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

Contents

List of Tables

List of Figures

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

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 were 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

$$\frac{\max(x - k_1, 0)^3 - \max(x - k_3, 0)^3}{k_3 - k_1} - \frac{\max(x - k_2, 0)^3 - \max(x - k_3, 0)^3}{k_3 - k_2},$$

where x is the value of the LCI variable at CT, and k_1, k_2, k_3 are the corresponding knot positions. 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 number of MBW- and chest CT-examinations performed at a certain age.

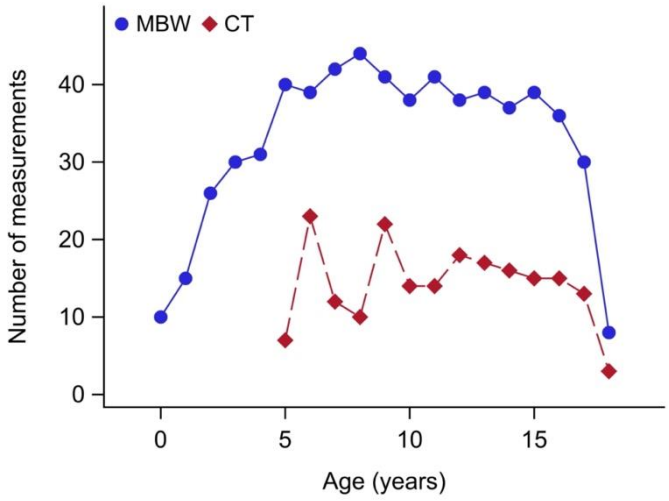


Figure E2. Estimated percentiles of total airway disease (%Dis) at six and 17 years of age *vs* LCI_{adj} at CT (+/- 3 days) (A, B), BLUP of LCI_{adj} at CT (C, D), and BLUP of mean LCI_{adj} up to CT (E, F). The BLUP is the best linear unbiased prediction using all available MBW data up and including 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.

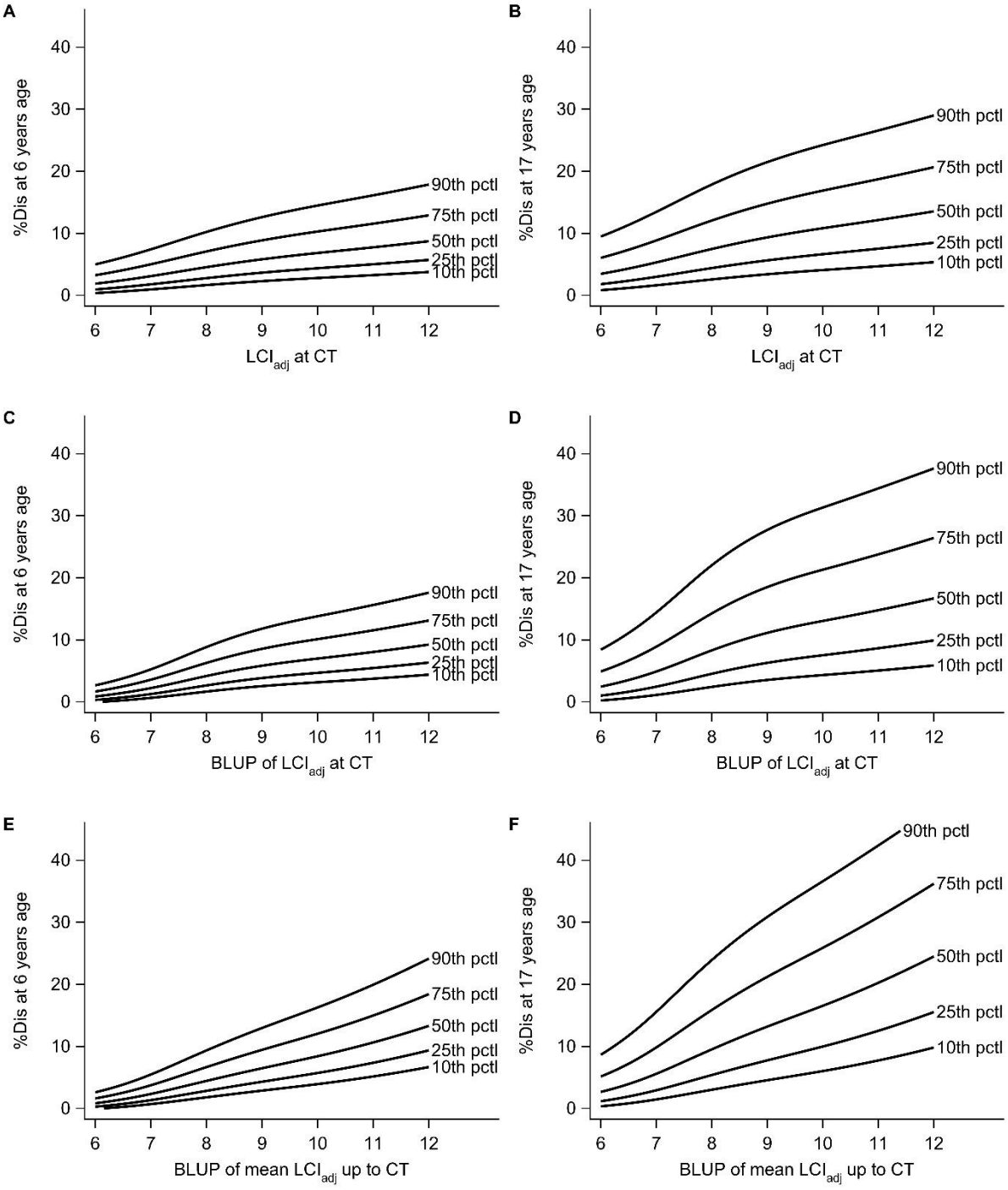


Figure E3. Estimated percentiles of bronchiectasis (%Be) at six years and 17 years of age *vs* LCI_{adj} at CT (+/- 3 days) (A, B), BLUP of LCI_{adj} at CT (C, D), and BLUP of mean LCI_{adj} up to CT (E, F). The BLUP is the best linear unbiased prediction using all available MBW data up and including 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.

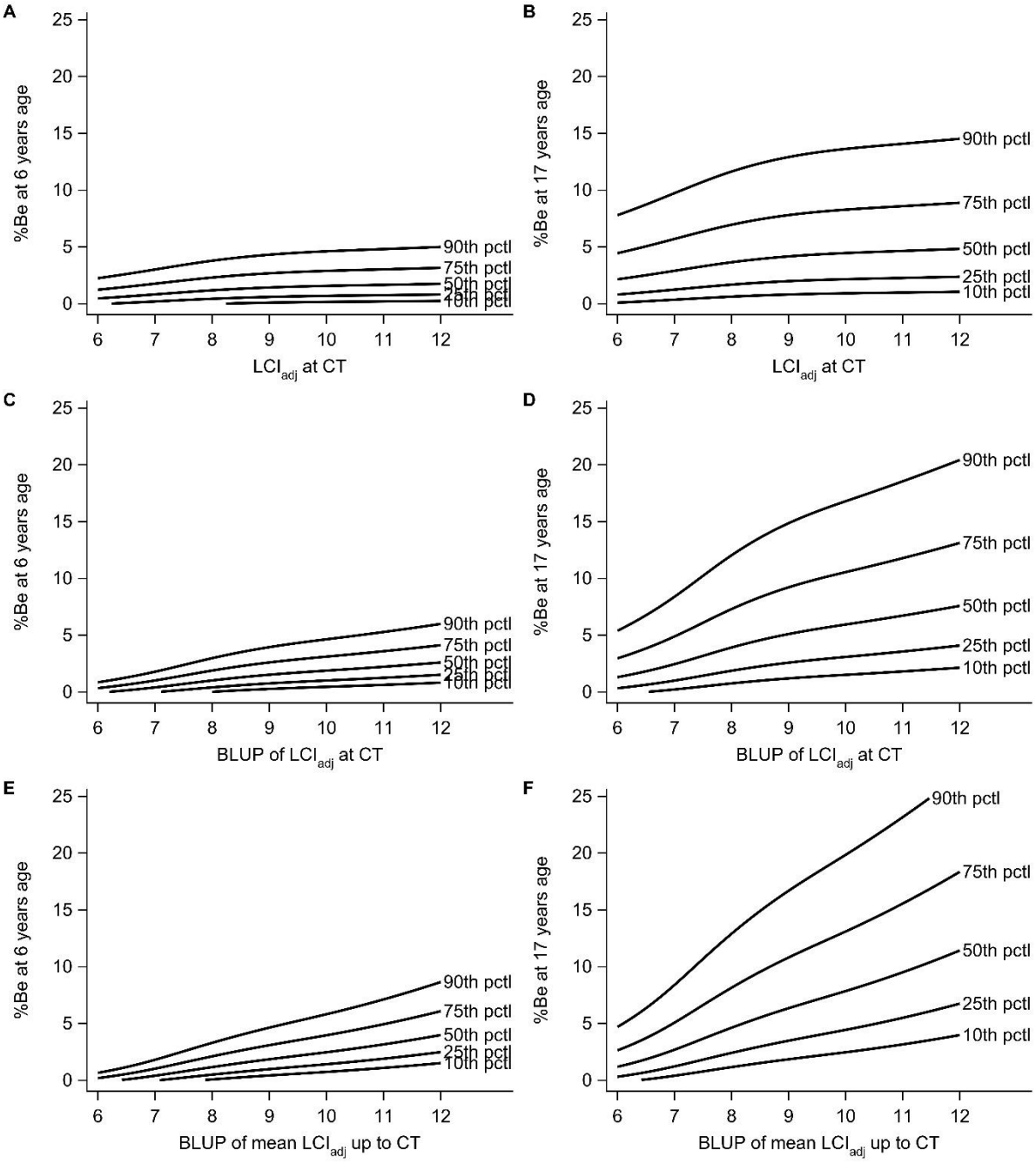


Figure E4. Model diagnostics for %Dis in logit-linear mixed effects model.

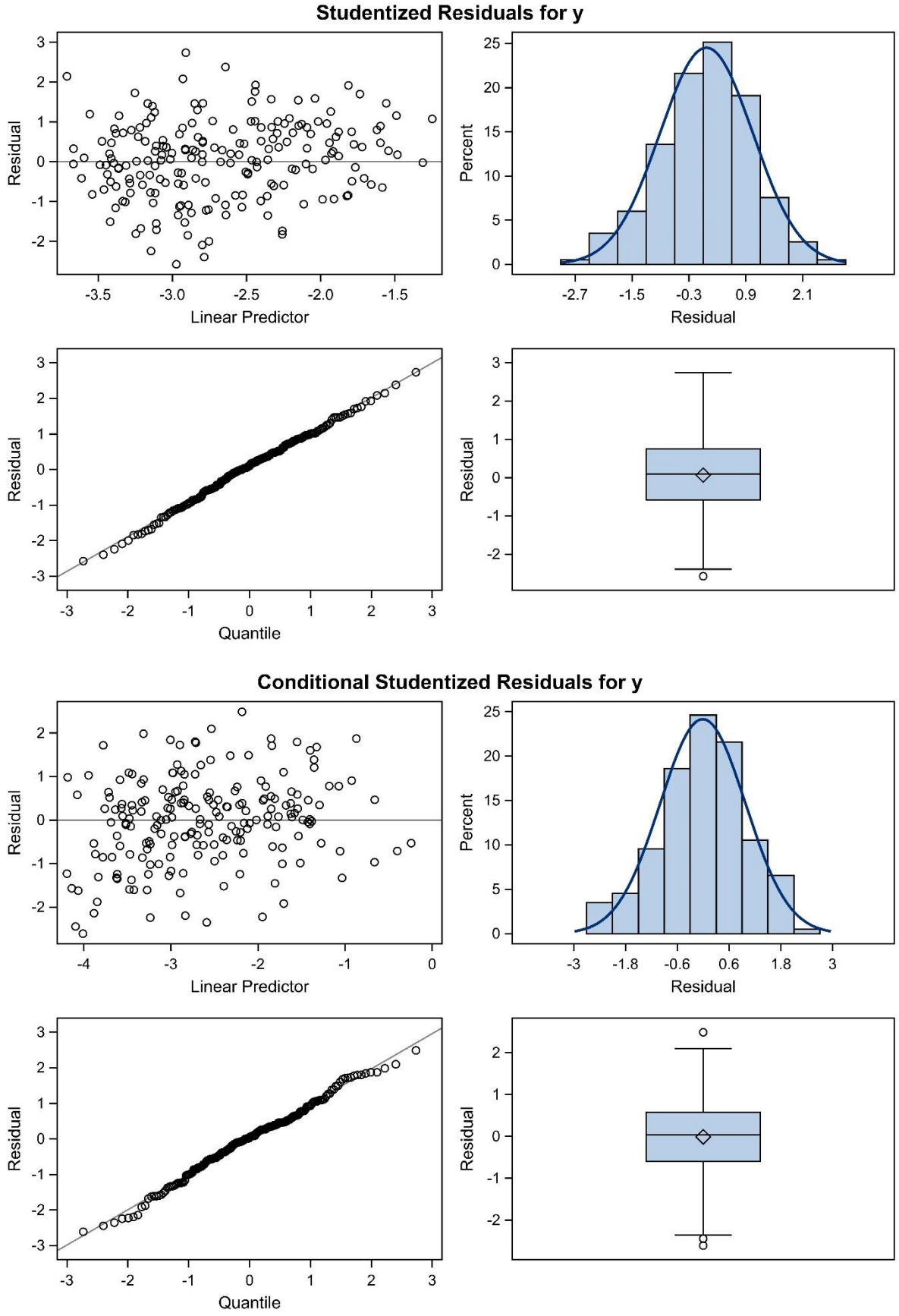
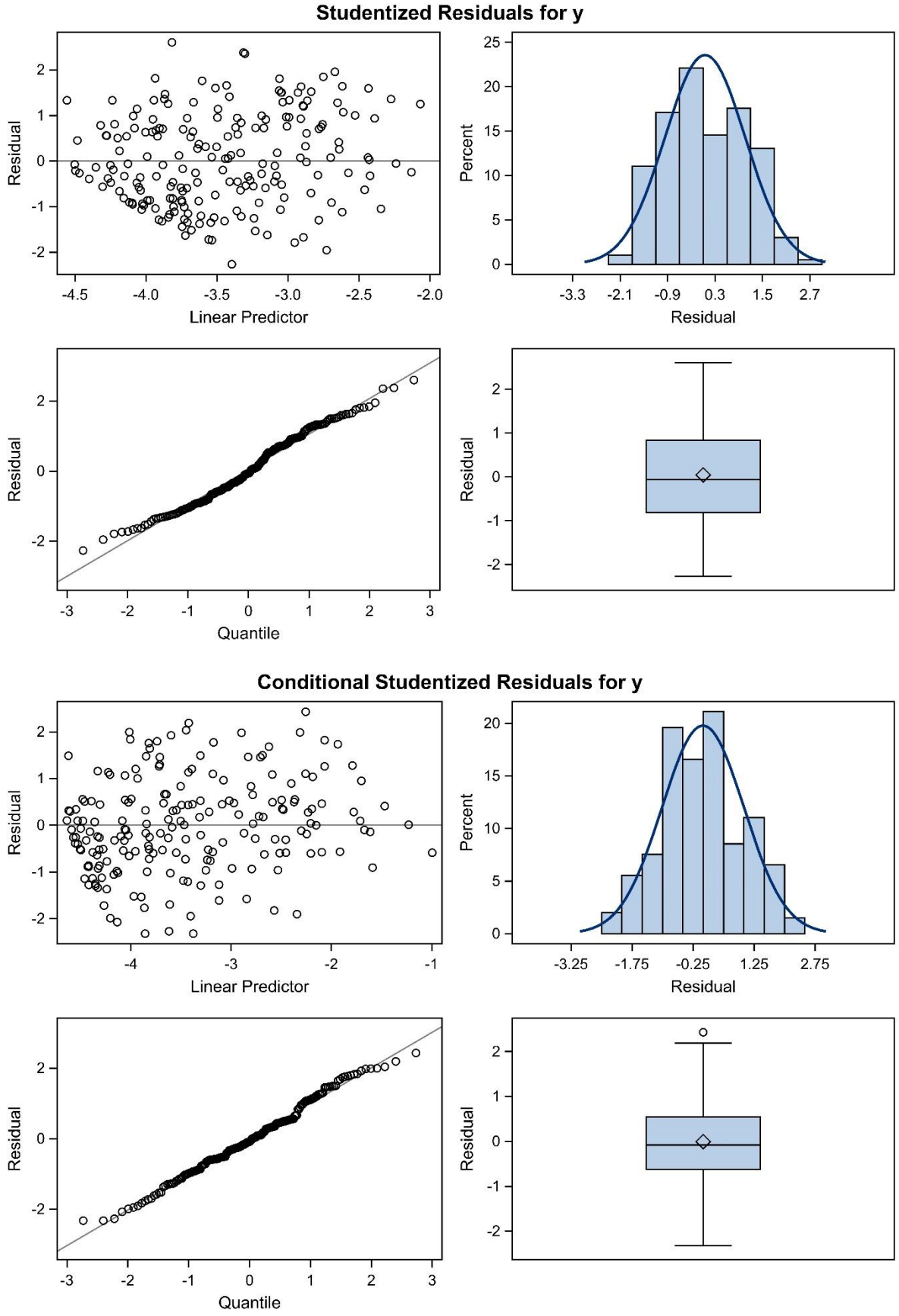


Figure E5. Model diagnostics for %Be in logit-linear mixed effects model.



References

1. Robinson PD, Latzin P, Verbanck S, et al. Consensus statement for inert gas washout measurement using multiple- and single- breath tests. *Eur Respir J* 2013;41(3):507-22. doi: 10.1183/09031936.00069712 [published Online First: 2013/02/12]
2. Lum S, Stocks J, Stanojevic S, et al. Age and height dependence of lung clearance index and functional residual capacity. *Eur Respir J* 2013;41(6):1371-7. doi: 10.1183/09031936.00005512 [published Online First: 2012/11/13]
3. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J* 2005;26(2):319-38. doi: 10.1183/09031936.05.00034805
4. Quanjer PH, Stanojevic S, Cole TJ, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *Eur Respir J* 2012;40(6):1324-43. doi: 10.1183/09031936.00080312