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

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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).

1

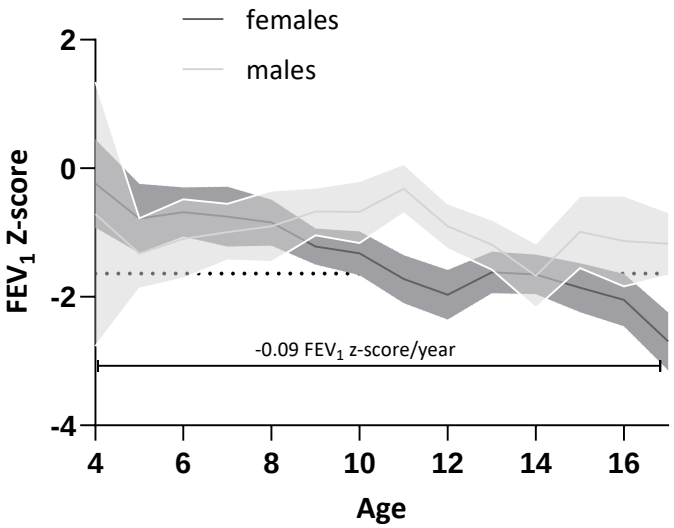

Figure E1: FEV_1 z-scores decrease over time without adjustments. The decrease in FEV_1 z-scores is shown over age and found, on average, to be -0.09 FEV_1 z-score/year (95% CI -0.14 - -0.05). During preschool age (3 – 5 years), the increase was found to be -0.16 FEV_1 z-score/year (95% CI -0.5; 0.21); 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

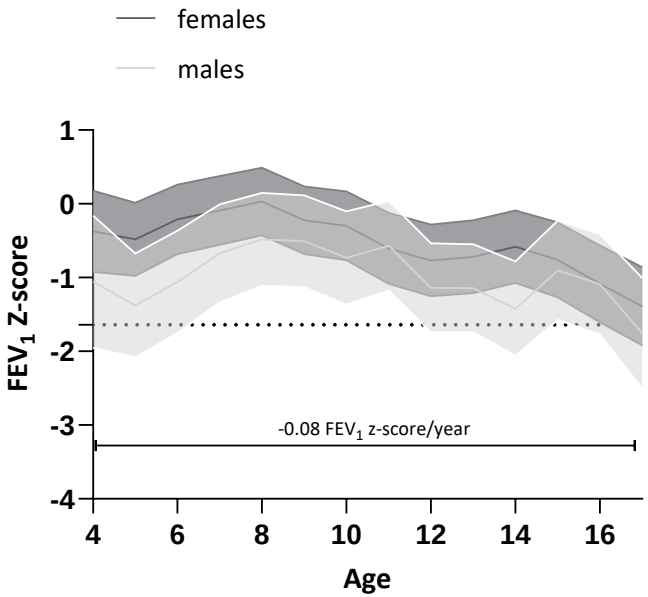

Figure E2: **FEV_1 z-scores decrease over time with adjustments for risk factors.** The decrease in FEV_1 z-scores after adjusting for risk factors (full model presented in table E5) is shown over age and found, on average, to be -0.08 FEV_1 z-scores/year (95%CI -0.13 - -0.04). During preschool age (3 - 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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