Early markers of cystic fibrosis structural lung disease: follow-up of the ACFBAL cohort

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In children with cystic fibrosis, airways disease severity on chest computed tomography at age 5 years increased the risk of bronchiectasis in adolescence and its extent was predicted by poorer nutrition, airway inflammation, and atelectasis <http://bit.ly/2Nnk8LW>

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ABSTRACT Little is known about early predictors of later cystic fibrosis (CF) structural lung disease. This study examined early predictors of progressive structural lung abnormalities in children who completed the Australasian CF Bronchoalveolar Lavage (ACFBAL) clinical trial at age 5-years and participated in an observational follow-up study (CF-FAB).

Eight Australian and New Zealand CF centres participated in CF-FAB and provided follow-up chest computed-tomography (CT) scans for children who had completed the ACFBAL study with baseline scans at age 5-years. CT scans were annotated using PRAGMA-CF scoring. Ordinal regression analysis and linear regression were used to investigate associations between PRAGMA-CF (Perth–Rotterdam Annotated Grid Morphometric Analysis for CF) outcomes at follow-up and variables measured during the ACFBAL study.

99 out of 157 ACFBAL children (mean±SD age 13±1.5 years) participated in the CF-FAB study. The probability of bronchiectasis at follow-up increased with airway disease severity on the baseline CT scan. In multiple regression (retaining factors at $p < 0.05$) the extent of bronchiectasis at follow-up was associated with baseline atelectasis (OR 7.2, 95% CI 2.4–22; $p \leq 0.001$), bronchoalveolar lavage (BAL) log₂ interleukin (IL)-8 (OR 1.2, 95% CI 1.05–1.5; $p = 0.010$) and body mass index z-score (OR 0.49, 95% CI 0.24–1.00; $p = 0.05$) at age 5 years. Percentage trapped air at follow-up was associated with BAL log₂ IL-8 (coefficient 1.3, 95% CI 0.57–2.1; $p < 0.001$) at age 5 years.

The extent of airway disease, atelectasis, airway inflammation and poor nutritional status in early childhood are risk factors for progressive structural lung disease in adolescence.

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Introduction

The lungs of patients with cystic fibrosis (CF) are thought to be structurally normal at birth. However, lower airway bacterial infection and chronic neutrophilic inflammation begin shortly afterwards [1, 2]. Both contribute to early structural lung disease [3, 4] that progresses with age, resulting in variable proportions of trapped air/hypoperfusion, airway wall thickening, mucus plugging and bronchiectasis [5, 6], and a later decline in lung function [7]. Early in life the extent of bronchiectasis is generally mild, but airway wall thickening, mucus plugging and trapped air are more prominent and detected even during infancy [3, 8]. It has been suggested that children with more severe lung disease as pre-schoolers may experience accelerated progression of structural lung disease during their school years [9], but the relationship between early life factors and later structural lung disease has not yet been well established.

The Australasian CF Bronchoalveolar Lavage (ACFBAL) study (Australian Clinical Trials Registry: ACTRN0126050006656639) was a randomised controlled trial conducted during 1999–2009. The ACFBAL study was designed to examine the clinical value of using bronchoalveolar lavage (BAL) to diagnose lower airway infection in infants and pre-school children with CF [10]. Subjects were followed for the first 5 years of life with detailed prospective collection of clinical data, including birthweight, respiratory microbiology and pulmonary exacerbations [11]. Children underwent anthropometric measurements (weight, height), a chest computed tomography (CT) scan, spirometry (forced expiratory volume in 1-s (FEV₁)) and BAL when they completed the study at the age of 5 years. The CF-FAB study was a longitudinal observational follow-up study of the ACFBAL cohort (CF-FAB study; ACTRN12613000778785) and was conducted during 2013–2017 to assess the clinical and psychosocial changes over late childhood and adolescence, and to examine early life determinants of long-term clinical outcomes in CF.

The aim of this paper was to examine in children who participated in the ACFBAL study the association between i) clinical factors and ii) a range of measures of lung health in early childhood (up to 5 years of age) and structural lung disease in early adolescence as established by chest CT scans performed for the CF-FAB study.

Methods

Study participants, setting and data sources

Participants had completed the ACFBAL study at age 5 years and undergone a follow-up chest CT scan as part of the CF-FAB study (supplementary methods). Potential explanatory variables from the ACFBAL study included clinical and microbiological data, and age 5 years anthropometric, BAL, spirometry and CT scan outcome variables. The first chest CT scan undertaken as part of the CF FAB study between May 2013 and April 2016 when aged 9.4–15.8 years was used (figure 1). Ethics committees at each site approved the ACFBAL and CF-FAB studies and consent was obtained from parents or carer for each participant.

CT scans

ACFBAL protocols involved sequential low-dose, high-resolution CT scans without contrast. Expiratory CT slices were at three equally spaced levels. The majority of ACFBAL scans were done in children who were awake and trained to breath-hold for the scans. 18 children had scans performed under general anaesthesia for clinical or logistical reasons. CF-FAB protocols employed low-dose, spirometer-controlled inspiratory and expiratory volumetric CT scans without contrast and were all performed in children who were awake.

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FIGURE 1 Timeline for collection of data and relationship between the ACFBAL and CF-FAB studies. ACFBAL: Australasian Cystic Fibrosis Bronchoalveolar Lavage study; BAL: bronchoalveolar lavage; CF-FAB: Follow-up of the Australasian Cystic Fibrosis Bronchoalveolar Lavage study; CT: computed tomography.

Image analysis

As different CT scan imaging techniques were used for the ACFBAL and CF-FAB studies, CT scan outcome variables were not directly comparable by visual inspection. We analysed the CT scans using the Perth–Rotterdam Annotated Grid Morphometric Analysis for CF (PRAGMA-CF) method. Briefly, with PRAGMA-CF [12] for inspiratory CT scans, a grid overlies 10 equally spaced axial slices. Grid cells were then annotated in hierarchical order: i) bronchiectasis; ii) mucus plugging; iii) airway wall thickening; iv) atelectasis; and v) normal. For expiratory scans, grid cells were annotated as trapped air, normal or as atelectasis. For each sub-score, the volume fraction was expressed as percentage of total lung volume. In addition, a composite score (%Disease) was computed reflecting all components related to airways disease by summing %Bronchiectasis, %Mucus plugging, and %Airway wall thickening where the hierarchical order no longer applied for this measure. ACFBAL scans were scored in random order by a single observer (observer 1) using PRAGMA-CF, while another (observer 2) scored the CF-FAB scans. Observers were blinded to clinical status and other CT scan results. 18 randomly selected ACFBAL scans were also scored by observer 2 to assess inter-observer agreement. Observer 2 also rescored 25 randomly selected CF-FAB scans 2 months after the first scoring to assess intra-observer reliability.

Statistical analyses

As different CT scan imaging techniques were employed for the ACFBAL and CF-FAB studies, CT scan outcome variables could not be analysed longitudinally using the absolute change between individual patient scans. In addition, baseline %Trapped air was not included as the technique using three slices for ACFBAL was less sensitive relative to the follow-up scans adopting a volumetric approach.

%Bronchiectasis at follow-up was collapsed into three categories: no; minimal ($\leq 1\%$); and $>1\%$ bronchiectasis. The 1% cut-off was the integer value closest to the median of the non-zero values. Z-scores were calculated for FEV₁ [13] and nutritional measures (2000 Centers for Disease Control Growth Reference Charts: www.cdc.gov/growthcharts/cdc_charts.htm).

Regression methods examined associations between the level of bronchiectasis (ordinal regression) and %Trapped air (linear regression) at follow-up, and baseline CT scan and clinical measures. Positively skewed explanatory variables were transformed to logarithmic base-2 scale, so that odds ratios and regression coefficients represent expected outcome change per doubling of the early life explanatory variable. Where extreme skewness was present, due to a large proportion of zeroes, baseline CT variables were dichotomised (bronchiectasis, atelectasis, mucus plugging) for analysis. Risk factors were examined separately adjusting for sex and age at follow-up. Those exhibiting association at p-values <0.1 were

assessed in multiple regression with backward selection, retaining explanatory variables with p-values <0.05 in the final model. Associations are presented with 95% confidence intervals and two-sided p-values. Predicted probabilities of bronchiectasis were calculated from an ordinal regression model for each factor (where $p < 0.05$) adjusting for age at follow-up and are presented with 95% confidence intervals for no bronchiectasis and $\geq 1\%$ bronchiectasis at follow-up, with age at follow-up held at its mean. As general anaesthesia may be associated with potential atelectasis seen on chest CT scans, a sensitivity analysis was undertaken excluding all the baseline scans performed under general anaesthesia to examine the association between atelectasis in the remaining baseline scans and later bronchiectasis.

Intra-class correlation coefficients (ICC) were calculated using one-way ANOVA to examine intra-observer reliability of CF-FAB scan outcome variables. Bland-Altman plots examined the inter-observer agreement for ACFBAL scan outcome variables.

Statistical analysis was performed using Stata version 15.1 (StataCorp, College Station, TX, USA).

Results

Study population

Of the 157 children who completed the ACFBAL study and were eligible to participate in the follow-up study, 99 were enrolled in CF-FAB and had a follow-up chest CT scan available at a mean $\pm$ SD age of 13 $\pm$ 1.5 years. In addition, of these 99 CF-FAB children, 73 had inspiratory scan data available at both baseline (at age 5 years) and follow-up time points (figure 2). Participants in the CF-FAB follow-up cohort were comparable to the original ACFBAL study cohort at the time of recruitment to ACFBAL (table 1). Follow-up characteristics of the CF FAB cohort are also summarised in table 1.

Distribution of CT-scan outcome variables at baseline and follow up

Figure 3 shows the composition of inspiratory PRAGMA-CF sub-scores at baseline and at follow-up sorted by decreasing %Disease. The largest component of structural lung disease at baseline was found to be %Airway wall thickening but, at follow-up, %Bronchiectasis and %Mucus plugging made the greatest contributions.

TABLE 1 Demographic and clinical characteristics comparing all the children who participated in ACFBAL and those who participated in the follow up CF-FAB study

Characteristics	ACFBAL (n=168)	CF-FAB (n=99)
At ACFBAL enrolment		
Patients assessed n	168	99
Age months	3.7±1.7	3.6±1.6
Males	88 (52)	51 (52)
Homozygous CFTR Phe508 deletion	111 (66)	69 (70)
Pancreatic insufficient	144 (86)	84 (85)
Meconium ileus	32 (19)	24 (24)
Weight kg	5.7±1.4	5.5±1.5
Weight z-score	-0.77±1.1	-0.87±1.2
Weight z-score <-2 SD	23 (14)	17 (17)
Sites		
New Zealand	26 (15)	17 (17)
Australia		
Queensland (two centres)	60 (36)	44 (44)
Victoria (two centres)	40 (24)	17 (17)
New South Wales (two centres)	37 (22)	21 (21)
South Australia	5 (3.0)	0 (0)
Highest education level of either parent		
Patients assessed n	156	92
Completed year 10	24±15	12±13
Completed secondary education	39±25	23±25
Trade	76±49	45±49
Completed tertiary education	17±11	12±13
Exposure to environmental tobacco smoke		
During pregnancy	35±21	19±19
Concurrent smoking in the household	n=167; 53 (32)	n=98; 29 (30)
Preterm delivery (<37-weeks)	n=166; 17 (10)	n=98; 12 (12)
Presence of respiratory symptoms		
Cough	73 (43)	46 (46)
Wheeze	n=166; 17 (10)	n=97; 10 (10)
At ACFBAL final outcome		
Patients assessed n	157	99
Age years	5.1±0.19	5.1±0.13
Weight z-score	-0.18±0.85	-0.19±0.75
BMI z-score	0.02±0.88	-0.02±0.76
FEV ₁ z-score	n=127; -0.58±1.3	n=81; -0.59±1.3
Pathogenic bacterial counts in BAL ≥10³ CFU·mL⁻¹		
Patients assessed n	151	95
None	49 (32)	27 (28)
<i>Pseudomonas aeruginosa</i>	16 (11)	15 (16)
Other bacterial pathogens	86 (57)	53 (56)
During ACFBAL study		
Ever had <i>Pseudomonas aeruginosa</i> eradication	83 (53)	56 (57)
At follow-up CT scan		
Age years	N/A	13±1.5
Weight z-score	N/A	-0.32±0.71
BMI z-score	N/A	-0.20±0.68
FEV ₁ z-score	N/A	-1.4±1.4
Pathogenic bacterial counts in sputum ≥10 ³ CFU·mL ⁻¹	N/A	
None		63 (64)
<i>Pseudomonas aeruginosa</i>		9 (9.1)
Other bacterial pathogens		27 (27)

Data are presented as mean±SD or n (%), unless otherwise stated. ACFBAL: Australasian Cystic Fibrosis Bronchoalveolar Lavage; BAL study: bronchoalveolar lavage; BMI: body-mass index; CF-FAB: Cystic Fibrosis-Follow-up of the ACFBAL study; CFTR: cystic fibrosis transmembrane conductance regulator; CFU: colony-forming units; CT: computed tomography; FEV₁: forced expiratory volume in 1 s; N/A: not applicable.

FIGURE 3 a) Overall distribution of computed-tomography scan outcome variables at baseline and at follow-up for inspiratory outcome variables. Each sub-score is expressed as a % of the total annotated lung volume. Subjects are sorted based on the sum of %Bronchiectasis, %Mucus plugging, %Airway wall thickening and %Atelectasis. Note that at baseline %Airway wall thickening is the most prominent structural change, while at follow-up this has shifted to %Bronchiectasis being more prominent. b) Distribution of %Bronchiectasis and %Airway wall thickening for paired data, sorted on decreasing %Airway wall thickening and then %Bronchiectasis at baseline. Note that at baseline %Airway wall thickening is the more prominent structural change, while at follow-up this has shifted to %Bronchiectasis.

Bronchiectasis at follow-up

Of the 96 FAB children with good quality follow-up CT scans able to be scored, 81 (84%) had radiographic evidence of bronchiectasis, with a similar proportion (81%) observed in the 73 children with paired baseline and follow-up CT-scans (supplementary table E1). 15 children had no radiographic signs of bronchiectasis at follow up (supplementary table E1) and the maximum lung volume affected by bronchiectasis was 12.2% with a median (interquartile range) lung volume affected by bronchiectasis of 0.88% (0.29–2.4).

Baseline atelectasis was strongly predictive of later %Bronchiectasis (table 2). Presence of atelectasis was associated with a seven-fold increase in the odds of higher levels of %Bronchiectasis at follow-up. In contrast, the odds of higher levels of %Bronchiectasis in late childhood and adolescence were halved for every standard

TABLE 2 Results from ordinal regressions for %Bronchiectasis at follow-up, adjusting for sex and age at follow-up

ACFBAL study explanatory variable	Univariable			Multivariable (n=69)	
	n	OR (95% CI)	p-value	OR (95% CI)	p-value
Bronchiectasis present	73	2.2 [0.89–5.3]	0.090		
Mucus plugging present	73	3.8 [1.2–12]	0.026		
%Airway wall thickening	73	1.2 [0.97–1.6]	0.088		
Atelectasis present	73	8.0 [2.9–22]	<0.001	7.2 [2.4–22]	<0.001
%Disease	73	1.3 [1.07–1.7]	0.012		
<i>Pseudomonas aeruginosa</i> ever during ACFBAL	96	1.6 [0.73–3.5]	0.248		
<i>Pseudomonas aeruginosa</i> eradication during ACFBAL study	96	1.7 [0.80–3.8]	0.159		
Bacteria $\geq 10^3$ CFU·mL⁻¹ in age 5-years BAL[#]	92		0.099		
None		Reference group			
<i>Pseudomonas aeruginosa</i>		3.6 [0.98–13]			
Other respiratory bacterial pathogens [¶]		2.3 [0.90–5.9]			
FEV₁ z-score at 5 years	78	0.73 [0.53–1.02]	0.064		
Birthweight kg	96	0.72 [0.35–1.5]	0.366		
Weight z-score at 5 years	96	0.78 [0.46–1.3]	0.346		
Height z-score at 5 years	96	1.2 [0.72–1.9]	0.511		
BMI z-score at 5 years	96	0.56 [0.33–0.96]	0.033	0.49 [0.24–0.999]	0.050
Log₂ of BAL total cell count at 5 years	88	0.96 [0.81–1.1]	0.651		
Log₂ of BAL neutrophil count at 5 years	83	1.01 [0.90–1.1]	0.898		
BAL neutrophil percentage at 5 years	86	1.7 [0.42–6.9]	0.450		
Log₂ of BAL IL-8 at 5 years	91	1.2 [1.08–1.4]	0.002	1.2 [1.05–1.5]	0.010
Log₂ of exacerbation rate in the first 2 years of life	96	1.07 [0.80–1.4]	0.637		
Log₂ of exacerbation rate during ACFBAL	96	1.2 [0.81–1.9]	0.314		
Log₂ of exacerbation rate resulting in hospital admission during ACFBAL	96	1.2 [0.95–1.6]	0.115		

Positively skewed explanatory variables were transformed to Log₂ scale, so that odds ratios represent expected outcome change per doubling of the early-life explanatory variable. ACFBAL: Australasian Cystic Fibrosis Bronchoalveolar Lavage study; BAL: bronchoalveolar lavage; BMI: body-mass index; CFU: colony-forming units; FEV₁: forced expiratory volume in 1 s; IL: interleukin. [#]: numbers of participants with bacterial counts $\geq 10^3$ CFU·mL⁻¹: *Pseudomonas aeruginosa* 15 (16%), other pathogens 52 (57%), no bacterial pathogens 25 (27%). [¶]: other bacterial pathogens were *Staphylococcus aureus*, including methicillin-resistant strains, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Aspergillus* spp., *Stenotrophomonas maltophilia*, *Moraxella catarrhalis* and other gram-negative bacilli. *Burkholderia cepacia* complex was not detected in ACFBAL participants at the age 5 years BAL [10].

deviation increase in body-mass index (BMI) at age 5 years, while there was a mean -0.18 (95% CI -0.33 – -0.03 ; $p=0.022$) z-score difference in BMI between the CF FAB and ACFBAL study time points (supplementary figure E1). A doubling of the interleukin (IL)-8 concentration in BAL fluid samples at 5 years of age increased the odds of higher levels of %Bronchiectasis later in life by approximately 20%. No evidence of confounding was identified for maternal smoking and education at the time of recruitment to the ACFBAL study, so these covariates were not considered further. In the multiple regression model, presence of atelectasis, BMI z-score, and BAL IL-8 remained independent predictors of later bronchiectasis, with atelectasis having the strongest evidence of association (table 2). The sensitivity analysis excluding baseline scans performed under general anaesthesia confirmed that the relationship between atelectasis and bronchiectasis was not a result of general anaesthesia increasing the risk of atelectasis. Indeed, after excluding these 18 scans, the odds of higher levels of bronchiectasis at follow-up went from a seven-fold to a nine-fold increase in the presence of atelectasis (OR 9.4, 95% CI 2.9–30; $p<0.001$).

Using the fitted models to derive predicted probabilities illustrates the strength of the association with baseline atelectasis: the probability of bronchiectasis at $>1\%$ was 0.77 (95% CI 0.58–0.89) in females (at the mean age of the study group) if atelectasis was present, compared with 0.29 (95% CI 0.14–0.49) if atelectasis was absent at baseline (with lower values but a similar difference in males). The predicted probability of remaining without bronchiectasis at follow-up was 0.25 (95% CI 0.13–0.44) in females

without baseline atelectasis compared with 0.04 (95% CI (0.01–0.11) in those with baseline atelectasis. Similarly, the fitted models showed the probability of bronchiectasis at >1% was 0.77 (95% CI 0.51–0.91) in females if mucus plugging was present compared with 0.46 (95% CI 0.30–0.63) if absent at baseline, and the predicted probability of no bronchiectasis at follow-up was 0.18 (95% CI 0.09–0.32) in females without baseline mucus plugging compared with 0.05 (95% CI 0.02–0.17) in those with baseline mucus plugging.

The predicted probabilities and 95% confidence intervals of follow-up bronchiectasis for each sex in the lowest (none) and highest (>1% of lung volume) categories are shown in figure 4 for integer values of %_{Disease}, BMI z-score and BAL log₂(IL-8) levels at age 5 years. The figure demonstrates the changes in absolute risk associated with the predictor variables, providing a more direct clinical interpretation than the odds ratios reported in table 2.

Trapped air at follow-up

Neutrophil percentage and BAL IL-8 levels at age 5 years were each associated with more severe/higher %_{Trapped air} (table 3). In the multiple regression model, only BAL IL-8 remained after selection at $p < 0.1$.

Reliability of scoring

Intra-observer reliability for the scoring of CF-FAB CT-scans was generally excellent with ICCs ≥ 0.9 for all CT outcome variables other than %_{Airway wall thickening} whose ICC was very good (0.73; 95% CI 0.53–0.92;

FIGURE 4 Predicted probabilities [95% CI] of no bronchiectasis and >1% bronchiectasis at follow-up for integer values of %_{Disease}, body mass index z-score and bronchoalveolar lavage log₂ interleukin-8 at baseline from regression for a) females and males and b) males and females combined adjusting for age at follow-up (each ignoring other clinical predictors). Age at follow-up is held at its mean. BMI: body-mass index; IL: interleukin.

TABLE 3 Results from linear regressions for %_{Trapped air} at follow-up, adjusting for sex and age at follow-up

ACFBAL study explanatory variable	Univariable		
	n	Coefficient (95% CI)	p-value
Bronchiectasis present	64	2.9 [−2.7–8.5]	0.298
Mucus plugging present	64	5.2 [−1.7–12]	0.136
% _{Airway wall thickening}	64	−0.25 [−1.8–1.3]	0.744
Atelectasis present	64	5.4 [−0.11–11]	0.055
% _{Disease}	64	0.35 [−0.98–1.7]	0.598
<i>Pseudomonas aeruginosa</i> ever during ACFBAL	87	3.8 [−1.1–8.8]	0.130
<i>Pseudomonas aeruginosa</i> eradication during ACFBAL	87	3.3 [−1.5–8.1]	0.179
Bacteria $\geq 10^3$ CFU·mL ^{−1} in age 5-years BAL [#]	83		0.036
None		Reference group	
<i>Pseudomonas aeruginosa</i>		7.2 [−0.58–15]	
Other respiratory bacterial pathogens [¶]		7.2 [1.6–13]	
FEV ₁ z-score at 5 years	71	−0.87 [−3.1–1.3]	0.429
Birthweight kg	87	−2.0 [−6.5–2.5]	0.377
Weight z-score at 5 years	87	−1.2 [−4.5–2.2]	0.491
Height z-score at 5 years	87	−1.1 [−4.3–2.0]	0.478
BMI z-score at 5 years	87	−0.32 [−3.7–3.0]	0.848
Log ₂ of BAL total cell count at 5 years	79	0.27 [−0.87–1.4]	0.635
Log ₂ of BAL neutrophil count at 5 years	75	0.81 [−0.08–1.7]	0.075
BAL neutrophil percentage at 5 years	78	11 [1.3–20]	0.026
Log ₂ of BAL IL-8 at 5 years	82	1.3 [0.57–2.1]	0.001
Log ₂ of exacerbation rate in the first 2 years of life	87	0.42 [−1.5–2.3]	0.669
Log ₂ of exacerbation rate during ACFBAL	87	−1.7 [−4.5–1.1]	0.232
Log ₂ of exacerbation rate resulting in hospital admission during ACFBAL	87	0.72 [−1.1–2.5]	0.423

Positively skewed explanatory variables were transformed to Log₂ scale, so that regression coefficients represent expected outcome change per doubling of the early-life explanatory variable. ACFBAL: Australasian Cystic Fibrosis Bronchoalveolar Lavage study; BAL: bronchoalveolar lavage; BMI: body-mass index; CFU: colony-forming units; FEV₁: forced expiratory volume in 1 s; IL: interleukin. #: numbers of participants with bacterial counts $>10^3$ CFU·mL^{−1}: *Pseudomonas aeruginosa* 13 [16%], other bacterial pathogens 47 [57%], no bacterial pathogens 23 [28%]. ¶: other bacterial pathogens were *Staphylococcus aureus*, including methicillin-resistant strains, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Aspergillus* spp., *Stenotrophomonas maltophilia*, *Moraxella catarrhalis* and other gram-negative bacilli. *Burkholderia cepacia* complex was not detected in ACFBAL participants at the age 5-year BAL [10].

supplementary table E2). There was more inter-observer variability with the scoring of ACFBAL CT-scans, especially for %_{Airway wall thickening} and %_{Trapped air} (supplementary figure E2).

Discussion

In this study, we observed a change in the pattern of potentially reversible structural lung disease characterised predominantly by airway wall thickening at the age of 5 years to mucus plugging and irreversible bronchiectasis in adolescence. Atelectasis at the age of 5 years increased both the probability and the odds of higher levels of bronchiectasis in late childhood and adolescence, while the extent of airway disease at age 5 years, as measured by %_{Disease}, increased the probability of more extensive bronchiectasis in adolescence, highlighting the importance of airway disease in early life. In contrast, a larger BMI z-score at age 5 years was associated with a lower probability of ensuing bronchiectasis. These findings suggest a potential window of opportunity to intervene in early life to prevent disease progression, which should be investigated further. Airway inflammation is recognised as being associated with the development of bronchiectasis [4, 14] and in this study a doubling of IL-8 levels in BAL at age 5 years increased the odds of a higher %_{Bronchiectasis} by 20%. Airway inflammation in early life was also associated with air trapping in adolescence highlighting the importance of early airway inflammation. These findings further emphasise the critical importance of establishing effective treatment in early childhood to prevent, or at least to minimise, progression of structural lung disease in children with CF.

Atelectasis is commonly seen when there is partial or complete bronchial obstruction. The association between early atelectasis and the later development of bronchiectasis is not unexpected when one

considers the well-recognised association between bronchiectasis and intraluminal obstruction from a foreign body [15] and with allergic bronchopulmonary aspergillosis in which bronchial obstruction, mucus plugging and atelectasis are commonly seen [16]. Animal studies have also suggested an association between bronchial obstruction and bronchiectasis, especially in the presence of airway infection and inflammation [17, 18].

The association between airway inflammation and later structural changes is well recognised [4, 14], however the exact pathophysiological mechanisms remain poorly understood. Similarly, while the relationship between improved nutritional status and long-term clinical outcomes, including positive effects upon survival and lung function is well established in CF [19–21], the link with bronchiectasis and air trapping and the mechanisms by which nutritional status might impact structural lung disease is unknown.

Potentially modifiable clinical factors of atelectasis, airway inflammation and poorer nutrition in early life were identified for the later development of bronchiectasis. There are, however, very few studies in young children examining the effects of improving mucus clearance on CF lung disease. It is possible that increasing airway clearance with inhaled mucoactive agents and physical therapies or by improving CF transmembrane conductance regulator (CFTR) function may lessen the risk of atelectasis. Modifying airway inflammation in CF is challenging because of the adverse effects associated with several anti-inflammatory agents, while also needing to maintain protective immunity in the setting of chronic airway infection. Nevertheless, airway inflammation as evidenced by elevated IL-8 levels in BAL samples at age 5 years was positively associated with both %Bronchiectasis and %Trapped air at follow-up, stressing the importance of early childhood airway inflammation as a potential biomarker for subsequent structural lung damage. Finally, improving nutritional status in CF is already recognised as being associated with better health outcomes and accordingly careful attention to nutrition is one of the cornerstones of early CF management.

This study did, however, have several limitations. Bronchiectasis might have been missed in the baseline CT-scans from quality differences and the protocol used, although it is unlikely that large areas of bronchiectasis would have been overlooked [5]. For the sensitive tracking of %Disease over time, the same volume and CT scan protocol should be used when follow-up scans are compared to those at baseline [22]. In the current study, this was not possible and the quality of CT scan images varied significantly between baseline and follow-up for several reasons. Participating centres had different CT scanners. Also, the scanning protocol for the ACFBAL baseline CT scan differed from the one at follow-up. ACFBAL used sequential inspiratory images and three expiratory images, while at follow-up, volumetric inspiratory and expiratory scans were obtained. This could lead to underestimating the severity of structural lung abnormalities at baseline, potentially diluting the evidence for association. Another important factor in CT scan quality was that at age 5 years, children cannot co-operate as well with the breathing requirements and breath-holds as they can in later childhood [23]. The age and technical differences may have been associated with variable inflation levels and an increase in the variability of the CT scan outcome variables. Without spirometer guidance, the median lung volume of inspiratory scans in children is 77% (range 55–106%) of measured total lung capacity and the volume levels of expiratory scans are even more variable, with a median volume level of 140% of measured residual volume (range 83–293%) [5]. The CT scan outcome %Airway wall thickening is influenced by the inflation level, as at full inspiration the airway expands and the walls of the airway become thinner, making it more difficult to compare longitudinally [24]. However, bronchiectasis remains more clearly visible, even on end-expiratory scans and is therefore more robust as a longitudinal outcome. PRAGMA-CF has a hierarchical scoring system and therefore some caution is required when interpreting the individual components of airway disease as predictors of later bronchiectasis. However, using %Disease at age 5 years takes the total components of airway disease into account and therefore avoids some of the potential concerns around differentiating between the various components of a hierarchical system. Finally, there was also a wide age range (9.4–15.8 years) for follow-up scans and so outcomes were adjusted for age [5].

Multicentre longitudinal studies involving imaging of children is challenging when comparing data over prolonged intervals, and the performance of the inspiratory and expiratory manoeuvres at different ages and CT scan quality remain important factors to be considered [22]. Automatic analysis of the scans could in the future replace manual annotations and visual scoring methods to quantify abnormal widening and thickening of the airways [25].

In conclusion, we investigated the predictive value of PRAGMA-CF at age 5 years and clinical variables over the first 5 years of life in children with CF for later school-age structural lung changes. We found that the probability of bronchiectasis in adolescence was positively related to the extent of early airway disease as measured by %Disease on chest CT scans at age 5 years. Structural changes, on the chest CT scan and

potentially modifiable clinical factors, including the BMI z-score and BAL IL-8 levels at age 5 years were independently associated with risk of bronchiectasis in adolescence. We also found that BAL IL-8 levels at 5 years of age were associated with greater levels of trapped air at follow-up. It is important to recognise that these are associations and causality cannot be inferred from this type of study. Properly conducted clinical trials however are warranted to examine whether reducing airway inflammation and atelectasis, and improving mucociliary clearance of mucus in early life, may result in less bronchiectasis in the long-term, while improving the nutritional status of children with CF may also lead to less lung damage later in life.

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