ORIGINAL ARTICLE

Aspergillus and progression of lung disease in children with cystic fibrosis

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ABSTRACT

Background The impact of *Aspergillus* on lung disease in young children with cystic fibrosis is uncertain.

Aims To determine if positive respiratory cultures of *Aspergillus* species are associated with: (1) increased structural lung injury at age 5 years; (2) accelerated lung function decline between ages 5 years and 14 years and (3) to identify explanatory variables.

Methods A cross-sectional analysis of association between *Aspergillus* positive bronchoalveolar lavage (BAL) cultures and chest high-resolution CT (HRCT) scan findings at age 5 years in subjects from the Australasian Cystic Fibrosis Bronchoalveolar Lavage (ACFBAL) study was performed. A non-linear mixed-effects disease progression model was developed using FEV_1% predicted measurements at age 5 years from the ACFBAL study and at ages 6–14 years for these subjects from the Australian Cystic Fibrosis Data Registry.

Results Positive *Aspergillus* BAL cultures at age 5 years were significantly associated with increased HRCT scores for air trapping (OR 5.53, 95% CI 2.35 to 10.82). However, positive *Aspergillus* cultures were not associated with either FEV_1% predicted at age 5 years or FEV_1% predicted by age following adjustment for body mass index z-score and hospitalisation secondary to pulmonary exacerbations. Lung function demonstrated a non-linear decline in this population.

Conclusion In children with cystic fibrosis, positive *Aspergillus* BAL cultures at age 5 years were associated contemporaneously with air trapping but not bronchiectasis. However, no association was observed between positive *Aspergillus* BAL cultures on FEV_1% predicted at age 5 years or with lung function decline between ages 5 years and 14 years.

Key messages

What is the key question?

► Are positive respiratory cultures of *Aspergillus* in young children with cystic fibrosis associated with structural lung damage and more impaired lung function with increasing age?

What is the bottom line?

► *Aspergillus* species cultured from bronchoalveolar lavage samples at age 5 years are associated with air trapping detected by high-resolution CT (HRCT) scans but not with bronchiectasis or with FEV_1% predicted measurements, either at age 5 years or with FEV_1% predicted values between 5 years and 14 years of age.

Why read on?

► Increased prevalence of *Aspergillus* in respiratory cultures in young children with cystic fibrosis is being recognised more frequently and is associated with some early structural changes in CF lung disease at age 5 years but not with subsequent progressive deterioration in lung function following adjustment for pulmonary exacerbations and body mass index.

INTRODUCTION

Chronic endobronchial infection with respiratory pathogens, especially *Pseudomonas aeruginosa*, is associated with increased morbidity and mortality in cystic fibrosis (CF).[1] Long-term use of inhaled antibiotics and increased patient survival has been accompanied by the emergence of other potential pathogens, particularly *Aspergillus fumigatus*,[2 3] which is the predominant filamentous fungus isolated from respiratory cultures of patients with CF[4 5] and whose prevalence appears to be increasing.[4] Furthermore, the persistence of *Aspergillus* has been associated with more frequent pulmonary exacerbations in older children with CF.[6] In contrast, detecting *Aspergillus* in infants and preschool-aged children with CF is considered uncommon. However, in the Australasian Cystic Fibrosis Bronchoalveolar Lavage (ACFBAL) study, (Australian New Zealand Clinical Trials Registry, ACTRN12605000665639), which was a randomised controlled trial of bronchoalveolar lavage (BAL)-directed therapy in children diagnosed through new-born screening and followed until age 5 years,[7] 28/156 (17.9%) subjects undergoing an end-of-study bronchoscopy grew *Aspergillus* from their BAL fluid specimens. This observation raised questions of whether early exposure to *Aspergillus* is associated with progressive structural lung injury and/or impaired lung function.

Chest high-resolution CT (HRCT) scans are an important imaging modality for detecting structural lung injury, and scoring systems have been developed to evaluate the extent and severity of CF-specific airway disease.[8 9] As these scans can reveal early lung damage in patients with CF,[10] they may allow monitoring of progressive structural lung disease.[11] This is important in the context of

the current study as there are recognised associations between more severe bronchiectasis in adults with CF and having positive sputum cultures for *Aspergillus*,[12] as well as impaired lung function in children aged >5 years and exhibiting persistently positive *A. fumigatus* respiratory cultures.[13] Longitudinal lung function data addressing the influence of *Aspergillus* on CF lung function, however, are limited[14] and show inconclusive evidence of accelerated pulmonary decline.[6 15] It therefore remains uncertain whether *Aspergillus* is a marker or an independent cause of accelerated CF lung disease.

To further explore the possible role of *Aspergillus* in early CF lung disease, we were able to take advantage of data from 156 subjects in the ACFBAL study, which included longitudinal data available on BAL specimens from 80 children randomised to the BAL arm of the trial. Additional longitudinal data were also accessed from the Follow-up ACFBAL (CF FAB) study (ACTRN12613000778785), which at the time of the current study involved 79/156 (50.6%) children from the original study cohort. Our study aimed to determine associations between: (1) *Aspergillus* and structural CF lung disease at age 5 years using chest HRCT scan data; (2) *Aspergillus* in the first 5 years of life cultured from BAL fluid, and any time afterwards cultured from either sputum or BAL fluid, and lung function changes with increasing age using a non-linear mixed-effects model; and (3) potential explanatory variables for accelerating lung function decline in children with CF aged between 5 years and 14 years. We hypothesised that: (1) having positive *Aspergillus* cultures at age 5 years would be associated with more severe structural lung damage at this age and (2) positive *Aspergillus* cultures in early childhood would be associated with impaired lung function at age 5 years and with accelerated decline in lung function later childhood.

METHODS

Subjects, study design and data collection

The ACFBAL study recruited infants detected by new-born screening and possessing classic CF features from eight centres in Australia and New Zealand.[7] Subjects were randomised to either a BAL-directed therapy arm or a standard care arm where clinical judgement and oropharyngeal swabs helped guide treatment of pulmonary exacerbations in the first 5 years of life. Children in the BAL-directed therapy arm had a BAL at baseline before age 6 months, during hospitalisation for an exacerbation, with any positive oropharyngeal culture for *P. aeruginosa* and after any *P. aeruginosa* eradication therapy. All ACFBAL participants underwent an elective end-of-study BAL at 5 years of age. Data on *Aspergillus* species from BAL cultures, subject characteristics and clinical information from birth until age 5 years were collected from all children completing the ACFBAL study. At age 5 years, all children underwent HRCT scan and spirometry assessment. Standard methods were used for BAL and HRCT scan protocols, while spirometry was performed according to American Thoracic Society criteria.[16] FEV_1 percentage predicted values were calculated using the global lung function initiative equations.[17] Respiratory pathogens were cultured in selective and non-selective media and identified by standard microbiological techniques (see online supplementary text for details).[18]

Data from 5 years of age were obtained from the Australian CF Data Registry (ACFDR) for 79 subjects who participated in the ACFBAL study and who had been recruited subsequently into the CF FAB study at the time of this analysis. ACFDR data collected from consented subjects included FEV_1% predicted measurements, BAL and sputum culture data and clinical information from birth until their current age.

HRCT scans

Chest HRCT scans performed at age 5 years as part of the ACFBAL study were evaluated using a validated Brody-II score.[7] Evidence of structural lung disease was measured by the total HRCT score, reported as a percentage of the maximum possible total score of 234 points. The HRCT score was divided into five components: air trapping, mucous plugging, airway wall thickening, parenchymal disease and bronchiectasis. The lowest point score of every component was 0 indicating absence of structural lung abnormalities. The maximal point scores were: air trapping: 18, mucous plugging: 36, airway wall thickening: 54, parenchymal disease: 54 and bronchiectasis: 72, indicating maximal abnormalities. All points were reported as percentages of the maximum total score. The distribution of abnormalities on HRCT scans have been reported previously.[7] HRCT abnormalities were mostly mild and only air trapping had a broad distribution. All HRCT component were categorised as present (score >0%) or absent (score=0%).[7]

Lung function

FEV_1 (L) measurements at 5 years of age were obtained from the ACFBAL study and from the ACFDR for ages 6–14 years. These were transformed into the percentage predicted values using the global lung function reference equation.[17] FEV_1 measurements from the ACFDR database were encounter based. All available FEV_1% predicted measurements for each child were included.

Positive *Aspergillus* cultures

Any growth of *Aspergillus* in BAL fluid from ACFBAL study subjects,[7] and their BAL or sputum specimens between ages 6 years and 14 years recorded in the ACFDR database, were defined as positive cultures. Additional positive *Aspergillus* cultures were defined as recurrent detection episodes if the previous respiratory specimen had not grown the fungus.

Analysis

Cross-sectional analysis

The influence of having positive *Aspergillus* BAL cultures at age 5 years on HRCT scan evidence of underlying structural lung disease at this time point was assessed with the following as potential explanatory variables. These included categorical variables of sex, meconium ileus at birth, *P. aeruginosa* BAL cultures $>10^3$ colony-forming units (cfu)/mL at age 5 years and *Staphylococcus aureus* BAL cultures $>10^3$ cfu/mL at baseline. Continuous covariates included body mass index (BMI) z-score at age 5 years, number of hospitalisations secondary to pulmonary exacerbation in the first 5 years of life, cumulative dosage (mg) of drugs (inhaled and intravenous tobramycin) received prior to their HRCT scans and maximum and minimum annual temperatures in their geographic region of birth (0^C). Structural lung disease was identified using chest HRCT component scores independently. A logistic regression analysis was used for all HRCT score components using SPSS software with p value <0.05 considered significant. The multivariable model was evaluated with a non-parametric bootstrap (n=1000) (see online supplementary text for details).

Longitudinal analysis

This evaluated the impact of *Aspergillus* on lung function changes with increasing age. Initially a linear and non-linear mixed-effect

Table 1 Characteristics of children with CF who completed the ACFBAL study and from whom BAL (n=156) and HRCT (n=155) data were available and of the subgroup of children who provided additional clinical, microbiological and lung function data beyond 5 years of age (n=79)

Characteristics	Median (IQR) or number of children in each category (%)		P value <0.05
	n=156	n=79	
Female	77 (49.4)	37 (46.8)	0.89
Number of children with meconium ileus at birth	32 (20.5)	17 (21.3)	0.87
Pancreatic insufficiency at age 5 years	149 (95.5)	75 (94.9)	1.00
BMI z-score at age 5 years	0.15 (–0.39 to 0.56)	0.01 (–0.56 to 0.47)	0.39
BAL study arm in ACFBAL	80 (51.2)	42 (53.2)	0.45
Site involved in ACFBAL study			
New Zealand	31 (19.9)	3 (3.8)	0.001
New South Wales	32 (20.5)	22 (27.8)	0.14
Queensland	56 (35.9)	37 (46.8)	0.03
South Australia	3 (1.9)	0 (0.0)	0.55
Victoria	33 (21.1)	13 (16.4)	0.39
Australian Capital Territory	1 (0.64)	1 (1.3)	1.00
Age at end-of-study BAL	5.05 (4.99–5.13)	5.06 (5.02–5.16)	0.79
Positive *Aspergillus* BAL culture (any growth) at end-of-study BAL	28 (17.9)	14 (17.7)	1.00
A. fumigatus	25 (16.0)	12 (15.2)	
A. terreus	2 (1.3)	2 (2.5)	
A. niger	1 (0.64)	0 (0.0)	
Received itraconazole during the first 5 years of life	13 (8.3)	6 (7.6)	0.82
Pseudomonas aeruginosa infection (≥10^3 cfu/mL) in BAL at age 5 years	17 (10.9)	14 (17.7)	0.16
Staphylococcus aureus BAL infection (≥10^3 cfu/mL) before age 6 months	25 (16.0)	11 (13.9)	0.71
Received oral *Staphylococcus aureus* prophylaxis before first birthday	33 (21.2)	22 (27.8)	0.42
Cum. intravenous tobramycin dose (mg) not for ET	0.00 (0.0–2003.8)	200.0 (0.0–2057.5)	0.34
Cum. intravenous tobramycin (mg) for ET	755.0 (0.0–2897.5)	1910.0 (0.0–3557.5)	0.81
Cum. inh. tobramycin (mg) for ET	33 000.00 (0.0–65 400)	34 2000 (0.0–68 400)	0.66
Cum. intravenous and inh. tobramycin (mg) for ET	32 225.00 (0.0–69 037.9)	36 000.00 (0.0–72 745.0)	0.65
Cum. macrolide (mg)	0.0 (0.0–108.8)	0.0 (0.0–0.0)	0.59
Cum. gentamicin (mg)	0.0 (0.0–74.3)	0.0 (0.0–65.0)	0.44
Number of hospitalisations secondary to PE	13 (9–16)	12 (10–15.5)	0.72
Max. temperature (°C)	24.8 (21.6–28.1)	24.1 (21.6–26.5)	0.15
Min. temperature (°C)	11.6 (8.8–14.9)	12.2 (9.5–15.4)	0.02
Interleukin-8 (ng/mL) in BAL at age 5 years	2.59 (0.67–7.42)	2.11 (0.52–10.26)	0.61
Bronchiectasis	87 (55.8)	38 (48.1)	0.41
Airway wall thickening	46 (29.5)	21 (26.6)	1.00
Parenchymal disease	90 (57.7)	41 (51.9)	0.58
Mucous plugging	64 (41.0)	30 (38.0)	0.89
Air trapping	70 (44.9)	36 (45.6)	0.58

*Number of scans of sufficient quality that were able to receive a score.
ACFBAL, Australasian Cystic Fibrosis Bronchoalveolar Lavage; BAL, bronchoalveolar lavage; BMI, body mass index; cfu, colony-forming units; cum., cumulative dosage (mg) of drugs prior to chest HRCT scan; ET, *P. aeruginosa* eradication therapy received prior to 5 years of age; HRCT, high-resolution CT; inh, inhaled tobramycin; IQR, interquartile range; max. temperature, maximum annual temperature of geographic area at birth (°C); min. temperature, minimum annual temperature of geographic area at birth (°C); n, number of children contributing data; OP, oropharyngeal; PE, pulmonary exacerbation requiring hospitalisation from birth until age 5 years.

model describing lung function changes over time, quantified by FEV_1% predicted, was developed using NONMEMV.7.4.[19] Structural model parameter estimates, between-subject variability (BSV) and residual unexplained variability were obtained by first-order conditional estimation with interaction.

The non-linear structural model was represented by a sigmoidal E_{max} function, which assumes lung function changes over age to have a natural maximal and minimal limit[20] according to equation 1:

$$FEV_1\%\left(t\right) = FEV_1\%_{baseline} - \frac{\Delta \max_{FEV_1}\% \times t^{\gamma}}{t_{50\%max}^{\gamma} + t^{\gamma}}, \quad \text{Equation (1)}$$

where the population average lung function progression over the child's age $\left(t\right)$ in years is determined by the sum of the baseline

$FEV_1\%$ predicted at 5 years $(FEV_1\%_{baseline},\ [\%])$ and a recovery function. The recovery function included the typical maximum change in lung function over the subject's lifetime from 5 years until end of life $(\Delta max_{FEV_1\%},\ [\%])$, age at which half of the maximal change in lung function occurs $(t_{50\%max},\ [years])$ and a Hill coefficient (γ), which determines the steepness of $FEV_1\%$ predicted changes over age (see online supplementary text for further details on lung disease progression model building).

As a potential influential factor on CF lung disease progression, recurrent positive *Aspergillus* cultures in BAL fluid in the first 5 years of life were tested on baseline $FEV_1\%$ predicted at age 5 years ($FEV_1\%_{baseline}$). *Aspergillus* in BAL or sputum cultures between the current and the last $FEV_1\%$ predicted measurement at any time from 5 years to 14 years of age on lung function changes were defined as a categorical variable ('yes' or 'no'), and *Aspergillus* was considered as a time-varying factor. The impact of *Aspergillus* in BAL or sputum cultures was evaluated on the rate of lung function decline (α) in the linear and on $\Delta max_{FEV_1\%}$, $[\%]$, $t_{50\%max}$, $[years]$ and γ in the non-linear structural model.

In addition to positive *Aspergillus* cultures, other explanatory variables, such as chest HRCT scan components scores, BMI z-score, hospitalisation secondary to pulmonary exacerbation and *P. aeruginosa* positive BAL or sputum cultures (see online supplementary text for details) were also tested on baseline $FEV_1\%$ predicted at age 5 years ($FEV_1\%_{baseline}$) and lung function changes from 5 years to 14 years of age.

An explanatory factor was retained in the model and defined as a significant influence when the fit of the model to the data improved and if biologically plausible. The improvement in the fit was measured by a decrease in the objective function value (OFV) generated by NONMEM. The difference in OFV between two hierarchical models is approximately X^2 distributed and can be tested for significance with $X^2_{1,0.05} = 3.84$.[21 22] Stepwise inclusion of explanatory factor analysis was performed for both structural models. The impact of the significant explanatory factors in both final models was explored via graphics in Rstudio.[23] Model selection and evaluation was further supported using goodness-of-fit plots, prediction and variance-corrected visual predictive checks[24] and a non-parametric bootstrap (n=1000).[25]

RESULTS

The clinical characteristics of the 156 subjects who completed the ACFBAL study and had HRCT and BAL data available at age 5 years are shown in table 1. Also shown in table 1 are the characteristics of the subgroup of 79 children who, up until the age of 14 years, provided clinical and microbiological data from birth and lung function data from 5 years of age. Other than for their distribution across the different sites, the subgroup was representative of the original study cohort. In the BAL-directed arm of the ACFBAL study, 36 children had one or more positive *Aspergillus* cultures in BAL fluid cultures during the first 5 years of life (online supplementary figure E1).

Aspergillus and lung structure at age 5 years

At 5 years of age, 28/156 (17.9%) patients had positive BAL cultures for *Aspergillus* at completion of the ACFBAL study (online supplementary table E1), and 155 chest HRCT scans were available for scoring. Distributions of total chest HRCT scores in children with and without positive *Aspergillus* BAL cultures at age 5 years are shown in online supplementary figure E2. Children with positive *Aspergillus* BAL cultures at 5 years of age had an increased probability of HRCT scores for air trapping (OR=5.53 (95% CI 2.35 to 10.85)). After adjusting for prior intravenous, inhaled and oral antibiotic exposure, and interleukin-8 values ≥250 ng/mL, positive *Aspergillus* cultures in BAL fluid collected at 5 years of age remained significantly associated with contemporaneous HRCT scan scores for air trapping. Those with positive *P. aeruginosa* BAL cultures at age 5 years had an increased probability of having airway wall thickening and bronchiectasis as shown in the multivariable model in online supplementary table E2.

Table 2 Longitudinal data for subjects consented into the study from age 6 years to 14 years with data obtained from the ACFBAL and ACFDR databases (n=79)

	Median (IQR) or number of children in each category (%)
Age (years)	9.1 (7.6–10.8)
Duration of follow-up per patient (years)	8.5 (7.8–8.8)
Total number of $FEV_1\%$ predicted measurements	2651
Number of $FEV_1\%$ predicted measurements per child	33 (23–43)
Total number of cultures per child	30 (20–46)
Number of sputum cultures per child	28.5 (19.0–43.0)
Number of BAL cultures per child	1.7 (1.0–2.0)
Number of cultures per child per year	4.2 (2.9–5.1)
Total number of positive *Aspergillus* cultures per child per year	2.7 (0.0–3.3)
Total number of positive *Aspergillus* cultures per total cultures per child	0.62 (0.0–4.0)
Number of years with positive *Aspergillus* cultures	2.4 (0.0–2.0)
Number of children with positive *Aspergillus* cultures	42 (53.2)
Number of children who never had positive *Aspergillus* culture	37 (46.8)
Number of children who had only one positive *Aspergillus* culture	16 (20.3)
Number of children who had recurrent positive *Aspergillus* culture	26 (32.9)

ACFBAL, Australasian Cystic Fibrosis Bronchoalveolar Lavage study; ACFDR, Australian Cystic Fibrosis Data Registry; BAL, bronchoalveolar lavage; n, number of children contributing data.

Aspergillus and lung function at age 5 years and subsequent lung function progression

Of the 79 subjects in the ACFBAL study who consented for their ACFDR data to be used, 37 had been in the standard arm and 42 in the BAL-directed therapy arm of the ACFBAL study. Overall, 2651 $FEV_1\%$ predicted measurements were obtained over a median period of 8.0 (IQR: 7–9) years from ages 4.8–14.4 years (table 2). The median (IQR) $FEV_1\%$ predicted measurement was 88.6% (76.1%–97.5%). The distribution of $FEV_1\%$ predicted measurements in children with CF included in the analysis is shown in figure 1. At age 5 years, all had a BAL culture result and from 6 years to 14.4 years, 2554 culture results were available, 2414 (94.5%) from sputum and 140 (5.5%) from BAL specimens.

Structural lung function progression model

The non-linear structural model provided an improved fit to the data compared with the linear model according to the OFV

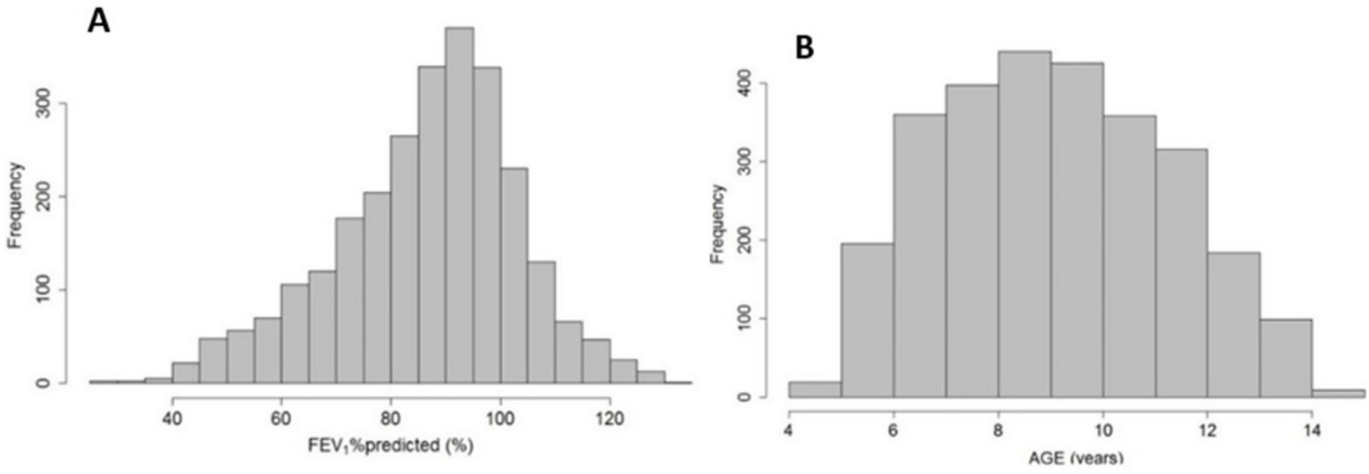

Figure 1 (A) FEV_1% predicted measurements obtained from 79 children included in the longitudinal analysis and (B) age (years) distribution at the time of the FEV_1% predicted measurements included in the longitudinal analysis of 79 subjects.

(ΔOFV=−20.5, p=0.001) and was deemed to be more plausible. Parameter estimates for non-linear progression model of FEV_1% predicted over age are presented in online supplementary table E3. The estimated population average $FEV_1\%_{baseline}$ (BSV) estimate was 99.7% (13.3%). The estimated population average age $t_{50\%max}$ at which 50% of the maximum change in FEV_1%predicted from baseline occurred was 8.38 years (BSV 30.0%), which aligned with literature values.[14] The estimated Hill coefficient (BSV) was 3.08 (52.5%), and the BSV for Δ $max_{FEV_1\%}$ was 63.6%. Online supplementary figure E3 shows the prediction and variance corrected visual predictive checks for the non-linear model, which shows a good agreement of the median predicted lung function decline and that of the median observed data. Slightly increased CIs with increasing age were noted, as data points became sparser with increasing age. Online supplementary figure E4 provides a visualisation of the data available for the lung function progression study, model evaluation and diagnostic comparisons. Model evaluation plots shown in online supplementary figure E4 all demonstrate good model fit.

Aspergillus on FEV_1% predicted at age 5 years ($FEV_1\%_{baseline}$)

Having had recurrent or intermittently positive *Aspergillus* BAL cultures in the first 5 years of life did not influence $FEV_1\%_{baseline}$. Inclusion of *Aspergillus* as a potential influential factor did not improve the model fit with ΔOFV=−0.05, p>0.05 (online supplementary table E4).

Aspergillus and FEV_1% predicted progression

After other more significant explanatory variables (BMI z-score and hospitalisation secondary to pulmonary exacerbation) were accounted for in the model, having a positive *Aspergillus* culture between a current and a previous FEV_1% predicted measurement any time between 5 years and 14 years of age did not influence disease progression; ΔOFV=−2.99, p>0.05 (online supplementary table E4).

HRCT scan component scores and lung function at age 5 years and lung function progression

Patients with air trapping at age 5 years had an average model predicted reduction in $FEV_1\%_{baseline}$ by 4.17 (95% CI 1.1% to 8.9%) $F_{Airtrapping(severe)\_B}$, ΔOFV=−39 at age 5 years compared with peers. HRCT scan scores indicating bronchiectasis, airway wall thickening, parenchymal disease and mucous plugging had no significant influence on lung function at age 5 years or on subsequent lung function decline.

DISCUSSION

Although adults with CF and *Aspergillus* in their sputum have more severe bronchiectasis,[12] in this study positive *Aspergillus* in BAL cultures at age 5 years was not associated with bronchiectasis in young children with CF. It is possible that this reflects the mild nature of bronchiectasis and the underlying lung disease in this age group that may have led to bronchiectasis being either missed or even overdiagnosed here. Nevertheless, positive *Aspergillus* BAL cultures at age 5 years were associated with an increased probability of air trapping, which in young children with CF correlates with small airways structural lung disease. Moreover, a recent report suggested that air trapping on expiratory chest HRCT scans (also known as mosaic attenuation) may result from both hypoventilation and hypoperfusion, the latter secondary to local low oxygen concentrations and predates irreversible structural lung injury,[26] while another found air trapping predicted the development of bronchiectasis.[27] It is possible that *Aspergillus* may either have a direct role in early lung injury, manifested by air trapping, or act as a marker for other pathological events, which ultimately lead to irreversible structural airway wall damage and bronchiectasis.

Aspergillus had no significant impact on lung function status or decline in our population of children diagnosed with classic CF. We also found no association of having recurrent positive *Aspergillus* cultures with the lung function estimate at age 5 years $FEV_1\%_{baseline}$ or with subsequent lung function changes between ages 5 years and 14 years. While *Aspergillus* improved the fit of the model significantly in the first steps of the model building process (see online supplementary text), the effect of *Aspergillus* diminished throughout the model building process once more significant factors were incorporated in a stepwise manner prior to including *Aspergillus*.

In contrast, we observed air trapping was associated with significantly reduced lung function at age 5 years. This suggests that any early structural lung changes resulting in air trapping might predict reduced lung function in young children with CF and extends a previous study in older CF subjects aged 4–19 years showing a correlation between lung function and radiographic scores.[28]

Chest HRCT scores are more sensitive than spirometry at detecting early and progressive CF lung disease in children.[10]

A model describing lung structural changes in children with CF using chest HRCT scans scores over time is warranted to further determine if there is a long-term effect of *Aspergillus* on structural lung injury. Furthermore, the clinical benefit of treating *Aspergillus* in early life may need to be established. In this study, we noted that 13 children received the antifungal agent, itraconazole, in the first 5 years of life, although no evidence of allergic bronchopulmonary aspergillosis was seen in this cohort. The effects of this treatment cannot be determined in this study as the numbers are small, and therapeutic drug monitoring was not undertaken systematically, which is essential for the drug in this population.[29] A study of itraconazole in adults with positive respiratory cultures failed to show a beneficial effect, although it was limited by its small size and failure to achieve therapeutic drug concentrations in many of its subjects.[30]

This study does, however, have several limitations that need to be considered. FEV_1 has low sensitivity for detecting early or mild CF lung disease and more reliable tests are needed to track lung function in this group of patients.[31] The second cohort was selected from those patients who were consented for the longitudinal follow-up study from the original cohort. However, at the time of our analysis, recruitment for the CF FAB study was incomplete. A dataset of more than 50–100 subjects has been recommended previously[32] when using a non-linear mixed effects approach to result in precise parameter estimates. Data collection of FEV_1% predicted from the ACFDR varied between participating centres. Some supplied encounter-based reporting, while others submitted annual reporting of best values. This unbalanced observation of repeated measured data points was accounted for using a non-linear mixed effects approach modelling approach.[33] Lung function measurements were only available between 5 years and14 years of age and not over the full lifespan of the patients, which limited the ability to estimate $\Delta max_{FEV_1\%}$. Instead prior information from a published review[14] was used, and a sensitivity analysis supported using 40% for $\Delta max_{FEV_1\%}$.

Other limitations included only using positive *Aspergillus* BAL cultures in this analysis to test the influence of *Aspergillus* on $FEV_1\%_{baseline}$ at age 5 years, which limited the number of patients contributing information and may have reduced the power to include this factor as an explanatory variable.[34] However, for other parts of the analysis, both BAL and sputum culture results were used, as ACFDR data relied mainly upon sputum cultures. Three of the eight sites did not use specific fungal media routinely for CF respiratory specimens, potentially limiting rates of detection.[35] However, no site bias was found for culturing fungi. The geographic variation observed with subjects consented for CF FAB resulted from delays in obtaining local ethics and site-specific approvals for that particular study. Consequently, adjustment for centre site was made in both the cross-sectional and the longitudinal analyses. Variable data were missing from only three (3.7%) of the follow-up cohort, while dropout secondary to death, lung transplant or loss to follow-up was not considered thus far, as none of these events had occurred in the population at the time of the data collection. Finally, as this was an observational study, causation cannot be inferred. Moreover, whether *Aspergillus* is a cause or a marker for worse lung disease is difficult to determine as risk factors for *Aspergillus* are also associated with worse outcomes in CF. Consequently, this can only be resolved by a randomised controlled trial of antifungal therapy to eradicate *Aspergillus* in children with CF and then to determine if this provides a measurable clinical benefit.

In conclusion, we found positive *Aspergillus* BAL cultures in early childhood were associated with the air trapping but not with bronchiectasis. No association was found between positive *Aspergillus* BAL cultures in early childhood on lung function at age 5 years and lung function decline between 5 years and 14 years of age. A non-linear model best described the non-constant decline in FEV_1% predicted over age in children with CF. Nutritional status and hospitalisation secondary to pulmonary exacerbations were found to be the major factors adversely affecting lung function in young patients with CF rather than the presence of *Aspergillus*.

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Contributors All authors meet the criteria of authorship. SNH was responsible for collecting and analysing the data and drafting the manuscript. CEW participated in concept development, supported data collection and reviewed and edited the manuscript. KG participated in concept development, reviewed and edited the manuscript. SH participated in concept development, reviewed and supported the analyses and reviewed and edited the manuscript.

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Patient consent Obtained.

Ethics approval Ethics committees at each of the eight study sites approved the ACFBAL study, and ethics committees in Australia (HREC/12/QRCH/171/AM01) and New Zealand (13/NTB/102) approved the CF FAB study.

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Data sharing statement The Australasian Cystic Fibrosis Bronchoalveolar Lavage research group—listed in the authorship—potentially have access to the ACFBAL data. Any decisions regarding the use of the data or publications within the group and/or access to the data from outside the group must be discussed and agreed by a core panel designated by the ACFBAL researchers.

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