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Original research

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

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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 CT has also been shown to be a sensitive marker to of early SLD in CF children, but with the disadvantage of accumulation of ionising radiation, which limits a more frequent utilisation.[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 and 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]

Key messages

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 with a higher lung clearance index.

Why read on?
► To better understand how longitudinal lung clearance index may be used in clinical practice.

METHOD

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 time point 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.

Lung function 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 6 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

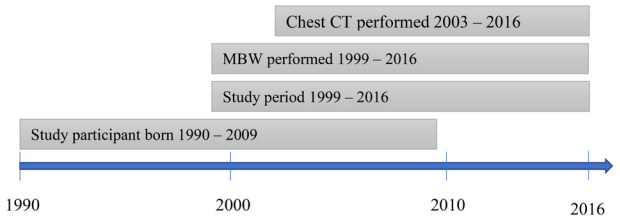

Figure 1 Longitudinal overview of the cohort and the main outcomes of the study. Multiple breath washout (MBW) was performed annually between 1999–2012 and every 6 months between 2013 and 2016. Chest CT was performed from the age of 6 years and repeated every third year.

reanalysed 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 supplemental material 1 for further details.

Chest CT acquisition and analysis

High-resolution chest CTs were performed in clinical stable CF subjects every third year from 6 years of age between 2003 and 2016. The Perth-Rotterdam Annotated Grid Morphometric Analysis for Cystic Fibrosis method was used to score SLD.[16] 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.[17]

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.[17] 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.

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 SEs 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 predictionsof 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, preschoolers and school-age children, all LCI values in children with CF were age-adjusted LCI values (LCI_{adj}) according to the formula

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

as derived from the healthy reference population.

Statistical analyses were performed using SAS software, V.9.4 (SAS Institute, Cary,North Carolina). For more information about the statistical methodology, see OSM.

RESULTS

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 reanalysed. 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

Table 1 Demographics and descriptive statistics of the CF cohort

Variable	n (%)/median (range)
Female sex	24 (32%)
Pancreatic insufficiency	67 (89%)
dF508/dF508, dF508/other, other/other	38 (51%)/34 (45%)/3 (4%)
Age at diagnosis (years)	0.8 (0.0–9.0)
Children treated with CFTR modulators	1 (1%)
Children with CF-related diabetes mellitus	2 (3%)
Chronically infected with Pa during study period	22 (29%)
Age at onset of chronic Pa infection (years)	13.0 (3.1–18.9)
Children with ABPA	3 (4%)
Follow-up time* (years)	9.0 (1.0–14.1)
Number of MBW examinations/child	11 (1–18)
Number of chest CTs/child	3 (1–5)
Chest CTs at MBW examinations (±3 days)	146 (73%)

*Time between the first and the last multiple breath washout or the last chest CT.
ABPA, allergic bronchopulmonary aspergillosis; CFTR, cystic fibrosis transmembrane conductance regulator; MBW, multiple breath washout; Pa, P. aeruginosa.

Table 2 Cross-sectional overview of CF disease progression for the cohort, including the upper reference limit of normal LCI (ULN) derived from 140 individuals with no lung disease. LCI values for the CF cohort are also presented as age adjusted LCI (LCI_{adj})

Variable	Age 2 years (n=27)	Age 5 years (n=41)	Age 7 years (n=44)	Age 12 years (n=44)
LCI	7.7 (6.3–13.8)	7.4 (6.2–12.2)	7.5 (5.7–12.4)	7.9 (5.6–11.0)
LCI_{adj}	7.2 (5.8–13.3)	7.3 (6.1–12.1)	7.5 (5.7–12.4)	7.9 (5.6–11.0)
ULN LCI	7.4	7.0	7.0	7.0
FEV_1 (z-score)	–	−0.1 (-2.0–2.1)*	−0.1 (-3.1–2.7)	−0.4 (-3.8–2.6)
Chest CT (%Dis)	–	–	3.8 (0–17.5)†	5.4 (0.9–32.7)‡
Chest CT (%Be)	–	–	0.8 (0–7.6)†	2.0 (0–17.5)‡
BMI (z-score)	−0.1 (−1.7–1.8)	−0.3 (−2.8–1.4)	−0.2 (−1.9–2.65)	−0.4 (−2.0–1.2)

Data are presented as median (range).
*30 of 41 subjects performed technically acceptable spirometry.
†35 of 44 subjects had a chest CT between age 6 and 8 years.
‡30 of 44 subjects had a chest CT between age 11 and 13 years.
%Be, bronchiectasis (%); BMI, body mass index; %Dis, total airway disease (%); FEV, forced expiratory volume; LCI, lung clearance index; LCIadj, age adjusted lung clearance index; ULN, upper limit of normal.

are presented in table 2. Detailed results regarding the progression of SLD in this cohort have been reported elsewhere.[17]

LCI in healthy children

In the healthy reference population, LCI decreased by 0.12 units per year (95% CI −0.16 to −0.09, p<0.0001) up to 6 years of age, after which no further decrease was observed (p=0.21, figure 2A,B). The age-specific mean and 95% upper prediction limit (upper limit of normal) are presented in OSM Table E1.

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 to 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 to 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 (online supplemental table E2).

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 to 0.84) for %Dis, r=0.65 (95% CI 0.46 to 0.85) for %Be, both p<0.0001) (figure 3A and C). One unit increase in mean LCI_{adj} was associated with 0.34 (95% CI 0.27 to 0.41) percentage points faster progression rate of %Dis/year and 0.24 (95% CI 0.19 to 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 to 0.76), p=0.004 for %Dis, r=0.41 (95% CI 0.09 to 0.72), p=0.011 for %Be) (figure 3B and D). The correlations were qualitatively similar but somewhat attenuated when adjusting for age at diagnosis, sex, birth cohort and concomitant infections with Pa, Sa and Asp (online supplemental table E3).

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<0.0001) and 35% of the variation in %Be (p=0.020) throughout the study period (online supplemental table E4). However, an individual LCI value beyond LCI_{adj}=9.0 was only weakly associated with increased SLD (online supplemental figures E2,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<0.0001) and 52% of the variation in %Be throughout the study period

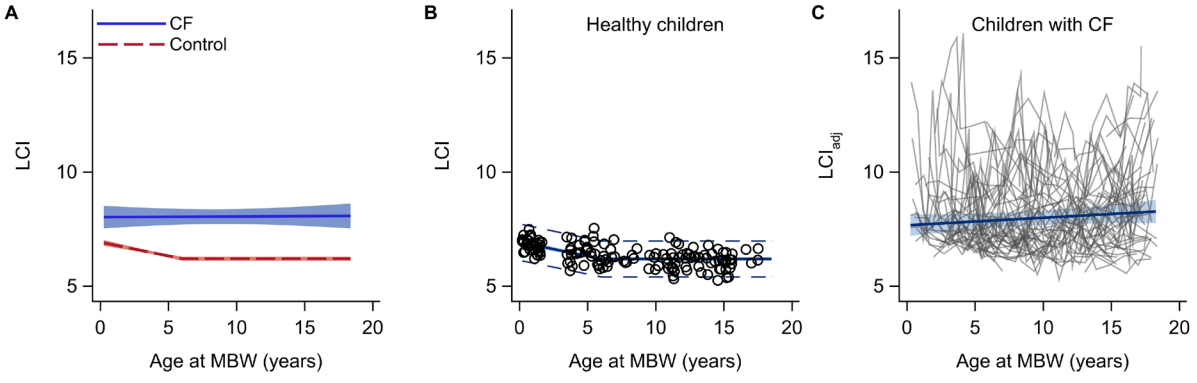

Figure 2 Mean LCI versus age in the CF cohort (n=75) and a healthy reference population (n=140) (A), LCI versus age in the healthy reference population only (B), and longitudinal progression of age-adjusted LCI in the CF cohort (C). Circles and grey lines are individual data. The blue lines with shaded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

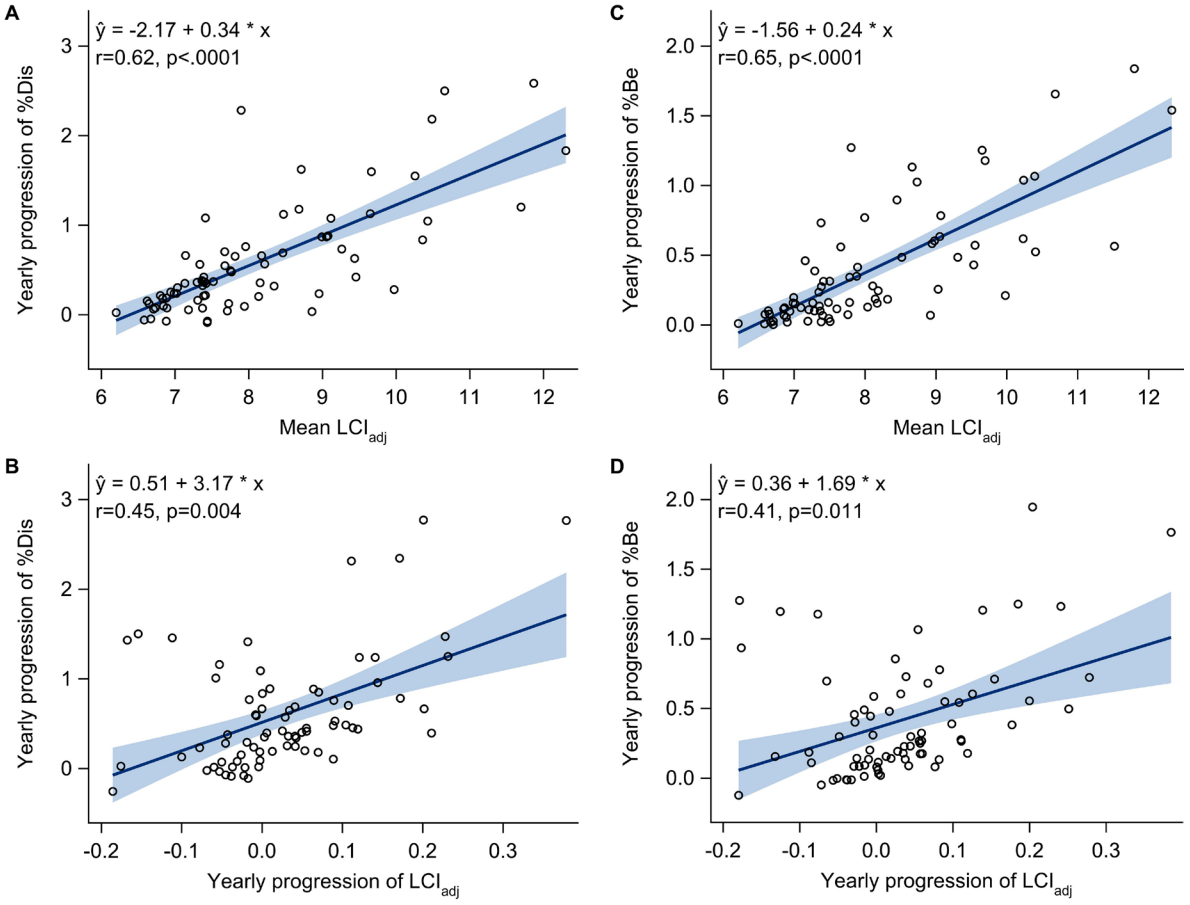

Figure 3 Yearly progression of total airway disease (A, B) and bronchiectasis (C, D) throughout the study period versus mean LCI_{adj} (A, C) and progression of LCI_{adj} (B, D) over the same period. Circles show the best linear unbiased predictions (BLUPs) of each subject's progression and mean, estimated 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. LC, lung clearance index; MBW, multiple breath washout.

($p<0.0001$) (figure 4, online supplemental figure E2,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 (online supplemental table E4).

SLD percentile curves

Percentile curves of %Dis and %Be versus mean LCI_{adj} at 6 and 17 years of age are presented in figure 5, and additionally for %Dis and %Be versus LCI_{adj} at CT in OSM Figure E2 and E3. A

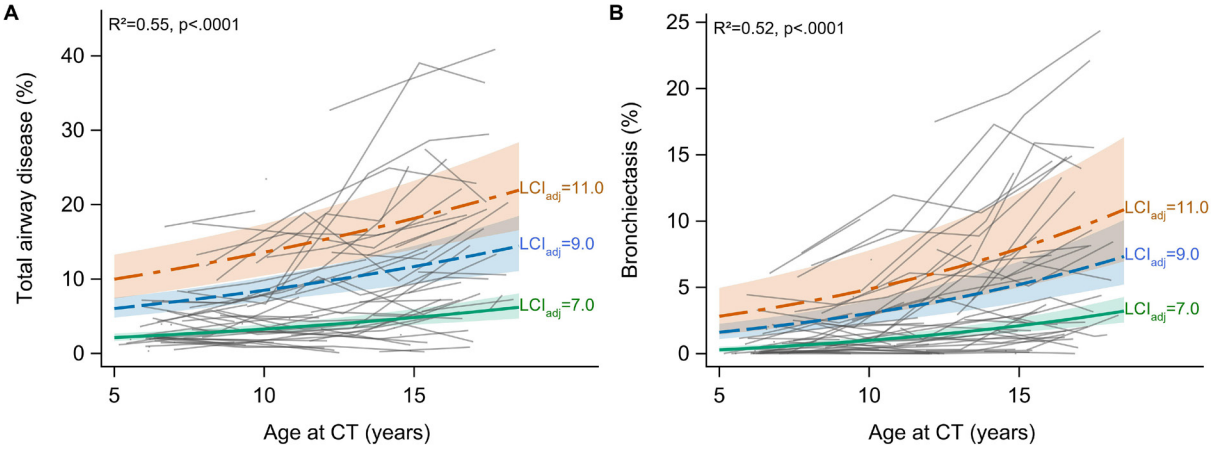

Figure 4 Progression of total airway disease (A) and bronchiectasis (B) in the CF cohort and relation to longitudinal LCI. Grey lines are individual data. The thick lines and shaded bands represent estimated median trends with 95% confidence limits, assuming a constant LCI_{adj} throughout childhood.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

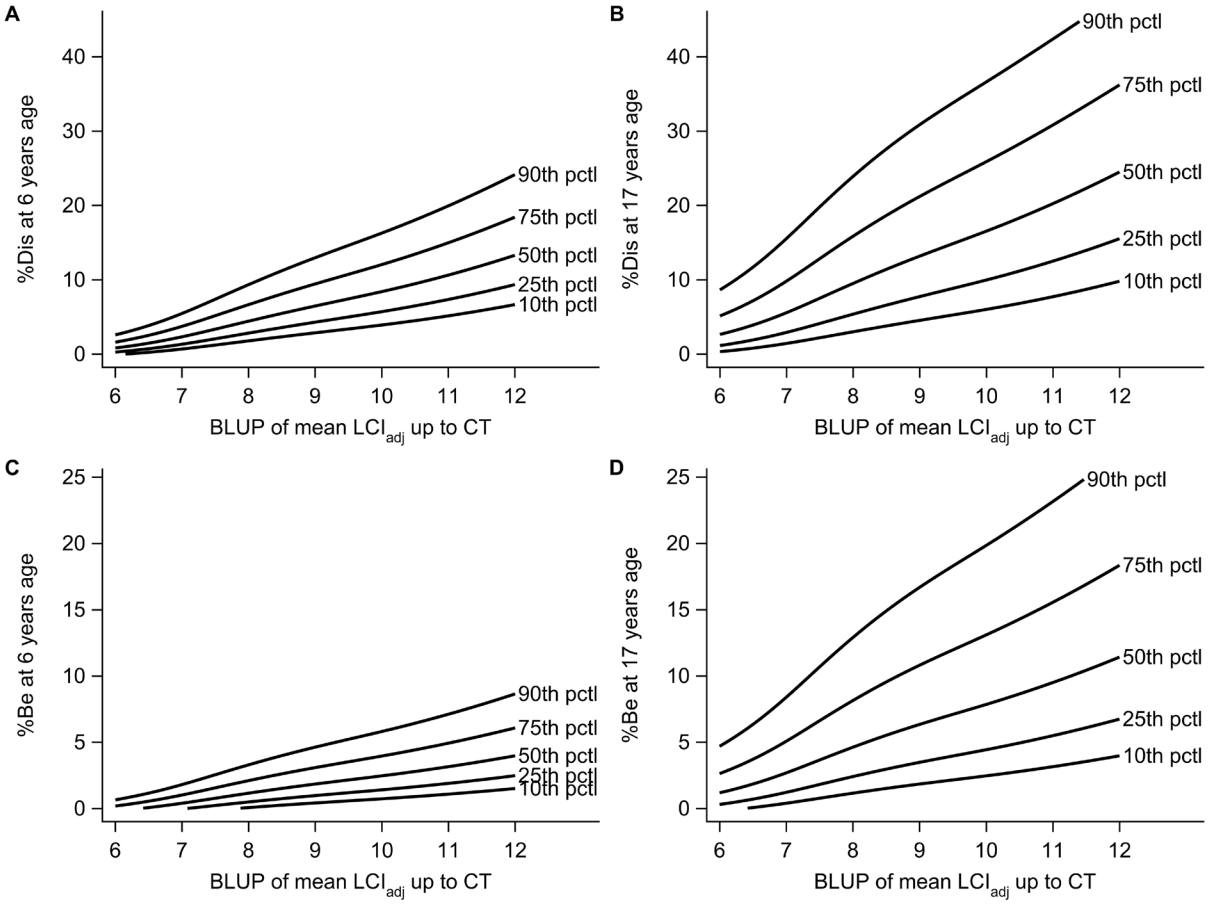

Figure 5 Estimated percentiles of total airway disease (%Dis) (A, B) and bronchiectasis (%Be) (C, D) at 6 years and 17 years of age versus longitudinal mean LCI_{adj} at CT. The BLUP is the best linear unbiased prediction using all available MBW data up and including the time-point of chest CT. Percentile curves were derived from mixed effects models on logit-transformed SLD outcomes, using all available longitudinal MBW and chest CT data. BLUP, best linear unbiased prediction; MBW, multiple breath washout; SLD, structural lung damage.

non-pathological mean LCI (LCI_{adj}=7.0) during preschool age corresponded to a median (10th–90th percentile) %Dis of 2.3% (0.7 to 5.5) and %Be of 0.4% (0.0 to 1.8) at 6 years of age. A two units higher LCI (LCI_{adj}=9.0) corresponded to a median (10th–90th percentile) %Dis of 6.5% (2.9 to 13.0) and %Be of 1.8% (0.4 to 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.[12] 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.[19] A Swiss paediatric CF cohort, who also used Exhalyzer D over a longer period of time, revealed a slow increase in 0.1 LCI units per year in the younger school children and a more pronounced annual LCI progression rate in the adolescences.[20] 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.[21] 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 22 23] 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 and 1999, but not in the children born after 2000. This finding most probably reflects the constant improvement in the CF healthcare 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.[24 25] 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 clinically stable patients with CF as well as sudden deteriorations or improvements in LCI caused by nearby pulmonary infections or interventions.[6 7 20 22 23] 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 with cross-sectional measurements of risk factors have also been found in other contexts, for example, risk prediction of future cardiovascular disease.[26] 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 preschool ages corresponded to relatively low extent of SLD at the age of 6 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 twofold 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.[16 27 28] 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 preschool and/or school age may be used as an indicator to perform chest CT scans less frequently. 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.[29] 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 preschool ages, we may limit the SLD progression rate as well as preserve the subject's lung function at early school age.[19 30 31]

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 patients with CF from MBW obtained at various ages and by different technical procedures, by removing the age trend observed in healthy preschool children. We used age-adjusted LCI_{adj} rather than LCI z-score since age-adjusted LCIs are easier to interpret and visualise 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.[32] 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 do not 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,[33] but this information was not available during the study period. Chest CTs were analysed and blinded to patient identifiers with a fully quantitative scoring system with good reproducibility.[17] A limitation is the absence of CTs during infancy or preschool ages. The pulmonary status at the time of initial CF diagnosis is variable in our cohort, as new-born screening 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 new-born screening, but still our results may be affected by lack of new-born screening.[10]

CONCLUSION

A low LCI during childhood was associated with less SLD and a slower SLD progression rate compared with 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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Contributors 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. AL is responsible for content in this article.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval The study was approved by the local Ethics Committee (Dnr. 206–18).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request.

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Online Data Supplement to the article "Longitudinal lung clearance index and association with structural lung damage in children with cystic fibrosis"

Marcus Svedberg, Henrik Imberg, Per Gustafsson, Harm Tiddens, Gwyneth Davies, Anders Lindblad

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1. Lung function acquisition and analysis

1.1 Multiple breath washout in the CF cohort

Infants and pre-schoolers (0–2 years) performed multiple breath washout (MBW) sedated with chloral hydrate in a supine position using a face mask. Pre-schoolers and school-aged children with CF performed MBW awake in a sitting position using a facemask or a mouthpiece together with a nose clip. A dry air mixture containing 4% sulphur hexafluoride (SF_6) was used during the wash-in period until the expiratory and inspiratory concentration of SF_6 was stable (4%). During the washout period, room air was inhaled during tidal breathing until the expiratory SF_6 concentration reached well below 0.1% ($1/40^{th}$ of the SF_6 starting concentration). A respiratory mass spectrometer (AMIS 2000, Innovision A/S, Odense, Denmark) was used to measure the expiratory gas concentrations. A minimum of two technically acceptable MBW tests were considered acceptable.

The MBW examinations between 1999–2016 assessed as acceptable were available for re-evaluation and analysis with the software LabVIEW. All MBW available were re-analysed according to the consensus statement by one paediatric pulmonologist (MS) [1]. The patient's identity was not blinded or in random order. To minimize bias, the MBW examinations were also re-analysed continuously or upon request by two other paediatric pulmonologists experienced in the MBW-analysis procedures.

1.2 Multiple breath washout in healthy subjects

A total of 140 healthy infants, pre-schoolers and school-age children served as a healthy reference population for the LCI values. Infants (n=30) and pre-schoolers (n=40) with no known history of lung disease performed MBW in London, UK, using identical equipment, procedures and test gas as described in the CF cohort [2]. Original MBW traces were re-

analysed by the same person (MS) using the software LabVIEW. Healthy school-aged children (n=70), with a normal spirometry and no known history of lung disease performed MBW using the same MBW-equipment at Skovde paediatric hospital and were re-analysed by PG using the same software. The healthy reference population was used to calculate upper reference limits of normal LCI, and further derive an age adjusted LCI (LCI_{adj}) variable for CF patients. Thus, LCI values of CF patients from MBW performed at various ages were transformed to a common scale by removing the age trend observed in healthy children. The age-adjustment was employed to account for natural non-CF related age trends, and for differences in technical procedures between infants, pre-schoolers and school-aged children.

1.3 Spirometry

Spirometry tests (Jaeger AG, Würzburg, Germany) were performed according to the ATS/ERS recommendations at the annual evaluation [3]. Spirometry results are expressed as Z-scores according to the Global Lung Initiative reference equations [4].

1.4 Airway pathogens and antibiotic treatment

During the study period children with CF had regular visit at Gothenburg CF centre and/or their local hospital every 6th week throughout childhood (0–17 years). The aim was to obtain a respiratory secretion sample (sputum sample or laryngeal suction in non-sputum producing individuals) from each CF-patient at least every third month. Prophylactic antibiotic (Flucloxacillin) was normally used till the age of 6 years. First line of treatment for mild pulmonary exacerbation (defined clinically by new or worsening in airway symptoms over a shorter period of time) was normally oral antibiotics (e.g., Amoxicillin/Clavulanic acid or trimethoprim sulphate) in 10–14 days and were normally initiated at home after contact the

CF-clinic or after a visit at the clinic. Chronic infections with *Pseudomonas aeruginosa* (Pa) was treated with a combination of iv antibiotics, inhaled antibiotics, and Azithromycin.

2. Additional statistical method details

2.1 LCI progression and subgroup analyses

We estimated the extent and progression of LCI during the study period for the entire study cohort and by subgroups formed by sex, pancreatic insufficiency, age at diagnosis (<1 *vs* >1 years) and birth cohort (born 1990–1999 *vs* 2000–2009). This was done using a linear mixed effects model with a random intercept and random age slope for each subject, assuming a general unstructured covariance matrix of the random effects. This model simultaneously describes the overall trend and subject specific progressions, and accounts for intra-individual correlations. Robust standard errors were used to account for the skewed distribution of the response variable. Subgroup differences in mean LCI and yearly progression in LCI were evaluated by adding the subgroup variable and subgroup with age interaction to the model. If the interaction effect was significant, the mean difference in LCI between subgroups were estimated at 7, 11 and 15 years of age from the corresponding linear combinations of the model parameters. If the interaction was non-significant, the age adjusted mean difference in LCI was estimated after removal of the interaction effect from the model.

2.2 Joint modelling of longitudinal SLD and LCI

Linear associations between longitudinal LCI measurements and SLD throughout childhood were analysed using joint modelling of all available longitudinal MBW and CT data. This was done using linear mixed effects models with LCI, %Dis and %Be as multivariate response variables. The model included an intercept and age slope per response variable as fixed effects, and random intercepts and random age slopes for each subject and response variable.

A general unstructured covariance matrix between all random effects within a subject was assumed. Correlations between the yearly progression of LCI and yearly progression of SLD were obtained from the model-based estimates of the correlations between the corresponding random effects. Similarly, we estimated the correlation between progression of SLD and mean LCI throughout childhood by omitting the fixed and random age slopes of LCI from the model. The significance of the correlation coefficients was evaluated using Wald-tests and corresponding confidence intervals were calculated from the estimated correlation coefficients and associated standard errors. We also estimated the best linear unbiased predictions (BLUPs) of each subject's mean LCI, progression of LCI and progression of SLD from the joint models described above and summarised the results graphically in scatter plots.

We also estimated adjusted correlation coefficients, accounting for age at diagnosis, sex, birth cohort, and colonisation of airway pathogens Pa, Sa and Asp. This was done by adding the subject characteristics, subject characteristic with age interactions, cumulative number of infections with Pa, Sa and Asp, and years chronically infected with Pa, as fixed effects in the model.

2.3 Non-linear associations between SLD and LCI

To understand the association between SLD and LCI at a more detailed level, we continued with non-linear mixed effects models of SLD related to LCI. Since both total airway disease (%Dis) and bronchiectasis (%Be) were measured at a continuous scale naturally bounded between 0 and 100 per cent, we employed a logistic-type transformation on the form

$$\log\left(\frac{1+x}{101-x}\right),$$

where x is the value of %Dis or %Be. After this transformation, approximately normally distributed and homoscedastic errors were obtained. The longitudinal transformed SLD data

was analysed using linear mixed effects models with age at CT and LCI as fixed effects, and with a random intercept and random age slope for each subject. A general unstructured covariance matrix of the random effects was assumed. The goodness of fit to the data was summarised by the fraction of variance (R^2) of the logit-transformed outcome explained by the fixed effects.

To account for repeated measures of LCI, we considered, in addition to LCI measured at CT (+/-3 days), 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 BLUPs of the LCI at CT were derived from mixed effects models with intercept and age at MBW as fixed effects, and with a random intercept and random age slope for each subject. The BLUPs of the mean LCI up to CT were derived from mixed effects models with only an intercept as fixed effect, and with a random intercept for each subject. A general unstructured covariance matrix of the random effects was assumed throughout. When calculating the BLUP of LCI or mean LCI for a particular subject and CT, only MBW performed prior to or at the timepoint of that CT were included for that subject, whereas the other subjects contributed with all their MBW data. We did not consider LCI metrics based on the change in LCI (e.g., progression in LCI during a specific period of time), since an association between the abovementioned LCI metrics and SLD in a longitudinal context also implies an association between change in LCI and change in SLD. Thus, temporal variations and trends in LCI were implicitly accounted for in the analyses.

In order to capture potential non-linear effects of LCI, the LCI variables were modelled by natural cubic splines with knots at the 10^{th}, 50^{th} and 90^{th} percentiles. This is equivalent to

including a linear term for LCI in the model, together with a non-linear term given by the transformed LCI variable

$$\frac{\max(\mathrm{x} - \mathrm{k}_1, 0)^3 - \max(\mathrm{x} - \mathrm{k}_3, 0)^3}{k_3 - k_1} - \frac{\max(\mathrm{x} - \mathrm{k}_2, 0)^3 - \max(\mathrm{x} - \mathrm{k}_3, 0)^3}{k_3 - k_2},$$

where x is the value of the LCI variable at CT, and k_1, k_2, k_3 are the corresponding knot positions. The significance of the association between LCI and SLD was assessed by an F-test on the linear contrast of model coefficients pertaining to LCI. The analyses were performed both unadjusted and adjusted for age at diagnosis, sex, birth cohort, and colonisation of airway pathogens Pa, Sa and Asp. The adjusted analyses were conducted by adding the subject characteristics, subject characteristic with age interactions, cumulative number of infections with Pa, Sa and Asp, and years chronically infected with Pa, as fixed effects in the model.

The logit-linear mixed effects models were subsequently used to derive median and percentile curves for various ages and LCI profiles, assuming normally distributed random effects and a normal distribution of the transformed outcomes. We used the unadjusted analyses for this purpose, since the adjusted analyses would require a separate set of plots for each patient profile of interest. Model diagnostics demonstrated a good fit to the data and supported the plausibility of the model assumptions, apart from the presence of exact zeroes in %Be (Figure E3 and E4). Model predicted percentiles below zero, which mainly were obtained at low ages and low levels of LCI, were interpreted as being equal to zero.

Table E1. LCI mean, SD and 95% upper prediction limit in 140 healthy children.

Age (years)	Mean	SD	ULN
0	6.87	0.40	7.66
1	6.74	0.40	7.54
2	6.62	0.40	7.41
3	6.50	0.40	7.28
4	6.38	0.40	7.16
5	6.25	0.40	7.04
≥6	6.19	0.40	6.98

LCI decreased by 0.12 units per year up to six years age (p<.0001).
There was no significant change in LCI after six years age (p=0.21).
A constant SD was assumed. Adding heteroscedastic errors did not improve model fit (p=0.11).
ULN is the 95% upper prediction limit.
Abbreviations: LCI, lung clearance index; SD, standard deviation; ULN, upper limit of normal.

Table E2. Progression of LCI_{adj} in the CF cohort, by subject characteristics.

Subgroup variable	Subgroup	Mean yearly progression (95% CI)	Interaction Subgroup*Age	Age adjusted mean difference (95% CI) between subgroups (last category is reference group)
All subjects		0.03 (-0.01 – 0.08) p=0.13		
Sex	**Girl (n=24)**	0.11 (0.03 – 0.20) p=0.011	p=0.020	At 7 years age: -0.19 (-0.93 – 0.55), p=0.61 At 11 years age: 0.28 (-0.52 – 1.07), p=0.50 At 15 years age: 0.74 (-0.28 – 1.76), p=0.15
	Boy (n=51)	-0.00 (-0.05 – 0.04) p=0.91		
Pancreatic status	**Sufficient (n=8)**	-0.01 (-0.07 – 0.05) p=0.75	p=0.21	-0.29 (-1.44 – 0.85) p=0.61
	Insufficient (n=67)	0.04 (-0.01 – 0.08) p=0.11		
Age at diagnosis	**<1 year (n=38)**	0.07 (0.01 – 0.12) p=0.016	p=0.06	-0.43 (-1.07 – 0.21) p=0.19
	>1 year (n=37)	-0.01 (-0.07 – 0.05) p=0.73		
Birth cohort	**Born 1990–1999**	0.07 (0.01 – 0.12) p=0.025	0.036	At 7 years age: 0.32 (-0.35 – 0.99), p=0.35 At 11 years age: 0.70 (-0.02 – 1.41), p=0.06 At 15 years age: 1.07 (0.17 – 1.98), p=0.02
	Born 2000–2009	-0.03 (-0.09 – 0.04) p=0.41		

Abbreviations: CI, confidence interval; LCI_{adj}, age adjusted lung clearance index.

Table E3. Longitudinal correlation between progression SLD with mean LCI_{adj} and yearly progression of LCI_{adj} throughout childhood using joint modelling of all available MBW and CT data.

		Correlation coefficient (95% CI)	
LCI parameter	**CT parameter**	**Unadjusted**	**Adjusted***
Mean LCI_{adj}	Yearly progression of %Dis	0.62 (0.40–0.84) p<.0001	0.47 (0.17–0.76) p=0.002
Mean LCI_{adj}	Yearly progression of %Be	0.65 (0.46–0.85) p<.0001	0.42 (0.14–0.71) p=0.004
Yearly progression of LCI_{adj}	Yearly progression of %Dis	0.45 (0.15–0.76) p=0.004	0.33 (-0.05–0.72) p=0.09
Yearly progression of LCI_{adj}	Yearly progression of %Be	0.41 (0.09–0.72) p=0.011	0.38 (0.01–0.75) p=0.045

*Adjusted for age at diagnosis, sex, birth cohort and concommitant infections of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Aspergillus species*.

Table E4. Regression analysis results for the longitudinal association between SLD and LCI_{adj} using logit-linear mixed effects models.

		Unadjusted				Adjusted*			
Outcome	**LCI variable**	**Linear LCI coefficient (SE)**	**Non-linear LCI coefficient (SE)**	**F-test**	**R^2**	**Linear LCI coefficient (SE)**	**Non-linear LCI coefficient (SE)**	**F-test**	**R^2**
%Dis	LCI_{adj} at CT +/-3 days	0.369 (0.078)	-0.060 (0.024)	p<.0001	0.49	0.327 (0.081)	-0.048 (0.025)	p<.0001	0.57
	BLUP of LCI_{adj} at CT	0.561 (0.099)	-0.143 (0.045)	p<.0001	0.52	0.505 (0.101)	-0.131 (0.045)	p<.0001	0.58
	BLUP of mean LCI_{adj} up to CT	0.622 (0.124)	-0.130 (0.059)	p<.0001	0.55	0.553 (0.134)	-0.113 (0.063)	p<.0001	0.61
%Be	LCI_{adj} at CT +/-3 days	0.223 (0.098)	-0.045 (0.033)	p=0.020	0.35	0.183 (0.089)	-0.037 (0.030)	p=0.038	0.47
	BLUP of LCI_{adj} at CT	0.417 (0.134)	-0.102 (0.062)	p<.0001	0.46	0.339 (0.127)	-0.090 (0.059)	p=0.003	0.55
	BLUP of mean LCI_{adj} up to CT	0.543 (0.143)	-0.120 (0.072)	p<.0001	0.52	0.411 (0.146)	-0.083 (0.073)	p<.0001	0.58

Statistical analyses were performed using linear mixed effects models with the LCI variable and age at CT as fixed effects, and with subject specific intercept and age as random effects.
The response variables (total airway disease or bronchiectasis, %) were transformed prior to analysis using a logit-type transformation log((1+x)/(101-x)).
The effect of the LCI varibles on the transformed outcomes were modelled using natural cubic splines with knots at the 10^{th}, 50^{th} and 90^{th} percentiles.
The linear LCI coefficient is the regression coefficient correponding to the actual value of the LCI variable at CT.
The non-linear LCI coefficient is the regression coefficient corresponding to the transformed LCI variable $(\max(x - k_1, 0)^3 - \max(x - k_3, 0)^3) / (k_3 - k_1) - (\max(x - k_2, 0)^3 - \max(x - k_3, 0)^3) / (k_3 - k_2)$, where x is the value of the LCI variable at CT and k_1, k_2, k_3 are the corresponding knot positions.
F-test is the test of association between LCI and the response variable constructed using linear contrasts of the model coefficients pertaining to LCI.
R^2 is the fraction of the logit-transformed response variable explained by the fixed effects included in the model.
The BLUP of LCI_{adj} at CT was derived from linear mixed models of longitudinal age-adjusted LCI with subject specific random intercept and age slope, using all data available up to the timepoint of CT.
The BLUP of mean LCI_{adj} up to CT was derived from linear mixed models of longitudinal age-adjusted LCI with subject specific random intercept, using all data available up to the timepoint of CT.
*Adjusted for age at diagnosis, sex, birth cohort and concommitant infections of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Aspergillus species*.
Abbreviations: Be, Bronchiectasis (%); BLUP, best linear unbiased prediction; CT, computed tomography; %Dis, total airway disease (%); LCI, lung clearance index; LCI_{adj}, age adjusted lung clearance index; SE, standard error.

Figure E1. Total MBW- and chest CT-examinations performed at a certain age.

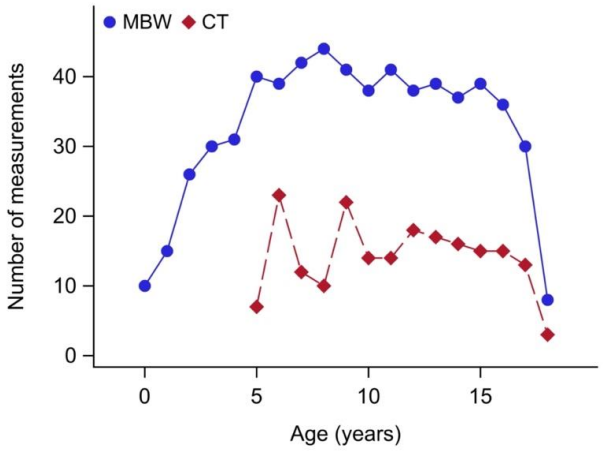


Figure E2. Estimated percentiles of total airway disease (%Dis) at six and 17 years of age *vs* LCI_{adj} at CT (+/- 3 days) (A, B), BLUP of LCI_{adj} at CT (C, D), and BLUP of mean LCI_{adj} up to CT (E, F). The BLUP is the best linear unbiased prediction using all available MBW data up and including the time-point of CT. Percentile curves were derived from mixed effects models on logit-transformed outcomes, using all available longitudinal MBW and CT data.

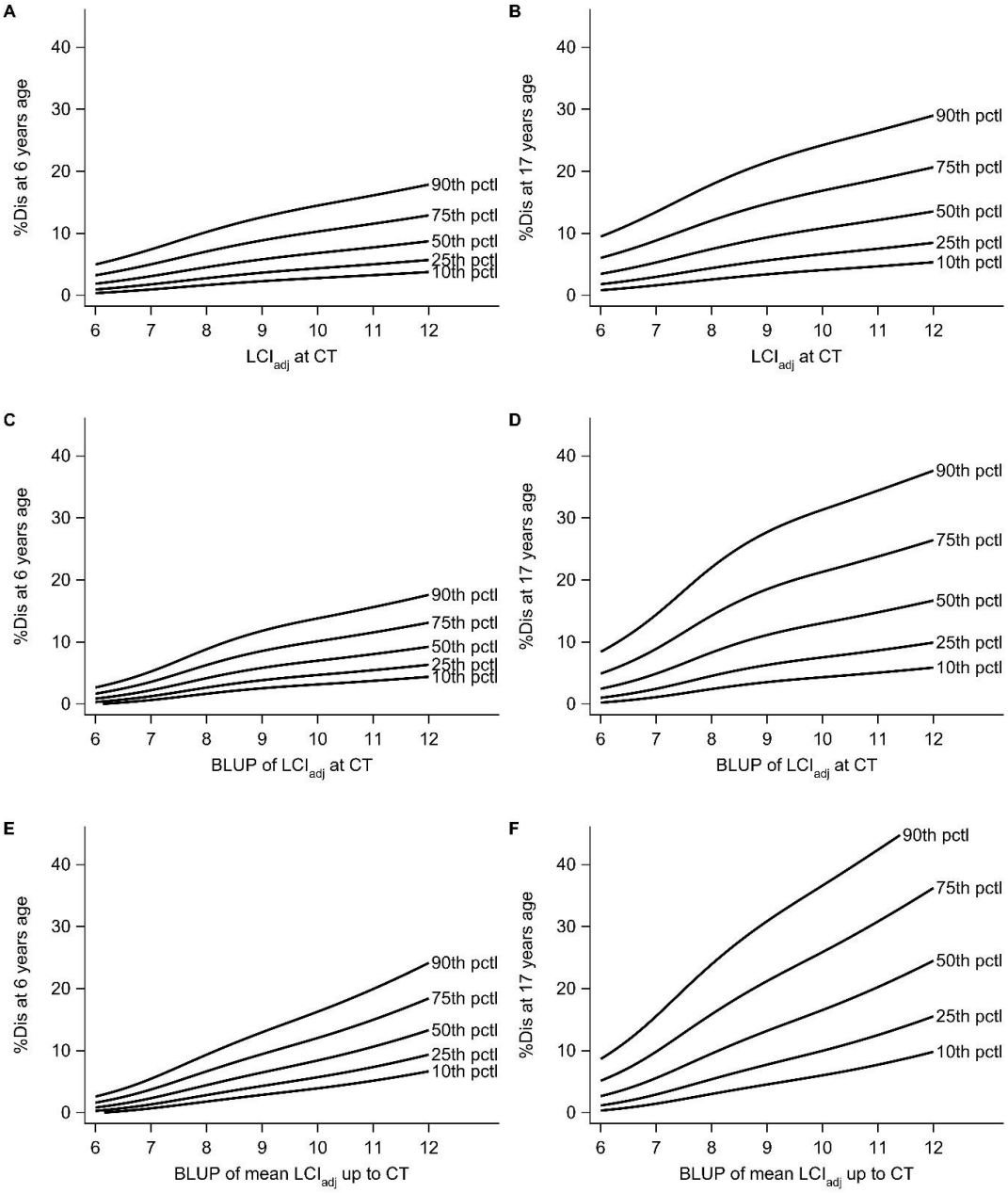


Figure E3. Estimated percentiles of bronchiectasis (%Be) at six years and 17 years of age *vs* LCI_{adj} at CT (+/- 3 days) (A, B), BLUP of LCI_{adj} at CT (C, D), and BLUP of mean LCI_{adj} up to CT (E, F). The BLUP is the best linear unbiased prediction using all available MBW data up and including the time-point of CT. Percentile curves were derived from mixed effects models on logit-transformed outcomes, using all available longitudinal MBW and CT data.

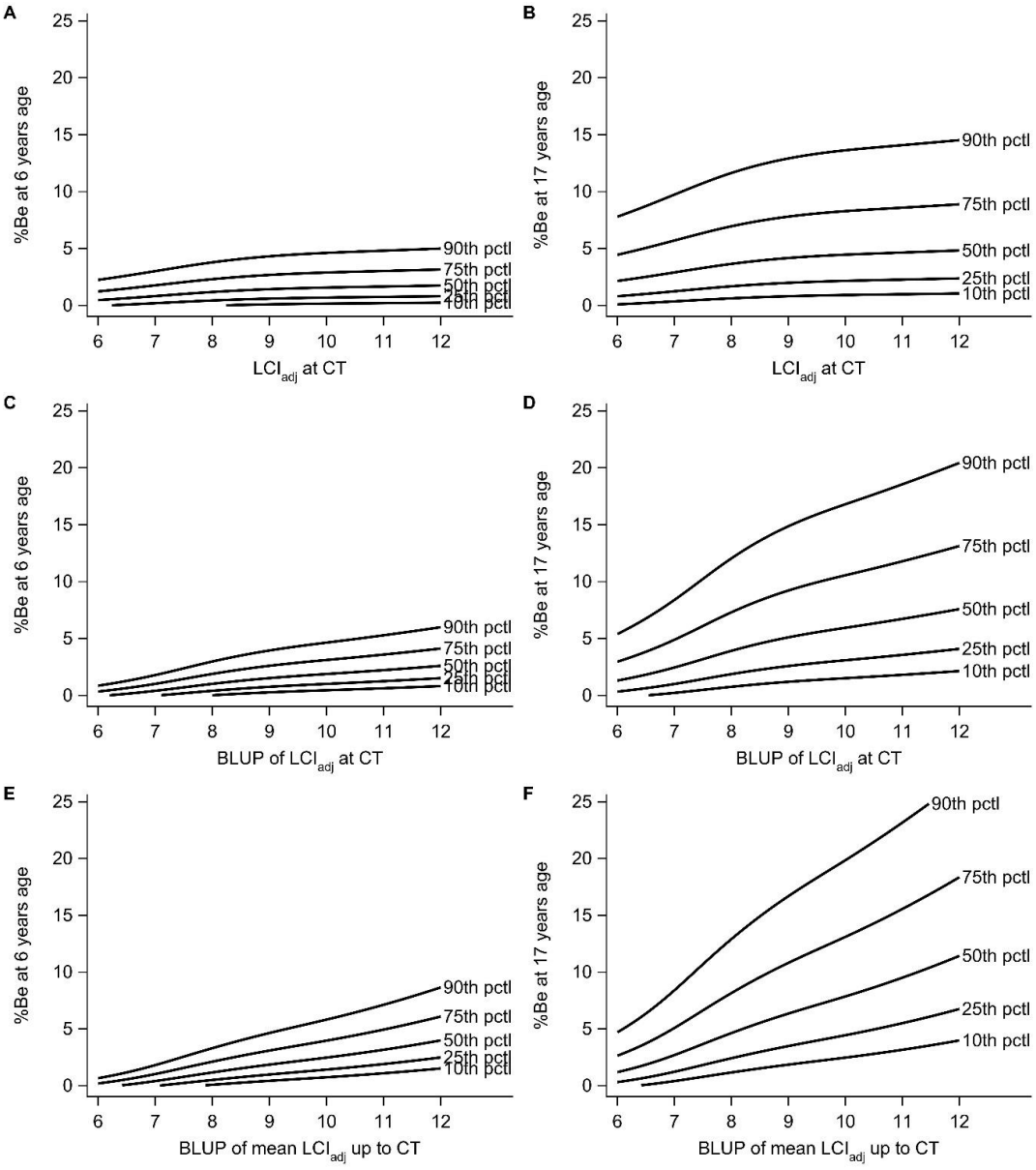


Figure E4. Model diagnostics for %Dis in logit-linear mixed effects model.

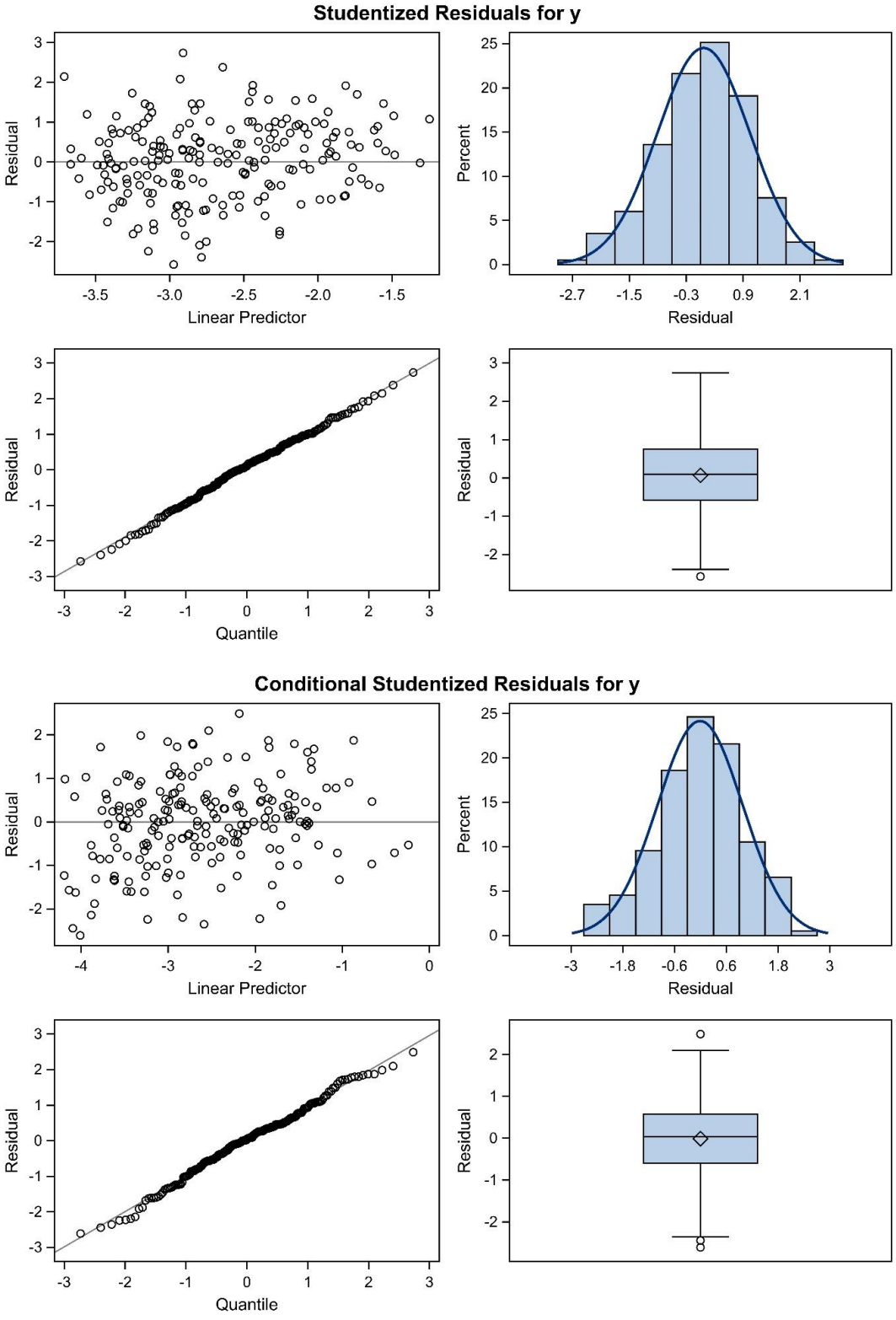
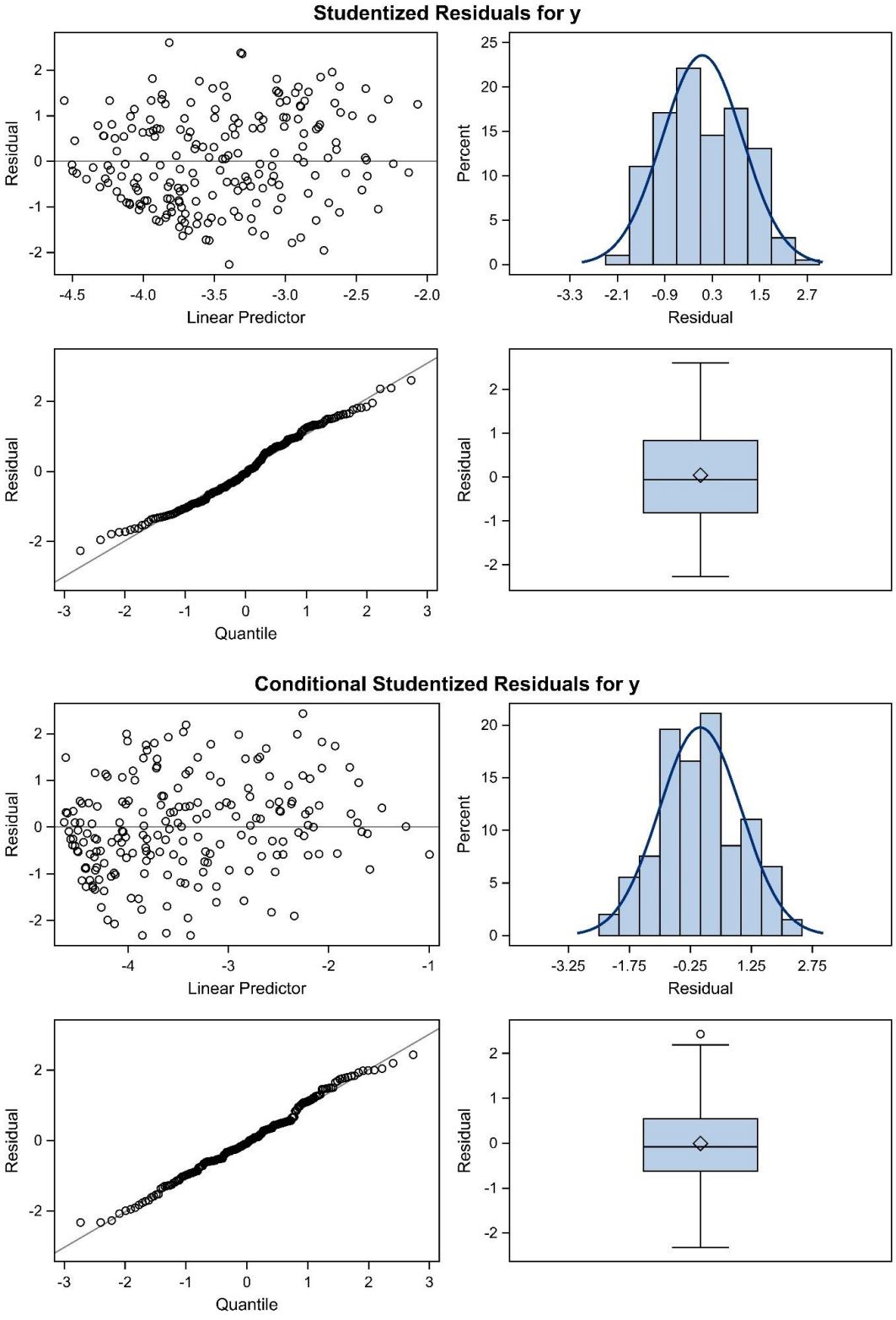


Figure E5. Model diagnostics for %Be in logit-linear mixed effects model.



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Spirometry tests (Jaeger AG, Würzburg, Germany) were performed according to the ATS/ERS recommendations at the annual evaluation [3]. Spirometry results are expressed as Z-scores according to the Global Lung Initiative reference equations [4].

1.4 Airway pathogens and antibiotic treatment

During the study period children with CF had regular visit at Gothenburg CF centre and/or their local hospital every 6th week throughout childhood (0–17 years). The aim was to obtain a respiratory secretion sample (sputum sample or laryngeal suction in non-sputum producing individuals) from each CF-patient at least every third month. Prophylactic antibiotic (Flucloxacillin) was normally used till the age of 6 years. First line of treatment for mild pulmonary exacerbation (defined clinically by new or worsening in airway symptoms over a shorter period of time) was normally oral antibiotics (e.g., Amoxicillin/Clavulanic acid or trimethoprim sulphate) in 10–14 days and were normally initiated at home after contact the

CF-clinic or after a visit at the clinic. Chronic infections with *Pseudomonas aeruginosa* (Pa) was treated with a combination of iv antibiotics, inhaled antibiotics, and Azithromycin.

2. Additional statistical method details

2.1 LCI progression and subgroup analyses

We estimated the extent and progression of LCI during the study period for the entire study cohort and by subgroups formed by sex, pancreatic insufficiency, age at diagnosis (<1 *vs* >1 years) and birth cohort (born 1990–1999 *vs* 2000–2009). This was done using a linear mixed effects model with a random intercept and random age slope for each subject, assuming a general unstructured covariance matrix of the random effects. This model simultaneously describes the overall trend and subject specific progressions, and accounts for intra-individual correlations. Robust standard errors were used to account for the skewed distribution of the response variable. Subgroup differences in mean LCI and yearly progression in LCI were evaluated by adding the subgroup variable and subgroup with age interaction to the model. If the interaction effect was significant, the mean difference in LCI between subgroups were estimated at 7, 11 and 15 years of age from the corresponding linear combinations of the model parameters. If the interaction was non-significant, the age adjusted mean difference in LCI was estimated after removal of the interaction effect from the model.

2.2 Joint modelling of longitudinal SLD and LCI

Linear associations between longitudinal LCI measurements and SLD throughout childhood were analysed using joint modelling of all available longitudinal MBW and CT data. This was done using linear mixed effects models with LCI, %Dis and %Be as multivariate response variables. The model included an intercept and age slope per response variable as fixed effects, and random intercepts and random age slopes for each subject and response variable.

A general unstructured covariance matrix between all random effects within a subject was assumed. Correlations between the yearly progression of LCI and yearly progression of SLD were obtained from the model-based estimates of the correlations between the corresponding random effects. Similarly, we estimated the correlation between progression of SLD and mean LCI throughout childhood by omitting the fixed and random age slopes of LCI from the model. The significance of the correlation coefficients was evaluated using Wald-tests and corresponding confidence intervals were calculated from the estimated correlation coefficients and associated standard errors. We also estimated the best linear unbiased predictions (BLUPs) of each subject's mean LCI, progression of LCI and progression of SLD from the joint models described above and summarised the results graphically in scatter plots.

We also estimated adjusted correlation coefficients, accounting for age at diagnosis, sex, birth cohort, and colonisation of airway pathogens Pa, Sa and Asp. This was done by adding the subject characteristics, subject characteristic with age interactions, cumulative number of infections with Pa, Sa and Asp, and years chronically infected with Pa, as fixed effects in the model.

2.3 Non-linear associations between SLD and LCI

To understand the association between SLD and LCI at a more detailed level, we continued with non-linear mixed effects models of SLD related to LCI. Since both total airway disease (%Dis) and bronchiectasis (%Be) were measured at a continuous scale naturally bounded between 0 and 100 per cent, we employed a logistic-type transformation on the form

$$\log\left(\frac{1+x}{101-x}\right),$$

where x is the value of %Dis or %Be. After this transformation, approximately normally distributed and homoscedastic errors were obtained. The longitudinal transformed SLD data

was analysed using linear mixed effects models with age at CT and LCI as fixed effects, and with a random intercept and random age slope for each subject. A general unstructured covariance matrix of the random effects was assumed. The goodness of fit to the data was summarised by the fraction of variance (R^2) of the logit-transformed outcome explained by the fixed effects.

To account for repeated measures of LCI, we considered, in addition to LCI measured at CT (+/-3 days), 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 BLUPs of the LCI at CT were derived from mixed effects models with intercept and age at MBW as fixed effects, and with a random intercept and random age slope for each subject. The BLUPs of the mean LCI up to CT were derived from mixed effects models with only an intercept as fixed effect, and with a random intercept for each subject. A general unstructured covariance matrix of the random effects was assumed throughout. When calculating the BLUP of LCI or mean LCI for a particular subject and CT, only MBW performed prior to or at the timepoint of that CT were included for that subject, whereas the other subjects contributed with all their MBW data. We did not consider LCI metrics based on the change in LCI (e.g., progression in LCI during a specific period of time), since an association between the abovementioned LCI metrics and SLD in a longitudinal context also implies an association between change in LCI and change in SLD. Thus, temporal variations and trends in LCI were implicitly accounted for in the analyses.

In order to capture potential non-linear effects of LCI, the LCI variables were modelled by natural cubic splines with knots at the 10^{th}, 50^{th} and 90^{th} percentiles. This is equivalent to

including a linear term for LCI in the model, together with a non-linear term given by the transformed LCI variable

$$\frac{\max(\mathrm{x} - \mathrm{k}_1, 0)^3 - \max(\mathrm{x} - \mathrm{k}_3, 0)^3}{k_3 - k_1} - \frac{\max(\mathrm{x} - \mathrm{k}_2, 0)^3 - \max(\mathrm{x} - \mathrm{k}_3, 0)^3}{k_3 - k_2},$$

where x is the value of the LCI variable at CT, and k_1, k_2, k_3 are the corresponding knot positions. The significance of the association between LCI and SLD was assessed by an F-test on the linear contrast of model coefficients pertaining to LCI. The analyses were performed both unadjusted and adjusted for age at diagnosis, sex, birth cohort, and colonisation of airway pathogens Pa, Sa and Asp. The adjusted analyses were conducted by adding the subject characteristics, subject characteristic with age interactions, cumulative number of infections with Pa, Sa and Asp, and years chronically infected with Pa, as fixed effects in the model.

The logit-linear mixed effects models were subsequently used to derive median and percentile curves for various ages and LCI profiles, assuming normally distributed random effects and a normal distribution of the transformed outcomes. We used the unadjusted analyses for this purpose, since the adjusted analyses would require a separate set of plots for each patient profile of interest. Model diagnostics demonstrated a good fit to the data and supported the plausibility of the model assumptions, apart from the presence of exact zeroes in %Be (Figure E3 and E4). Model predicted percentiles below zero, which mainly were obtained at low ages and low levels of LCI, were interpreted as being equal to zero.

Table E1. LCI mean, SD and 95% upper prediction limit in 140 healthy children.

Age (years)	Mean	SD	ULN
0	6.87	0.40	7.66
1	6.74	0.40	7.54
2	6.62	0.40	7.41
3	6.50	0.40	7.28
4	6.38	0.40	7.16
5	6.25	0.40	7.04
≥6	6.19	0.40	6.98

LCI decreased by 0.12 units per year up to six years age (p<.0001).
There was no significant change in LCI after six years age (p=0.21).
A constant SD was assumed. Adding heteroscedastic errors did not improve model fit (p=0.11).
ULN is the 95% upper prediction limit.
Abbreviations: LCI, lung clearance index; SD, standard deviation; ULN, upper limit of normal.

Table E2. Progression of LCI_{adj} in the CF cohort, by subject characteristics.

Subgroup variable	Subgroup	Mean yearly progression (95% CI)	Interaction Subgroup*Age	Age adjusted mean difference (95% CI) between subgroups (last category is reference group)
All subjects		0.03 (-0.01 – 0.08) p=0.13		
Sex	**Girl (n=24)**	0.11 (0.03 – 0.20) p=0.011	p=0.020	At 7 years age: -0.19 (-0.93 – 0.55), p=0.61 At 11 years age: 0.28 (-0.52 – 1.07), p=0.50 At 15 years age: 0.74 (-0.28 – 1.76), p=0.15
	Boy (n=51)	-0.00 (-0.05 – 0.04) p=0.91		
Pancreatic status	**Sufficient (n=8)**	-0.01 (-0.07 – 0.05) p=0.75	p=0.21	-0.29 (-1.44 – 0.85) p=0.61
	Insufficient (n=67)	0.04 (-0.01 – 0.08) p=0.11		
Age at diagnosis	**<1 year (n=38)**	0.07 (0.01 – 0.12) p=0.016	p=0.06	-0.43 (-1.07 – 0.21) p=0.19
	>1 year (n=37)	-0.01 (-0.07 – 0.05) p=0.73		
Birth cohort	**Born 1990–1999**	0.07 (0.01 – 0.12) p=0.025	0.036	At 7 years age: 0.32 (-0.35 – 0.99), p=0.35 At 11 years age: 0.70 (-0.02 – 1.41), p=0.06 At 15 years age: 1.07 (0.17 – 1.98), p=0.02
	Born 2000–2009	-0.03 (-0.09 – 0.04) p=0.41		

Abbreviations: CI, confidence interval; LCI_{adj}, age adjusted lung clearance index.

Table E3. Longitudinal correlation between progression SLD with mean LCI_{adj} and yearly progression of LCI_{adj} throughout childhood using joint modelling of all available MBW and CT data.

		Correlation coefficient (95% CI)	
LCI parameter	**CT parameter**	**Unadjusted**	**Adjusted***
Mean LCI_{adj}	Yearly progression of %Dis	0.62 (0.40–0.84) p<.0001	0.47 (0.17–0.76) p=0.002
Mean LCI_{adj}	Yearly progression of %Be	0.65 (0.46–0.85) p<.0001	0.42 (0.14–0.71) p=0.004
Yearly progression of LCI_{adj}	Yearly progression of %Dis	0.45 (0.15–0.76) p=0.004	0.33 (-0.05–0.72) p=0.09
Yearly progression of LCI_{adj}	Yearly progression of %Be	0.41 (0.09–0.72) p=0.011	0.38 (0.01–0.75) p=0.045

*Adjusted for age at diagnosis, sex, birth cohort and concommitant infections of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Aspergillus species*.

Table E4. Regression analysis results for the longitudinal association between SLD and LCI_{adj} using logit-linear mixed effects models.

		Unadjusted				Adjusted*			
Outcome	**LCI variable**	**Linear LCI coefficient (SE)**	**Non-linear LCI coefficient (SE)**	**F-test**	**R^2**	**Linear LCI coefficient (SE)**	**Non-linear LCI coefficient (SE)**	**F-test**	**R^2**
%Dis	LCI_{adj} at CT +/-3 days	0.369 (0.078)	-0.060 (0.024)	p<.0001	0.49	0.327 (0.081)	-0.048 (0.025)	p<.0001	0.57
	BLUP of LCI_{adj} at CT	0.561 (0.099)	-0.143 (0.045)	p<.0001	0.52	0.505 (0.101)	-0.131 (0.045)	p<.0001	0.58
	BLUP of mean LCI_{adj} up to CT	0.622 (0.124)	-0.130 (0.059)	p<.0001	0.55	0.553 (0.134)	-0.113 (0.063)	p<.0001	0.61
%Be	LCI_{adj} at CT +/-3 days	0.223 (0.098)	-0.045 (0.033)	p=0.020	0.35	0.183 (0.089)	-0.037 (0.030)	p=0.038	0.47
	BLUP of LCI_{adj} at CT	0.417 (0.134)	-0.102 (0.062)	p<.0001	0.46	0.339 (0.127)	-0.090 (0.059)	p=0.003	0.55
	BLUP of mean LCI_{adj} up to CT	0.543 (0.143)	-0.120 (0.072)	p<.0001	0.52	0.411 (0.146)	-0.083 (0.073)	p<.0001	0.58

Statistical analyses were performed using linear mixed effects models with the LCI variable and age at CT as fixed effects, and with subject specific intercept and age as random effects.
The response variables (total airway disease or bronchiectasis, %) were transformed prior to analysis using a logit-type transformation log((1+x)/(101-x)).
The effect of the LCI varibles on the transformed outcomes were modelled using natural cubic splines with knots at the 10^{th}, 50^{th} and 90^{th} percentiles.
The linear LCI coefficient is the regression coefficient correponding to the actual value of the LCI variable at CT.
The non-linear LCI coefficient is the regression coefficient corresponding to the transformed LCI variable $(\max(x - k_1, 0)^3 - \max(x - k_3, 0)^3) / (k_3 - k_1) - (\max(x - k_2, 0)^3 - \max(x - k_3, 0)^3) / (k_3 - k_2)$, where x is the value of the LCI variable at CT and k_1, k_2, k_3 are the corresponding knot positions.
F-test is the test of association between LCI and the response variable constructed using linear contrasts of the model coefficients pertaining to LCI.
R^2 is the fraction of the logit-transformed response variable explained by the fixed effects included in the model.
The BLUP of LCI_{adj} at CT was derived from linear mixed models of longitudinal age-adjusted LCI with subject specific random intercept and age slope, using all data available up to the timepoint of CT.
The BLUP of mean LCI_{adj} up to CT was derived from linear mixed models of longitudinal age-adjusted LCI with subject specific random intercept, using all data available up to the timepoint of CT.
*Adjusted for age at diagnosis, sex, birth cohort and concommitant infections of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Aspergillus species*.
Abbreviations: Be, Bronchiectasis (%); BLUP, best linear unbiased prediction; CT, computed tomography; %Dis, total airway disease (%); LCI, lung clearance index; LCI_{adj}, age adjusted lung clearance index; SE, standard error.

Figure E1. Total MBW- and chest CT-examinations performed at a certain age.

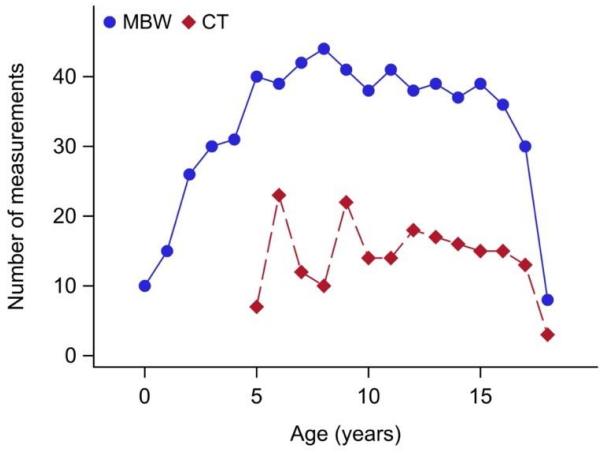


Figure E2. Estimated percentiles of total airway disease (%Dis) at six and 17 years of age *vs* LCI_{adj} at CT (+/- 3 days) (A, B), BLUP of LCI_{adj} at CT (C, D), and BLUP of mean LCI_{adj} up to CT (E, F). The BLUP is the best linear unbiased prediction using all available MBW data up and including the time-point of CT. Percentile curves were derived from mixed effects models on logit-transformed outcomes, using all available longitudinal MBW and CT data.

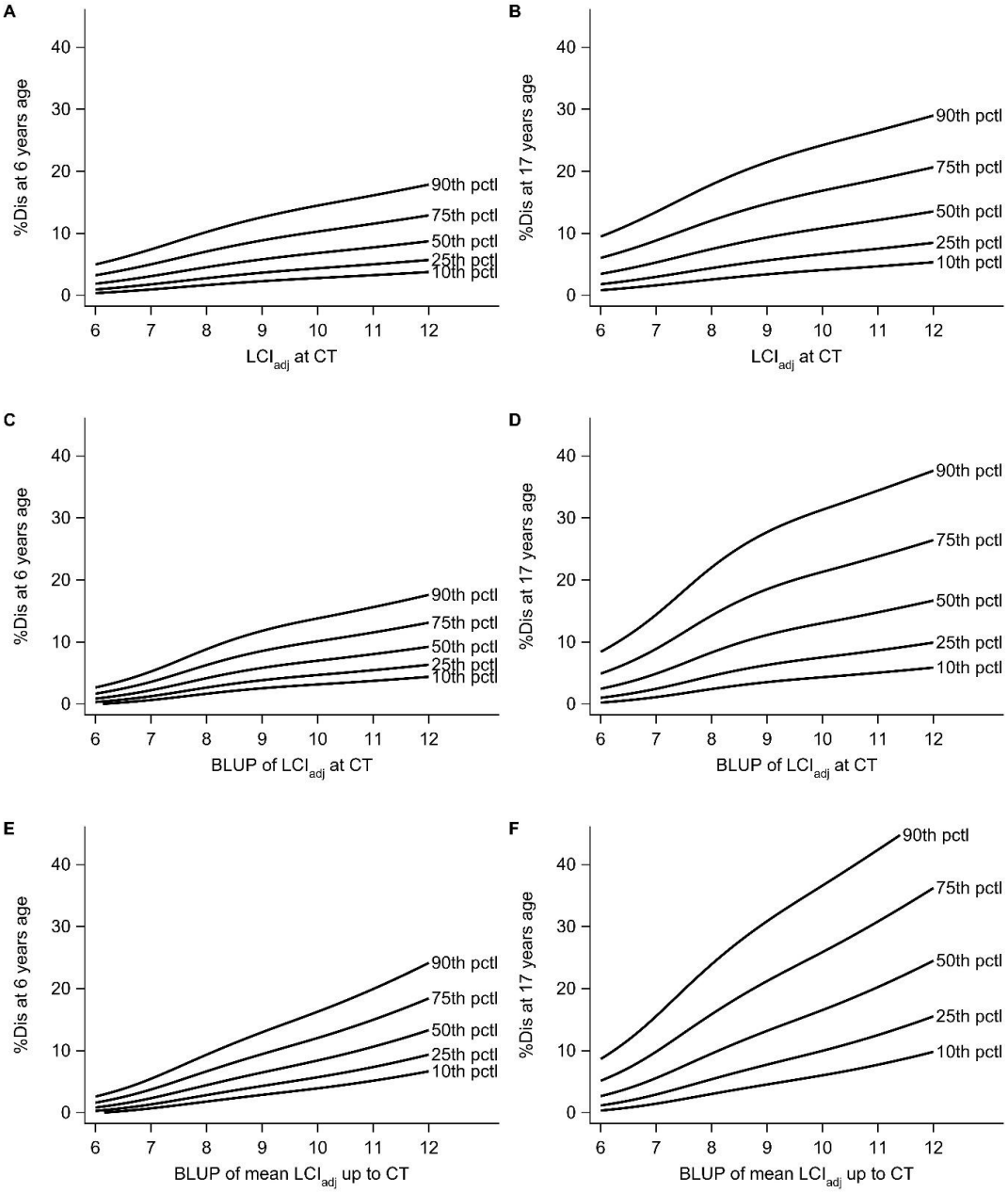


Figure E3. Estimated percentiles of bronchiectasis (%Be) at six years and 17 years of age *vs* LCI_{adj} at CT (+/- 3 days) (A, B), BLUP of LCI_{adj} at CT (C, D), and BLUP of mean LCI_{adj} up to CT (E, F). The BLUP is the best linear unbiased prediction using all available MBW data up and including the time-point of CT. Percentile curves were derived from mixed effects models on logit-transformed outcomes, using all available longitudinal MBW and CT data.

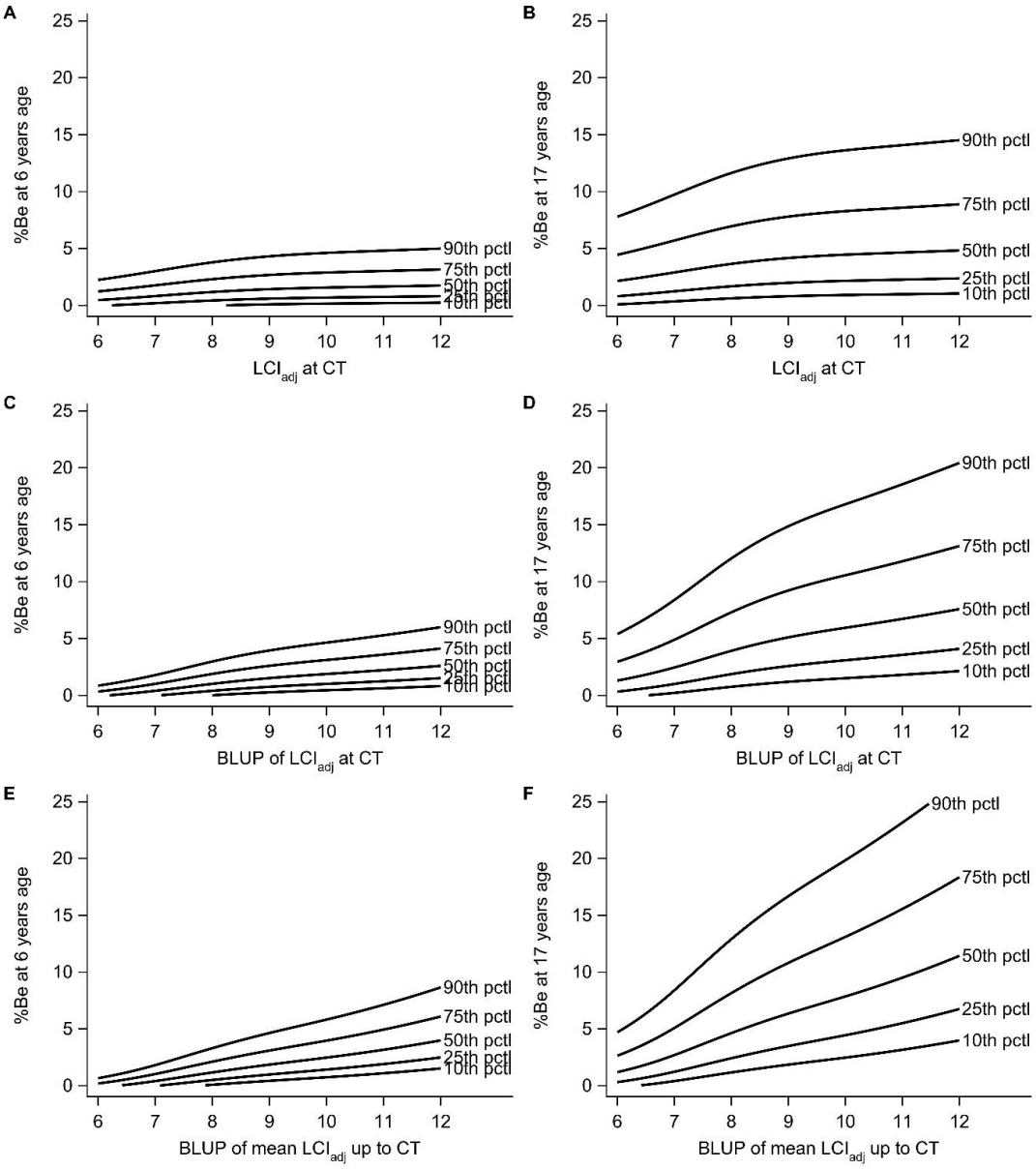


Figure E4. Model diagnostics for %Dis in logit-linear mixed effects model.

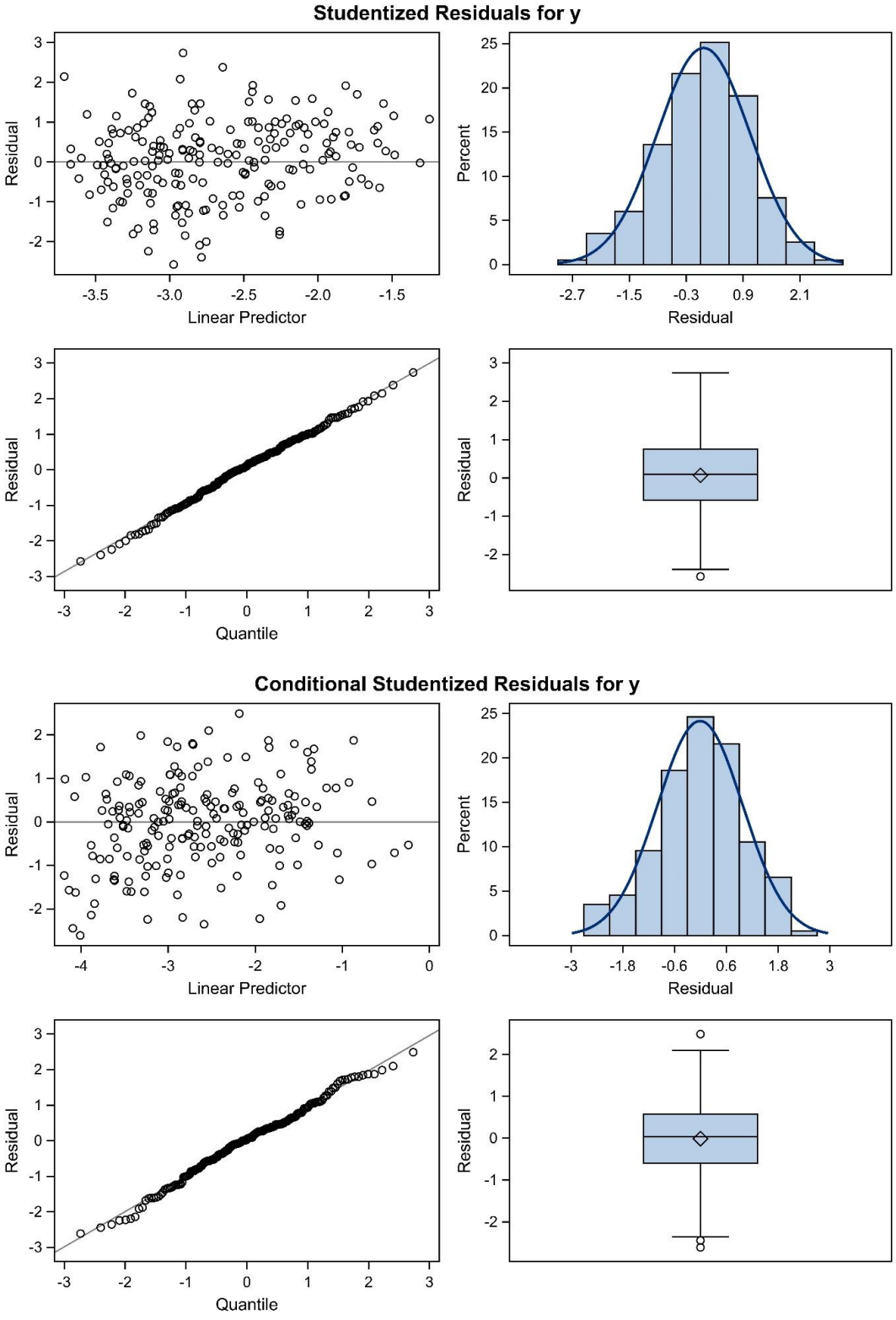
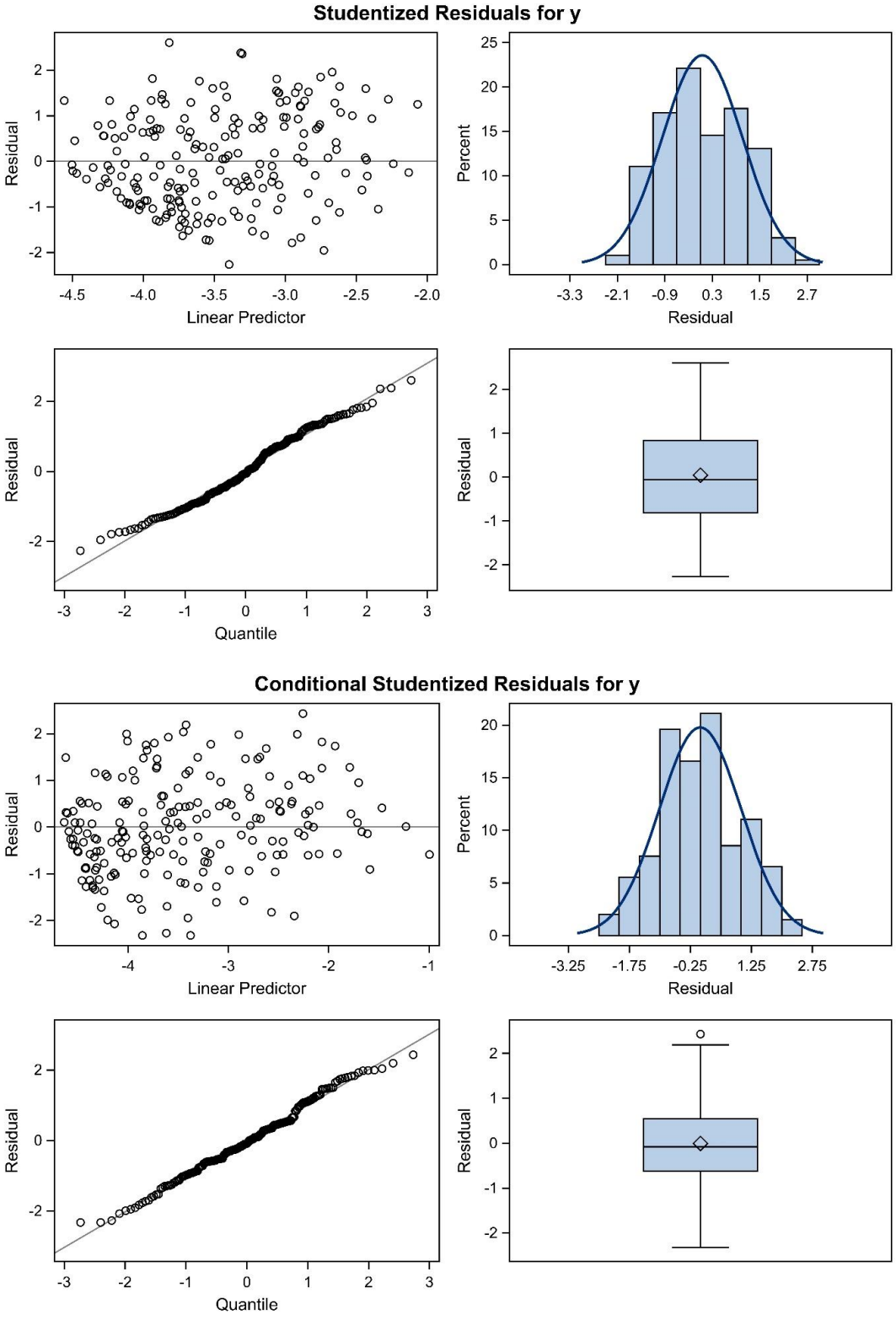


Figure E5. Model diagnostics for %Be in logit-linear mixed effects model.



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