Title: Longitudinal lung clearance index and association with structural lung damage in children with cystic fibrosis

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Author contributions: MS, PG, AL and HI were responsible for the design of the study. MS re-analysed all MBW examination in children with CF under supervision from PG and AL.

MBW-data from healthy infants and pre-schoolers were made accessible by GD. MS performed all CT scoring supervised by HT. The statistical analyses were performed by HI. MS, AL and HI drafted the manuscript. All authors revised the manuscript, contributed with conceptual content, and approved the final manuscript.

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ABSTRACT

Background

The clinical utility of multiple breath washout (MBW) in children with cystic fibrosis (CF) is still unclear. The aim was to evaluate the longitudinal association between lung clearance index (LCI), a lung function parameter derived from MBW, and the extent of structural lung damage (SLD) in children with CF.

Methods

MBWs were performed annually in 75 children with CF. Age-adjusted LCI values (LCI_{adj}) were calculated from 140 healthy controls. Children with CF underwent a minimum of one chest computed tomography (CT) scan, and structural lung damage (SLD) was measured as total airway disease (%Dis) and bronchiectasis (%Be). The association between SLD and LCI was analysed with joint modelling and logit-linear mixed effects models. SLD percentile curves were calculated for various ages and LCI-profiles.

Results

A total of 777 MBW examinations and 199 chest CT scans were included in the analysis with an average follow-up period of 8.4 years. There was no significant annual increase in LCI_{adj} in the CF-cohort. One unit increase in mean LCI_{adj} throughout childhood was associated with 0.34 (95% CI: 0.27–0.41) percentage points faster progression of %Dis/year and 0.24 (95% CI: 0.19–0.29%) percentage points faster progression of %Be/year. A mean LCI_{adj} of 7.0 and 9.0 during the preschool years corresponded to a median (10^{th}–90^{th} percentile) %Dis of 2.3% (0.7–5.5) and 6.5% (2.9–13.0), and %Be of 0.4% (0.0–1.8) and 1.8% (0.4–4.6) at six years of age.

Conclusions

A low LCI during childhood is associated with less SLD and a slower SLD progression rate.

Key words: cystic fibrosis, multiple breath washout, lung clearance index, chest computed tomography, children

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What is the key question?

- Can longitudinal multiple breath washout examination estimate the extent and the progression rate of CF lung disease measured with chest CT?

What is the bottom line?

- A low lung clearance index during childhood is associated with a lower extent and slower progression rate of structural lung damage compared to a higher LCI.

Why read on?

- To better understand how longitudinal lung clearance index may be used in clinical practice

1.1 INTRODUCTION

Children with cystic fibrosis (CF) now often go through childhood with only subtle upper and lower airway related symptoms and have well-preserved lung function assessed by spirometry[1]. Despite this improvement, irreversible structural lung damages (SLD) start early in life and progress even in the absence of symptoms[2 3]. An important clinical challenge in CF care is to predict the extent and foresee the progression of CF lung disease. This issue is most appropriately addressed by longitudinal studies with repeated measurements of relevant outcomes, which reflect the situation encountered in clinical practice. Multiple Breath Washout (MBW) has been shown to be a sensitive, non-invasive, feasible method in all ages for repeated measurements over time to track early CF-lung disease[4 5]. The Lung Clearance Index (LCI) is the most commonly used outcome from MBW examinations and reflects the global ventilation inhomogeneity [4]. Several studies have demonstrated that LCI responds to interventions and it is suggested that MBW may be a useful complementary tool in routine clinical care to detect and evaluate treatment responses[6 7]. Chest computed tomography (CT) has also been shown to be a sensitive marker to of early SLD in CF children, but with the disadvantage of accumulation of ionizing radiation which limits a more frequent utilization[8]. Several studies, most of them cross sectional, have compared the sensitivity for both methods suggesting relatively similar sensitivity to detect early CF lung disease[9-12]. Even though chest CT and MBW are considered complementary markers of the CF lung disease, longitudinal studies are needed to understand the potential use of LCI as a predictor of the extent and the progression of SLD.

The aims of this study were to: A) describe the longitudinal progression of LCI in a Swedish cohort with CF between the ages 0–17 years. B) investigate the association between the magnitude and progression of longitudinal LCI-measurements with the levels and progression

rates of SLD measured with chest CT. C) evaluate if longitudinal LCI-measurement in preschool and school-age children can predict SLD magnitude assessed by chest CT at school-age. The LCI trend and the association between chest CT and longitudinal LCI measurements have partly been reported in the form of an abstract[13].

METHOD

2.1 Study population

We performed a longitudinal, retrospective, observational study of Swedish children diagnosed clinically with CF. A healthy cohort of children from Sweden and the UK with cross sectional MBW measurements served as controls. All subjects with CF included in the study were born between 1990 and 2009 and had performed at least one routine chest CT and one routine MBW examination (Figure 1). All chest CT measurements with at least one MBW performed before or at the timepoint of chest CT were included in the analyses. No other exclusion criteria were applied. Demographic data for each subject with CF were retrieved from the Swedish CF Registry. The study was approved by the local Ethics Committee (Dnr. 206-18)

2.2 Lung functions acquisition and analysis

MBW examinations were performed annually between 1999 and 2016 in clinically stable CF subjects at the paediatric Clinical Physiology laboratory at Queen Silvia Children's, Gothenburg and at the Paediatric department at the Central Hospital, Skovde. Additional interim MBW measurements were performed every six months from 2013 to 2016. A respiratory mass spectrometer (AMIS 2000, Innovision A/S, Odense, Denmark) was used to measure the expiratory gas concentrations of sulphur hexafluoride (SF_6)[4 14]. All MBW examinations that earlier were considered clinically acceptable were re-analysed according to

consensus statement by one paediatric pulmonologist (MS)[15]. A total of 140 MBW examinations in 140 healthy subjects aged 0–17 years with no history of pulmonary illness were also analysed to understand the relation between age and LCI in a healthy population, and to adjust LCI values in the CF cohort accordingly. The healthy cohort used the same equipment, gas mixes and procedures as in the subjects with CF. See online-supplement material (OSM) for further details.

2.3 Chest CT acquisition and analysis

High resolution chest CT's were performed in clinical stable CF subjects every third year from six years of age between 2003 and 2016. The Perth-Rotterdam Annotated Grid Morphometric Analysis for Cystic Fibrosis (PRAGMA-CF) method, was used to score SLD[17]. The primary outcomes for the chest CT scans were bronchiectasis (%Be) and the composite score total airways disease (%Dis). The longitudinal progression of SLD in this cohort has been presented in a previous publication and inter-rater reproducibility for both outcomes were considered excellent[16].

2.4 Airway pathogens

The incidence of airway pathogens *Pseudomonas aeruginosa* (Pa), *Staphylococcus aureus* (Sa) and *Aspergillus species* (Asp) for the CF cohort was analysed from sputum cultures and cultures from laryngeal suctions[16]. Chronic infection with Pa was defined according to the Leeds criteria[18]. See OSM for more information about general antibiotic treatment strategies at Gothenburg paediatric CF-centre.

2.5 Statistical methods

For descriptive purposes, the data are presented as medians and ranges for continuous variables and as numbers (%) for categorical variables. Statistical analyses were performed using parametric statistical methods and the plausibility of model assumptions was assessed by visual inspection of residual diagnostic plots

We analysed the cross-sectional relation between LCI and age in healthy children using a piecewise linear regression model. The yearly progression of LCI in the CF cohort was estimated from longitudinal MBW data using linear mixed models with a random intercept and age slope for each subject. Robust standard errors were used to account for the skewed distribution of LCI in children with CF.

To evaluate the association between longitudinal LCI measurements and SLD, we performed two different analyses. First, the correlation between the progression of SLD and progression of LCI throughout childhood was estimated by joint modelling of all available longitudinal MBW and CT data, using linear mixed effects models with correlated subject specific random intercepts and age slopes. Similarly, we estimated the correlation between progression of SLD and mean LCI throughout childhood by omitting the age slope of LCI from the model.

To understand the association between SLD and LCI at a more detailed level, we continued with non-linear mixed effects models for SLD, with age and LCI as explanatory variables. A logit-type transformation of %Dis and %Be on the form $\log\left(\frac{1+x}{101-x}\right)$ was used to obtain approximately normally distributed and homoscedastic errors. To account for repeated measures of LCI, we estimated the best linear unbiased predictions (BLUPs) of LCI at chest CT and mean LCI up to chest CT, using mixed effects models of LCI on all available MBW data up to and including the time-point of chest CT. The results of the logit-linear mixed

effects models were subsequently used to derive SLD percentile curves at 6 and 17 years of age.

To account for natural age-trends in LCI between infants, pre-schoolers, and school-age children, all LCI values in children with CF were age adjusted (LCI_{adj}) according to the formula

$$LCI_{adj} = \begin{cases} LCI - 0.12 \times age, & if\ age < 6.0 \\ LCI, & if\ age \geq 6.0 \end{cases} \quad (Equation\ 1),$$

as derived from the healthy reference population.

Statistical analyses were performed using SAS software, Version 9.4 (SAS Institute, Cary, NC, USA). For more information about the statistical methodology, see OSM.

RESULTS

3.1 Study population

The cohort included 75 of 75 eligible subjects with CF from Gothenburg paediatric CF centre (Table 1). A total of 785 MBW examinations from the cohort were accessible and re-analysed. Eight of the 785 MBW examinations that had previously been considered acceptable were excluded following quality control according to consensus statement[15]. In total 777 MBW examinations together with 199 chest CT scans were included in the study (for more information see OLS Figure E1). Cross sectional data of the CF disease progression during preschool and school-ages are presented in Table 2. Detailed results regarding the progression of SLD in this cohort have been reported elsewhere[16].

3.2 LCI in healthy children

In the healthy reference population, LCI decreased by 0.12 units per year (95% CI: -0.16 – -0.09, p<.0001) up to six years of age, after which no further decrease was observed (p=0.21, Figure 2A and 2B). The age-specific mean and 95% upper prediction limit (upper limit of normal, ULN) are presented in OSM Table E1.

3.3 The progression of LCI in children with CF

Overall, there was a weak but non-significant progression of age-adjusted LCI (LCI_{adj}) in the CF cohort (+0.03 units/year, 95% CI: -0.01–0.08, p=0.13, Figure 2C). There was a significant progression in LCI_{adj} of 0.11 units/year in girls (95% CI: 0.03–0.20, p=0.011) and of 0.07 units/year (0.01–0.12, p=0.025) in children born 1990–1999. No significant subgroup differences in mean LCI_{adj} or progression in LCI_{adj} were observed with respect to pancreatic sufficiency or age at diagnosis (OSM Table E2).

3.4 Joint modelling of longitudinal SLD and LCI

There was a positive correlation between mean LCI_{adj} and progression rate of SLD throughout childhood (r=0.62 (95% CI: 0.40–0.84) for %Dis, r=0.65 (95% CI: 0.46–0.85) for %Be, both p<.0001) (Figure 3A and 3C). One unit increase in mean LCI_{adj} was associated with 0.34 (95% CI 0.27–0.41) percentage points faster progression rate of %Dis/year and 0.24 (95% CI: 0.19–0.29) percentage points faster progression rate of %Be/year. A slightly weaker correlation existed between progression rate of LCI_{adj} and the progression rate of SLD (r=0.45 (95% CI: 0.15–0.76), p=0.004 for %Dis, r=0.41 (95% CI: 0.09–0.72), p=0.011 for %Be) (Figure 3B and 3D). The correlations where qualitatively similar but somewhat attenuated

when adjusting for age at diagnosis, sex, birth cohort, and concomitant infections with Pa, Sa and Asp (OSM Table E3).

3.5 Non-linear associations between SLD and LCI

Logit-linear mixed effects models of SLD showed that LCI_{adj} measured at CT (+/-3 days) together with age at CT explained 49% of the variation in %Dis ($p<.0001$) and 35% of the variation in %Be ($p=0.020$) throughout the study period (OSM Table E4). However, an individual LCI-value beyond $LCI_{adj}=9.0$ was only weakly associated with increased SLD (OSM Figure E2 and E3). In contrast, an increase in longitudinal LCI was steadily associated with more SLD and explained up to 55% of the variation in %Dis ($p<.0001$) and 52% of the variation in %Be throughout the study period ($p<.0001$) (Figure 4, OSM Figure E2 and E3). Similar associations between LCI and SLD were observed when accounting for age at diagnosis, sex, birth cohort and concomitant infections with Pa, Sa and Asp (OSM Table E4).

3.6 SLD percentile curves

Percentile curves of %Dis and %Be *vs* mean LCI_{adj} at 6 and 17 year of age are presented in Figure 5, and additionally for %Dis and %Be *vs* LCI_{adj} at CT in OSM Figure E2 and E3. A non-pathological mean LCI ($LCI_{adj}=7.0$) during pre-school age corresponded to a median (10^{th}–90^{th} percentile) %Dis of 2.3% (0.7–5.5) and %Be of 0.4% (0.0–1.8) at six years of age. A two units higher LCI ($LCI_{adj}=9.0$) corresponded to a median (10^{th}–90^{th} percentile) %Dis of 6.5% (2.9–13.0) and %Be of 1.8% (0.4–4.6).

DISCUSSION

In this study we have explored how longitudinal and cross sectional LCI measurements can be used in clinical practice to estimate the extent and the progression rate of SLD in a paediatric

CF population. Evaluation of LCI in a longitudinal context resulted in stronger associations with SLD measured by chest CT than only considering the most recent LCI value. A mean LCI within the normal range during childhood corresponded to a low extent and slow progression of SLD, whereas a higher mean LCI resulted in more SLD and a higher SLD progression rate. Our results support the clinical use of regular MBW examinations in children with CF with the aim of maintaining or pursuing a low LCI.

There was a weak but non-significant progression of LCI in our CF cohort. The knowledge about longitudinal changes in LCI over time in children with CF is still limited but recently a few studies have discussed this topic. A 2-year longitudinal Danish study in children with CF aged 6–18 years using Exhalyzer D and nitrogen as inert gas, revealed a progression of 0.35 LCI-units per years[19]. Another 2-year longitudinal study in children with CF aged 5–10 years from North America using the same MBW equipment as in the Danish study showed no annual progression in LCI [20]. A Swiss paediatric CF-cohort, who also used Exhalyzer D over a longer period of time revealed a slow increase of 0.1 LCI units per year in the younger school children and a more pronounced annual LCI progression rate in the adolescences[21]. Results from the London CF Collaboration using the same MBW equipment as in our study revealed a deterioration of 0.18 LCI-units per year in children with CF[22]. In the past 10 years we gained a lot of experience about the biological variability of LCI between LCI measurements and how pathogens and therapeutic interventions affects the course of LCI[5 7 23 24]. It is reasonable to believe that this information also has changed the course of how we use LCI in clinical practice in different CF-centres, which might be reflected in the longitudinal trends of the LCI progression rate. An increase in LCI in our clinic was often followed by an intervention with the aim to restore LCI to the patient's LCI-baseline, which might be one possible explanation to the absent of a significant LCI progression in our cohort. There was a

significant progression in the children born between 1990–1999, but not in the children born after 2000. This finding most probably reflects the constant improvement in the CF health care over time. We also observed a significant progression in girls. A gender disparity has also been described in studies using alternative methods to track the CF lung disease[25 26]. Our study further supports the hypothesis that CF lung disease progression is greater in girls but was not designed to assess this further.

In this study we have demonstrated that both LCI at chest CT and longitudinal LCI measurements prior to and at chest CT are positively associated with SLD in a paediatric CF population. Longitudinal LCI measurements prior to and at chest CT explained 55% of the variability in %Dis, and 52% of the variability in %Be throughout the study period. Single LCI measurements (cross sectional at chest CT) were less associated with SLD. This finding may partly be explained by the biological variability of LCI between MBW measurements in clinical stable patients with CF, as well as sudden deteriorations or improvements in LCI caused by nearby pulmonary infections or interventions[6 7 21 23 24]. Thus, a single LCI value is sensitive to random fluctuation and needs to be interpreted with caution and put in a clinical perspective, including when and why the MBW-examination was performed. Our results indicate that longitudinal LCI measurements are more robust and reliable than single LCI measurements to track SLD. Similar findings regarding the benefit of longitudinal compared to cross sectional measurements of risk factors have also been found in other contexts, e.g., risk prediction of future cardiovascular disease[28]. Nonetheless, single LCI values may be useful to capture recent or ongoing lung damages, as a sudden deterioration (increase in LCI) may indicate an early sign of progression the CF-lung disease[4]. In clinical practice both the present and longitudinal LCI measurements should be considered to better understand the CF lung disease.

Longitudinal LCI measurements can be used to discriminate between SLD severity in children with CF. A normal LCI (mean LCI_{adj}=7.0) during infancy and the pre-school ages corresponded to relatively low extent of SLD at the age of six years (median = 2.3 for %Dis and 0.8 for %Be). In comparison, a two-units higher mean LCI_{adj} during early childhood resulted in more than a two-fold higher SLD. This relationship between longitudinal LCI values and SLD measured with chest CT was observed throughout the entire paediatric ages. Longitudinal mean LCI also demonstrated a positive correlation with the SLD progression rate. We know from earlier studies that there is an association between the degree of airway inflammation and LCI as well as between the airway inflammation and the SLD progression rate in children with CF[17 29 30]. Accordingly, a high mean LCI may reflect a high degree of airway inflammation over a longer period of time, which could explain the relationship between a high mean LCI and a faster progression rate of the CF lung disease. In clinical practice, a normal mean LCI during pre-school and/or school age may be used as an indicator to perform chest CT scans less frequently. Magnet resonance imaging (MRI) of the lungs may also be an alternative, as cross sectional MRI studies have shown a good correlation between LCI and structural airways abnormalities in younger children with CF [31]. We speculate that a high stable LCI over time should be considered as a risk factor for faster SLD progression and followed by interventions with the aim to pursue a lower LCI. By keeping LCI as low as possible throughout infancy and pre-school ages we may limit the SLD progression rate as well as preserve the subject's lung function at early school age[20 32 33].

Strengths and weaknesses

This study included all available individuals at Gothenburg CF centre, with MBW and chest CT performed over a long period of time. The study also included MBW from a healthy

reference cohort, all of which were performed with similar equipment and analysed with the same software and settings as used in the CF cohort. The availability of a healthy reference population enabled us to adjust LCI values of the CF patients from MBW obtained at various ages and by different technical procedures, by removing the age trend observed in healthy pre-school children. We used age-adjusted LCI values rather than LCI z-score since age-adjusted LCI are easier to interpret and visualize for the reader. Apart from loss of interpretability, the results would be identical if z-scores derived from the same model and population had been used.

LCI-values derived from different MBW equipment or different inert gases are not considered interchangeable[15]. A recent publication in 2021 revealed a systematic software error from the gas sensors of the Exhalyzer D, that is commonly used in many MBW-studies[27]. This error resulted in an overestimation of LCI when using nitrogen as an inert gas. This finding will affect the earlier differences seen between N2 and SF6 measurements as well as in comparison with other MBW equipment when the new software is released[15]. Despite challenges in comparison between different MBW-equipment and inert gases, results from our study can still provide useful information to understand the relationship between other measurements to track the CF lung disease.

This study was a retrospective, real-world study and we acknowledge several limitations. None of the data from the lung functions tests or the chest CTs were blinded for the clinician. The study stretches over many years and we don’t have information about changes in medication or treatment regimens that might have affected the results. Thus, existence of unobserved confounding factors cannot be excluded due to the observational nature of the study. MBW performed in a supine position throughout all paediatric ages may further have

improved the associations between LCI and SLD[34], but this information was not available during the study period. Chest CTs were analysed blinded to patient identifiers with a fully quantitative scoring system with good reproducibility[16]. A limitation is the absence of CTs during infancy or pre-school ages. The pulmonary status at the time of initial CF diagnosis is variable in our cohort, as new-born screening (NBS) is not yet implemented in the Swedish CF-care. There were no significant differences in results regarding LCI in the cohort with early or late CF-diagnoses and our results regarding the extent of SLD at early school age are similar to countries that have NBS, but still our results may be affected by lack of NBS[10].

Conclusion

A low LCI during childhood is associated with less SLD and a slower SLD progression rate compared to a higher longitudinal mean LCI. This study further strengthens the clinical utility of regular MBW examinations throughout childhood, with the aim of pursuing a low LCI.

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FIGURE LEGENDS

Figure 1. Longitudinal overview of the cohort and the main outcomes of the study. Multiple breath washout was performed annually between 1999–2012 and every six months between 2013–2016. Chest CT was performed from the age of six years and repeated every third year.

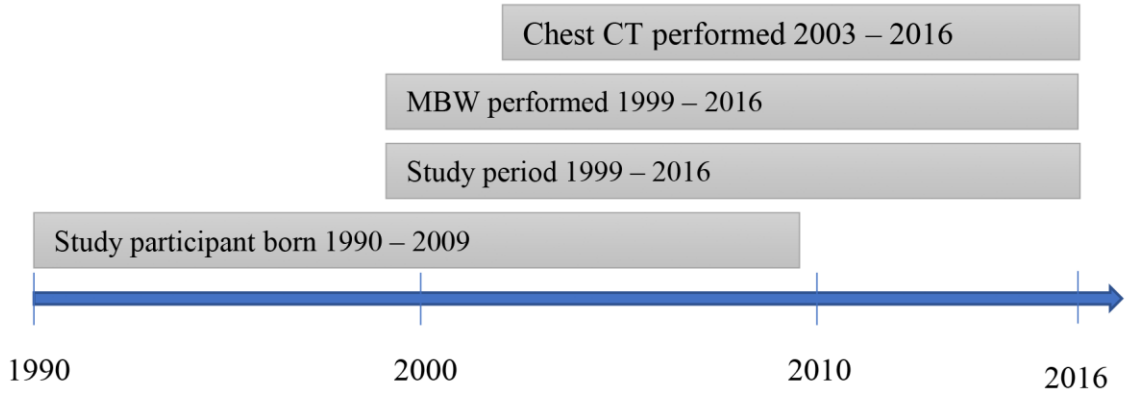

Figure 2. Mean LCI *vs* age in the CF cohort (n=75) and a healthy reference population (n=140) (A), LCI *vs* age in the healthy reference population only (B), and longitudinal progression of age-adjusted LCI in the CF cohort (C). Circles and grey lines are individual data. The blue lines with shaded bands show the mean trends with 95% confidence limits. Dashed lines are 95% prediction limits.

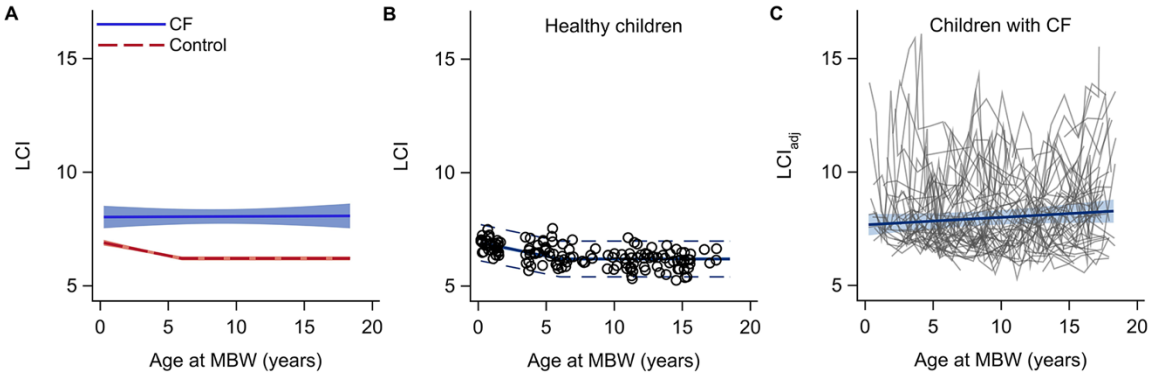

Figure 3. Yearly progression of total airway disease (A, B) and bronchiectasis (C, D) throughout the study period *vs* mean LCI_{adj} (A, C) and progression of LCI_{adj} (B, D) over the same period. Circles show the best linear unbiased predictions (BLUPs) of each subject's

progression and mean, estimated by joint modelling of all longitudinal chest CT and MBW data. “r” is the corresponding correlation coefficient. The solid lines with shaded bands are fitted regression lines with 95% confidence limits.

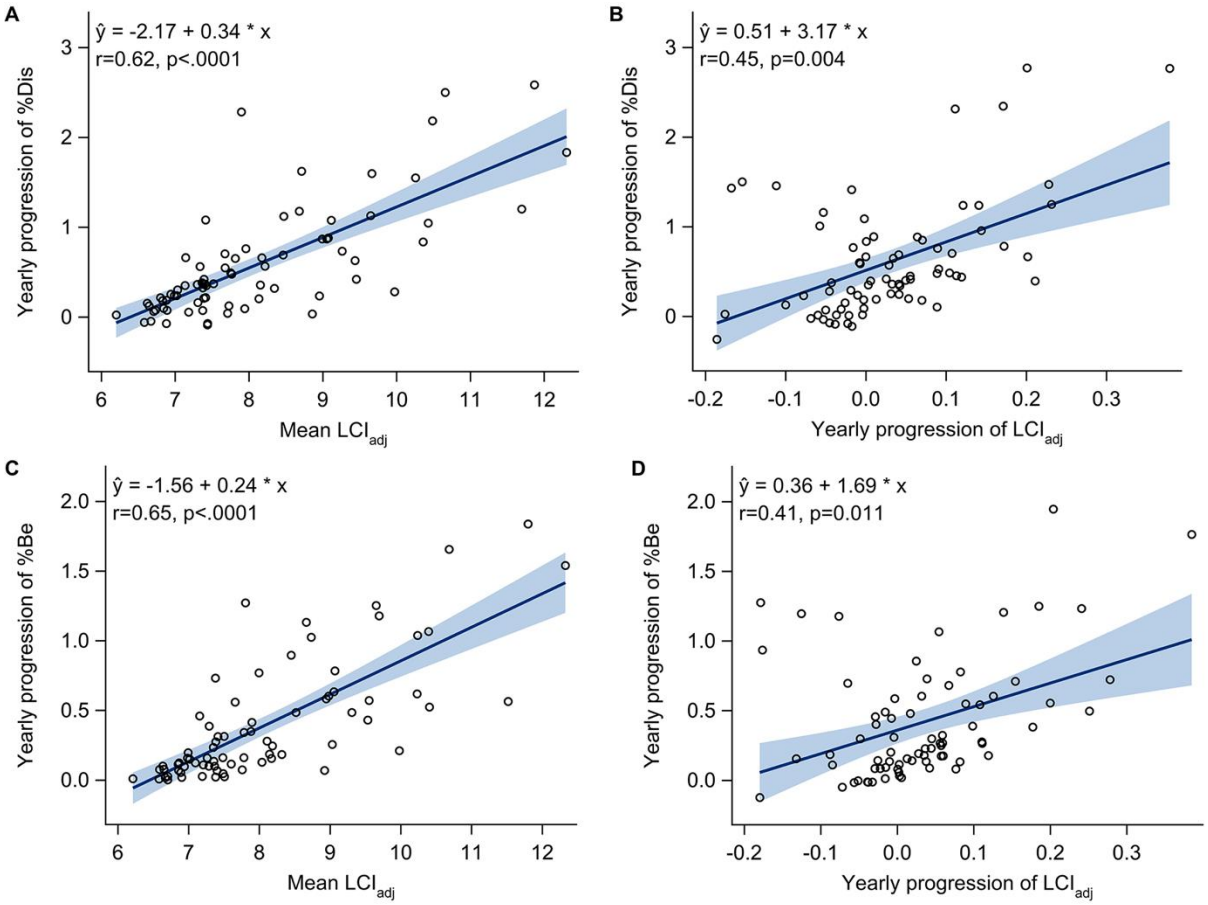

Figure 4. Progression of total airway disease (A) and bronchiectasis (B) in the CF cohort and relation to longitudinal LCI. Grey lines are individual data. The thick lines and shaded bands represent estimated median trends with 95% confidence limits, assuming a constant LCI_{adj} throughout childhood. Regression curves were derived from mixed effects models on logit-transformed SLD outcomes, with age at CT and the best linear unbiased prediction of the mean LCI at CT as explanatory variables.

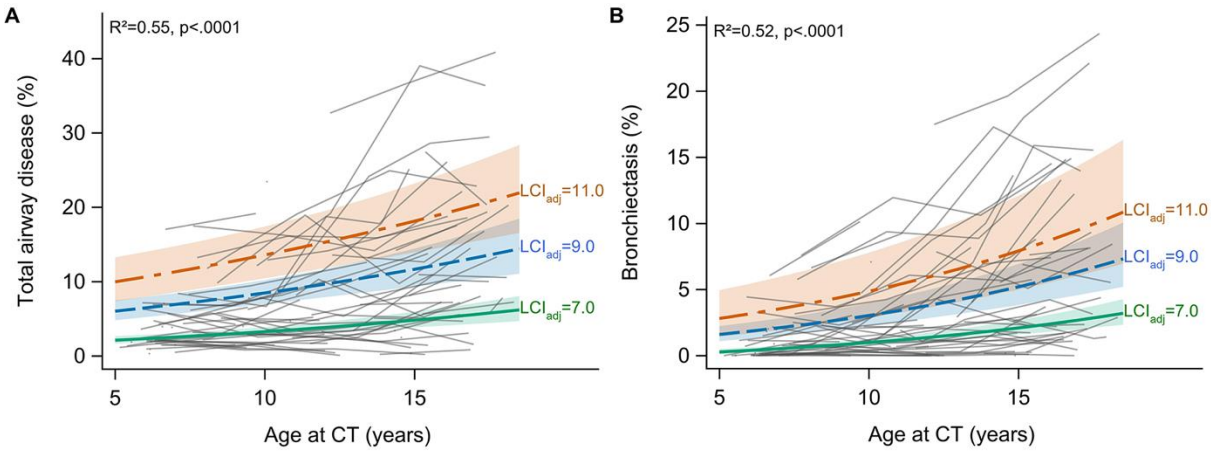

Figure 5. Estimated percentiles of total airway disease (%Dis) (A, B) and bronchiectasis (%Be) (C, D) at six years and 17 years of age *vs* longitudinal mean LCI_{adj} at CT. The BLUP is the best linear unbiased prediction using all available MBW data up and including the time-point of chest CT. Percentile curves were derived from mixed effects models on logit-transformed SLD outcomes, using all available longitudinal MBW and chest CT data.