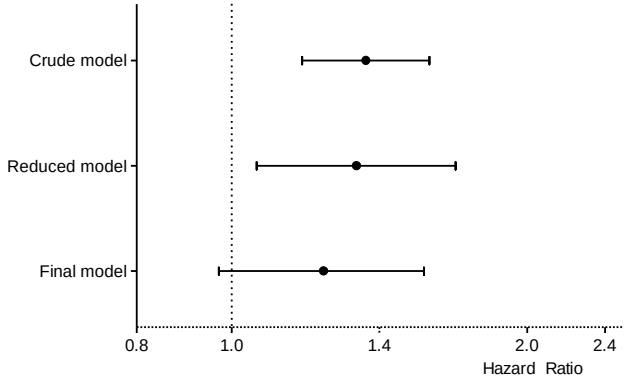


Figure 3. Respiratory survival in individuals with CF according to baseline LCI. Individuals with baseline LCI value ≤ study population median of 7.3 z-score, n = 119 are highlighted in blue. Individuals with baseline LCI value > study population median of 7.3 z-score, n = 118 are highlighted in red. *Definitions:* p = 0.50: 50% of the individuals in each group died or received LTX. CF = Cystic fibrosis, LCI = Lung clearance index.

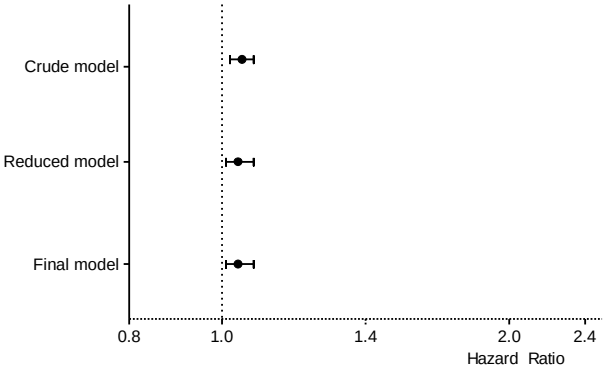
a) LCI in individuals with >= 3 measurements



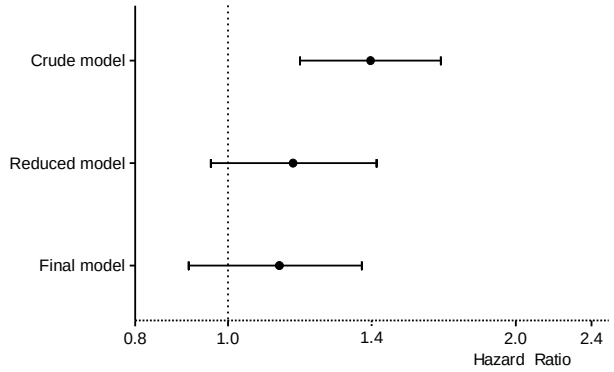
b) FEV1 in individuals with >= 3 measurements



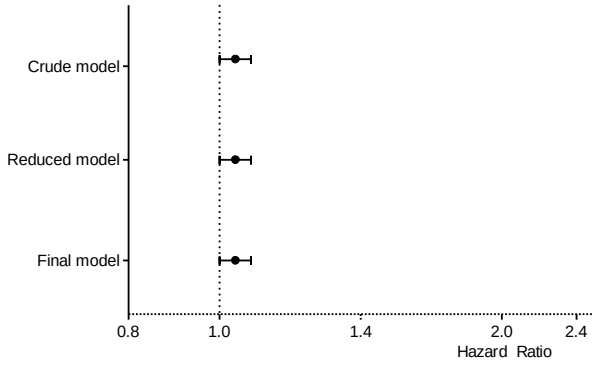
c) LCI in children <=16 years of age



d) FEV1 in children <= 16 years of age



e) LCI in individuals born after 1987



f) FEV1 in individuals born after 1987

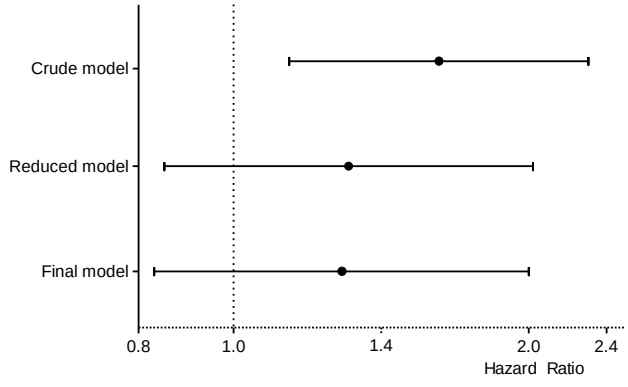


Figure 4. Risk of death of lung transplantation in the sensitivity analysis. Crude and adjusted Hazard Ratios [95% CI] for the risk of death or lung transplantation in the sensitivity analyses: a) Per one z-score increase in LCI and b) per one z-score decrease in FEV_1 in individuals ≥ 3 MBW measurements within three years after study entry using the average of the first three available LCI and corresponding FEV_1 values as baseline (n = 188). c) Per one z-score increase in LCI and d) per one z-score decrease in FEV_1 in children ≤ 16.0 years of age (n = 168) using the first available LCI and corresponding FEV_1 value as baseline. e) Per one z-score increase in LCI and f) per one 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 closed circles display the estimate (HR) and the horizontal lines indicate 95% CI, X-axis shows log-transformed HR. Additive inverse of FEV1 (-FEV1) was used to allow better comparison of LCI with FEV1. *Definitions*: Crude model: unadjusted HR per one z-score increase in LCI and one z-score decrease in FEV_1, Reduced model: Adjusted HR per one z-score increase in LCI and one z-score decrease in FEV_1, adjusted for the selected variables sex, age, BMI, year of birth, number of hospitalisations; Final model: HR per one z-score increase in LCI and per one z-score decrease in FEV_1, adjusted mutually in addition to the aforementioned variables. BMI = Body mass index, CF = Cystic fibrosis, HR = Hazard Ratio, LCI = Lung clearance index, LTX = Lung transplantation.

Tables

Table 1. Population characteristics.

Baseline	
n [females, %]	237 [113, 51.1]
Year of birth	1983 [10.7; 1952 – 2000]
Median age at CF diagnosis [IQR, range]	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]
F508del homozygous, n [%]	136 [57.4]
F508del heterozygous, n [%]	74 [31.2]
Other, n [%]	27 [11.4]
Pancreatic insufficiency, n [%]	200 [84.4]
CFRD, n [%]	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, n [%]	173 [73.0]
Staphylococcus aureus, n [%]	137 [57.8]
ABPA, n [%]	19 [8.0]
Individuals hospitalized at least once during baseline, n (%)	107 [45.2]
Number of hospitalisations per individual during baseline	2.2 [1.7; 1.0 – 9.0]

Antibiotic treatment (oral, inhaled, intravenous), n (%)	201 [84.1]
Inhaled medication (mucolytics, bronchodilators, steroids), n (%)	204 [86.1]
Follow-up duration (years) until outcome event or end of study	16.1 [6.6; 0.3 – 32.3]
Outcome (Death or LTX), n [%]	94 [39.7]
Death, n [%]	41 [17.3]
males, n [%]	22 [53.7]
females, n [%]	19 [46.3]
LTX, n [%]	53 [22.4]
males, n [%]	27 [50.9]
females, n [%]	26 [49.1]
Loss to follow-up, n [%]	15 [6.3]
Person-years at risk	3813.3

Legend Table 1: Data presented as mean [SD, range], unless indicated otherwise. *Definitions:* Baseline = First available LCI (= study entry) and corresponding FEV_1 values; Demographic and clinical data derived within the first three years after study entry. Study entry = Date of third LCI value within the first three years after study entry; Follow-Up: Duration (years) from study entry until outcome event or end of study in 2018/12/30. *Abbreviations***:** ABPA = Allergic bronchopulmonary aspergillosis, BMI = Body mass index, CF = Cystic fibrosis, CFRD = Cystic fibrosis-related diabetes, FEV_1 = Forced expired volume in the first second, LCI = Lung clearance index, LTX = Lung transplantation.

Table 2. Risk of death or lung transplantation according to baseline lung function.

A)	**LCI (z-score)**	**FEV_1 (z-score)**
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
B)	**LCI (SD-score)**	**FEV_1 (SD-score)**
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

Legend Table 2: Crude and adjusted Hazard Ratios [95% CI], p-values for the risk of death or lung transplantation per (A) one z-score increase in LCI or per one z-score decrease in FEV_1 and (B) one SD-score increase in LCI or per one SD-score increase in FEV_1, in 237 individuals with CF using the first available LCI and corresponding FEV_1 value as baseline. *Definitions*: Crude model: unadjusted HR per one z-score increase in LCI and one z-score decrease in FEV_1, Reduced model: Adjusted HR per one z-score increase in LCI and one z-score decrease in FEV_1, adjusted for the selected variables sex, age, BMI, year of birth, number of hospitalisations; Final model: HR per one z-score increase in LCI and per one z-score decrease in FEV_1, adjusted mutually in addition to the aforementioned variables. Results for the *Mutual* and *Complete* models are provided in Table S3, OLS. *Abbreviations***:** BMI = Body mass index, CF = Cystic fibrosis, CI = Confidence interval, FEV_1 = Forced expired volume in the first second, LCI = Lung clearance index, LTX = Lung transplantation.

Table 3. Population characteristics in the sensitivity analyses.

	Individuals with ≥ 3 LCI/ FEV_1 measurements	**Children ≤ 16.0 years of age**	**Individuals born after 1987**
n [females, %]	188 [96, 51.1]	168 [78, 46.4]	102 [49, 48.0]
Year of birth	1984 [10.4; 1952 – 2000]	1988 [6.7; 1971–2000]	1992 [3.7; 1987–2000]
Median age at CF diagnosis [IQR, range]	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), n [%]	70 [37.2]	54 [32.1]	13 [13.7]
Loss to follow-up, n [%]	9 [4.8]	8 [5.4]	4 [4.2]

Legend Table 3: Data presented as mean [SD; range], unless indicated otherwise. *In individuals with ≥ 3 MBW 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. *Abbreviations*: CF = Cystic fibrosis, FEV_1 = Forced expired volume in the first second, LCI = Lung clearance index, LTX = Lung transplantation.

Table 4. Risk of death or lung transplantation in the sensitivity analyses.

	Individuals with ≥ 3 LCI/ FEV_1 measurements within 3 years	**Children ≤ 16.0 years of age**	**Individuals born after 1987**
n	188	168	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

Legend Table 4: Crude and adjusted Hazard Ratios [95% CI], p-values for the sensitivity analyses using the average LCI and average FEV_1 of the first three available LCI and corresponding FEV_1 measurements as baseline in individuals with ≥ 3 MBW measurements within three years, and in age strata using the first available LCI and corresponding FEV_1 as

baseline. *Definitions*: Crude model: unadjusted HR per one z-score increase in LCI and one z-score decrease in FEV_1; Reduced model: Adjusted HR per one z-score increase in LCI and one z-score decrease in FEV_1, adjusted for the selected variables sex, age, BMI, year of birth, number of hospitalisations; Final model: HR per one z-score increase in LCI and per one z-score decrease in FEV_1, adjusted mutually in addition to the aforementioned variables. *Abbreviations***:** ABPA = Allergic bronchopulmonary aspergillosis, BMI = Body mass index, CF = Cystic fibrosis, CI = Confidence interval, FEV_1 = Forced expired volume in the first second, HR = Hazard ratio, LCI = Lung clearance index, LTX = Lung transplantation.

ONLINE SUPPLEMENT

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], Florian Singer[1]

1. Division of Respiratory Medicine, Department of Pediatrics, Inselspital University Hospital Bern, University of Bern, Bern, Freiburgstrasse 15, 3010 Bern, Switzerland
2. Graduate School for Health Sciences, University of Bern, Bern, Switzerland
3. Institute of Social and Preventive Medicine, University of Bern, Mittelstrasse 43, 3012 Bern, Switzerland
4. Quartier Bleu, Lindenhofspital, Bremgartenstrasse 117, 3012 Bern, Switzerland

Tables

Table S1: Definitions of variables in baseline and sensitivity analyses.

Sex	Gender, binary (0: female, 1: male)
Age at CF diagnosis	Individuals age at CF diagnosis in years
Mutation	Individuals homozygote for F508del (0), *individuals heterozygote for F508del (1),* individuals with any other mutation (2)
Test date	Date (yyyy/mm/dd) of the third MBW measurement out of the three MBW measurements within the first three years after study entry; begin of crude mortality time
Age, height, weight, BMI	Values from test date (age: years, height: meters, weight: kg, BMI: z-score)
Person-years	Person-years takes the number of individuals and the amount of time each single individual spent under risk of experiencing the outcome, death or LTX, into account. As CF is a genetic disorder, the origin of "time under risk" started at birth (zero years of age) in this study
LCI	*Baseline*: First available MBW measurement as baseline value *Sensitivity analysis:* Mean of the first three LCI values within the first three years after study entry (= first available MBW measurement) as baseline value and exclusion of individuals with less than three measurements within the first three years after study entry
FEV_1	*Baseline*: FEV_1 value that was reported corresponding to the first available LCI value (FEV_1 and LCI were measured at the same day) Sensitivity analysis: Mean of the three FEV_1 values that were reported

	corresponding to the first three available LCI values within the first three years after study entry
Hospitalisations	Count of all hospitalisations reported in the electronic patient charts within the first three years after study entry
Exacerbations	Count of all exacerbations reported in the electronic patient charts within the first three years after study entry
Pancreatic insufficiency	Pancreatic insufficiency at least once reported/ diagnosed within the frist three years after study entry "yes – 1", otherwise "no - 0"
CFRD	CFRD at least once reported/ diagnosed within the first three years after study entry "yes – 1", otherwise "no - 0"
ABPA	ABPA at least once reported/ diagnosed within the first three years after study entry "yes – 1", otherwise "no - 0"
Microbiology	Pseudomonas aeruginosa at least once reported/ diagnosed within the first three years after study entry "yes – 1", otherwise "no – 0"
	Staphylococcus aureus at least once reported/ diagnosed within the first three years afters study entry "yes – 1", otherwise "no – 0"
Medication	At least once treated with any antibiotics (oral, iv, inhaled) within the first three years after study entry "yes – 1", otherwise "no – 0"
	At least once treated with any inhaled medication (mucolytics, bronchodilators, steroids) within the first three years after study entry "yes – 1", otherwise "no – 0"
Outcome (death or LTX)	Date (yyyy/mm/dd) of death or of lung transplantation as reported in the electronic patient charts (1) versus survival (0). Survival means the patient was alive until the end of the study in 2018/12/31, e.g. when the patient had at least one follow-up visit after 2018/12/31)

Legend Table S1: *Abbreviations***:** ABPA = Allergic bronchopulmonary aspergillosis, BMI = Body mass index, CF = Cystic fibrosis, CFRD = Cystic fibrosis-related diabetes, FEV_1 = Forced expired volume in the first second, LCI = Lung clearance index, LTX = Lung transplantation

Table S2: Population characteristics of individuals stratified by endpoint.

	Death	**LTX**	**Alive**	**Lost to follow-up**
n [females, %]	41 [22, 53.7]	53 [27, 50.9]	128 [54, 42.2]	15 [10, 66.7]
LCI (units)	17.2 [7.3; 3.9 – 46.6]	18.9 [8.4; 5.6 – 59.8]	16.9 [6.6; 3.5 – 47.2]	19.0 [9.2; 9.3 – 41.2]
LCI (z-score)	8.3 [7.3; - 5.0 – 37.7]	10.0 [8.4; -3.3 – 50.9]	8.0 [6.6; -5.4 – 38.3]	10.1 [9.2; 0.4 – 32.3]
FEV_1 (z-score)	-3.7 [1.9; -6.3 – 0.8]	-3.1 [1.8; -6.1 – 0.4]	-1.6 [1.6; -5.6 – 1.7]	-3.5 [1.9; -6.4 – -0.5]
Year of birth	1974 [9.4; 1952 – 1991]	1980 [8.7; 1960 – 1997]	1988 [9.8; 1955 – 2000]	1980 [7.7; 1963 – 1990]
Age at baseline (years)	19.7 [9.7; 6.0 – 41.0]	15.0 [7.4; 5.9 – 34.2]	11.4 [7.1; 5.6 – 40.2]	15.8 [6.4; 6.9 – 26.1]
BMI (z-score)	-1.3 [1.2; -4.0 – 0.3]	-1.2 [1.0; -4.0 – 1.4]	-0.5 [1.0; -3.4 – 2.4]	-1.4 [1.1; -4.0 – 0.0]
Age at outcome (years)	32.0 [10.8; 9.0 – 50.0]	28.8 [9.0; 15.0 – 47.0]	30.4 [9.8; 18.0 – 63.0]	25.3 [11.4; 8.0 – 44.0]

Legend Table S2: Data presented as mean [SD; range], unless indicated otherwise. *Abbreviations:* BMI = Body mass index, FEV_1 = Forced expired volume in the first second, LCI = Lung clearance index.

Table S3. Risk of death or lung transplantation according to baseline lung function.

	LCI	**FEV_1**
Crude model	1.04 [1.01 – 1.06]	1.25 [1.11 – 1.41],
Mutual model	1.03 [1.00 – 1.06]	1.22 [1.08 – 1.38]
Complete model	1.03 [1.00 – 1.06]	1.15 [0.97 – 1.36]
Reduced model	1.04 [1.01 – 1.07]	1.18 [1.01 – 1.38]
Final model	1.04 [1.01 – 1.07]	1.12 [0.95 – 1.33],

Legend Table S3: Crude and adjusted Hazard Ratios [95% CI] for the risk of death or lung transplantation using the first available LCI and corresponding FEV_1 value as baseline. *Definitions:* Crude model: unadjusted HR per one z-score increase in LCI and one z-score decrease in FEV_1; Mutual model: HR per one z-score increase in LCI and one z-score decrease in FEV_1, adjusted mutually; Complete model: HR per one z-score increase in LCI and per one z-score decrease in FEV_1 adjusted separately for all demographic and clinical variables (sex, age, BMI, year of birth, number of hospitalisations, number of exacerbations, mutation, pancreatic insufficiency, CFRD, ABPA, microbiology, medication), Reduced model: Adjusted HR per one z-score increase in LCI and one z-score decrease in FEV_1, adjusted for the selected variables sex, age, BMI, year of birth, number of hospitalisations; Final model: HR per one z-score increase in LCI and per one z-score decrease in FEV_1, adjusted mutually in addition to the aforementioned variables. *Abbreviations*: ABPA = Allergic bronchopulmonary aspergillosis, BMI = Body mass index, CF = Cystic fibrosis,

CFRD = Cystic fibrosis-related diabetes, CI = Confidence interval, FEV_1 = Forced expired volume in the first second, HR = Hazard ratio, LCI = Lung clearance index, LTX = Lung transplantation.

Table S4: Population characteristics in individuals with normal FEV_1 and in adults.

	Individuals with normal FEV_1 (≥ - 1.96 z-score)	**Adults > 16.0 years of age)**
n [females, %]	108 [55, 50.9]	69 [35, 50.7]
Year of birth	1988 [9.2; 1955 – 2000]	1970 [6.8; 1952–1988]
Median age at CF diagnosis [IQR, range]	1.0 [0.0 – 2.0; 0.0 – 14.0]	1.0 [0.0–3.0; 0.0–28.0]
Age (years)	10.4 [6.2; 5.6 – 40.2]	24.8 [6.5; 16.1–41.0]
BMI at study entry (z-score)	-0.4 [1.0; -4.2 – 2.4]	-1.1 [1.2; -4.2–0.4]
LCI (z-score)	7.3 [7.5; -5.4 – 50.9]	8.3 [6.8; -5.0 – 32.3]
FEV_1 (z-score)	-0.56 [0.9; -1.9 – 1.7]	-3.8 [1.8; -6.4–0.4]
Outcome (Death or LTX), n [%]	24 [22.2]	40 [58.0]
Loss to follow-up, n [%]	4 [3.7]	7 [10.1]

Legend Table S4: Data presented as mean [SD, range], unless indicated otherwise. *Definitions:* Baseline = First available LCI and corresponding FEV_1 value demographic and clinical data derived within the first three years after study entry; *Abbreviations***:** BMI = Body mass index, CF = Cystic fibrosis, FEV_1 = Forced expired volume in the first second, LCI = Lung clearance index, LTX = Lung transplantation.

Table S5: Risk of death or lung transplantation individuals with normal FEV_1 and in adults.

	Individuals with normal FEV_1 (≥ - 1.96 z-score)	**Adults (> 16.0 years of age)**
n	108	69
LCI		
Crude model	1.02 [0.97 – 1.08]	1.01 [0.96 – 1.06]
Mutual model	1.03 [0.98 – 1.08]	0.98 [0.92 – 1.03]
Complete model	1.01 [0.96 – 1.07]	1.01 [0.94 – 1.09]
Reduced model	1.02 [0.97 – 1.07]	1.00 [0.95 – 1.06]
Final model	1.03 [0.98 – 1.09]	0.97 [0.91 – 1.04]
FEV_1		
Crude model	0.99 [0.61 – 1.61]	1.33 [1.07 – 1.65]
Mutual model	0.96 [0.58 – 1.57]	1.37 [1.09 – 1.72]
Complete model	0.68 [0.35 – 1.31]	1.22 [0.92 – 1.62]
Reduced model	0.76 [0.43 – 1.36]	1.26 [0.96 – 1.64]
Final model	0.71 [0.40 – 1.29]	1.33 [0.99 – 1.79]

Legend Table S5: Crude and adjusted Hazard Ratios [95% CI] for the risk of death or lung transplantation in individuals with normal FEV_1 (≥ - 1.96 z-score) and adults (≥ 16.0 years of age) using the first available LCI and corresponding FEV_1 value as baseline. *Definitions:* Crude model: unadjusted HR per one z-score increase in LCI and one z-score decrease in FEV_1; Mutual model: HR per one z-score increase in LCI and one z-score decrease in FEV_1, adjusted mutually; Complete model: HR per one z-score increase in LCI and per one z-score decrease in FEV_1 adjusted separately for all demographic and clinical variables (sex, age, BMI, year of birth, number of hospitalisations, number of exacerbations, mutation, pancreatic

insufficiency, CFRD, ABPA, microbiology, medication), Reduced model: Adjusted HR per one z-score increase in LCI and one z-score decrease in FEV_1, adjusted for the selected variables sex, age, BMI, year of birth, number of hospitalisations; Final model: HR per one z-score increase in LCI and per one z-score decrease in FEV_1, adjusted mutually in addition to the aforementioned variables. *Abbreviations***:** ABPA = Allergic bronchopulmonary aspergillosis, BMI = Body mass index, CF = Cystic fibrosis, CFRD = Cystic fibrosis-related diabetes, CI = Confidence interval, FEV_1 = Forced expired volume in the first second, HR = Hazard ratio, LCI = Lung clearance index, LTX = Lung transplantation.

Table S6: Fully adjusted Cox proportional hazards regression model.

	HR	**P**	**[95% CI]**
LCI (z-score)	1.033	0.030	1.003 – 1.064
FEV_1 (z-score)	1.099	0.294	0.921 – 1.310
Sex (0 = female, 1 = male)	0.486	0.003	0.302 – 0.783
Age (years)	0.892	0.002	0.830 – 0.958
BMI (z-score)	0.771	0.034	0.606 – 0.980
Year of birth	0.947	0.104	0.886 – 1.011
Age at CF-diagnosis	0.998	0.952	0.936 – 1.064
Mutation (0 = F508del homozygous, 1 = F508del heterozygous, 2 = Other)	1.027	0.910	0.652 – 1.615
Number of exacerbations	1.075	0.277	0.944 – 1.224
Number of hospitalisations	1.129	0.196	0.939 – 1.357
ABPA (1= yes, 0 = no)	1.595	0.238	0.734 – 3.466
Pseudomonas aeruginosa (1= yes, 0 = no)	1.148	0.637	0.606 – 2.176
Staphylococcus aureus (1= yes, 0 = no)	0.679	0.122	0.415 – 1.109
CFRD (1= yes, 0 = no)	0.873	0.693	0.444 – 1.715
Pancreas insufficiency (1= yes, 0 = no)	0.895	0.866	0.245 – 3.272
Antibiotic medication (1 = yes, 0 = no)	1.079	0.911	0.287 – 4.058

Legend Table S6: Estimates for the fully adjusted Cox proportional hazard regression model using the first available LCI and corresponding FEV_1 value as baseline. *Abbreviations:* ABPA = Allergic bronchopulmonary aspergillosis, BMI = Body mass index, CF = Cystic fibrosis, CFRD = CF-related diabetes, CI = Confidence interval, FEV_1 = Forced expired volume in the first second, HR = Hazard ratio, LCI = Lung clearance index, P = P-value.

Table S7: Final Cox proportional hazards regression model.

	HR	**P**	**[95% CI]**
LCI (z-score)	1.037	0.011	1.008 – 1.066
FEV_1 (z-score)	1.124	0.164	0.953 – 1.327
Sex (0 = female, 1 = male)	0.570	0.011	0.370 – 0.878
Age (years)	0.907	0.005	0.847 – 0.971
BMI (z-score)	0.772	0.027	0.613 – 0.971
Year of birth	0.952	0.109	0.896 - 1.011
Number of hospitalisations	1.117	0.151	0.961 – 1.300

Legend Table S7: Estimates for the final Cox proportional hazards regression model using the first available LCI and corresponding FEV_1 value as baseline. Definition: Final model. HR per one z-score increase in LCI and one z-score decrease in FEV_1 adjusted mutually in addition to the selected variables (sex, age, BMI, birth year, number of hospitalisations). *Abbreviations:* BMI = Body mass index, CI = Confidence interval, FEV_1 = Forced expired volume in the first second, HR = Hazard ratio, LCI = Lung clearance index, P = P-Value

Figure legends

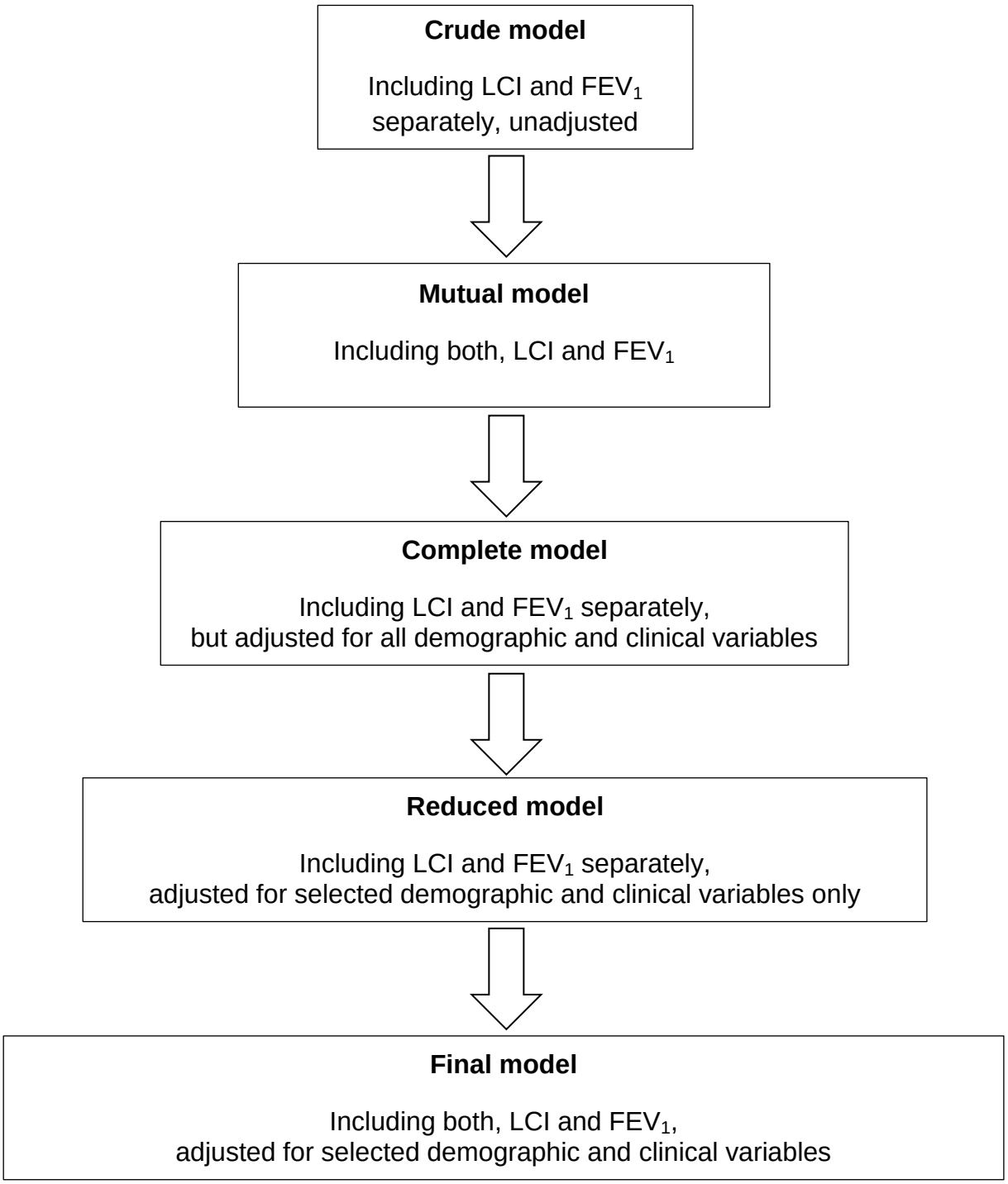


Figure S1. Analysis steps. *Crude model:* Unadjusted HRs per z-score increase in LCI and decrease in FEV_1. *Mutual model:* HR per z-score increase in LCI and decrease in FEV_1, adjusted mutually; *Complete model:* HR per z-score increase in LCI and decrease in FEV_1,

adjusted separately for all demographic and clinical variables (sex, age, BMI, year of birth, mutation, age at CF diagnosis, infection burden, CFRD, ABPA, pancreas function, medication, number of exacerbations and hospitalisations); *Reduced model:* HR per z-score increase in LCI and decrease in FEV_1, adjusted separately for selected variables only (sex, age, BMI, year of birth, number of hospitalisations); *Final model:* HR per z-score increase in LCI and decrease in FEV_1, adjusted mutually in addition to selected variables (sex, age, BMI, year of birth, number of hospitalisations). ABPA = Allergic bronchopulmonary aspergillosis, BMI = Body mass index, CF = Cystic fibrosis, CFRD = Cystic fibrosis-related diabetes, FEV_1 = Forced expired volume in the first second, HR = Hazard ratio, LCI = Lung clearance index.

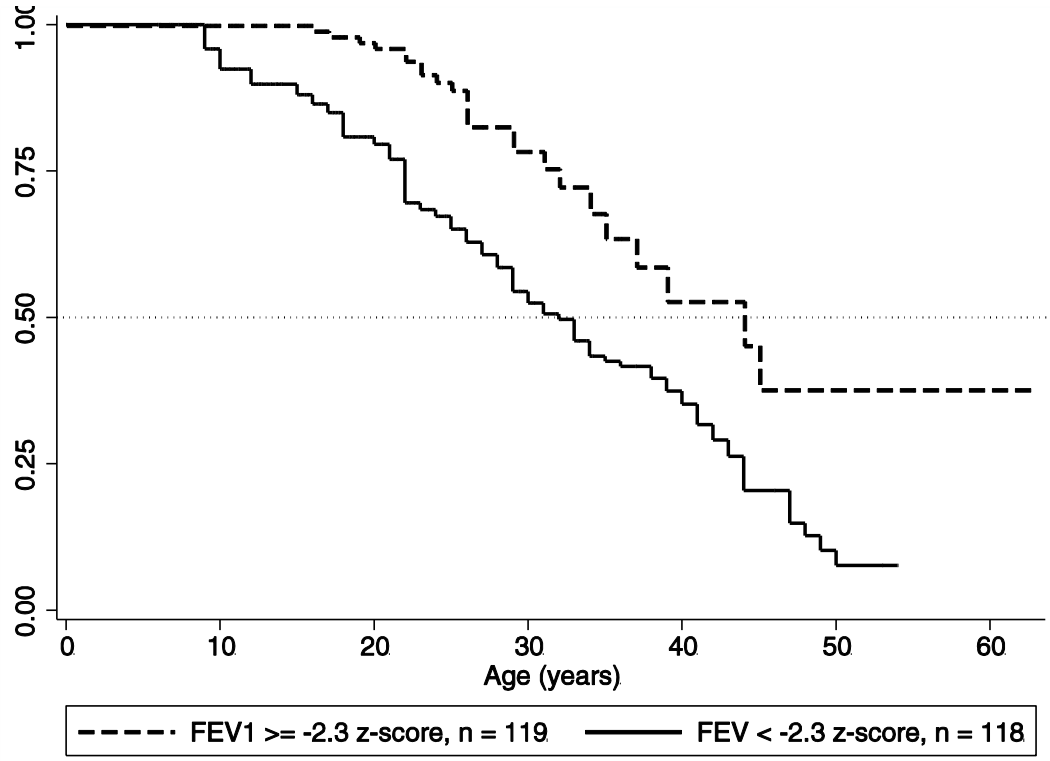


Figure S2. Respiratory survival in individuals with CF according to baseline FEV_1. Individuals with baseline FEV_1 values ≥ study population median of -2.3 z-score, n = 119 (dashed line) vs. individuals with baseline FEV_1 values < study population median of -2.3 z-score, n = 118 (solid line) using the first available FEV_1 value as baseline. CF = Cystic fibrosis, FEV_1 = Forced expired volume in the first second, LTX = Lung transplantation, p = 0.50: 50% of the individuals in each group died or received LTX.

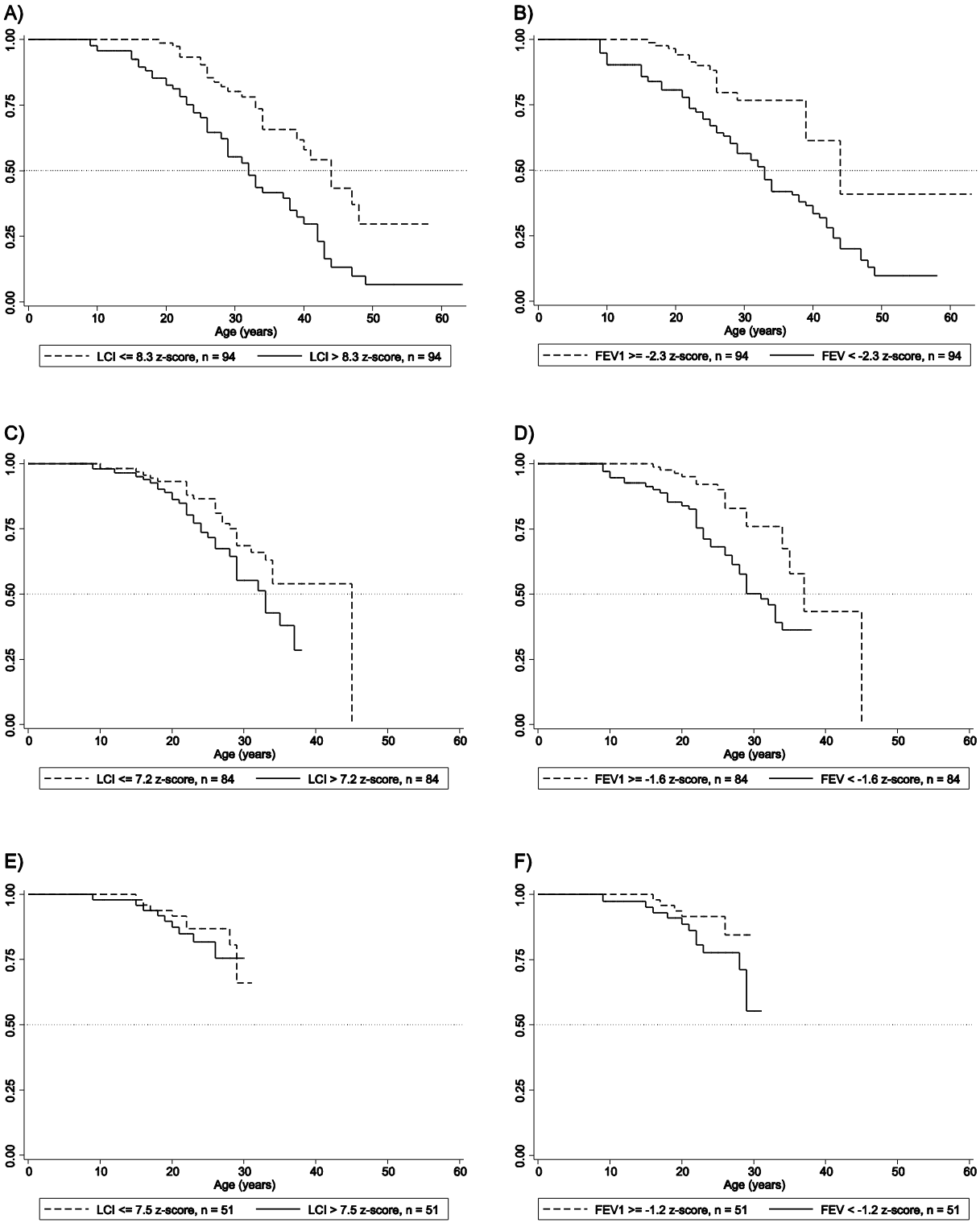


Figure S3. Respiratory survival in the sensitivity analyses. A) Individuals with ≥ 3 MBW measurements within 3 years and average baseline LCI values ≤ study population median of 8.3 z-score, n = 84 (dashed line) vs. individuals with average baseline LCI values > study

population median of 8.3 z-score, n = 84 (solid line). *B)* Individuals with ≥ 3 MBW measurements within 3 years and average baseline FEV_1 values ≥ study population median of -2.3 z-score, n = 94 (dashed line) vs. adults with average baseline FEV_1 values < study population median of -2.3 z-score, n = 94 (solid line); *C)* Children (≤ 16.0 years of age) with baseline LCI value ≤ study population median of 7.2 z-score, n = 84 (dashed line) vs. children with baseline LCI value > study population median of 7.2 z-score, n = 84 (solid line); *D)* Children (≤ 16.0 years of age) with baseline FEV_1 value ≥ study population median of -1.6 z-score, n = 84 (dashed line) vs. children with baseline FEV_1 value < study population median of -1.6 z-score, n = 84 (solid line). *E)* Individuals born after 1987 with baseline LCI value ≤ study population median of 7.5 z-score, n = 51 (dashed line) vs. individuals with baseline LCI value > study population median of 7.5 z-score, n = 51 (solid line). *F)* Individuals born after 1987 with baseline FEV_1 value ≥ study population median of -1.2 z-score, n = 51 (dashed line) vs. individuals with baseline FEV_1 value < study population median of -1.2 z-score, n = 51 (solid line). CF = Cystic fibrosis, FEV_1 = Forced expired volume in the first second, LCI = Lung clearance index, LTX = Lung transplantation, p = 0.50: 50% of the individuals in each group died or received LTX.

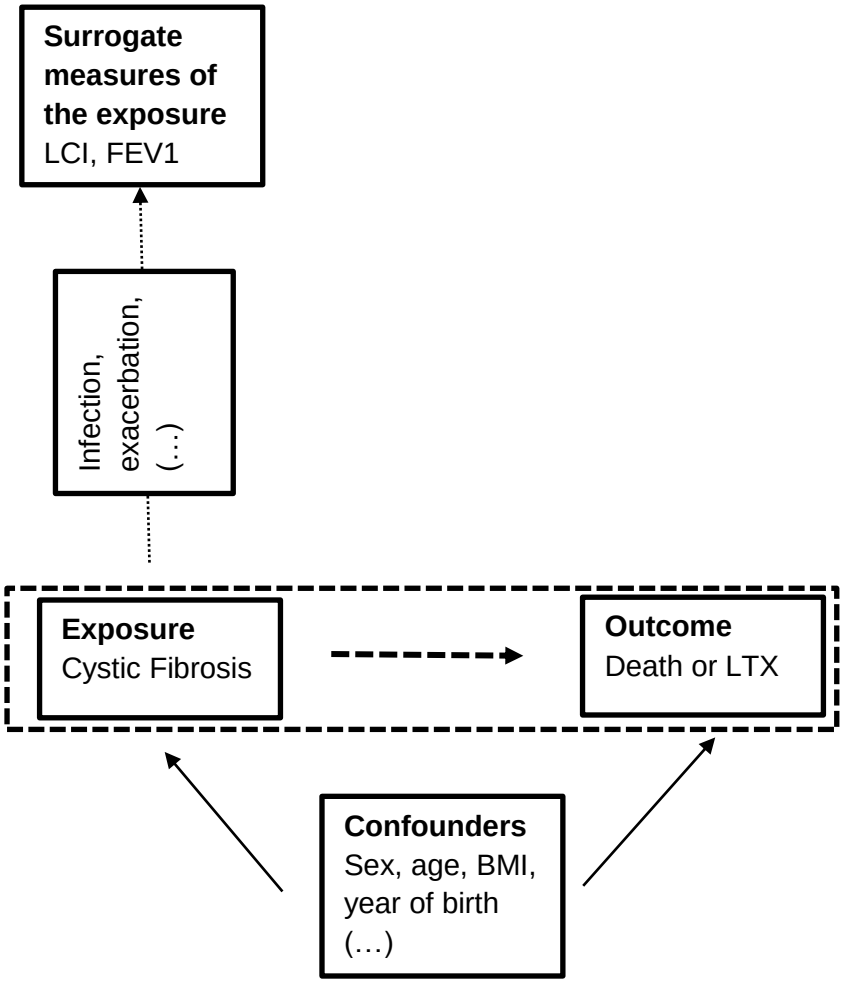


Figure S4. Directed acyclic graph (DAG) of a causal structure possibly underlying the associations of LCI and FEV_1 with survival in individuals with CF. CF influences LCI and FEV1 (dotted arrow) and also death or LTX (dashed arrow), but LCI and FEV1 are not a cause of death or LTX, therefore there is no arrow between LCI and FEV1 and death and LTX.