Determinants of lung disease progression measured by lung clearance index in children with cystic fibrosis.

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Take home message: Lung clearance index measured during preschool years is a major determinants of school age LCI. These findings further support that the preschool years are critical for early intervention strategies.

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Abstract:

Background: The lung clearance index (LCI) measured by the multiple breath washout (MBW) test is sensitive to early lung disease in children with cystic fibrosis (CF). While LCI worsens during the preschool years in CF, there is limited evidence to clarify whether this continues during the early school age years, and whether the trajectory of disease progression as measured by LCI is modifiable.

Methods: A cohort of children (healthy (HC) and CF) previously studied for 12 months as preschoolers were followed during school age (5-10 years). LCI was measured every three months for a period of 24 months using the Exhalyzer® D MBW nitrogen washout device. Linear mixed effects regression was used to model changes in LCI over time.

Results: A total of 582 MBW measurements in 48 healthy subjects and 845 measurements in 64 CF subjects were available. The majority of children with CF had elevated LCI at the first preschool and first school age visits (57.8% (37/64)), whereas all but six had normal forced expiratory volume in 1 second (FEV_1) values at the first school age visit. During school age years, the course of disease was stable (-0.02 units/year (95% CI -0.14; 0.10). LCI measured during preschool years, as well as the rate of LCI change during this time period, were important determinants of LCI and $FEV_{1,}$ at school age.

Conclusion: Preschool LCI was a major determinant of school age LCI; these findings further support that the preschool years are critical for early intervention strategies.

Introduction:

Early diagnosis (e.g. newborn screening) and improved treatments targeted at preventing disease progression in Cystic Fibrosis (CF) have resulted in improved outcomes and milder respiratory symptoms during childhood.[1-5] Increasingly, adolescents with CF transition to adult programs with normal spirometric indices.[6] Although spirometry may be normal, lung imaging studies have consistently demonstrated the presence of significant and progressive disease, including air trapping and bronchiectasis.[7-12] Consequently, more sensitive measures of lung function are needed to guide the clinical management of young children with CF.

The lung clearance index (LCI) measured by the multiple breath washout (MBW) test is a sensitive physiological marker of ventilation inhomogeneity and can detect early signs of lung disease in children with CF. The LCI can differentiate between health and disease[5, 13-15], track disease progression[12, 16, 17] and identify clinically relevant changes[18] in preschool age children with CF. In a cohort of children with CF, diagnosed based on clinical symptomatology, Aurora et al. reported that LCI during preschool years was predictive of LCI and FEV_1 during the school age years;[17] these findings were supported in a more recent Australian study.[19] As these studies were limited to single measurements at both time periods, there is currently no evidence delineating how LCI trajectories during preschool years affect lung function in the school age years, and whether LCI trajectories during the early school age years are similar to those during the preschool period.

In this study, we aimed to describe the trajectories of LCI in early school-age years and to determine whether there are modifiable factors that influence LCI during this time period.

Methods:

A cohort of children with CF and age-matched healthy controls originally included in a longitudinal observational study with repeated assessments during preschool years (<6 years) [16] was approached for an additional two years of follow-up during school age years (5-10 years); two of the original three sites which contributed the majority of subjects to the initial cohort participated in the school age follow-up study (Hospital for Sick Children, Toronto; Riley Children's Hospital, Indianapolis). The third site, which contributed the least number of subjects to the study, was not included for logistical reasons. All subjects provided informed consent to participate in the study and the study was approved by the research ethics committees at the Hospital for Sick Children and Riley Children's Hospital.

The LCI was measured every three months for a period of 24 months using the Exhalyzer® D multiple breath nitrogen washout device. Research study visits coincided with routine clinic visits; however, not all subjects attended all quarterly visits and in some instances scheduling conflicts precluded the measurement of research study outcomes. Overall, the MBW testing protocol during the school age years was similar to the preschool study with the exception that preschool children were measured with a mask and a 18.6 mL bacterial filter (Medisoft MED59), whereas school age children were measured with a mouthpiece and a 33.3 mL bacterial filter (Air Safety Pediatric Slimline). To account for this, LCI was calculated at the gas-sampling point (uncorrected for equipment dead space (Vd) between the airway opening and the gas sampling point) at both preschool and school age visits (**see Supplement for details**).[20] A sub-set performed MBW using both preschool and school age interfaces at three consecutive visits to determine the impact of changing interface on interpretation of results (**see Supplement for details**). Spirometry was performed according to American Thoracic Society/European

Respiratory Society standards and reported as both percent predicted and z-scores using Global Lung Function Initiative reference equations.[21]

Clinical details at each study visit were recorded including concomitant medications, pulmonary exacerbations treated with either oral or intravenous antibiotics, and respiratory symptoms at the visit. Pancreatic status was defined as use of pancreatic enzyme replacement therapy. In addition to the previously collected medical history from diagnosis to the last preschool study visit, a detailed chart review was conducted between the last preschool study visit and the first school age study visit to identify hospitalizations and record bacterial culture results of respiratory tract specimens. The frequency of routine clinic visits and bacterial culture samples was similar between the two participating sites.

Statistical Analyses

Descriptive statistics were used to compare the healthy group with the CF cohort at the first school age visits, and to compare the subjects lost to follow-up. To determine the effect of changing the MBW interface, measurements within the same subject using the two interfaces were compared using Bland-Altman plots.

Linear regression was used to identify the factors associated with higher (worse) LCI at the first school age visit. A random intercept, random slope mixed effects linear regression using preschool measurements only was used to estimate a slope and intercept for each subject during the preschool years; regression coefficients were estimated using bootstrap procedures (n=500) to account for the additional uncertainty. A mixed-effects linear regression (random intercepts/random slopes) was used to model all school-age LCI measurements. To understand the factors that are associated with a steeper deterioration in LCI during school age years, an

interaction between each time-independent variable and age was fitted in the model. All analyses were repeated with FEV_1 as the outcome.

Reproducibility of LCI during the school-age years was defined using limits of agreement, coefficient of reproducibility and both absolute and relative changes between consecutive visits.[22]

Results:

Summary of the Study Population

Seventy-five percent of the original cohort (48/72 healthy control (HC) and 64/78 CF) were followed-up during the school age years (**Figure 1**). The fifteen subjects from University of North Carolina at Chapel Hill who participated in the original preschool study did not participate in this study for logistical reasons; of the remaining subjects, families of six healthy subjects did not continue follow-up visits and four subjects with CF moved out of the area. One subject with CF, and five healthy subjects withdrew from the study during the school-age follow-up period; two healthy subjects developed physician-diagnosed asthma and were excluded. The subjects with CF that were not included in this follow-up study were slightly older and had higher LCI values at their first preschool visit (**Table S1**). There were no differences in the healthy children lost to follow-up compared to those that remained in the study. The average age of the healthy group and the CF group at the first preschool visit was 4.0 years (Range 2.5 – 6.0) and 4.3 years (Range 2.6-5.9), respectively. On average, there was a 2.3-year gap between the last preschool visit and the first school age visit (minimum 1.2 years, maximum 3.6 years) with no differences in this gap between the healthy controls and CF group (mean 2.3 and 2.3 years, respectively; p=0.96). Measurements between preschool and school age MBW interfaces were not interchangeable; measurements made using the preschool interface were on average 0.28 units (95% CI 0.07 – 0.50; 95% limits of agreement -0.81, 1.37; p=0.01) higher than with the school-age interface (**Table S2; Figure S1; Figure S2).** Analyses were separated by preschool or school age to address the methodological differences.

During the two-year school age study period, 341 MBW measurements in healthy children and 540 measurements in CF children were performed. The MBW testing feasibility

was 99% in healthy subjects and 95% in CF. Overall, between the two sites, there were 582 MBW measurements in HC and 845 measurements in CF subjects available from both preschool and school-age visits. On average the healthy control group had 13 visits (IQR 11; 15) during the two study periods, whereas the CF group had 14 visits on average (IQR 12-16). As this was an observational study, not all subjects routinely attended quarterly visits, and MBW testing was not always logistically feasible to complete as clinical care always took priority. All measurements, whether symptomatic or stable were included in the analysis unless otherwise stated.

A summary of the study population is presented in **Table 1**. At the first school-age visit, the majority of subjects with CF (70.3%, n=45) had LCI values above the upper limit of normal (the upper 2.5th centile (+1.96 z-scores); 7.85 units), defined by the mean and standard deviation of the first measurement in healthy subjects in the study population. We used our previously defined upper limit of normal (the upper 2.5th centile (+1.96 z-scores)) from the same group of healthy children during preschool years (LCI = 8.1 units [16]) to interpret the preschool measurements. More than half of the CF group (57.8% (37/64)) were above the upper limit of normal at both preschool and school age visits, 18.8% (12/64) remained in the normal range, whereas 12.5% (8/64) deteriorated and were above the upper limit of normal (7.85 units) at the first school-age visit; 10.9% (7/64) improved and were below the upper limit of normal. Only 7/64 subjects with CF had an abnormal FEV_1 (i.e. below the lower limit of normal the lower 5th centile (-1.65 z-scores)) at the first school age visit, all of whom also had an abnormal (elevated) LCI.

The proportion of subjects on mucus clearance therapies and CFTR modulators increased between the preschool and school age years; 42% (n=27) had initiated hypertonic saline as preschoolers and 40% (n=26) were on dornase alfa. By school age years, 55% (n=35) were on

hypertonic saline and 56% (n=36) were on dornase alfa. One subject initiated Ivacaftor during the preschool period, three initiated this drug in the intervening years between study periods and a total of five subjects were on treatment by the school age period. Lumacaftor/Ivacaftor (LUM/IVA) was not available during the preschool study; 27% (n=17) were started on LUM/IVA during the intervening years and a total of 30% (n=19) were on either LUM/IVA or tezacaftor/ivacaftor at the end of the two-year school-age follow-up period. Two subjects were on LUM/IVA intermittently. The vast majority (all but 2 subjects on LUM/IVA or tezacaftor/ivacaftor) initiated therapy either before or during the first year of the two-year school-age follow-up.

Risk Factors for Elevated LCI at First School Age visit

In the CF group, none of the demographic or diagnostic characteristics including age, sex, diagnosis by newborn screening, or pancreatic status were associated with an elevated (worse) LCI at the first school age visit **(Table 2)**. The three children with a class IV-V mutation had significantly lower LCI compared to those in the class I-III class group. Children with a history of one hospitalization before the first school age visit had LCI values that were on average 1.6 units higher (95%CI 0.51; 2.78) than children without a history of any hospitalizations. In the small number of children with a history of *A. fumigatus* (n=6) and chronic inhaled antibiotic use (n=4) the LCI was significantly higher. None of these factors were associated with the first school age FEV_1 measurement (**Table 2)**.

In CF subjects, a higher LCI value at the first preschool visit was associated with a higher LCI value at the first school age visit (**Figure 2a; Table 2**). The first school age measurement was required to be a stable measurement; therefore represents a non-exacerbation visit. In addition, a steeper deterioration in LCI during the preschool years was also associated with

worse LCI at the school age visit in CF (**Figure 2b; Table 2**). FEV_1 measured in the preschool years was also associated with school-age LCI but the confidence intervals were much wider. A lower FEV_1 during preschool years, and a steeper rate of decline in FEV_1 were both associated with lower FEV_1 values during school age years. Preschool LCI values (initial value and slope) were predictive of FEV_1 at the school age visit, whereas preschool FEV_1 values (initial value and slope) were not predictive of school age LCI values (**Table 2).**

In healthy children, LCI at the first school age visit was independent of sex (mean difference between boys and girls -0.24 (95% CI: -0.52; 0.04)), age at first school age visit (slope 0.08 (95% CI: -0.04; 0.20)), as well as the first LCI measured during the preschool years (slope 0.19 (95% CI: -0.09; 0.46)).

Lung Function Trajectories:

The LCI was stable during the school-age follow-up years in the healthy group (slope -0.02; 95% CI -0.05; 0.10; **Figure S4**). In the CF group, the rate of change in LCI including all visits (stable and symptomatic) during the two-year school age follow-up period was also stable (-0.02 (95% CI -0.14; 0.10) (**Figure 3a**). The rate of change over the two-year school-age period was independent of newborn screening diagnosis, pancreatic status, functional class of the genetic mutation, or the history of hospitalizations (i.e. the interaction term between each variable and time was not statistically significant). Subjects with a higher LCI at the first school age visit, compared to those with a lower LCI, were more likely to improve over the study period. The rate of change of LCI during the preschool period (i.e. the preschool slope) was associated with the initial LCI at the school age visit (Table 2). There was a significant within-subject correlation between the rate of LCI change during preschool and the rate of LCI change during school age (Pearson correlation 0.732 (95% CI 0.593; 0.829). Within an individual, the

preschool slope (0.39 (95%CI 0.35; 0.43)) was also steeper on average than during school age (slope 0.06 units/year (95% CI 0.04; 0.08). In addition, the rate of change was worse in the second year of observation (slope 0.44 (95% CI 0.02; 0.85)) compared with the first (slope = -0.11 (95%CI 0.44; 0.23).

We also investigated factors associated with a higher LCI during the school-age follow-up, specifically investigating the time-varying factors (**Table 3**); colonization with *S. aureus* or *A. fumigatus* or treatment with either oral or IV antibiotics were associated with higher LCI at visits where these factors were present; conversely, higher BMI was associated with better LCI.

During school age, the rate of change in FEV_1 was similar between healthy controls (slope -0.10; 95% CI -0.17; 0.04) and CF (slope -0.06; 95% CI -0.14; 0.02 **Figure 3b**). None of the investigated factors modified the rate of change in FEV_1 during the school age period in the CF group. During school age years, the LCI slope was weakly correlated (Spearman r = -0.35 (95% CI -0.40; -0.30) with the FEV_1 slope**.**

LCI reproducibility during school age

Reproducibility analyses limited to asymptomatic, clinically stable visits in both healthy controls and CF are presented in **Table 4**. Between two consecutive visits (average 3 months (maximum 4 months)), the average change in LCI in healthy controls was zero, with limits of agreement between -14% and 14%. The between-visit reproducibility was much wider between stable visits in subjects with CF (Limits of Agreement -25% to 26%). The magnitude of the variability was proportional to the mean LCI, especially in the group with CF. In other words, children with a worse LCI had greater variability between study visits.

Discussion:

In this comprehensive longitudinal study of children with CF and age-matched healthy controls, we tracked lung function including LCI from the preschool years throughout early school age years. During the early school-age period, the LCI remains stable in both healthy controls and CF children, but was elevated above the normal range in the vast majority of children with CF. Compared to the preschool years, the rate of LCI deterioration was slower during school-age years. A major determinant of abnormal LCI during school-age years was abnormal lung function during the preschool years, and the rate of deterioration of LCI during the preschool time period. These data further support that the period before and during preschool years is important for early interventions to modulate lung disease progression in children with CF.

Our results are consistent with Aurora et al.,[17] and Hardaker et al.,[19] which both highlighted that elevated LCI in preschoolers is a predictor of lung function in school age years. These earlier studies did not include repeated measurements during the preschool years and therefore could not define the effect of the rate of LCI changes on future outcomes. Our study highlights that both the initial preschool LCI and the rate of deterioration observed during preschool years are important predictors of school age LCI. During early school age years, LCI remained stable and within individuals the rate of change was slower compared with the preschool period. Subjects with a higher LCI at the first school age visit were more likely to improve over the study period. This could reflect both the natural course of lung disease progression and/or the effect of initiating treatments such as dornase alfa, hypertonic saline, and CFTR modulators. Although treating physicians were blind to the LCI results, compared to the preschool years, treatment with mucolytic and modulator therapies was more common in the

early school age years. During the two-year school-age follow-up period, the rate of LCI deterioration was steeper in the second year of follow-up compared with the first year. More subjects had CFTR modulator therapy initiated in the first year of the two-year follow-up, which likely explains the slower rate of decline compared to both the preschool period and the second year of the two-year follow-up. It is also possible that the stability of LCI during school-age years may reflect regression to the mean or the limitations of LCI to detect changes in more subtle ventilatory defects. In a longitudinal study of children with CF, Smith et al. reported progression of ventilation defects in 100% of children when measured by magnetic resonance imaging using hyperpolarized gas ventilation, compared with only 79% of children using LCI.[23] Therefore, while LCI was stable during early school age years, we cannot assume that there was no disease progression; even more sensitive measures might be needed in the modern era of CF care to track early lung disease.[12]

We have reported clinimetric properties of LCI values for school-age children. The range of values observed in healthy controls were consistent with recently published normative ranges using similar equipment and protocols [24]. Among healthy children, the limits of reproducibility during school age years were similar to those previously reported in this cohort as preschoolers,[25] and other studies in school age children.[26, 27] Among children with CF, the limits of reproducibility were slightly wider than a previously published study of school age children with CF.[28] As CF lung disease is highly variable between patients as a result of genetic heterogeneity, disease severity, as well as clinical management, the differences observed may be multifactorial. The determination of a clinically meaningful threshold of change should account for the range of LCI values observed in health, as well as disease severity. Furthermore,

the variability of LCI both within and between test-occasions within the same individual may provide further insight into disease progression and warrants further investigation.

A history of hospitalization was associated with a worse LCI during school age years. We would expect to observe a trend in the effects of cumulative hospitalizations; however, only a few patients had multiple hospitalizations in this study population. A comprehensive analysis of the clinical characteristics of respiratory symptoms and treatment decisions in this cohort provides further insight into the complexities of classifying pulmonary exacerbations and is reported elsewhere.[29] Furthermore, thresholds for treatment and hospitalization may vary between centers and physicians; therefore, hospitalizations *per se* may not adequately reflect disease severity. The LCI was also elevated during school age years in a very small number of subjects with a history of *A. fumigatus* and in those treated with chronic inhaled antibiotics (a proxy for chronic *P.aeruginosa* infection). Although this is consistent with previous studies [30, 31] these results are difficult to interpret since the number of observations is so small. Although we did not find any factors that modified an individual's school age trajectory, LCI measured when a participant had a positive culture with *S. aureus* or *A. fumigatus* or were treated with either oral or IV antibiotics was significantly elevated compared to visits when these factors were not present.

This observational study was not designed to measure the effectiveness of intensified treatment (e.g. mucolytics and modulators) to improve LCI and it is likely that our observations were biased by indication. Evidence from a randomized control study of hypertonic saline in preschool children would suggest that early treatment with mucolytics has the potential to improve LCI, albeit the magnitude of treatment effect was modest.[32] As highly effective modulator therapies become available for younger children, LCI may also be able to identify

patients who will benefit from this intervention early on; thereby serving as a useful tool for monitoring treatment efficacy.

These longitudinal data also provide practical considerations for future longitudinal studies and clinical practice. Measurements made using the preschool interface (mask and small filter) are not interchangeable with the school age interface (mouthpiece and standard filter). There is no ideal time to switch interfaces; children should be measured on the same interface for all study visits whenever possible. Although it is feasible to measure LCI in preschool children using a mouthpiece, available mouthpieces are not always well tolerated by this age group, which can increase testing time.[33, 34]

We must also acknowledge several limitations of this work. This study was limited to children with CF from two large tertiary centers and the characteristics of the subjects as well as the treatment decisions of the physicians at these sites may not be generalizable. As this is an observational study, we are unable to make any causal inferences about the associations observed. Our sample size was limited to conduct comprehensive multivariable analyses, and we also cannot rule out potential confounding effects that were not measured but may have influenced the results. The observation period during preschool and school age was different by design; however, this did not influence the overall interpretation of results. The group of subjects that was not included in the school age follow-up period were slightly older and had worse LCI values; therefore, the results presented here are not directly comparable to our original preschool reports. In a sensitivity analysis we repeated the original preschool analysis excluding these subjects and the results were comparable (data not shown). The exclusion of these subjects, with slightly worse LCI at the start of the preschool period may have strengthened the observations at school age, since the preschool values are an important predictor of later lung function. Finally,

our study did not include lung images to verify whether the stability of LCI correlated with structural changes.

Conclusion: LCI measured during the early school age years is abnormal in the majority of CF children, but remains stable when studied over a 2-year period. Given that both elevated LCI at the beginning of the preschool period and the rate of deterioration during the preschool years are major predictors of lung function during early school age years, these findings further support that the preschool years are critical for early intervention strategies.

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Figure legends:

Figure 1. Flow diagram summarizing longitudinal follow-up of original preschool cohort

Figure 2. Relationship between a) first preschool LCI and first school-age (vertical line represents the preschool age upper limit of normal; horizontal line represents the school age upper limit of normal) and b) Rate of change in LCI during the preschool years (LCI units/year) and first school-age LCI.

Figure 3. Tracking changes in a) LCI and b) $zFEV_t$ over the two-year school age period in the CF group. Average change over time LCI -0.02 (95% CI -0.14; 0.10) and zFEVt (-0.02 (95% CI -0.13; 0.09) were estimated from a linear mixed effects model.

Table 1. Participant characteristics at the first clinically stable school age visit.

Demographics	**Healthy (n=48)**	**CF (n=64)**
Demographics		
Male n (%)	21 (43.8)	32 (50)
White n (%)	38 (79.2)	56 (87.5)
Age (yrs), mean (SD)	8.1 (1.1)	8.4 (1.3)
Height-for-age centile, mean (SD)	58.7 (28.8)	41.4 (26.5)*
Weight-for-age centile, mean (SD)	59.5 (26.3)	43.9 (27.4)*
BMI-for-age centile, mean (SD)	57.6 (26.9)	49.1 (25.3)
Lung Function		
LCI, mean (SD)	6.81 (0.49)	9.01 (1.84)*
FEV_1 % Predicted, mean (SD)	102.7 (13.0)	97.0 (13.2)*
$zFEV_1$, mean (SD)	0.22 (1.1)	-0.25 (1.1)*
Diagnostic Characteristics		
Diagnosed by newborn screening		52 (81.3)
Mutation Class		
I - III		61 (95.3)
IV – V		3 (4.7)
Pancreatic Sufficient		6 (9.4)
Pancreatic Insufficient		58 (90.6)
Hospitalization history prior to first school age visit		
0 hospitalizations		29 (45.3)
1 hospitalization		35 (54.7)
Microbiology at first school age visit		
P. aeruginosa		2 (3.4)
S. aureus		26 (44.1)
MRSA		6 (10.2)
S. maltophilia		0 (0)
H. influenzae		4 (6.8)
B. cepacia complex		2 (3.4)
A. fumigatus		0 (0)

*statistically significant difference ($p<0.05$) between healthly controls and CF groups.

Table 2. Univariable associations between demographic and clinical characteristics and first stable school age LCI or FEV_1 in children with CF.

Demographic Characteristics	N	LCI coefficient (95% CI)	$zFEV_1$ coefficient (95% CI)
Age at first school age visit	64	0.26 (-0.08; 0.61)	-0.07 (-0.28; 0.13)
Gender			
Male	32	Reference	Reference
Female	32	0.07 (-0.86; 0.99)	0.15 (-0.40; 0.71)
Newborn Screening			
No	12	Reference	Reference
Yes	52	0.25 (-0.93; 1.43)	0.08 (-0.63; 0.79)
Mutation Class			
I - III	61	Reference	Reference
IV – V	3	**-2.37 (-4.48; -0.26)**	-0.16 (-1.47; 1.16)
Pancreatic Sufficient	6	Reference	Reference
Pancreatic Insufficient	58	1.40 (-0.15; 2.94)	0.12 (-0.83; 1.08)
Hospitalization history prior first school age			
0 hospitalizations	29	Reference	Reference
1 + hospitalization	35	**1.11 (0.23; 2.01)**	-0.42 (-0.97; 0.12)
History of Positive culture prior first school age (ever/never)			
P. aeruginosa	14	0.68 (-0.42; 1.79)	0.04 (-0.63; 0.72)
S. aureus	54	-0.26 (-1.53; 1.00)	0.02 (-0.74; 0.79)
MRSA	10	1.13 (-0.11; 2.37)	-0.04 (-0.81; 0.72)
S. maltophilia	8	0.53 (-0.86; 1.92)	0.45 (-0.39; 1.28)
H. influenzae	31	-0.46 (-1.38; 0.45)	0.03 (-0.52; 0.59)
B. cepacia complex	4	0.94 (-0.95; 2.84)	-0.50 (-1.64; 0.64)
A. fumigatus	6	**2.07 (0.57; 3.57)**	-0.52 (-1.47; 0.42)
Treatment History prior to first school age (ever/never)			
Dornase alfa	32	0.63 (-0.29; 1.53)	-0.19 (-0.75; 0.36)
Hypertonic saline	34	0.49 (-0.43; 1.41)	-0.30 (-0.85; 0.25)
Ivacaftor	5	-1.34 (-3.03; 0.35)	0.31 (-0.72; 1.35)
Lumacaftor/Ivacaftor; Tezacaftor/Ivacaftor	17	0.89 (-0.13; 1.91)	-0.23 (-0.85; 0.40)
Chronic inhaled antibiotics	4	**1.95 (0.11; 3.81)**	-0.27 (-1.41; 0.88)
Preschool Lung Function			
LCI at first preschool visit	64	**5.79 (3.95; 7.58)**	**-1.78 (-3.11; -0.44)**
LCI slope during preschool (per 1.0 unit increase)	64	**4.28 (3.05; 5.51)**	**-1.82 (-2.69; -0.95)**
zFEVt at first preschool visit	64	-0.70 (-1.42; 0.02)	**0.94 (0.53; 1.35)**
zFEVt slope during preschool (per 0.1 unit increase)	64	-2.34 (-5.33; 0.65)	**3.41 (2.18; 4.63)**

Bold indicates statistically significant at a significance level of 0.05

Table 3. Mixed-effects regression to identify time-varying factors associated with progression of LCI during school age years. All associations reported are adjusted for age only. N represents the number of subjects with at least one visit during the school age period with the exposure.

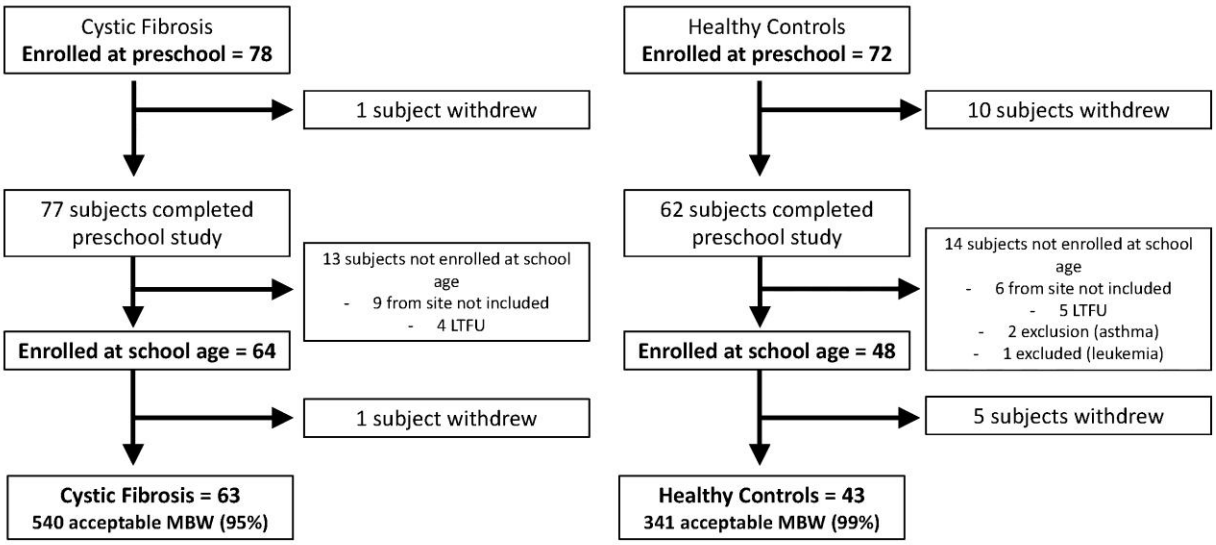
	N	Univariable
Age	64	0.10 (-0.03; 0.23)
LCI at first school age visit	64	**0.75 (0.65; 0.85)**
BMI at each visit	64	-0.01 (-0.02; -0.001)
Dornase alfa	36	0.13 (-0.31; 0.57)
Hypertonic saline	35	-0.04 (-0.53; 0.44)
Ivacaftor	5*	-0.84 (-1.87; 0.20)
Orkambi/Symdeko	17	-0.44 (-0.97; 0.09)
P. aeruginosa	15	-0.07 (-0.55; 0.42)
S. aureus	54	**0.25 (0.01; 0.49)**
MRSA	12	0.22 (-0.33; 0.78)
S. maltophilia	8*	0.26 (-0.36; 0.88)
H. influenzae	28	0.29 (-0.05; 0.63)
B. cepacia complex	5*	-0.09 (-0.85; 0.66)
A. fumigatus	8*	**1.12 (0.58; 1.66)**
Pulmonary Exacerbation	48	**0.59 (0.34; 0.82)**

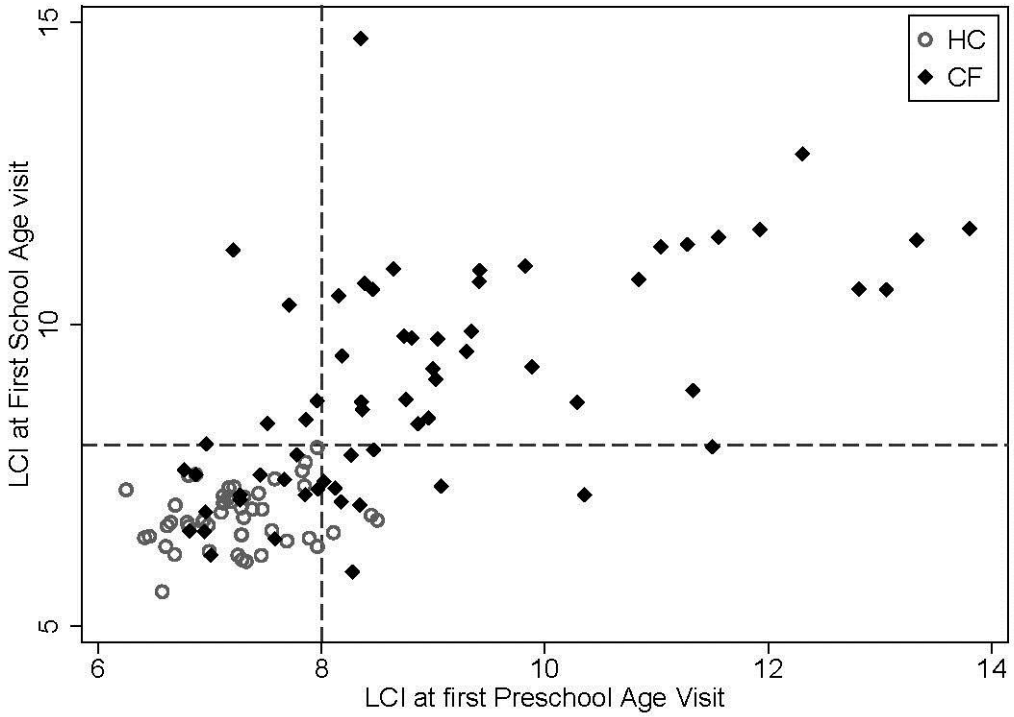
Bold indicates statistical significance at $p<0.05$

*****risk factors with smaller sample sizes need to be interpreted cautiously

Table 4. Summary of LCI reproducibility at 3 month intervals (+/- 1 months) during school age from clinically stable visits.

	Healthy (visits=240)	Cystic Fibrosis (visits=179)
Absolute difference	0.00	-0.02
Percent change (95% limits)	0.18 (-14, 14)	0.77 (-25, 26)
Within-subject between-test SD	0.26	0.56
Coefficient of variation	3.8%	6.5%
Bland-Altman Limits of Agreement	-0.9 to 0.9	-2.1 to 2.1
Coefficient of reproducibility	0.71	1.55
Intra-class coefficient	0.29	0.74


Cystic Fibrosis
Enrolled at preschool = 78
1 subject withdrew
77 subjects completed preschool study
13 subjects not enrolled at school age
- 9 from site not included
- 4 LTFU
Enrolled at school age = 64
1 subject withdrew
Cystic Fibrosis = 63
540 acceptable MBW (95%)
Healthy Controls
Enrolled at preschool = 72
10 subjects withdrew
62 subjects completed preschool study
14 subjects not enrolled at school age
- 6 from site not included
- 5 LTFU
- 2 exclusion (asthma)
- 1 excluded (leukemia)
Enrolled at school age = 48
5 subjects withdrew
Healthy Controls = 43
341 acceptable MBW (99%)


HC
CF
LCI at First School Age visit
LCI at first Preschool Age Visit
5
10
15
6
8
10
12
14

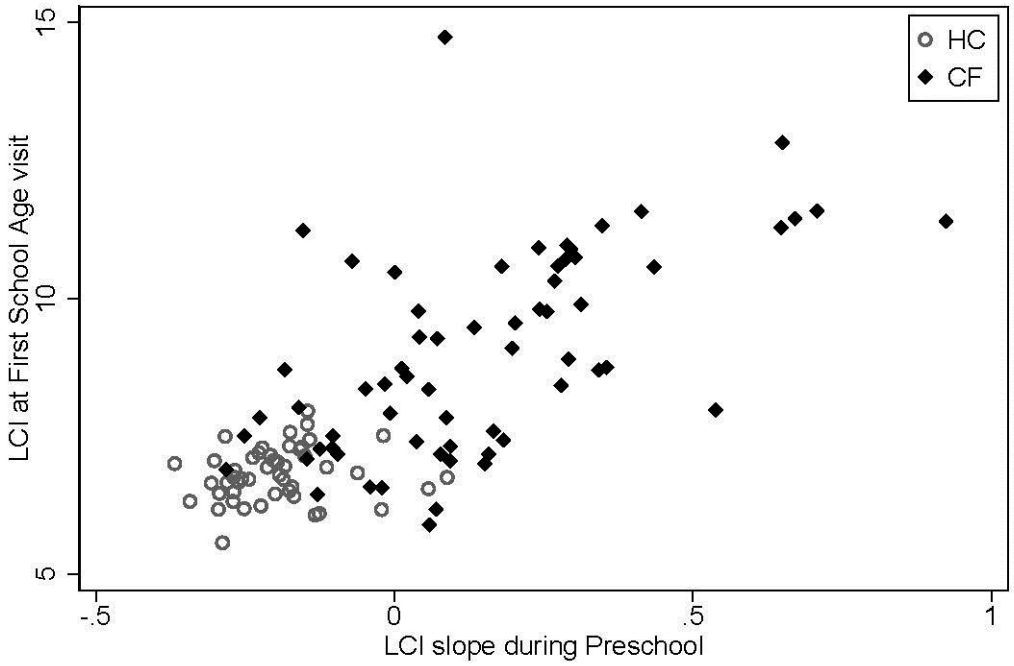


Figure 2b. Relationship between rate of change in LCI during the preschool years (LCI units/year) and first school-age LCI.

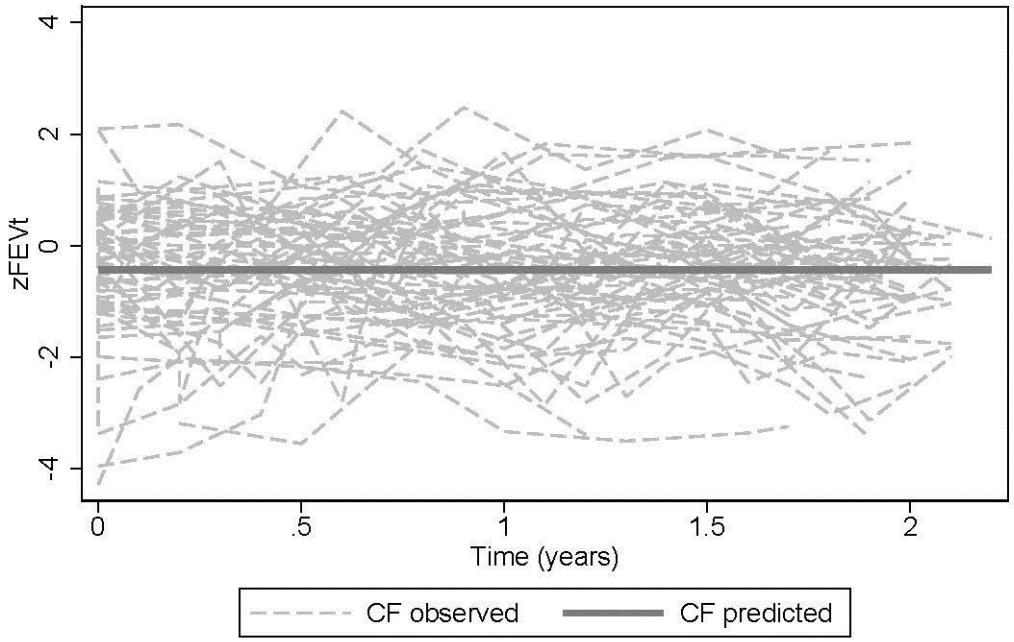


Figure 3b. Tracking changes in zFEVt over the two-year school age period in the CF group. Average change over time of zFEVt (-0.02 (95% CI -0.13; 0.09) were estimated from a linear mixed effects model.

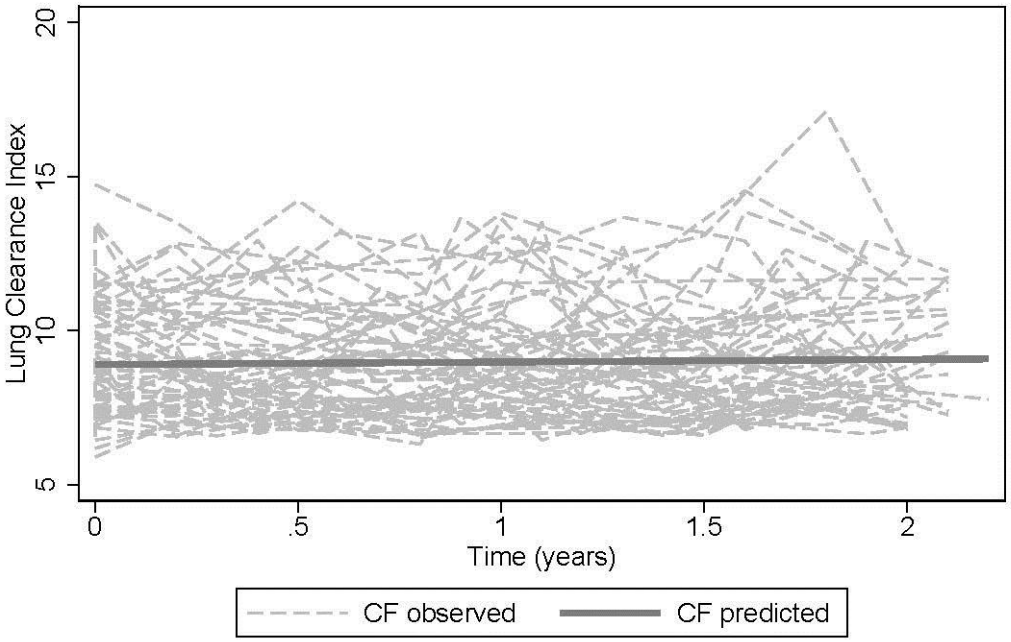


Figure 3a. Tracking changes in LCI over the two-year school age period in the CF group. Average change over time LCI -0.02 (95% CI -0.14; 0.10) were estimated from a linear mixed effects model.

Determinants of lung function progression measured by lung clearance index in children with cystic fibrosis.

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Table S1. Comparison of subjects Lost to Follow-up (LTFU) and those included in school-age follow-up.

	Healthy Controls (n=72)			**CF (n=78)**		
	TRACK (n=48)	**LTFU (n=24)**	**p-value**	**TRACK (n=64)**	**LTFU (n=14)**	**p-value**
Male, n (%)	21 (44%)	12 (50%)	0.62	32 (50%)	7 (50%)	0.76
White, n (%)	38 (79%)	17 (71%)	0.43	56 (87%)	13 (93%)	0.57
Age, mean (range)	4.0 (2.5, 5.9)	4.2 (3, 5.9)	0.30	**4.2 (2.6, 5.9)**	**4.9 (2.7, 5.9)**	**0.02**
Centiles, mean (range)						
Height-for-age	61.1 (8.2, 99.8)	61.3 (6.7, 98.7)	0.97	44.7 (2.1, 97.5)	41.9 (0.8, 89.2)	0.73
Weight-for-age	61.6 (3.8, 99.6)	64.4 (8.8, 99.8)	0.64	43.5 (2.0, 94.7)	41.1 (2.2, 80.7)	0.77
BMI-for-age	59.5 (1.3, 99.1)	63.7 (17.6, 99.9)	0.54	47.2 (1.0, 94.9)	48.7 (15.9, 91.0)	0.85
MBW						
LCI, mean (range), n=103	7.1 (6.1, 8.1)	7.2 (6.8, 7.7)	0.34	**8.6 (6.7, 13.6)**	**9.9 (6.4, 13.0)**	**0.04**
Spirometry						
$FEV_{0.75}$ % predicted, n=71	97.0 (69.1, 147.0)	96.8 (79.6, 119.5)	0.99	92.4 (73.6, 122.3)	81.6 (42.0, 100.6)	0.08
FEV_1 % predicted, n=89	101.2 (73.3, 129.9)	102.7 (79.3, 136.4)	0.76	93.5 (76.6, 119.3)	84.5 (42.5, 105.9)	0.13
FEF_{25-75} % predicted, n=54	103.0 (71.3, 139.3)	91.8 (66.8, 135.5)	0.19	**98.6 (49.6, 152.9)**	**65.0 (43.5, 79.8)**	**0.02**
$zFEV_t$, mean (range), n=94	0.1 (-2.4, 3.3)	0.1 (-1.5, 2.3)	0.96	-0.6 (-2.1, 1.8)	-1.4 (-4.3, 0.0)	0.07

Preschool vs. School age Interface

To ascertain any bias due to interface differences between the preschool and school age visits, twenty-nine subjects (14 HC and 15 CF) performed repeated measurements using both the preschool (mask + small dead space filter) and school age (mouthpiece + standard filter) interface at the same study visit for three consecutive visits. LCI was calculated at the gas sampling point for both interface set-ups. Within an individual subject the difference in LCI between preschool and school age interfaces from all visits pooled was 0.28 units higher for all preschool set interface (95% CI 0.07 – 0.50; 95% limits of agreement -0.81, 1.37; p=0.01). The difference was similar for all consecutive visits, and the observed differences were similar in health and CF which supported pooling of the data (**Table S2; Figure S1**). The analysis accounted for the repeated measurements in the same individual. The difference in LCI between interfaces was also not dependent on subject height or weight (**Figure S2**). Since LCI values were not interchangeable between interfaces, a 'interface' covariate was included in all analyses to interpret longitudinal measurements.

Table S2. Summary of LCI differences between preschool and school age interface (preschool – school age). There were 14 HC and 15 CF subjects; not all had successful measurements at all 3 visits.

	Visit 1	Visit 2	Visit 3
HC	0.42 (0.15, 0.70)	0.33 (0.15, 0.52)	0.24 (0.02, 0.47)
CF	0.26 (-0.07, 0.60)	-0.03 (-0.39, 0.34)	0.57 (0.37, 0.77)

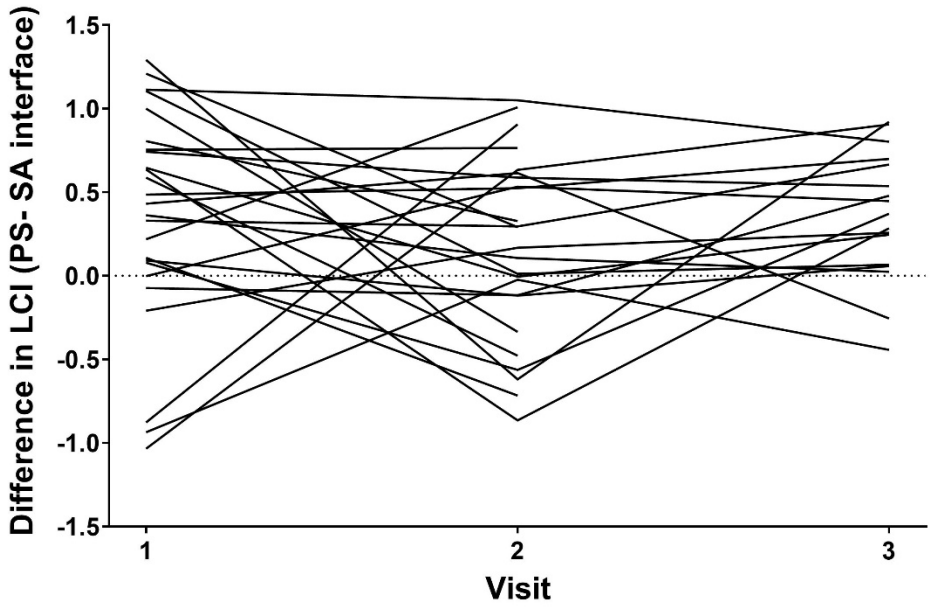

Figure S1. Differences in LCI between preschool and school age interface between 3 consecutive visits including both healthy controls and the CF group.

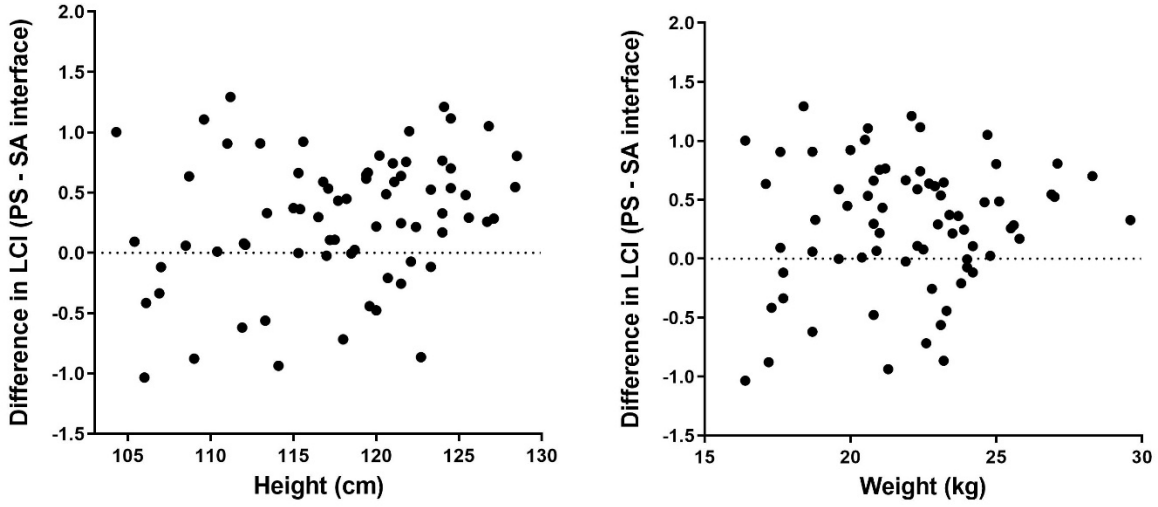

Figure S2. Relationship between difference in LCI between preschool and school age interface and height (left) and weight (right) including both healthy and CF subjects.

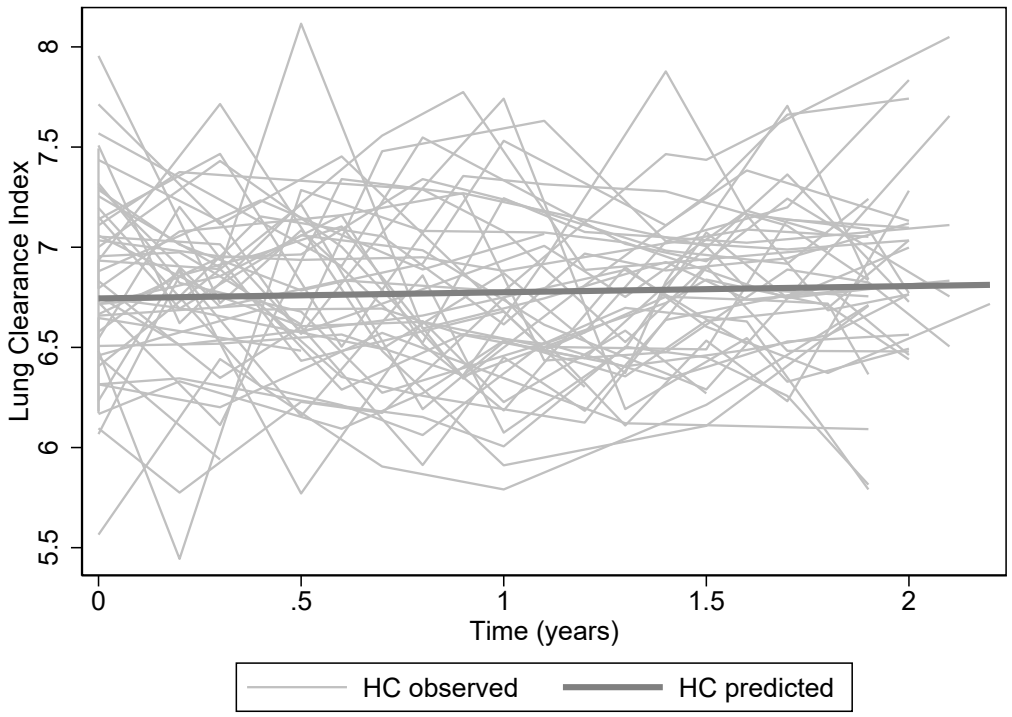

Figure S3. Tracking changes in LCI over the two-year school age period in the HC group. Average change over time for LCI was 0.02 (95% CI -0.05; 0.10) and was estimated from a linear mixed effects model.