Early Markers of Cystic Fibrosis Structural Lung Disease:

Long-Term Follow-Up of the ACFBAL Cohort

Online Supplementary Material

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METHODS

The Australasian Cystic Fibrosis Bronchoalveolar Lavage (ACFBAL) Study

The ACFBAL study was a multi-centre randomised controlled trial (Australian Clinical trials registry ACTRN0126050006656639 http://www.actr.org.au/) and has been described in detail previously (1, 2). It was designed to examine the clinical value of using bronchoalveolar lavage (BAL) to diagnose lower airway infection in infants and young children with cystic fibrosis (CF). The trial found that BAL-directed therapy did not result in less structural lung abnormalities or a lower prevalence of *Pseudomonas aeruginosa* infection (defined as $\geq 10^3$ colony-forming units (CFU)/mL of BAL fluid) at 5-years of age when compared with standard management based on clinical judgement and oropharyngeal culture results (2).

The ACFBAL cohort consisted of infants diagnosed with CF through newborn screening. Recruitment occurred in eight CF centres in Australia (states of New South Wales, Queensland, South Australia and Victoria) and New Zealand between June 1999 and April 2005 inclusive. Infants eligible to enter the trial were aged <6-months with a confirmed diagnosis of classic CF (presence of two of the following: two CF mutations, sweat chloride level >60mmol/L, pancreatic insufficiency, or meconium ileus). In brief, infants were randomly assigned in a 1:1 ratio, stratified by site and sex to receive either BAL-directed therapy or standard management up until age 5-years. Routine clinic review occurred 3-monthly. Children had oropharyngeal cultures performed at enrolment, during a pulmonary exacerbation and after eradication treatment for *P. aeruginosa*. Those receiving BAL-directed therapy had in addition a BAL at enrolment, during hospitalisation for pulmonary exacerbations, with any isolation of *P. aeruginosa* from oropharyngeal cultures

and after completing *P. aeruginosa* eradication therapy. Pulmonary exacerbations were defined as any change from baseline respiratory status.

Upon completing the trial after reaching their fifth birthday, all study participants underwent a series of final outcome investigations undertaken during a period of clinical stability. These included weight and height measurements, a chest high-resolution computed-tomography (CT)-scan (Baseline CT-scan), BAL and spirometry.

Potential baseline explanatory variables for structural CF lung disease in adolescence

Potential baseline explanatory variables from the ACFBAL study used in this study included: (i) **at recruitment**: birthweight, maternal education level and smoking history; (ii) **at any time during the ACFBAL study**: pulmonary exacerbation rate and respiratory-related hospitalisation, *P. aeruginosa* infection as determined by its detection in oropharyngeal swabs in the standard treatment group or when cultured at $\geq 10^3$ CFU/mL in BAL fluid, and courses of *P. aeruginosa* eradication therapy; and (iii) t**he final outcome investigations at age 5-years**: anthropometric measures (height, weight, body-mass index), spirometry (forced expiratory volume at 1-second), BAL fluid culture and inflammatory indices (total cell count, absolute neutrophil counts and percentages, interleukin-8), and chest CT-scan outcome variables (see below)

Follow-Up of the ACFBAL Study (CF-FAB) Study

Children who had taken part in the ACFBAL study were invited to participate in the follow-up study CF-FAB (Australian Clinical trials registry ANZCTR 12613000778785

http://www.actr.org.au/) between May 2013 and April 2016. Children had two reviews while clinically stable, which included clinical assessment, a chest CT-scan, spirometry, induced sputum analysis, health-related quality of life questionnaires, assessment of mental health, and nutritional assessment with a minimum time of 12-months between reviews. For this study however only the first visit CT-scan outcome variables from the CF-FAB study were used.

Imaging

ACFBAL Baseline CT-Scans

Chest CT-scans were performed on 155 ACFBAL participants at the age of 5-years, when they had completed the trial and were stable: (ie. not receiving intravenous antibiotics and not having had an acute pulmonary exacerbation in the preceding 4-weeks). Children from the four smaller centres travelled to the four main centres (Royal Children's Hospital Brisbane, Royal Children's Hospital Melbourne, Westmead Children's Hospital New South Wales and Starship Hospital New Zealand) for a chest CT-scan to ensure consistency in technique and scan quality. A low-dose, high-resolution CT-scan of the chest (1-mm collimation scans at 10-mm intervals, 120kVp, 50mA, 1.0 seconds and high spatial frequency reconstruction algorithm) was used. The scans were made without contrast and prior to bronchoscopy and BAL. Expiratory CT slices at three equally spaced levels from the top of the aortic arch to 1cm above the diaphragm were used to assess the degree of trapped air. In contrast with other centres, the New Zealand centre used general anaesthesia without a standardised protocol for pressure-controlled breath-hold manoeuvres to obtain inspiratory and expiratory scans.

CF-FAB Follow-up CT-Scans

Only the first follow-up scan was examined for this study. These scans were performed between May 2013 and April 2016 when the children were aged 9.4-15.8 years. In order to ensure consistency in technique and scan quality, the scans were undertaken at the same four main centres as for the ACFBAL study. Low-dose spirometer-controlled inspiratory and expiratory volumetric chest CT-scans using a standard protocol were performed without contrast. CT-scanner settings across the centres were standardised to optimise image quality and radiation dose using a phantom image (QRM Quality Assurance in Radiology and Medicine, Bayern, Germany).

Imaging analysis

CT-scans were anonymised, randomised and divided into batches before annotating. The ACFBAL baseline scans were sent to the LungAnalysis Lab (Erasmus Medical Center Sophia Children's Hospital) in Rotterdam and were re-scored using Perth-Rotterdam Annotated Grid Morphometric Analysis for CF (PRAGMA-CF) software (3) by a single certified experienced observer (observer-1) in 2016. The follow-up CF-FAB scans were scored by another certified experienced observer (observer-2) in 2017 using the same software. Observers were blinded to the child's clinical status and to the results of any previous scans or tests to detect infection or inflammation. Eighteen randomly selected ACFBAL scans were also scored by observer-2 to assess the inter-observer agreement. The intra-observer agreement was assessed on a randomly selected batch of 25 CF-FAB scans, which observer-2 rescored 2-months after first scoring the scans.

The scoring software computes the volume fraction of scored items using a square grid overlaying 10 equally spaced axial slices between the lung apex and base of a volumetric chest CT-scan. The grid is subsequently subdivided into grid cells and adjusted for lung size by having the grid cell size equal to 5% of the lung width at the carina (3). Each grid cell containing at least 50% lung tissue is scored according to a hierarchical system providing a subscore for each category from the highest to lowest priority for the inspiratory scans as follows: **Bronchiectasis**: an outer edge bronchus to artery cross-sectional area ratio >1.0; **Mucus plugging**: a high-density airway occlusion or tree-in-bud appearance, as well as consolidation, which is also scored as mucus plugging; **Airway wall thickening**: bronchial wall thickening (airway walls that are thicker or have increased signal intensity relative to normal airways, assessed subjectively); **Atelectasis**: collapsed lung; and **Normal**: where there is only normal lung architecture. **Trapped air:** was assessed similarly on expiratory scans and deemed present if trapped air represented >50% of the lung field within the grid cell.

Each subscore was expressed as a percentage of total lung volume. Hence, small numbers for %Bronchiectasis, %Mucus plugging and %Air wall thickness can represent a large number of abnormal airways. The percentage of lung with airway disease (%Disease) was the number of cells annotated with one of the three disease parameters (bronchiectasis, mucus plugging or air wall thickness) divided by the total number of annotated cells, excluding cells with atelectasis. The cells with atelectasis contain lung parenchyma that cannot be annotated due to collapse and were therefore excluded from the total lung volume (3).

Ethics Statement

Ethics Committees from each participating centre approved the ACFBAL and CF-FAB studies. Written informed caregiver consent was given prior to enrolment for each of the two studies, and participants also provided assent for the CF-FAB study.

REFERENCES

1. Byrnes CA, Vidmar S, Cheney JL, Carlin JB, Armstrong DS, Cooper PJ, Grimwood K, Moodie M, Robertson CF, Rosenfeld M, Tiddens HA, Wainwright CE; ACFBAL Study Investigators. Prospective evaluation of respiratory exacerbations in children with cystic fibrosis from newborn screening to 5 years of age. Thorax. 2013; 68:643-651.
2. Wainwright CE, Vidmar S, Armstrong DS, Byrnes CA, Carlin JB, Cheney J, Cooper PJ, Grimwood K, Moodie M, Robertson CF, Tiddens HA; ACFBAL Study Investigators. Effect of bronchoalveolar lavage-directed therapy on Pseudomonas aeruginosa infection and structural lung injury in children with cystic fibrosis: a randomized trial. JAMA. 2011;306:163-171.
3. Rosenow T, Oudraad MC, Murray CP, Turkovic L, Kuo W, de Bruijne M, Ranganathan SC, Tiddens HA, Stick SM; Australian Respiratory Early Surveillance Team for Cystic Fibrosis (AREST CF). PRAGMA-CF. A Quantitative Structural Lung Disease Computed Tomography Outcome in Young Children with Cystic Fibrosis. Am J Respir Crit Care Med. 2015;191:1158-1165.

Table E1. Three categories of bronchiectasis at follow-up.

a. N=96 children with follow-up computed-tomography bronchiectasis data

Bronchiectasis level, CF-FAB	**No. (%)**
None	15 (16)
>0 to 1%	37 (39)
>1%	44 (46)

b. N=73 children with computed-tomography bronchiectasis data at both time points

Bronchiectasis level, CF-FAB	**No. (%)**
None	14 (19)
>0 to 1%	25 (34)
>1%	34 (47)

CF-FAB, Follow-up of the Australasian Cystic Fibrosis Bronchoalveolar Lavage study

Table E2. Intra-observer reliability for Follow-up of the Australasian Cystic Fibrosis Bronchoalveolar Lavage study computed-tomography scans (n=25)

	Intra-class correlation coefficient	95% confidence interval
%Bronchiectasis	0.99	(0.98, 1.00)
%Mucus plugging	0.94	(0.89, 0.99)
%Air wall thickening	0.73	(0.53, 0.92)
%Atelectasis	0.90	(0.83, 0.98)
%Normal	0.98	(0.96, 1.00)
%Disease	0.98	(0.96, 1.00)
%Trapped air	0.99	(0.98, 1.00)

Figure Legends

Figure E1. Box plot for BMI z-score at ACFBAL and CF FAB time points and also for the paired difference between the two time points.

Figure E2. Bland-Altman plots showing differences in the PRAGMA-CF scores of Australasian Cystic Fibrosis Bronchoalveolar Lavage study computed-tomography scan outcome variables between two observers versus the mean of the paired scores (n=18).

Figure E1.

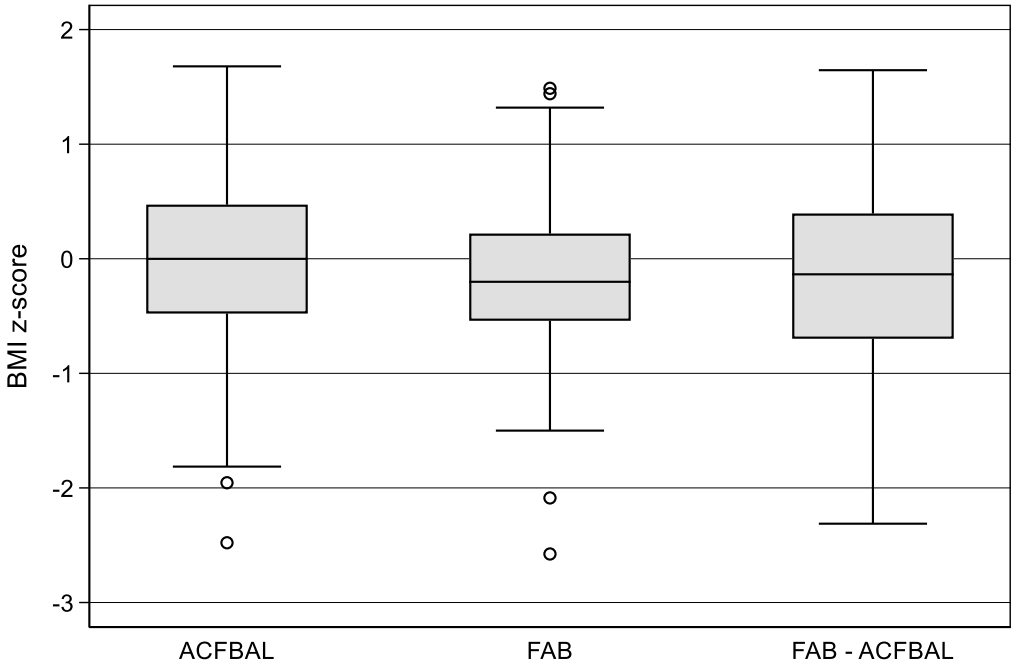
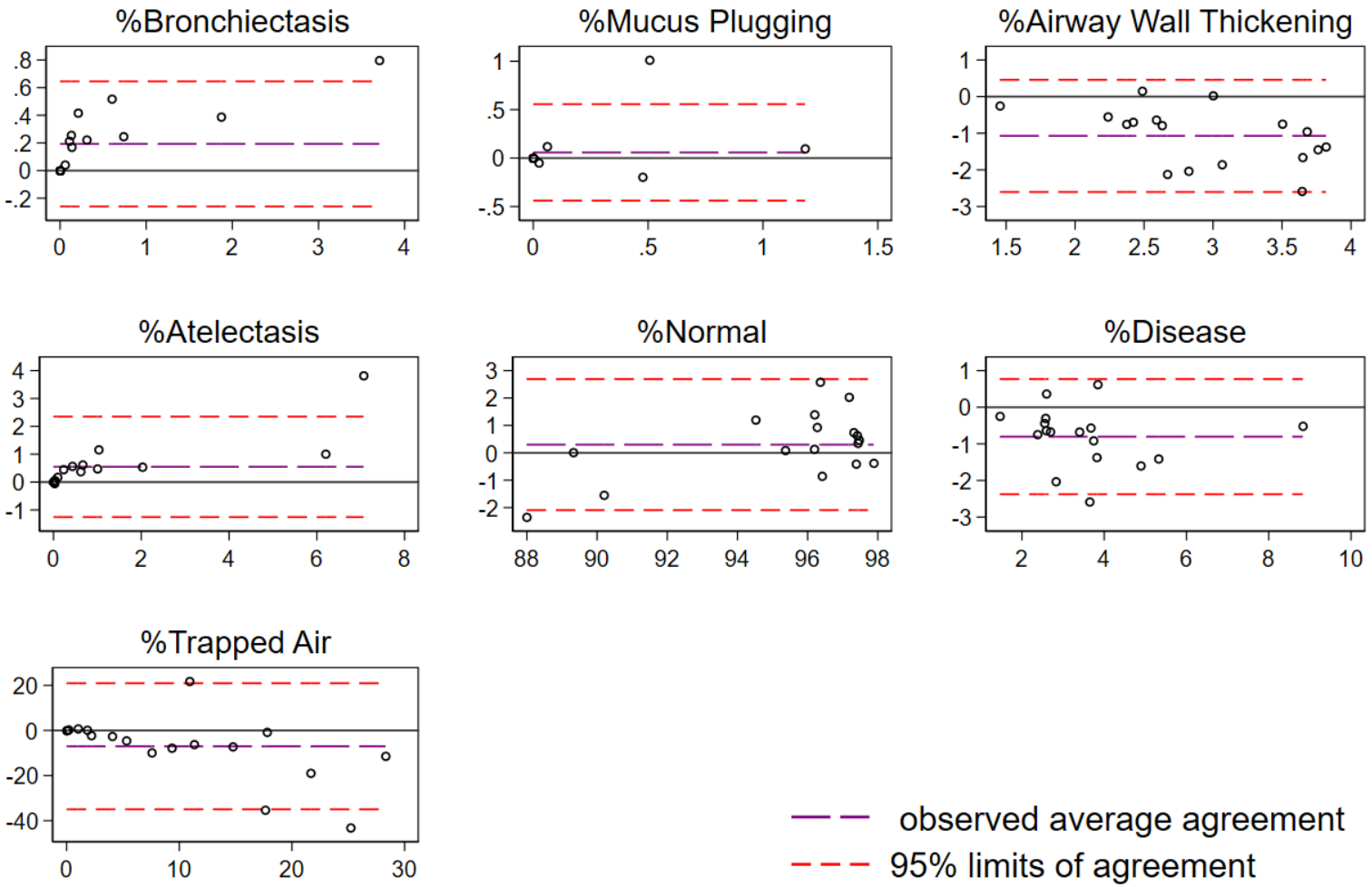


Figure E2


%Bronchiectasis
%Mucus Plugging
%Airway Wall Thickening
%Atelectasis
%Normal
%Disease
%Trapped Air
observed average agreement
95% limits of agreement