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Longitudinal Course of Clinical Lung Clearance Index in Children with Cystic Fibrosis

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**Take home message (250 characters)**

Lung clearance index (LCI) is sensitive to assess lung disease progression in children with cystic fibrosis in clinical routine. An increased change in LCI should prompt further diagnostic intervention to determine the underlying pathological process.

**Author contributions:** BF, KR, SY, and PL were responsible for the conception and design of this study. Data acquisition was conducted by BF, SB, LK, and SY. BF, KR, PL, and BS were responsible for data interpretation. BS supported the statistical analysis which was conducted by BF. BF, KR, and PL drafted the manuscript and all authors revised and approved the manuscript for intellectual content before submission.

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**Key words:** Cystic fibrosis, multiple breath washout, lung clearance index, children, adolescence

**Total word count: 3222 words**

This article has an Online Data Supplement

**Abstract**

*Rationale*

While lung clearance index (LCI) is a sensitive marker of small airway disease in individuals with cystic fibrosis (CF), less is known about longitudinal changes in LCI during routine clinical surveillance.

*Objectives*

To describe the longitudinal course of LCI in children with CF during routine clinical surveillance and assess influencing factors.

*Methods*

Children with CF aged 3 - 18 years performed LCI measurements every three months as part of routine clinical care between 2011-2018. We recorded clinical data at every visit. We used a multilevel mixed-effect model to determine changes in LCI over time and identify clinical factors that influence LCI course.

*Measurements and Main Results*

We collected LCI from 1204 visits (3603 trials) in 78 participants, of which 907 visits had acceptable LCI data. The average unadjusted increase in LCI for the entire population was 0.29 LCI units/year (95% CI 0.20 - 0.38). The increase in LCI was more pronounced in adolescence, with 0.41 units/year (95% CI 0.27 - 0.54). Colonization with either Pseudomonas aeruginosa or Aspergillus fumigatus, pulmonary exacerbations, CF-related diabetes, and bronchopulmonary aspergillosis were associated with a higher increase in LCI over time. Adjusting for clinical risk factors reduced the increase in LCI over time to 0.24 LCI units/year (95% CI 0.16 – 0.33).

*Conclusion*

LCI measured during routine clinical surveillance is associated with underlying disease progression in children with CF. An increased change in LCI over time should prompt further diagnostic intervention.

**Word Count Abstract:** 236

## Introduction

Lung disease in cystic fibrosis (CF) starts early in life with bacterial infection and pulmonary inflammation leading to structural abnormalities [1, 2]. Damage to the lung during sensitive periods of lung growth and development can influence the trajectory of lung disease [1-3]. Therefore, early and sensitive monitoring of lung disease progression is essential. Conventional surveillance strategies have several limitations. Lung function monitoring using conventional methods such as spirometry is less sensitive to assess small airway pathology [4, 5]. Computed tomography (CT) leads to radiation exposure [6], bronchoalveolar lavage (BAL) requires sedation, and the invasive nature of both measures should limit their application to exigent clinical problems. Therefore, the multiple breath washout (MBW) technique has gained increasing interest as a non-invasive and sensitive tool to detect early CF lung disease [4, 5].

The lung clearance index (LCI) is a global measure of ventilation inhomogeneity from MBW that is sensitive to early, peripheral lung disease in children with CF [4, 5]. In healthy individuals, LCI ranges between 6 and 8, an increase in LCI above these limits indicates worsening lung function [7]. LCI correlates with the extent of structural disease [8-10] and pulmonary inflammation [11] and is responsive to interventions [12, 13] in children with CF. Together, these data suggest that LCI is an appropriate clinical surveillance outcome in young children with CF.

However, before LCI can be used to guide clinical decisions, further knowledge into the longitudinal course of LCI during routine clinical surveillance is needed. To date, studies into longitudinal changes in LCI have rarely extended beyond twelve months [14, 15], were performed in research settings [16], or used equipment that is currently not available [17, 18]. At the Bern University Children's Hospital, MBW measurements have been part of the 3-

monthly routine clinical surveillance of pediatric participants with CF since 2011 using a commercially available MBW device. We have utilized this unique dataset of LCI measurements from early childhood to adolescence to 1) assess the longitudinal course of LCI in children with CF during routine clinical surveillance, 2) identify the clinical factors associated with changes in LCI over time, and 3) assess the different abilities of LCI and $FEV_1$ to capture disease progression.

## Methods

### Study design

This study was a longitudinal, observational, single-center study in children with CF aged three to 18 years attending 3-monthly outpatient clinical surveillance visits at the Bern University Children's Hospital between 01/01/2011 and 31/12/2018. Clinical data was assessed retrospectively by structured chart review. Written informed consent was obtained from participants and caregivers at study entry and the study was approved by the local ethics committee in Bern.

### Clinical data

Clinical data were collected at 3-monthly outpatient visits conducted by a pediatric pulmonologist using a standardized questionnaire. At each visit, symptoms and treatment review of the past three months, clinical examination, microbiological sampling (throat swab or sputum), and lung function testing (MBW, spirometry) were performed. Pulmonary exacerbations were assessed at each visit and respiratory symptoms classified according to the modified Fuchs criteria [19] (see Online Supplement). The influence of pro-inflammatory pathogens (Pseudomonas aeruginosa (P. aeruginosa), Aspergillus fumigatus (Aspergillus), Staphylococcus aureus (S.aureus), Haemophilus influenzae (H. influenzae), Streptococcus pneumoniae (S. pneumoniae) [20]) on LCI was examined as an acute effect at every visit and by the overall colonization status over the entire study period. Colonization status was considered chronic if the pathogen was present in at least 50% of the samples and intermittent if present less than 50% but at least once. This approach takes into account varying lengths of study participation [21]. Further, we assessed the influence of new acquisition of a pathogen on the course of LCI. Severe exacerbations were defined if the

modified Fuchs criteria were fulfilled with subsequent need for intravenous (i.v.) antibiotics and considered as number per year to avoid overestimation of this factor in participants with longer follow-up. CF-related diabetes was defined according to ISPAD Guidelines (online supplement) and assessed as acute effect by the time of clinical evidence [22]. Acute bronchopulmonary aspergillosis (ABPA) was defined as occurring at least once over the study period or never. For data analysis, only participants with at least three clinical visits and acceptable lung function data were included. A detailed summary of variable definition is available in the online supplement.

**Lung function**

Nitrogen MBW tests were performed using the Exhalyzer D MBW device (Eco Medics AG, Duernten, Switzerland) and Spiroware software with settings according to current consensus [23] (Supplemental). Testing was performed using a mouthpiece and dead space adjusted according to the participant's weight. As different software versions were used for data collection during the study period (3.1.3, 3.1.6, 3.2.1), MBW trials were reloaded into the latest Spiroware version 3.2.1 to ensure comparability. Quality control was performed according to current guidelines [23-26] and tests with at least two acceptable MBW trials were included in our analysis. Spirometry (Jaeger MasterScreen, CareFusion, Hochberg, Germany) was performed after MBW according to ATS/ERS guidelines [27-30]. Results for spirometry are expressed as z- score values calculated from the Global Lung Initiative reference equations [31].

**Statistical analysis**

We used a mixed-effects linear regression model to assess the mean rate of change in LCI and spirometry indices with age included as linear term. We included a participant-specific random intercept and random slope to account for between-participant variability, different observation periods for each participant, and unequal numbers of study visits [32]. The baseline model was adjusted for sex and BMI. We then adjusted the final model for predefined clinically most relevant covariates. Next, we assessed all potentially influencing covariates on LCI course first in a univariate analysis and second in the fully adjusted model. We distinguished between time-invariant characteristics (sex, pathogen colonization, severe exacerbations, ABPA) and time-varying characteristics that were visit-specific (acute pathogen sampling, acute exacerbations, CF-related diabetes, BMI). *Time-invariant* covariates were included as main effects and interaction terms with age to assess whether covariates were associated with a steeper slope in LCI over time. V*isit-specific* covariates were included as main effects only to assess the absolute differences in LCI associated with the presence of the characteristic at a given time point. Non-parametric summaries are presented for skewed data; for normally distributed characteristics parametric summaries are used. Statistical analyses were performed using Stata 16.0 (StataCorp 2019, College Station; TX). Figures were created using Stata 16.0 or Graph Pad Prism (Prism G 2018, La Jolla; California).

## Results

### Study population

Seventy-eight children (44 female) with CF between three and 18 years of age were monitored clinically and had MBW measurements performed between January 2011 and December 2018. Overall, 3603 MBW trials were reloaded and quality controlled in Spiroware 3.2.1 and 1375 (38%) trials were excluded due to quality or technical issues (Figure 1). Feasibility by age group is summarized in Table E1. In total, 907 visits from 71 participants satisfied the inclusion criteria of at least three visits per participant with acceptable MBW data and matched clinical data. Demographical characteristics are summarized in Table 1 and E2.

### LCI course over time without adjustments for risk factors

First, we studied the increase in LCI over time without adjusting for clinical risk factors. As shown in Figure 2, LCI increased with age (mean slope of 0.29 LCI units/year, 95% confidence interval (CI) 0.2 - 0.38; $p < 0.001$). LCI was stable during the preschool years (slope = -0.4 LCI units/year (95% CI -1.1 – 0.33)) and started to increase at school age (0.21 (0.07 – 0.35)). The highest increase in LCI was observed during adolescence (0.41 (0.27 – 0.54). There was a significant interaction between age and LCI slope (p-value for interaction = 0.02) (Table 2).

The pattern of LCI slope was different between males and females (Figure 2). Females displayed an earlier and more consistent increase in LCI over time than males, whereas males had a rapid increase in LCI between the ages of 10 and 14 years. Over the entire period there was no difference in the unadjusted increase in LCI between females (0.5 LCI units/year, 95% CI 0.27 – 0.63) and males (0.42 LCI units/year, 95% CI 0.21 – 0.64; p-value for interaction 0.86).

**Covariates associated with an increase in LCI over the study period**

The influence of clinical covariates on LCI course was first assessed in a univariate analysis and then in the full adjusted model (Table 3). We found that colonization with any proinflammatory pathogen was associated with a steeper increase in LCI over time as indicated by the significant interaction with LCI slope (Table 3). Aspergillus colonization was individually associated with a steeper increase in LCI, with a higher LCI slope in those chronically colonized compared with never colonized (Table 3, Figure 3). There was also a trend towards a higher LCI slope in those with chronic P. aeruginosa colonization compared with never colonized, which was not statistically significant (Figure 3). For S.aureus and H. influenzae colonization we found no significant interaction with LCI slope. Further, new acquisition of Aspergillus or P. aeruginosa was associated with a steeper increase in LCI slope compared with before colonization (Supplemental Table E3). Severe exacerbations and experiencing ABPA during the study period were associated with a steeper increase in LCI over time. The increase in LCI over time was independent of baseline characteristics (baseline LCI, follow-up time, comorbidities, and medication use) (Supplemental Table E4).

**Covariates associated with acute changes in LCI**

We also assessed visit-specific covariates that could result in acute changes in LCI (Table 4). Acute exacerbations and clinical evidence of CF-related diabetes were associated with acute changes in LCI. A higher BMI was associated with a better (lower) LCI. Acute, visit-specific pathogen colonization (any proinflammatory pathogen, Aspergillus, P. aeruginosa S.aureus, H. influenza), was not associated with acute LCI changes (Supplemental Table E4).

**Increase in LCI over time with adjustments for risk factors**

When adjusting our model for the predefined clinically most relevant covariates sex, BMI, PsA- and Aspergillus-colonization, CF-related diabetes, acute and severe exacerbations, the increase in LCI over time diminished from 0.29 (0.2 – 0.38) (Figure 2) to 0.24 (0.16 – 0.33) LCI units/year (Figure 4, Supplemental Table E5). The change in LCI over time in the fully adjusted model indicates that also in absence of risk factors (e.g. colonization with P.aeurginosa), LCI would still increase substantially over time.

After adjustment for clinical risk factors, the pattern of change in LCI at different ages was comparable to the unadjusted model, however, the overall increase in LCI was less pronounced (Figure 4, Table 2). The steeper increase in LCI observed in females during adolescence was due to a combination of different prevalences of risk factors and different effect sizes of covariates between males and females (Supplemental Table E6). After adjusting for all covariates in the final model, the increase in LCI over time was similar between females and males (Figure 4).

**Lung function over time for spirometry indices**

For spirometry indices, $FEV_1$ and $FEF_{25-75}$, the pattern of lung function decline for the entire cohort was comparable to LCI (Figure E1). The unadjusted decrease was found to be -0.09 $FEV_1$ z-score/year (95% CI -0.14; -0.05); -0.09 $FEF_{25-75}$ z-scores /year (95% CI -0.14; -0.04) and -0.3 FVC z-scores/year (95% CI -0.07; 0.01) , with no differences in slope between preschool, school-age and adolescence (Table E7).

The association of time-invariant risk factors for spirometry indices was less pronounced than for LCI (Table E8 and E9). For time-dependent risk factors, only acute pulmonary exacerbation was associated with the rate of change of all spirometry indices (Table E10). The decrease in lung function over time after adjusting for risk factors remained similar to the unadjusted model ($FEV_1$: -0.08 z-score/year (95%CI -0.13; -0.04); $FEF_{25-75}$: -0.07 z-score/year (95%CI -0.11; -0.03); FVC -0.04 z-score/year (95%CI -0.08; 0.01).

## Discussion

We found that LCI measured during routine clinical surveillance increased over time in children with CF, even after adjusting for clinical risk factors. LCI starts to increase at school-age followed by a steeper increase during adolescence. LCI course was primarily influenced by pulmonary exacerbations, pathogen colonization, CF-related diabetes, and ABPA.

### *Comparison with literature*

We report an increase in LCI over time between 0.24 and 0.29 LCI units/year, depending on which risk factors were adjusted for in the model. Only few studies have assessed the course of LCI over 12 months or longer, and have reported increases between 0.18 and 0.61 LCI units/year [14-18]. However, comparability of absolute LCI values may be limited due to the different methodologies and study designs.

In our cohort, LCI remained stable during the preschool years (3 – 5 years) and did not significantly increase until school age and adolescence. This is contrary to findings of Stanojevic *et al.*, who reported an increase in LCI of 0.4 LCI units/year over a two-year follow-up of preschool children with CF aged 2.5 to 6 years [16]. The cause of these divergent findings is unclear but likely to be influenced by these differences: i) cohort characteristics: mostly newborn screened CF patients in the Stanojevic study vs mostly clinically diagnosed in our cohort and ii) study design differences: research setting with standardized enrolment and follow-up compared with a clinical surveillance setting with different periods of enrolment and follow-up. Further, one major difference in our study was that the clinicians in our centre were not blinded to LCI. Therefore, preschool children with elevated LCI might have received more intensive treatment at a period where lung disease is still modifiable.

Interestingly, follow-up data of the Stanojevic cohort [33] revealed that LCI flattened during school-age years, which they hypothesized was due to intense periods of treatment during preschool years. Substantial therapeutic changes in the last few years with the availability of CFTR-modulators may be an additional explanation of the different LCI trajectories between these two cohorts. Our findings are similar to the reported increase in LCI of 0.18 LCI units/year from preschool to adolescence in patients with CF in the study of Davies *et al.* [18]. While there are methodological differences to our study, the cohort assessed is clinically the most comparable currently available. The different patterns of LCI increase between males and females during adolescence only observed in our study are most probably attributable to the different prevalence of risk factors between males and females.

Associations between LCI and disease markers have mostly been reported in cross-sectional or short-term longitudinal studies. A range of clinical factors, such as pulmonary inflammation, infection, and exacerbations have been shown to influence LCI [8, 11, 13, 34, 35]. We confirmed that these clinical variables also influence the long-term course of LCI. A novel finding was the strong association between Aspergillus colonization and increase in LCI independent of other risk factors and occurrence of ABPA. While associations between Aspergillus infection and clinical outcomes have been reported previously, the impact of Aspergillus infection, especially in absence of ABPA diagnosis, on lung disease progression is unclear [36-38]. Interestingly, detection of Aspergillus in BAL samples from children with CF was associated with progression of structural lung disease on chest CT over time [34]. We also found that colonization with any proinflammatory pathogen and new acquisition of P. aeruginosa was associated with an increased LCI slope [20]. The detrimental effect of pulmonary exacerbations on long-term lung function decline has been reported previously

for spirometry [39]. In our study, we could show that severe and acute pulmonary exacerbations were associated with acute and long-term effects on LCI course [13].

While spirometry indices $FEV_1$ and $FEF_{25-75}$ showed comparable patterns of lung function decline to LCI, the association with age was less pronounced. Except for acute exacerbations, none of the risk factors assessed were consistently associated with spirometry outcomes. While direct comparison between spirometry outcomes and LCI was not the objective of our study, our findings suggest that LCI is more sensitive to detect underlying clinical manifestations of lung disease in children with CF compared to spirometry [4, 17].

*Strengths and limitations*

One of the main strengths of the present study is the use of a longitudinal dataset of routinely measured LCI in a clinical cohort of children with CF. Despite an increasing number of studies using LCI as an endpoint, data on the long-term course of LCI are still limited. We used a commercially available MBW device and analyzed all data in the currently available software version to obtain high-quality MBW data. We performed rigorous quality control according to latest guidelines [23, 24, 26]. This approach led to a relatively high exclusion rate of MBW tests (24%). While these numbers appear high for an experienced center in MBW testing, it is important to note that MBW testing at our center started in 2011 before current quality control standards were available. Further, feasibility was lowest at preschool age, which is not unexpected and comparable to other studies (Supplemental Table E1)[40]. Thus, we believe our dataset is representative of LCI in the clinical setting as it incorporates all challenges associated with the use of LCI measurements in routine testing: limited measuring time, the broad age range of participants with varying disease severities, staff with different levels of testing experience and varying intervals between measurements. The

definition of pathogen colonization status and the sensitivity of different sampling methods likely influenced the interpretation of pathogen data in our study. However, there is no clear consensus on how to define colonization in the clinical setting and recent omics-based monitoring further questions our understanding of culture-based pathogen detection [41]. In general, one of the major limitations of observational studies is to disentangle the direct effects of exposure variables from potential cofounding characteristics of the participants. This issue could have been minimized with a healthy control population which was not available for our cohort.

*Clinical relevance*

We found that LCI increases over time in children with CF even in the absence of risk factors, indicating that LCI is sensitive to assess disease progression without overt clinical symptoms. A wide spectrum of different clinical factors, including microbiology, pulmonary, and endocrinological complications were associated with increasing LCI, which suggests that LCI is useful to monitor disease pathology in CF. An increased LCI in individual children with CF should prompt further diagnostic tests. Overall, our results highlight the clinical validity of LCI for several reasons: (i) known clinical risk factors of disease progression influenced LCI course over time, (ii) LCI increases also in absence of risk factors, and (iii) adjusting for risk factors minimized differences in LCI course between female and male participants.

*Outlook*

Two main questions remain to be addressed before LCI can be recommended for widespread clinical use: (i) is LCI responsive to detect and monitor treatment changes in clinical practice? and (ii) would LCI guided therapy improve outcomes in children with CF? To

answer these questions, randomized trials of LCI guided therapies [42] where clinicians are blinded to LCI results are needed. To better interpret and understand changes in LCI from a pathophysiological point of view, prospective longitudinal studies that compare LCI results with structural and/or functional imaging methods [43, 44] and direct comparison of conventional lung function parameters are required. Future longitudinal studies in cohorts of participants diagnosed by newborn screening and/or receiving novel CFTR-modulating treatments are needed to define the impact of these substantial diagnostic and therapeutic changes on LCI course.

*Conclusions*

These novel data from longitudinal clinical measurements provide further evidence that LCI is a sensitive measure to assess lung disease progression over time. An increased change in LCI should prompt further diagnostic intervention to determine the underlying pathological processes even in absence of overt clinical signs and symptoms.

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Figure 1: Flow chart of study population and MBW tests included. Multiple breath washout tests were considered acceptable if at least two trials passed quality control review. Percentages of accepted MBW trials are calculated as proportion of the total number of trials. Percentage of accepted visits are calculated as proportion of the total number of visits. Percentage of participants included are calculated as proportion of the total participants evaluated. Abbreviations: MBW: multiple breath washout.

Figure 2: LCI increase over time without adjustments. The increase in LCI is shown over age and was found, on average, to be 0.29 LCI units/year (95% CI 0.2; 0.38). Increase in preschool age was -0.4 LCI units/year (95%CI -1.1;0.33), in school-age 0.21 LCI units/year (95%CI 0.07; 0.35), in adolescence 0.41 LCI units/year (95%CI 0.27; 0.54) (p-value for interaction 0.02), with a steeper increase in females during adolescence. On the y-axis, LCI raw values are given. Line represents mean LCI 2.5% across all participants with available data at a given age. Shaded areas represent point-wise upper and lower 95% confidence intervals, the dotted line refers to an upper limit of normal for LCI of 8. Abbreviations: LCI: lung clearance index. CI: Confidence interval.

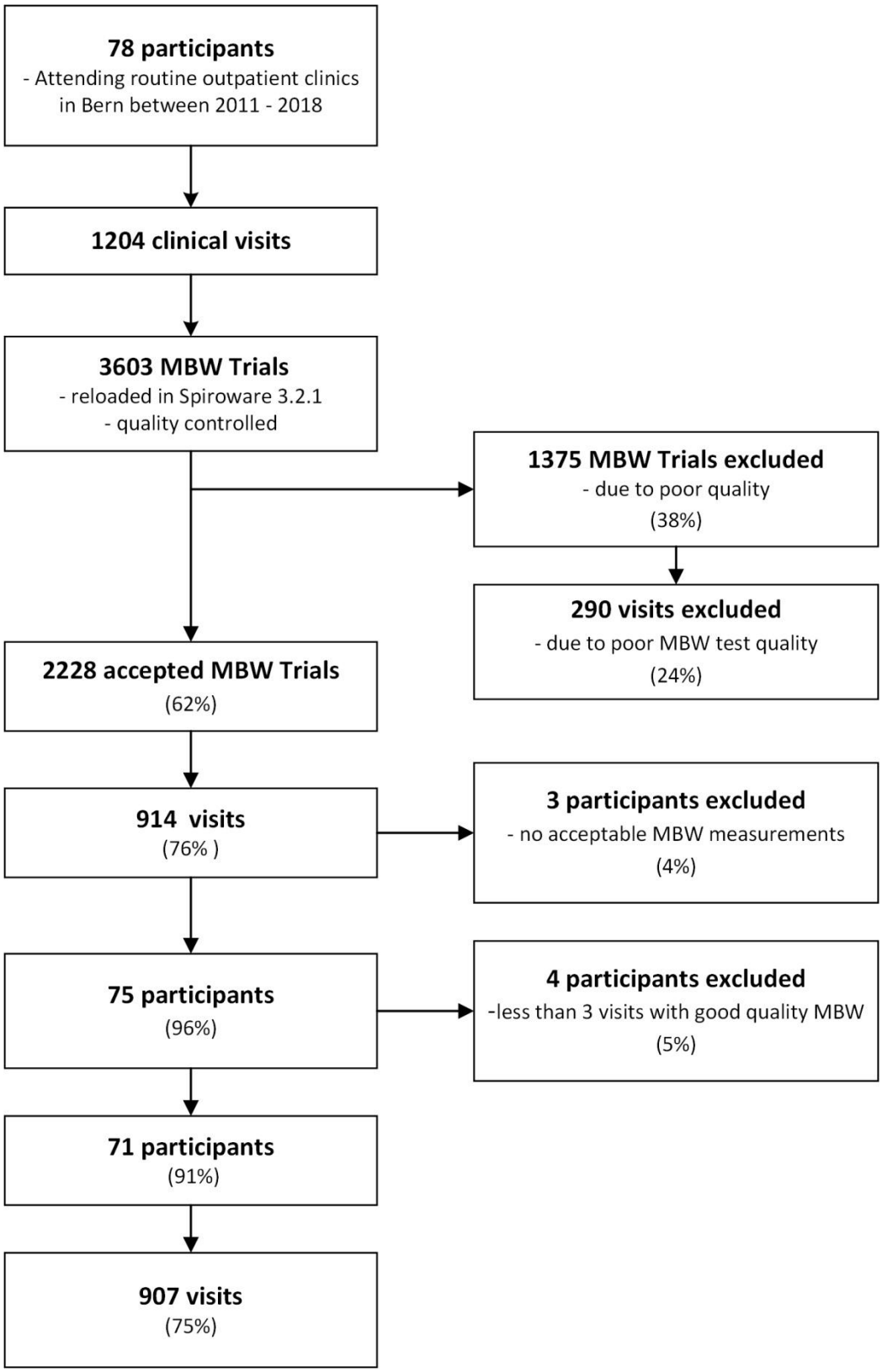
Figure 3 and b: Effect of pathogen colonization on LCI increase over time. Change in LCI over time with the corresponding confidence interval is given for participant groups that were colonized with Aspergillus or P.aeruginosa; either never, intermittent (1x – < 50% of the samples/observation time) or chronic (=50% of the samples/observation time). For Aspergillus (a), the slopes of LCI were significantly different within colonization groups and compared to the overall increase without pathogen colonization (p-value for interaction 0.04). For P.aeruginosa (b) colonization, the slope of LCI was only significantly different compared to the overall increase but not within colonization groups (p-value for interaction 0.2). P-values

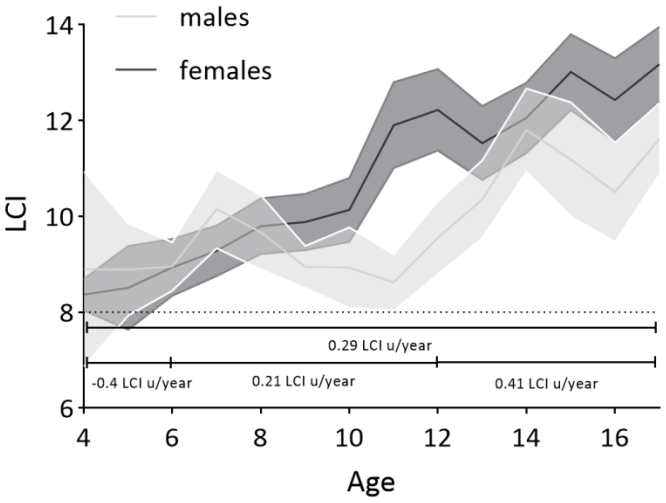
given refer to the comparison for each group to the never colonized group. Slope coefficient and predicted LCI are derived from the fully adjusted model (Supplemental Table E5). Circles, squares, and triangles represent the mean, whiskers upper, and lower 95% confidence interval. Abbreviations: P.aeruginosa: Pseudomonas aeruginosa; Aspergillus: Aspergillus fumigatus. LCI u/year: Lung clearance index units per year.

Figure 3 and b: Effect of pathogen colonization on LCI increase over time. Change in LCI over time with the corresponding confidence interval is given for participant groups that were colonized with Aspergillus or P.aeruginosa; either never, intermittent (1x – < 50% of the samples/observation time) or chronic (=50% of the samples/observation time). For Aspergillus (a), the slopes of LCI were significantly different within colonization groups and compared to the overall increase without pathogen colonization (p-value for interaction 0.04). For P.aeruginosa (b) colonization, the slope of LCI was only significantly different compared to the overall increase but not within colonization groups (p-value for interaction 0.2). P-values given refer to the comparison for each group to the never colonized group. Slope coefficient and predicted LCI are derived from the fully adjusted model (Supplemental Table E5). Circles, squares, and triangles represent the mean, whiskers upper, and lower 95% confidence interval. Abbreviations: P.aeruginosa: Pseudomonas aeruginosa; Aspergillus: Aspergillus fumigatus. LCI u/year: Lung clearance index units per year.

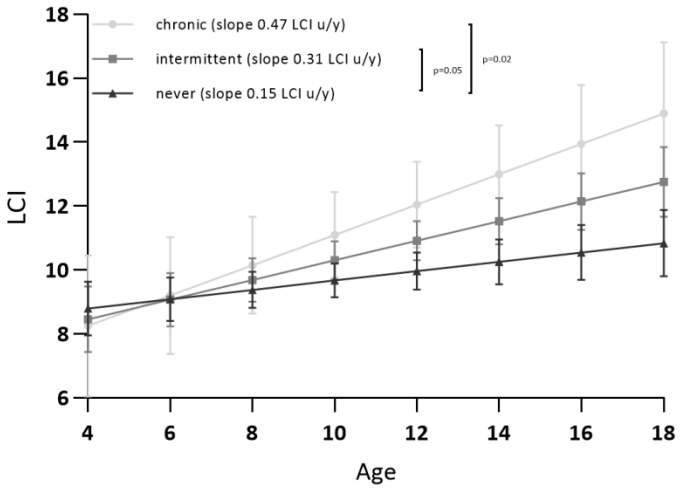
Figure 4: LCI increase over time with adjustments for risk factors. In the absence of risk factors, the increase in LCI is shown over age and was found to be 0.24 LCI units/year (95% CI 0.16; 0.33). Increase in preschool age was -0.48 LCI units/year (95%CI -1.2;0.22), in school age 0.16 LCI units/year (95%CI 0.03; 0.29), in adolescence 0.35 LCI units/year (95%CI 0.22; 0.49) (p-value for interaction 0.01),

with a similar pattern of increase between males and females. On the y-axis, LCI values are given derived from the fully adjusted model as described in the manuscript and Supplemental Table E5. Line represents the point-wise mean LCI 2.5% across all participants at a given age, shaded areas representing upper and lower 95% confidence intervals, the dotted line refers to an upper limit of normal for LCI of 8. Abbreviations: LCI u/year: lung clearance index units per year; CI: Confidence interval.

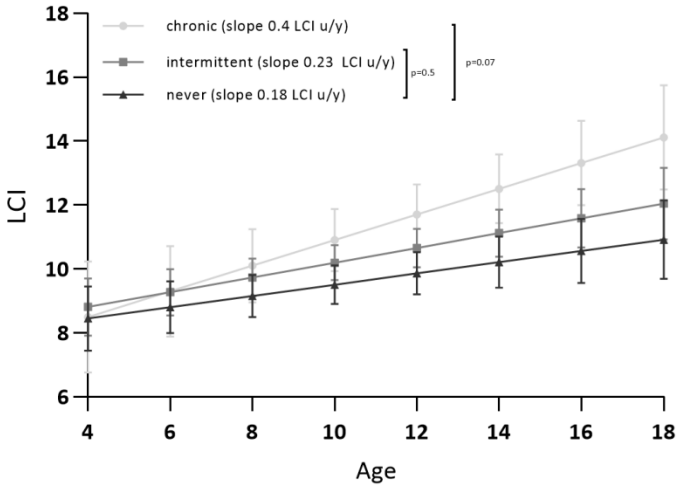

78 participants
- Attending routine outpatient clinics in Bern between 2011 - 2018
1204 clinical visits
3603 MBW Trials
- reloaded in Spiroware 3.2.1
- quality controlled
1375 MBW Trials excluded
- due to poor quality
(38%)
290 visits excluded
- due to poor MBW test quality
(24%)
2228 accepted MBW Trials
(62%)
914 visits
(76% )
3 participants excluded
- no acceptable MBW measurements
(4%)
75 participants
(96%)
4 participants excluded
-less than 3 visits with good quality MBW
(5%)
71 participants
(91%)
907 visits
(75%)

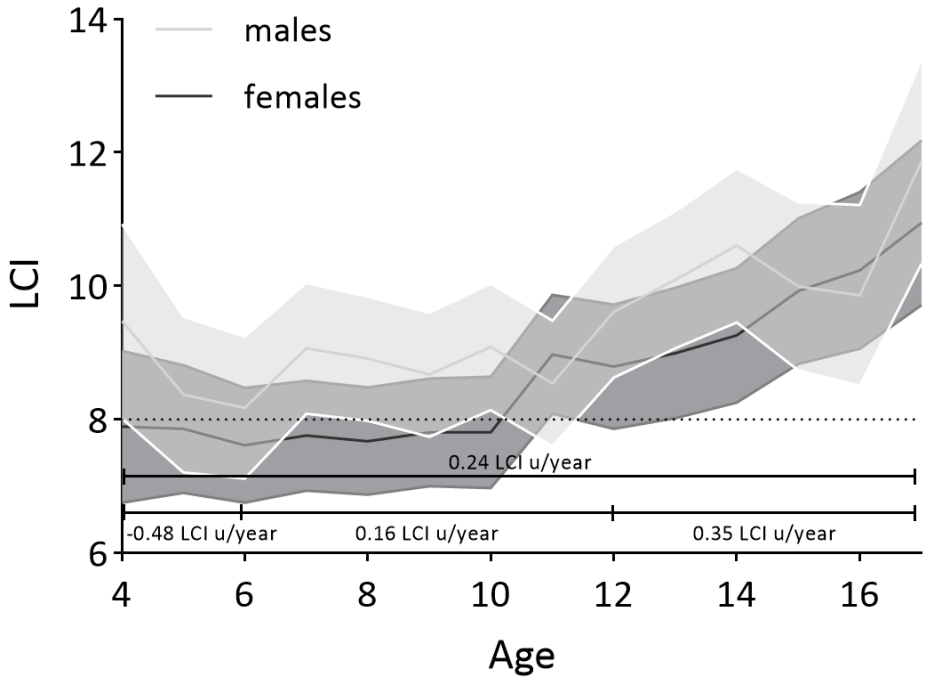

males
females
LCI
14
12
10
8
6
4
6
8
10
12
14
16
Age
0.29 LCI u/year
-0.4 LCI u/year
0.21 LCI u/year
0.41 LCI u/year

## Aspergillus colonization




## P.aeruginosa colonization




14
12
10
8
6
LCI
males
females
0.24 LCI u/year
-0.48 LCI u/year
0.16 LCI u/year
0.35 LCI u/year
4
6
8
10
12
14
16
Age

**Tables**

***Table 1: Patient demographics at baseline***

| | |
|---|---|
| **Patients (n)** | 71 |
| **Female: n (%)** | 44 (62) |
| **Age (years)*** | 8.2 (4; 15.21) |
| **Mutation status** | |
| **Class I-III** | 70 (99) |
| **Class IV-VI** | 1 (1) |
| **Pancreatic function** | |
| **Insufficient** | 66 (93) |
| **Sufficient** | 5 (7) |
| **Weight z-score** | -0.22 (-2.86; 1.76) |
| **Height z-score** | -0.1 (-3.3; 2.32) |
| **BMI z-score** | -0.01 (-1.85; 1.65) |
| **$FEV_1$ z-score** | -1.1 (-4.3; 1.49) |
| **LCI 2.5%** | 9.36 (6.45; 15.7) |
| **FRC (L)** | 1.1 (0.55; 2.8) |

**Demographic characteristics of included participants.** Data are presented as median (range) or n (%). Definition of abbreviations: BMI = Body mass index, $FEV_1$: forced expiratory volume in one second, LCI: Lung clearance index, FRC: Functional residual capacity. *Age range for inclusion in this study was 3-18 years, however, the youngest participant that could be included for further analysis was 4 years old.

***Table 2: Increase in LCI over time from preschool age to adolescence***

| | Baseline Model | | | Fully adjusted model | | |
|---|---|---|---|---|---|---|
| | Slope * (LCI u/year) | 95% CI | p-value † | Slope‡ (LCI u/year) | 95% CI | p-value † |
| 3 - 5 years (N= 17) | **-0.4** | -1.1; 0.33 | | **-0.48** | -1.2; 0.22 | |
| 6 – 11 years (N= 42) | **0.21** | 0.07; 0.35 | | **0.16** | 0.03; 0.29 | |
| 12 – 18 years (N= 12) | **0.41** | 0.27; 0.54 | **0.02** | **0.35** | 0.22; 0.49 | **0.01** |

**Increase in LCI over time by age groups when adjusting for baseline characteristics and for potential risk factors (see full adjusted model in Table E5).** LCI did not change during preschool years and started to increase at school age with the steepest increase at adolescence. Abbreviations: LCI u/year: LCI units per year, CI: Confidence interval.

* The slope coefficient is derived from the baseline model adjusted for sex and body mass index using age as the time variable and an interaction term for age group.

† P- value from the interaction of age group with LCI slope.

‡ The slope coefficient is derived from the fully adjusted model (Supplemental Table E5) using age as the time variable and an interaction term for age group.

A positive coefficient indicates a worsening LCI (higher LCI), whereas a negative coefficient indicates an improving LCI (lower LCI). Values in bold are statistically different from 0 at $p < 0.05$ significance level.

***Table 3: Covariates associated with an increased change in LCI over time***

| | Slope* (LCI u/years) | 95% CI | p-value for interaction[†] |
|---|---|---|---|
| Sex | | | |
| Males (N=27) | 0.21 | 0.07; 0.34 | |
| Females (N=44) | 0.27 | 0.15; 0.36 | 0.56 |
| Any proinflammatory pathogen[#] | | | |
| Intermittent (N=9) | **0.21** | 0.06; 0.37 | |
| Chronic (N=62) | **0.24** | 0.15; 0.32 | **0.001** |
| Aspergillus colonization | | | |
| Never colonized (N=40) | **0.15** | 0.03; 0.26 | |
| Intermittent (N=26) | **0.31** | 0.18; 0.43 | |
| Chronic (N=5) | **0.48** | 0.21; 0.73 | **0.04** |
| P. aeruginosa colonization | | | |
| Never colonized (N=31) | 0.18 | 0.04; 0.31 | |
| Intermittent(N=29) | 0.23 | 0.11; 0.35 | |
| Chronic (N=11) | 0.40 | 0.2; 0.6 | 0.2 |
| H. influenzae colonization | | | |
| Never colonized (N=18) | 0.41 | 0.25; 0.56 | |
| Intermittend (N=50) | 0.19 | 0.09; 0.28 | |
| Chronic (N=3) | 0.24 | -0.17; 0.66 | 0.07 |
| S.aureus colonization | | | |
| Never colonized (N=5) | 0.23 | -0.08; 0.54 | |

| | | | |
|---|---|---|---|
| Intermittend (N=17) | 0.07 | -0.10; 0.24 | |
| Chronic (N=49) | 0.29 | 0.19; 0.39 | 0.09 |
| Severe exacerbations (N/year) | | | |
| 0 /year (N=36) | **0.12** | -0.01; 0.24 | |
| ≥1x/year (N=35) | **0.34** | 0.23; 0.45 | **0.01** |
| No ABPA /study period (N=62) | **0.20** | 0.11; 0.28 | |
| ABPA / study period (N=9) | **0.40** | 0.26; 0.54 | **0.01** |

**Influence of time-invariant clinical covariates on LCI course in the fully adjusted analysis.**

Definitions of abbreviations: LCI u/year: Lung clearance index units per year; CI: Confidence interval; Aspergillus: Aspergillus fumigatus; P.aeruginosa: Pseudomonas aeruginosa; ABPA: acute bronchopulmonary aspergillosis.

Covariates assessed are time-invariant and considered in the model as stated over the entire study period. The slope coefficients are derived from a multilevel mixed effects model adjusted for the main effects of final selected covariates (fully adjusted model summarized in Supplemental Table E5).

* Slope represents the increase in LCI over time compared within the group characteristic. The 95% CI represents the comparison to zero increase in LCI for each subgroup separately. A positive coefficient indicates a worsening LCI (higher LCI), whereas a negative coefficient indicates an improving LCI (lower LCI). Values in bold are statistically different from 0 at $p < 0.05$ significance level.

† P- value is derived from the interaction within the group characteristic and LCI slope

# Proinflammatory pathogens were considered S.aureus, P.aeruginosa, H.Influenzae, S.pneumoniae and Aspergillus

***Table 4: Covariates associated with acute changes in LCI***

| | LCI units | 95% CI |
|---|---|---|
| Acute Exacerbations (N = 586/907) | **0.64** | 0.45; 0.83 |
| CF-related diabetes (N=84/907)* | **1.1** | 0.37; 1.75 |
| BMI z-score | **-0.60** | -0.84; -0.36 |

**Influence of visit specific clinical covariates on acute LCI changes in the fully adjusted analysis.** Definitions of abbreviations: LCI: Lung clearance index; CI: Confidence interval; CF: cystic fibrosis; BMI: Body mass index.

Coefficients represent the increase in LCI at a given timepoint compared to those without the characteristic (95% confidence interval) and are derived from a multilevel mixed effects model adjusted for the main effects of final selected covariates (fully adjusted model summarized in Supplemental Table E5). A positive coefficient indicates a worsening LCI (higher LCI), whereas a negative coefficient indicates an improving LCI (lower LCI). Values in bold are statistically different from 0 at $p < 0.05$ significance level.

*6 patients developed diabetes during the study period, 2 patients had diabetes at study entry; in total contributing to 84 visits with diabetes.

**Longitudinal course of clinical lung clearance index in children with cystic fibrosis**

Bettina S. Frauchiger, Severin Binggeli, Sophie Yammine, Ben Spycher, Linn Krüger, Kathryn A. Ramsey, Philipp Latzin

Online Data Supplement

## Methods

### Lung function testing

Nitrogen multiple breath washout (MBW) tests were performed using an unmodified Exhalyzer D Device (EcoMedics AG, Duernten, Switzerland) and associated software Spiroware with settings according to current consensus guidelines [1]. The main outcomes reported were the lung clearance index (LCI) and functional residual capacity (FRC) in liters. Testing was performed in an upright position using a mouthpiece and a nose clip to ensure a leak-free system. Dead-space was adjusted according to participant's weight. For participant's under 35 kg we used the dead space reducer set 2 and for heavier participants set 3, as recommended by the manufacturer. As measurements were collected in a clinical outpatient setting, roughly twenty minutes were spent per test and a minimum of two trials was collected. As MBW measurements were initiated in 2011 in our clinic, different Spiroware software versions (3.1.3, 3.1.6, 3.2.1) were used due to manufacturer-dependent improved software releases over the study period. To ensure the comparability of the outcomes, every MBW trial was reloaded manually in the latest software version Spiroware 3.2.1. Every trial was quality controlled by an experienced reviewer (BF) according to recent guidelines [1-3]. An MBW test was considered successful if at least two acceptable trials were available wherefrom the mean LCI and FRC were calculated for further analyses. Spirometry (Jaeger MasterScreen, CareFusion, Hochberg, German) was performed after MBW according to ATS/ERS guidelines with the primary outcome $FEV_1$ in liters [4].

**Pulmonary exacerbation assessment**

To assess pulmonary exacerbation status at each visit, symptoms were classified by the treating physician according to the modified Fuchs criteria provided by the EuroCareCF working group [5]: increased cough, increased quantity or change in sputum color, increased malaise or fatigue, less physical performance, increased dyspnea or shortness of breath, decreased appetite, loss of weight (> -5 percentiles or > 10% weight loss compared to the last visit) and decrease in $FEV_1$ percent predicted > 10% compared to the last visit. A pulmonary exacerbation was defined if at least two of the modified Fuchs criteria were present and were considered independent of treatment decision. Treatment was considered separately for mild pulmonary exacerbations treated with oral antibiotics and assessed as acute effects at every visit. Severe pulmonary exacerbations were defined if at least two of the modified Fuchs criteria were met along with the subsequent need for intravenous antibiotic therapy and hospitalization. Severe exacerbations were considered as number per year to avoid overestimation of this factor in participants with longer study participation.

**Microbiological sampling**

Microbiological review is performed at every visit either by throat swab or spontaneous sputum sampling. We assessed the influence of pro-inflammatory pathogens (Pseudomonas aeruginosa (P. aeruginosa), Aspergillus fumigatus (Aspergillus), Staphylococcus aureus (S.aureus), Haemophilus influenzae (H. influenzae), Streptococcus pneumoniae (S. pneumoniae) [6]) cumulative and for each pathogen separately. Influence of pathogens on LCI was considered as acute effect at every visit and summarized as colonization status over the entire study period. Colonization status was considered chronic if pathogen was present in at least 50% of the samples and intermittent if present less than 50% but at least once. This approach takes into account varying lengths of study participation [7]. Further, we

assessed the influence of acquisition of a pathogen on the slope of LCI. Herefore, we compared the slope of LCI before a pathogen was present to the slope after the first positive sampling.

**Genotype classification**

Genotype was grouped according to mutation classes, class I-III mutations representing the most severe phenotypes, class IV-VI mutations representing the milder phenotypes [8].

**Other outcomes studied**

We studied the effect of prophylactic treatment regimens, such as hypertonic saline inhalation, dornase alfa use, and chronically inhaled antibiotics. Participants were considered to be on the beforementioned treatments if adherence to the medication was present in at least 50% of the visits attended. CF-related diabetes (CFRD) was defined according to ISPAD Clinical Practice Consensus Guidelines and considered to be present if criteria for CFRD were met; either with or without fasting hyperglycemia [9]. Acute bronchopulmonary aspergillosis (ABPA) was evaluated if suspected by the treating physician by clinical signs and following serological analysis according to current guidelines [10]. Being a rare outcome, for further analysis in this study ABPA was considered as a binary outcome if at least one episode over the whole study period was present or if never occurred. To assess the potential influence of diagnosis before and after newborn screening was introduced in Switzerland, we studied if the increase in LCI over time differed between groups. Further, we assessed the effect of pancreatic insufficiency, total observation time in the study, and the year of diagnosis on the increase in LCI over time.

**Statistical analysis**

We used a mixed-effects linear regression model to assess the mean rate of change in LCI with age, which was included as linear term. We included a participant-specific random intercept which allows variation of the response variable LCI on the participant level (patient). The random slope for age accounts for between patient variability and handles the different observation times for each participant with unequal numbers of study visits [11]. Unstructured covariance was used to allow for different variance and covariance and correlation between slope and intercept; in other words, if LCI is correlated with the influencing variables. Our baseline model was adjusted for sex and BMI z-score. Sex was chosen due to different lung development pattern for females and males [12-14]. BMI- z-score was chosen as LCI is reported not to be independent of body size [15]. Using interaction terms, we tested for differences in LCI changes during adolescence (12-18 years) compared to school age (6-11 years) and preschool (3-5 years). To avoid variable selection only due to random fluctuation in the data [16], we predefined clinically deemed most relevant variables for the full adjusted model. The variables included in the final model were CF-related diabetes, acute pulmonary exacerbations, severe exacerbations, P.aeruginosa and Aspergillus colonization, and ABPA. Clinical covariates possibly influencing LCI course were first assessed in univariate analysis in the baseline model and then in the full adjusted model. We distinguished between time-invariant characteristics (sex, pathogen colonization, severe exacerbations, ABPA, medication, baseline characteristics) and time-varying characteristics that were visit-specific (acute exacerbations, CF-related diabetes, BMI, acute pathogen sampling). *Time-invariant* variables were included as main effects and in interaction terms with age. The latter allowed us to assess whether these variables were associated with trends in LCI (slope coefficient of age) over the whole study period. For *visit*-

*specific* covariates, we included only main effects, allowing us to assess acute changes in LCI associated with the presence of the characteristic at a given time point. Statistical analyses were performed using Stata 16.0 (StataCorp 2019) [17] and graphs created using either Stata 16.0 or Graph Pad Prism [18].

## Results

### Study population

Median age at enrollment was 8.7 years (range 4 - 15.2 years). Average time of study participation was 5 years (range 0.4 - 7.1 years), with similar distribution across all age groups (Table E1). Participants excluded (N=7) for this study were significantly younger (Table E10), however, for most of these participants, the first visit performing an MBW was assessed without any training possibility before. Most patients (89%) were diagnosed before newborn screening was introduced in Switzerland in 2011, median age at diagnosis was 3 months (range 0 - 124 months). Except for one participant, all patients were carriers of two disease-causing mutations (class I-III).

### Covariates associated with an increase in LCI over time

The main predictors of LCI are discussed in the main article. All variables that were not significantly associated with an increased rate of change in LCI are summarized in supplemental table E4.

*Baseline characteristics*

We found no association for baseline characteristics (mutation class, pancreatic insufficiency, and diagnosis by newborn screening) with an increase in LCI over time. Further, we found no long-term effect on LCI of preventive inhalation with hypertonic saline and dornase alfa but for an intensified treatment regimen by prescribing inhaled antibiotics (Table E4). These patients had a steeper slope of LCI, indicating that prescribing inhaled antibiotics is most probably a marker of disease severity.

At study entry, 93% of the patients were pancreatic insufficient and 89% of the children were diagnosed before newborn screening was introduced in Switzerland. For both covariates, the unequal sample sizes are most probably the reason why we found little

evidence for an association with an increase in LCI. Follow-up time was not associated with an elevated LCI either, ensuring that our results are not influenced by the length of observation time.

*Pathogen colonization*

The cumulative effect of colonization with any proinflammatory pathogen is presented in table 3. All patients were at least intermittent colonized with any proinflammatory pathogen (N=9) and the vast majority was colonized chronically with proinflammatory pathogens (N=62). Both, intermittent and chronic colonization with proinflammatory pathogens was associated with an increased slope of LCI. Aspergillus colonization was individually associated with a steeper increase in LCI, with a higher LCI slope in those chronically colonized compared with never colonized (Table 3, Figure 3). There was also a trend towards a higher LCI slope in those with chronic P. aeruginosa colonization compared with never colonized, which was not statistically significant (Figure 3). For S.aureus and H. influenzae colonization we found no significant interaction with LCI slope.

This association of pathogen colonization and LCI increase over the whole study period was further confirmed by the finding that acquiring these pathogens (Aspergillus; P. aeruginosa) was associated with a subsequent increase in LCI by the time of clinical evidence (Supplemental Table E3). For acute infection with any proinflammatory pathogen, we could not find an association with acute changes in LCI.

*Influence of sampling method*

In total, 678 throat swabs and 213 sputum samples were available. Further, participants producing sputum were significantly older with a mean age of 13.0 compared to 11.2 ($p<0.001$). Introducing the sampling type as a confounder in the model did not change the

slope for age (0.24 LCI units/year). Sensitivity analysis by introducing sampling method as confounder revealed no influence on pathogen colonization and the slope of LCI when adjusting the analysis for sampling method.

***Table E1: Feasibility of clinical LCI and follow-up times by age groups***

| | Total visits (N) | Visits with acceptable MBW (N) | Feasibility (%) | Years of follow-up | Study visits |
|---|---|---|---|---|---|
| 3 - 5 years (N= 25) | 125 | 64 | 51 | 2.7 (1.5; 5.9) | 10 (3; 18) |
| 6 – 11 years (N= 42) | 530 | 408 | 77 | 4.8 (0.4; 7.1) | 14 (3; 27) |
| 12 – 18 years (N= 11) | 546 | 442 | 81 | 4.5 (2.7; 6.2) | 15 (4; 22) |

**Summary of LCI feasibility after retrospective quality control and follow-up time in this study by age group.** Data are presented as median and range or numbers and percentages. Age group refers to the age at study entry (only their initial visit was considered for this summary). Years of study participation and number of study visits refer to the total time participated in the study.

***Table E2: Patient demographics for excluded patients***

| | Excluded | Included | p-value |
|---|---|---|---|
| **Patients (n)** | 7 | 71 | |
| **Female: n (%)** | 4 (57) | 44 (62) | |
| **Age (years)** | **4.3 (3.8 11.75)** | **8.2 (4; 15.21)** | **0.01** |
| **Mutation status** | | | |
| **Class I-III** | 7 (100)) | 70 (99) | |
| **Class IV-VI** | | 1 (1) | |
| **Pancreatic function** | | | |
| **Insufficient** | 5 (71) | 66 (93) | |
| **Sufficient** | 2 (19) | 5 (7) | |
| **Weight z-score** | -0.12 (-1.66; 1.05) | -0.22 (-2.86; 1.76) | 0.61 |
| **Height z-score** | 0.17 (-0.8; 0.9) | -0.1 (-3.3; 2.32) | 0.47 |
| **BMI z-score** | 0.01 (-2.1; 1.03) | -0.01 (-1.85; 1.65) | 0.80 |
| **$FEV_1$ z-score** | -0.8 (-4.3; 1.49) | -1.1 (-4.3; 1.49) | 0.97 |
| **LCI 2.5%** | 7.9 (6.45; 15.7) | 9.36 (6.45; 15.7) | 0.08 |
| **FRC (L)** | 0.8 (0.48; 2.4) | 1.1 (0.55; 2.8) | 0.67 |

**Characteristics for patients excluded for further analysis due to not having >3 good quality MBW compared to included patients.** Data are presented as median (range) or n (%). Definition of abbreviations: BMI = Body mass index, $FEV_1$: forced expiratory volume in one second, LCI: Lung clearance index, FRC: Functional residual capacity. P-value refers to the comparison of characteristics of included and excluded patients.

***Table E3: Influence of newly acquired pathogens on LCI course***

| | Slope * (LCI u/ years) | 95% CI | p-value for interaction |
|---|---|---|---|
| Aspergillus colonization | | | |
| Not colonized | **0.20** | 0.11; 0.29 | |
| Newly acquired | **0.28** | 0.17;0.39 | **0.02** |
| P. aeruginosa colonization | | | |
| Not colonized | **0.19** | 0.01; 0.29 | |
| Newly acquired | **0.29** | 0.19;0.29 | **0.02** |
| H. influenzae colonization | | | |
| Not colonized | 0.24 | 0.14; 0.34 | |
| Newly acquired | 0.24 | 0.15; 0.34 | 0.17 |
| S.aureus colonization | | | |
| Not colonized | 0.24 | 0.10. ; 0.39 | |
| Newly acquired | 0.26 | 0.18; 0.35 | 0.2 |

**Influence of newly acquired pathogen on the slope of LCI.** Results present the slope of LCI before participants were colonized compared to the slope of LCI when a pathogen was acquired and the participant had repeated positive samples and was therefore considered intermittent or chronically colonized.
* The slope coefficient is derived from the fully adjusted model (supplemental table E5) using age as the time variable and an interaction term for pathogen acquisition.
A positive slope indicates a worsening LCI, whereas a negative slope indicates an improving LCI. Values in bold are statistically different from 0 at $p < 0.05$ significance level.

***Table E4:*** **Covariates associated with changes in LCI derived from the full adjusted analysis**

| **Time invariant covariates** | Slope* (LCI u/years) | 95% CI | p-value for interaction |
|---|---|---|---|
| Pancreatic insufficient | | | |
| No (N=5) | 0.2 | 0.04; 0.35 | |
| Yes (N=66) | 0.24 | 0.16; 0.33 | 0.36 |
| Newborn screened | | | |
| No (N=63) | 0.23 | 0.15; 0.32 | |
| Yes (N=8) | 0.15 | -0.07; 0.36 | 0.18 |
| LCI at study entry | | | |
| <8 (N= 12) Age 7.7 (4.6 – 11.08) ‡ | 0.18 | 0.02; 0.37 | |
| > 8 (N=59) Age 8.7 (4 – 15.2) ‡ | 0.25 | 0.16; 0.34 | 0.5 |
| Time of follow-up (years) | -0.17 | -0.41; 0.08 | |
| Hypertonic saline | | | |
| Yes (N=61) | 0.27 | 0.18; 0.35 | |
| No (N=10) | 0.04 | -0.19; 0.27 | 0.07 |
| Dornase Alfa | | | |
| Yes (N=12) | 0.43 | 0.23; 0.64 | |
| No (N=59) | 0.23 | 0.12; 0.3 | 0.06 |
| Chronically inhaled antibiotics | | | |
| Yes (N=14) | **0.41** | 0.24; 0.58 | |
| No (N=57) | **0.19** | 0.1; 0.28 | **0.02** |
| **Visit specific covariates** | Coefficient (LCI units) † | 95% CI | |
| Any proinflammatory pathogen# | 0.05 | -0.18; 0.27 | |
| 1 proinflammatory pathogen# | 0.03 | -0.21; 0.27 | |
| >1 proinflammatory pathogens# | 0.09 | -0.18; 0.37 | |
| Acute P.aeruginosa | -0.16 | -0.43; 0.11 | |
| Acute Aspergillus | 0.22 | -0.10; 0.54 | |
| Acute S.aureus | 0.02 | -0.20; 0.24 | |
| Acute H.Influenzae | 0.07 | -0.19; 0.33 | |
| Oral antibiotics | 0.01 | -0.1; 0.13 | |

**Influence of clinical covariates on LCI course in the full adjusted analysis.**

Abbreviations: LCI u/year: LCI units per year, CI: Confidence interval, P.aeruginosa: Pseudomonas aeruginosa, S.aureus: Staphylococcus aureus, H.influenzae: Haemophilus influenzae

[*] **For *time-invariant* variables** we report interaction terms with age to assess trends in LCI (slope coefficient of age) over the whole study period. Slope and main effects are derived from the fully adjusted model presented in table E5.

[†]**For *visit-specific* covariates**, we report main effects to assess acute changes in LCI associated with the presence of the characteristic at a given time point.

[‡] The numbers refer to participants with an LCI below or above the upper limit of normal of 8 at study entry and corresponding the median age with its range.

[#] Proinflammatory pathogens were considered S.aureus, P.aeruginosa, H.Influenzae, S.pneumoniae and Aspergillus and assessed as any proinflammatory pathogen and as additive effect.

A positive coefficient indicates a worsening LCI (higher LCI), whereas a negative coefficient indicates an improving LCI (lower LCI). Values in bold are statistically different from 0 at $p < 0.05$ significance level.

***Table E5: Full adjusted model to define the increase in LCI over time***

| | Coefficient* (LCI u/year) | 95% CI |
|---|---|---|
| Age | **0.24** | 0.16; 0.33 |
| Sex | | |
| Male | baseline | |
| Female | 0.44 | -0.33; 1.2 |
| BMI z-score | **-0.6** | -0.84; -0.36 |
| P.aeruginosa | | |
| Never colonized | baseline | |
| Intermittend | 0.68 | -0.16; 1.52 |
| Chronic | **1.63** | 0.5; 2.8 |
| Aspergillus | | |
| Never colonized | baseline | |
| Intermittend | 0.71 | -0.16; 1.6 |
| Chronic | **1.6** | 0.16; 3.14 |
| CFRD | | |
| absent | baseline | |
| present | **1.1** | 0.35; 1.73 |
| Acute Exacerbations | **0.64** | 0.45; 0.82 |
| Severe exacerbations | | |
| 0 /year | baseline | |
| 1/year | 0.14 | -0.78; 1.05 |
| >1x/year | 0.18 | -0.7; 1.1 |
| No ABPA during study | baseline | |
| ABPA during study | **0.71** | 0.15 – 1.26 |
| constant | **5.68** | 4.6 ; 6.78 |

**Full adjusted model presented including all predefined covariates.** Definitions of abbreviations: LCI u/year: Lung clearance index units per year; CI: Confidence interval, P.aeruginosa: Pseudomonas aeruginosa; ABPA: acute bronchopulmonary aspergillosis.
*This table presents the covariates adjusted for in the final multilevel mixed effects model. Coefficient for age represents the slope for LCI increase per year of age. Coefficients for the other covariates represent the additional increase if the covariate is present that needs to be added to the constant plus the slope for age. A positive coefficient indicates a worsening LCI (higher LCI), whereas a negative coefficient indicates an improving LCI (lower LCI).
Values in bold are statistically different from 0 at $p < 0.05$ significance level.

***Table E6: Covariates associated with LCI changes differ between males and females***

| | N (%) | Slope * (LCI u/ years) | 95% CI | N (%) | Slope * (LCI u/ years) | 95% CI |
|---|---|---|---|---|---|---|
| | **Females** | | | **Males** | | |
| Aspergillus colonization | | | | | | |
| Never colonized (N=23) | 23 (52) | **0.12** | -0.02; 0.27 | 17 (63) | 0.14 | -0.02; 0.3 |
| Intermittent | 17 (39) | **0.33** | 0.19; 0.48 | 9 (33) | 0.24 | 0.03; 0.46 |
| Chronic | 4 (9) | **0.43** | 0.16; 0.7 | 1 (4) | 0.85 | 0.27; 1.4 |
| P.aeruginosa | | | | | | |
| Never colonized (N=17) | 17 (38) | 0.18 | -0.01; 0.36 | 14 (52) | 0.19 | 0.001; 0.37 |
| Intermittent | 21(48) | 0.28 | 0.13; 0.42 | 8 (30) | 0.11 | -0.12; 0.34 |
| Chronic | 6 (14) | 0.28 | 0.02; 0.53 | 5 (18) | 0.51 | 0.18; 0.84 |
| Severe exacerbations | | | | | | |
| 0 /year | 20 (45) | **0.06** | -0.1; 0.22 | 16 (59) | 0.17 | -0.02; 0.35 |
| 1/year | 20 (45) | **0.34** | 0.20; 0.47 | 11 (41) | 0.28 | 0.05; 0.50 |
| >1x/year | 4 (10) | **0.45** | 0.20; 0.72 | 0 | | |
| No ABPA/study period (N=37) | 37 (84) | **0.22** | 0.10; 0.33 | 25 (93) | **0.14** | 0.002; 0.28 |
| ABPA / study period | 7 (16) | **0.34** | 0.20; 0.51 | 2 (7) | **0.63** | 0.36; 0.90 |

**Prevalence and influence on LCI of risk factors for females and males**. Definitions of abbreviations: LCI u/year: Lung clearance index units per year; CI: Confidence interval; Aspergillus: Aspergillus fumigatus; P.aeruginosa: Pseudomonas aeruginosa; ABPA: acute bronchopulmonary aspergillosis.

Coefficients are derived from a multilevel mixed effects model adjusted for the main effects of final selected covariates (final model summarized in supplemental table E5). Covariates assessed are time-invariant and stable throughout the study period and analyses performed separately for females and males.

* Slope represents the increase in LCI over time compared within the group characteristic. The 95% CI represents the comparison to zero increase in LCI for each subgroup separately. A positive coefficient indicates a worsening LCI (higher LCI), whereas a negative coefficient indicates an improving LCI (lower LCI).

Values in bold had a statistically significant interaction at a significance level from 0 at $p < 0.05$ for the comparison of the slopes within the group characteristic.

***Table E7: Increase in spirometry indices over time from preschool age to adolescence when adjusting for baseline characteristics***

| | Slope* ($FEV_1$ z-scores /y) | 95% CI | p-value † | Slope* ($FEF_{25-75}$ z-scores /y) | 95% CI | p-value† | Slope* (FVC z-scores /y) | 95% CI | p-value † |
|---|---|---|---|---|---|---|---|---|---|
| Age | | | | | | | | | |
| 3 - 5 years (N= 17) | -0.16 | -0.5; 0.21 | | -0.07 | -0.49; 0.36 | | -0.06 | -0.43; 0.31 | |
| 6 – 11 years (N= 42) | -0.08 | -0.15; -0.01 | | -0.08 | -0.16; 0.01 | | -0.08 | -0.14; 0.01 | |
| 12 – 18 years (N= 12) | -0.11 | -0.18; -0.04 | 0.8 | -0.13 | -0.21; -0.05 | 0.7 | 0.18 | -0.4; 0.08 | 0.2 |

**Decrease in spirometry indices over time by age groups when adjusting for baseline characteristics (BMI and sex).** Indices reported were $FEV_1$ z-scores, $FEF_{25-75}$ z-scores and FVC z-scores. Abbreviations: $FEV_1$: Forced expiratory volume in one second ; $FEF_{25-75}$: Mean forced expiratory flow between 25% and 75% of FVC; FVC: forced vital capacity, CI: Confidence interval.

* The slope coefficient is derived from the baseline model adjusted for sex and body mass index using age as the time variable and an interaction term for age group.

† P- value is derived from the interaction within the group characteristic

A negative coefficient indicates worsening spirometry, whereas a positive coefficient indicates improving spirometry. Values in bold are statistically different from 0 at $p < 0.05$ significance level.

***Table E8: Covariates associated with a decrease in $FEV_1$ z-scores over time in the fully adjusted analysis***

| | Slope* ($FEV_1$ z-scores /years) | 95% CI | p-value for interaction |
|---|---|---|---|
| Age | | | |
| 3 - 5years (N= 17) | -0.11 | -0.5; 0.23 | |
| 6 – 11 years (N= 42) | -0.08 | -0.15; -0.01 | |
| 12 – 18 years (N= 12) | -0.08 | -0.15; -0.02 | 0.2 |
| Sex | | | |
| Males (N=27) | -0.04 | -0.12; 0.03 | |
| Females (N=44) | -0.1 | -0.16;-0.05 | 0.19 |
| Aspergillus colonization | | | |
| Never colonized (N=40) | -0.06 | -0.12; 0.01 | |
| Intermittent (N=26) | -0.11 | -0.18; -0.04 | |
| Chronic (N=5) | -0.09 | -0.23; 0.05 | 0.2 |
| P. aeruginosa colonization | | | |
| Never colonized (N=31) | -0.06 | -0.13; 0.01 | |
| Intermittent(N=29) | -0.08 | -0.15; -0.02 | |
| Chronic (N=11) | -0.13 | -0.24; -0.03 | 1.0 |
| Severe exacerbations (N/year) | | | |
| 0 /year (N=36) | -0.04 | -0.1 ; 0.02 | |
| 1/year (N=31) | -0.1 | -0.16; -0.04 | |
| >1x/year (N=4) | -0.23 | -0.4; -0.07 | 0.06 |
| No ABPA /study period (N=62) | -0.08 | -0.13; -0.04 | |
| ABPA / study period (N=9) | -0.08 | -0.16; 0.01 | 0.2 |

**Influence of clinical covariates on spirometry in the fully adjusted analysis.** Definitions of abbreviations: $FEV_1$: Forced expiratory volume in one second; CI: Confidence interval; Aspergillus: Aspergillus fumigatus; P.aeruginosa: Pseudomonas aeruginosa; I.v.: intravenous; ABPA: acute bronchopulmonary aspergillosis.

Covariates assessed are time-invariant and were considered over the entire study period. The slope coefficients are derived from a multilevel mixed effects model adjusted for the main effects of final selected covariates (final model summarized in supplemental table E5).

* Slope represents the increase in $FEV_1$ z-scores over time compared within the group characteristic. The 95% CI represents the comparison to zero increase in $FEV_1$ z-scores for each subgroup separately. A negative coefficient indicates a decrease in $FEV_1$ z-scores (lower lung function), whereas a positive coefficient indicates an increased $FEV_1$ z-score (better lung function).

Values in bold show a statistically significant difference from 0 at $p < 0.05$ significance level for the comparison of the slopes within the group characteristic.

***Table E9: Covariates associated with a decrease in $FEF_{25-75}$ and FVC over time in the fully adjusted analysis***

| | Slope* ($FEF_{25-75}$ z-scores /years) | 95% CI | p-value for interaction | Slope* (FVC z-scores /years) | 95% CI | p-value for interaction |
|---|---|---|---|---|---|---|
| Age | | | | | | |
| 3 - 5.9 years (N= 17) | **-0.04** | -0.44; 0.36 | | -0.02 | -0.40; 0.34 | |
| 6 – 11.9 years (N= 42) | **-0.06** | -0.14; 0.01 | | -0.08 | -0.15; -0.02 | |
| 12 – 18 years (N= 12) | **-0.09** | -0.16; -0.01 | 0.05 | 0.03 | -0.03; 0.09 | 0.1 |
| Sex | | | | | | |
| Males (N=27) | -0.02 | -0.1; 0.05 | | -0.01 | -0.07; 0.06 | |
| Females (N=44) | -0.10 | -0.15; -0.04 | 0.1 | -0.05 | -0.1; 0.01 | 0.3 |
| Aspergillus colonization | | | | | | |
| Never colonized (N=40) | **-0.05** | -0.11; 0.01 | | -0.02 | -0.08; 0.03 | |
| Intermittent (N=26) | **-0.11** | -0.18; -0.15 | | -0.02 | -0.08; 0.04 | |
| Chronic (N=5) | **0.01** | -0.13; 0.05 | 0.02 | -0.11 | -0.24; 0.02 | 0.2 |
| P. aeruginosa colonization | | | | | | |
| Never colonized (N=31) | -0.05 | -0.12; 0.03 | | -0.04 | -0.1; 0.03 | |
| Intermittent(N=29) | -0.06 | -0.13; -0.01 | | -0.03 | -0.09; 0.03 | |
| Chronic (N=11) | -0.11 | -0.22; -0.03 | 1.0 | -0.03 | -0.13; 0.07 | 0.6 |
| Severe exacerbations (N/year) | | | | | | |
| 0 /year (N=36) | -0.05 | -0.1; 0.01 | | **0.01** | -0.05; 0.07 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 1/year (N=31) | -0.06 | -0.13; 0.01 | | **-0.05** | -0.11; 0.01 | |
| >1x/year (N=4) | -0.16 | -0.3; 0.01 | 1.0 | **-0.16** | -0.31; -0.01 | 0.04 |
| No ABPA /study period (N=62) | **-0.06** | -0.13 ; -0.01 | | -0.04 | -0.09; 0.01 | |
| ABPA / study period (N=9) | **-0.09** | -0.18; -0.01 | 0.02 | 0.01 | -0.07; 0.08 | 0.15 |

**Influence of clinical covariates on spirometry in the fully adjusted analysis.** Definitions of abbreviations: $FEF_{25-75}$: Mean forced expiratory flow between 25% and 75% of FVC; FVC: forced vital capacity, CI: Confidence interval; Aspergillus: Aspergillus fumigatus; P.aeruginosa: Pseudomonas aeruginosa; I.v.: intravenous; ABPA: acute bronchopulmonary aspergillosis.

Covariates assessed are time-invariant and stable throughout the study period. The slope coefficients are derived from a multilevel mixed effects model adjusted for the main effects of final selected covariates (final model summarized in supplemental table E5).

* Slope represents the increase in $FEF_{25-75}$ / FVC z-scores over time compared within the group characteristic. The 95% CI represents the comparison to zero increase in $FEF_{25-75}$ / FVC z-scores for each subgroup separately. A negative coefficient indicates a decrease in $FEF_{25-75}$ / FVC z-scores (lower lung function), whereas a positive coefficient indicates an increased $FEF_{25-75}$ / FVC z-score (better lung function).

Values in bold show a statistically significant difference from 0 at $p < 0.05$ significance level for the comparison of the slopes within the group characteristic.

***Table E10: Covariates associated with acute changes in $FEV_1$ z-scores, $FEF_{25-75}$ z-scores, and FVC z-scores***

| | $FEV_1$ z-scores | 95% CI | $FEF_{25-75}$ z-scores | 95% CI | FVC z-scores | 95% CI |
|---|---|---|---|---|---|---|
| Acute Exacerbations (N = 586/907) | **-0.05** | **-0.56; -0.37** | **-0.42** | **-0.53; -0.31** | **-0.38** | **-0.48; -0.28** |
| CF-related diabetes (N=84/907) | 0.17 | -0.17**;** 0.52 | -0.38 | -0.76; 0.01 | 0.44 | -0.09; 0.79 |
| BMI z-score | **0.44** | **0.32; 0.56** | **0.39** | **0.25; 0.53** | 0.38 | 0.26; 0.50 |

**Acute effects of clinical covariates on spirometry in the fully adjusted analysis.** Definitions of abbreviations: $FEV_1$: Forced expiratory volume in one second; $FEF_{25-75}$: Mean forced expiratory flow between 25% and 75% of FVC; FVC: forced vital capacity, CI: Confidence interval; CF: cystic fibrosis; BMI: Body mass index.

Coefficients represent the increase in spirometry z-scores at a given timepoint compared to those without the characteristic (95% confidence interval) and are derived from a multilevel mixed effects model adjusted for the main effects of final selected covariates (final model summarized in supplemental table E5). A negative coefficient indicates a decrease in spirometry z-scores (lower lung function), whereas a positive coefficient indicates an increased z-score (better lung function).

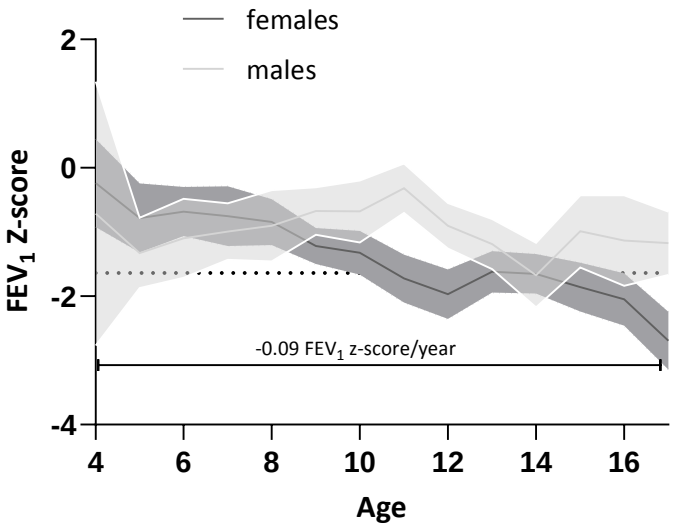



**Figure E1: $FEV_1$ z-scores decrease over time without adjustments.** The decrease in $FEV_1$ z-scores is shown over age and found, on average, to be -0.09 $FEV_1$ z-score/year (95% CI -0.14 - -0.05). During preschool age (3 – 5 years), the increase was found to be -0.16 $FEV_1$ z-score/year (95% CI -0.5; 0.21); during school age (6 – 11 years) -0.08 $FEV_1$ z-score/year (95% CI -0.15; -0.01); during adolescence (12-18 years) -0.11 $FEV_1$ z-score/year (95% CI -0.18; -0.04), p-value for interaction 0.8). On the y-axis, $FEV_1$ z-scores are given. Line represents mean $FEV_1$ z-score across all subjects with available data at a given age. Shaded areas represent point-wise upper and lower 95% confidence intervals. Dotted line represents the lower limit of normal of -1.64 z-scores. Abbreviations: $FEV_1$: forced expiratory volume in one second; CI: Confidence interval

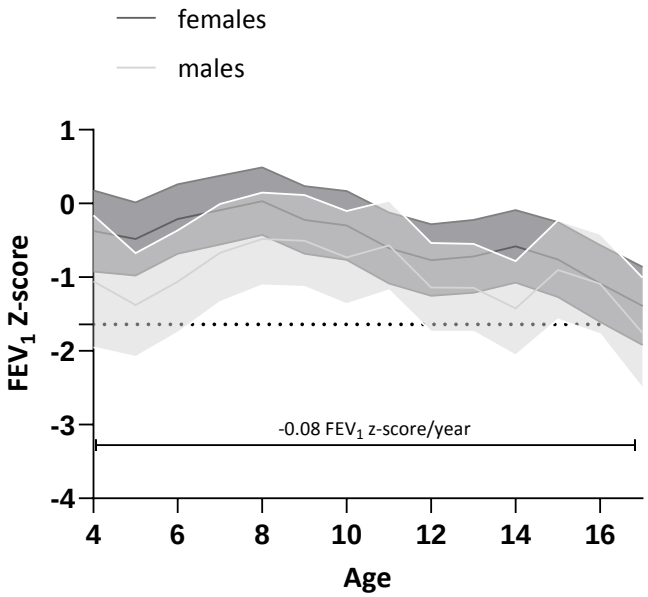



**Figure E2**: **$FEV_1$ z-scores decrease over time with adjustments for risk factors.** The decrease in $FEV_1$ z-scores after adjusting for risk factors (full model presented in table E5) is shown over age and found, on average, to be -0.08 $FEV_1$ z-scores/year (95%CI -0.13 - -0.04). During preschool age (3 - 5 years), the increase was found to be -0.11 $FEV_1$ z-score/year (95% CI -0.5; 0.23); during school age (6 – 11 years) -0.08 $FEV_1$ z-score/year (95% CI -0.15; -0.01); during adolescence (12-18 years) -0.08 $FEV_1$ z-score/year (95% CI -0.15; -0.02), p-value for interaction 0.2). On the y-axis, $FEV_1$ z-scores are given. Line represents mean $FEV_1$ z-score across all subjects with available data at a given age. Shaded areas represent point-wise upper and lower 95% confidence intervals. Dotted line represents the lower limit of normal of -1.64 z-scores. Abbreviations: $FEV_1$: forced expiratory volume in one second; CI: Confidence interval

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