The effect of azithromycin on structural lung disease in infants with cystic fibrosis (COMBAT CF): a phase 3, randomised, double-blind, placebo-controlled clinical trial

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Lancet Respir Med 2022; 10: 776–84

Published Online June 2, 2022 https://doi.org/10.1016/S2213-2600(22)00165-5

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Summary

Background Structural lung disease and neutrophil-dominated airway inflammation is present from 3 months of age in children diagnosed with cystic fibrosis after newborn screening. We hypothesised that azithromycin, given three times weekly to infants with cystic fibrosis from diagnosis until age 36 months, would reduce the extent of structural lung disease as captured on chest CT scans.

Methods A phase three, randomised, double-blind, placebo-controlled trial was done at eight paediatric cystic fibrosis centres in Australia and New Zealand. Infants (aged 3–6 months) diagnosed with cystic fibrosis following newborn screening were eligible. Exclusion criteria included prolonged mechanical ventilation in the first 3 months of life, clinically significant medical disease or comorbidities other than cystic fibrosis, or macrolide hypersensitivity. Participants were randomly assigned (1:1) to receive either azithromycin (10 mg/kg bodyweight orally three times per week) or matched placebo until age 36 months. Randomisation was done with a permuted block strategy and an interactive web-based response system, stratified by study site. Unblinding was done once all participants completed the trial. The two primary outcomes were the proportion of children with radiologically defined bronchiectasis, and the percentage of total lung volume affected by disease. Secondary outcomes included clinical outcomes and exploratory outcomes were inflammatory markers. Analyses were done with the intention-to-treat principle. This study is registered at ClinicalTrials.gov (NCT01270074).

Findings Between June 15, 2012, and July 10, 2017, 281 patients were screened, of whom 130 were enrolled, randomly assigned, and received first study dose. 68 participants received azithromycin and 62 received placebo. At 36 months, 88% (n=50) of the azithromycin group and 94% (n=44) of the placebo group had bronchiectasis (odds ratio 0·49, 95% CI 0·12 to 2·00; p=0·32), and total airways disease did not differ between groups (median difference –0·02%, 95% CI –0·59 to 0·56; p=0·96). Secondary outcome results included fewer days in hospital for pulmonary exacerbations (mean difference –6·3, 95% CI –10·5 to –2·1; p=0·0037) and fewer courses of inhaled or oral antibiotics (incidence rate ratio 0·88, 95% CI 0·81 to 0·97; p=0·0088) for those in the azithromycin group. For the preplanned, exploratory analysis, concentrations of airway inflammation were lower for participants receiving azithromycin, including interleukin-8 (median difference –1·2 pg/mL, 95% CI –1·9 to –0·5; p=0·0012) and neutrophil elastase activity (–0·6 µg/mL, –1·1 to –0·2; p=0·0087) at age 36 months, although no difference was noted between the groups for interleukin-8 or neutrophil elastase activity at 12 months. There was no effect of azithromycin on body-mass index at age 36 months (mean difference 0·4, 95% CI –0·1 to 0·9; p=0·12), nor any evidence of pathogen emergence with the use of azithromycin. There were few adverse outcomes with no differences between the treatment groups.

Interpretation Azithromycin treatment from diagnosis of cystic fibrosis did not reduce the extent of structural lung disease at 36 months of age; however, it did reduce airway inflammation, morbidity including pulmonary exacerbations in the first year of life and hospitalisations, and improved some clinical outcomes associated with cystic fibrosis lung disease. Therefore we suggest thrice-weekly azithromycin is a strategy that could be considered for the routine early management of paediatric patients with cystic fibrosis.

Funding Cystic Fibrosis Foundation.

Introduction

Cystic fibrosis in early life is progressive[1–4] and associated with mucous obstruction,[5] neutrophilic inflammation[6] and lower airway infection.[7,8] Worsening lung disease is associated with adverse clinical outcomes, including pulmonary exacerbations,[9] hospital admissions,[9] and reduced health-related quality of life.[10] Newborn screening for cystic fibrosis is now a common practice

Research in context

Evidence before this study

Before the COMBAT CF trial, strong evidence was accumulating that early progressive lung disease, assessed with chest CT, was associated with neutrophilic inflammation in the lower airways. We searched PubMed for reports with the terms "azithromycin" and "cystic fibrosis," "children", "inflammation", and "lung" for literature dated between Feb 1, 1998, and March 31, 2022. The bulk of the evidence was from reports by the Australian Respiratory Early Surveillance Team for Cystic Fibrosis and its collaborators. These studies reported longitudinal data from a unique clinical programme that obtained CT images and bronchoalveolar lavage fluid during a single anaesthetic annually from the age of 3 months. When the clinical trial was conceived, the conclusions from international workshops and a review of the literature suggested that CT was a potentially useful endpoint for clinical trials. The choice of intervention was based on data emerging before the study that the beneficial effects of azithromycin in children with cystic fibrosis appeared to be independent of its antimicrobial activity. Several mechanisms had been suggested, including well documented anti-inflammatory actions. Given the strong evidence linking progressive lung damage with neutrophilic inflammation, azithromycin was a strong candidate as a useful anti-inflammatory agent in young children.

Added value of this study

To the best of our knowledge, no previous randomised controlled studies have investigated the effects of an anti-inflammatory therapy to prevent progressive lung damage in a newborn-screened population of children with cystic fibrosis. This trial is the first to evaluate chest CT as an outcome measure in children with cystic fibrosis in a clinical trial of an intervention commencing at diagnosis. The prevalence of bronchiectasis and difference in total airways disease did not differ between the azithromycin and placebo groups. The trial provides further safety data for the long-term use of azithromycin in young children and shows a direct anti-inflammatory effect of azithromycin in the lower airways.

Implications of all the available evidence

During the study it emerged that muco-obstructive neutrophilic inflammation is a key driver of progressive early cystic fibrosis. Furthermore, airway hydration with hypertonic saline improves lung function and prevents structural damage in preschool children with cystic fibrosis. Therefore, the combination of azithromycin with hypertonic saline in young children might address cardinal factors in the early genesis and progression of lung damage in cystic fibrosis. Although chest CT is sensitive to track the progression of disease in an individual, the image resolution and validated assessment algorithms are currently insufficient to detect small differences in disease progression between individuals younger than 36 months with mild disease. The COMBAT CF biobank is a unique open repository of biospecimens that can be used to further explore pathogen–host microenvironment interactions in the lung during development with multiomics approaches.

worldwide and offers opportunities for early interventions.

The airways in patients with cystic fibrosis contain large numbers of neutrophils and high concentrations of inflammatory cytokines,[11] such as interleukin-8 (IL-8) and IL-17. Although neutrophils are an essential component of pulmonary antibacterial defences, neutrophil necrosis, excessive formation of neutrophil extracellular traps, and degranulation with release of neutrophil exosomes result in a plethora of chemoattractants and proinflammatory products that contribute to the vicious cycle of neutrophilic inflammation and immunopathology of cystic fibrosis airway disease,[11] culminating in progressive structural lung damage and ultimately respiratory failure. Neutrophilic airway inflammation is present in most infants with cystic fibrosis by 3 months of age,[12] is associated with progressive airway disease, bronchiectasis,[2,4] and can occur in the apparent absence of lower airway infection.[5] High-dose ibuprofen is the only recommended anti-inflammatory medication for cystic fibrosis and reduces lung function decline in paediatric patients.[13] However, it is not generally recommended for use in young children. Therefore, we investigated the efficacy of azithromycin, a macrolide antibiotic with anti-inflammatory properties,[14] to prevent the development of lung disease in infants newly diagnosed with cystic fibrosis after newborn screening. We hypothesised that continuous treatment with azithromycin after diagnosis with cystic fibrosis would reduce structural lung damage detected on chest CT scans at age 36 months. We anticipated that azithromycin would also reduce lower airway inflammation as indicated by the presence of free neutrophil elastase activity and concentrations of IL-8 in bronchoalveolar lavage fluid.

Methods

Study design and participants

A phase three, multicentre, randomised, double-blind, placebo-controlled, investigator-initiated trial was done across eight paediatric cystic fibrosis clinics in Australia and New Zealand. Eligibility criteria included infants aged 3–6 months with a confirmed diagnosis of cystic fibrosis after newborn screening (appendix p 5). Exclusion criteria included prolonged mechanical ventilation in the first 3 months of life, a clinically significant medical disease or comorbidity other than cystic fibrosis, and macrolide hypersensitivity. Participants were identified through participating cystic fibrosis clinics and approached by site study teams to participate in the trial. The trial was approved by site-specific hospital research ethics committees and written informed consent was obtained from parents and guardians.

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See **Online** for appendix

Randomisation and masking

Participants were randomly assigned (1:1) to the azithromycin group or placebo group, stratified by site. A permuted block strategy was used, with block size assigned on a site-by-site basis according to recruitment expectations. An interactive web-based response system was used to assign participants to treatment groups at each site, ensuring allocation concealment. Blinding was maintained until the last participant completed the trial, with unblinding done with the interactive system. Study medication was packaged in identical plastic bottles to ensure blinding was maintained.

Procedures

The study medication was taken orally three times per week (Monday, Wednesday, and Friday) from enrolment until age 36 months. Azithromycin and a matched placebo were supplied as powder for suspension and administered orally. Participants' weight was recorded at each study visit, and medication doses were adjusted and prescribed accordingly at 10 mg/kg of bodyweight. Following randomisation, diary cards were dispensed to parents or guardians at each visit to ascertain drug adherence, record concurrent medications, and adverse events. Diary cards were collected and reviewed by site staff at each study visit.

Participants completed 13 study visits over 36 months. Study visits were completed every 3 months, designed to coincide with routine clinical assessments. Study visit schedules were calculated on the basis of the participant's month of age, referenced to the actual birth date. Visits were done within the specified visit windows, with all out-of-window visits documented as protocol deviations. Apart from two participating cystic fibrosis centres, where annual bronchoalveolar lavage and chest CT scans are standard clinical care, all study procedures were done in addition to participants' routine clinical care regimens (appendix p 26).

Chest CT scans were done at age 12 months and 36 months under general anaesthesia with mechanical ventilation and breath-holding techniques, as previously described.[15] Images were centrally assessed by LungAnalysis (Erasmus Medical Centre; Rotterdam, Amsterdam) with the Perth-Rotterdam Annotated Grid Morphometric Analysis for Cystic Fibrosis (PRAGMA-CF) scoring algorithm.[16] Two trained observers annotated scans to ascertain the percentage of lung tissue affected by bronchiectasis, total airways disease, (the percentage of total lung volume affected by disease; includes bronchiectasis, mucus-plugging, and airway wall thickening), and trapped air.[16] Inter-rater and intra-rater correlation coefficients are described in the appendix (p 18).

Bronchoalveolar lavage was completed under general anaesthesia via a flexible bronchoscope at enrolment, and immediately after the CT scan at 12 months and 36 months, in accordance with usual clinical procedures used in Perth, WA, Australia, and Melbourne, VIC, Australia. Bronchoalveolar lavage fluid was analysed for microbiology and inflammatory markers. Isolation of pathogens in the fluid was reported by each site with methods used for routine clinical testing according to National Association of Testing Authorities (Australia) and International Accreditation New Zealand standards for medical testing laboratories. Free neutrophil elastase activity and IL-8 concentrations were analysed at a central laboratory.

Pulmonary exacerbations were defined as treatment with oral, inhaled, or intravenous antibiotics and fulfilment of one or more symptom criteria, including increased cough, increased work of breathing or respiratory rate, and new or increased adventitial sounds on chest examination (appendix p 7).

A health-related quality-of-life questionnaire was completed by parents at age 36 months to measure perceptions of wellbeing. The questionnaire was based on the validated Cystic Fibrosis Questionnaire-Revised, modified to be appropriate for the trial age group.[10] Health-related quality-of-life categories included physical health, treatment burden, and symptoms.

Outcomes

The two primary outcomes at 36 months were the proportion of children with radiologically defined bronchiectasis (prevalence of bronchiectasis) and the percentage of total lung volume affected by disease (airways disease severity).

At study initiation, the single primary outcome was the prevalence of bronchiectasis. Airways disease severity was added during the trial after the PRAGMA-CF algorithm was validated specifically for use in infants and preschool children aged 3–6 years with cystic fibrosis.[16] This amended approach was included in the final statistical analysis plan and reviewed by an independent data safety monitoring board before the dataset was unblinded.

Secondary efficacy outcomes included the extent and severity of bronchiectasis at age 36 months; the volume of trapped air at age 36 months, cystic fibrosis-related quality of life, time to first pulmonary exacerbation, proportion of participants with a pulmonary exacerbation, number of courses of inhaled or oral antibiotics, number of days of inhaled antibiotics, incidence of hospitalisations or accident and emergency department visits for an acute respiratory exacerbation, number of days hospitalised for an acute respiratory exacerbation, number of days of intravenous antibiotics, and body-mass index at age 36 months. Additional exploratory outcomes included markers of inflammation (neutrophil elastase and IL-8) measured in bronchoalveolar lavage fluid (appendix p 6). Adverse events were reported from the time written informed consent was given until 28 days after the final study visit.

Statistical analysis

Based on data in 2011, projecting approximately 100 new diagnoses of cystic fibrosis via newborn screening each

year, the goal recruitment rate was 75% of eligible children per year. To calculate the required sample size, the prevalence of bronchiectasis in the placebo group was assumed to be 50%. To detect a 50% reduction in bronchiectasis prevalence at 36 months with 80% power (α=0·05), 116 children (58 per group) were required to complete the study. Anticipating 10% attrition, we aimed to recruit 130 children.

The statistical analysis plan was approved by the study investigators and the data safety monitoring board before analysis. Two interim analyses were undertaken: when 50% of anticipated enrolments had completed the 12-month CT scan, and when all participants had completed the 12-month CT scan. The interim results did not meet predetermined stopping criteria as reviewed by the data safety monitoring board. Analysis was completed with the intention-to-treat principle, where all participants who were randomly assigned and had evaluable data for the endpoints under investigation were included in the analysis, regardless of compliance to their allocated treatment. Study sites were not included as a covariable in analyses, only for stratification during randomisation to allow for balance between the two groups. The safety population was defined as all participants who received at least one dose of their allocated medication.

Continuous variables are summarised as mean (SD) or median (IQR) as appropriate, and categorical variables as frequency (percentage rounded to nearest whole number). For the primary outcomes, airways disease severity was analysed with median regression, and prevalence of bronchiectasis was analysed with logistic regression. Regression models included treatment group (azithromycin or placebo) as the main effect. Between-group differences for secondary outcomes measured on the interval scale were compared with linear regression, although when model assumptions did not hold, median regression was used. Time-to-event outcomes were analysed with Cox proportional hazards regression. Count outcomes were prespecified to be analysed with Poisson regression, but due to data overdispersion were analysed with negative binomial regression. Analyses used Stata statistical software version 14.1. A p value less than 0·05 was considered significant. The trial is registered on ClinicalTrials.gov (NCT01270074).

Role of the funding source

The COMBAT CF trial was designed jointly by consultant experts and the trial sponsors. Data were collected by site principal investigators and their respective study teams. The trial was funded by the Cystic Fibrosis Foundation (CFF) in Maryland, USA. The CFF had no role in study design, data collection, data analysis, data interpretation, or the writing of the report. The CFF assembled a data safety monitoring board who provided an ongoing independent review of data from the trial to address any safety concerns.

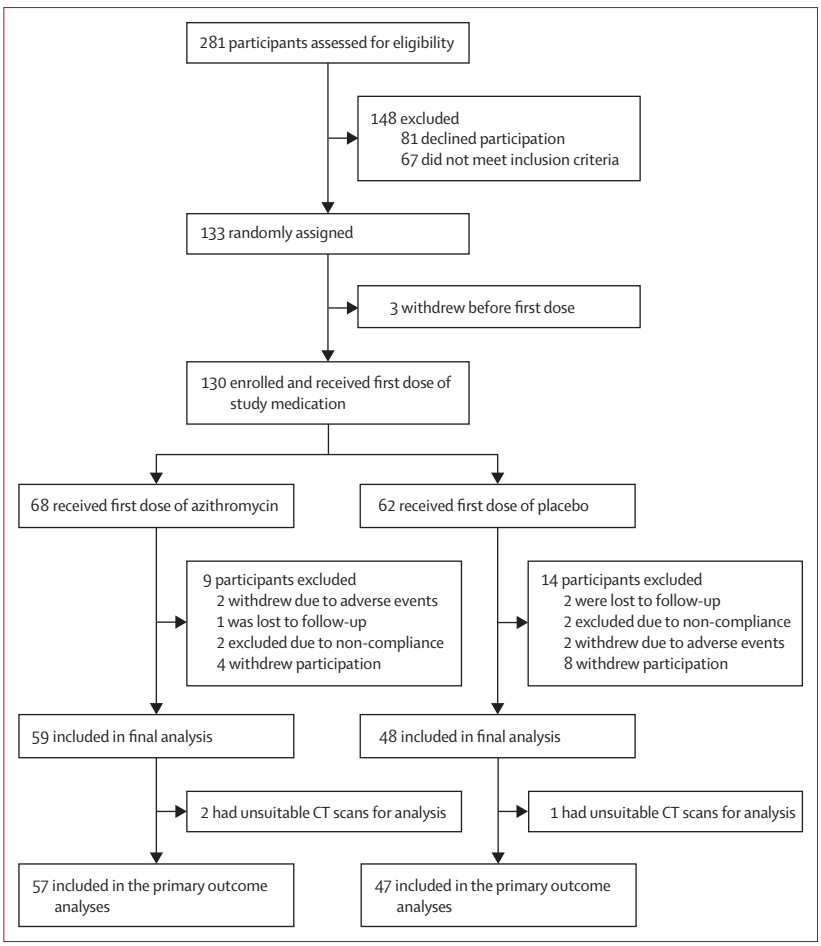

Figure 1: **Trial profile**

	Azithromycin group (n=68)	Placebo group (n=62)
Mean age, months (SD)	3·6 (1·2)	3·6 (1·3)
Sex		
Female	30 (44%)	22 (35%)
Male	38 (56%)	40 (65%)
First born child	36 (53%)	27 (44%)
Tobacco smoking inside house	1 (1%)	3 (5%)
Hypertonic saline use	1 (1%)	1 (2%)
Median IL-8 concentration (IQR), pg/mL*	222 (74–1082)	291 (123–918)
Detectable neutrophil elastase†	8/60 (13%)	11/58 (19%)
Pancreatic insufficiency	62 (91%)	57 (92%)
Mean BMI (SD)	16·7 (3·0)	16·3 (2·7)
Genotype		
p.Phe508del homozygous	34 (50%)	37 (60%)
p.Phe508del heterozygous	29 (43%)	24 (39%)
Other	5 (7%)	1 (2%)

Data are n (%), unless stated otherwise. *Items missing for nine participants (eight azithromycin and one placebo). †Items missing for 12 participants (eight azithromycin and four placebo).

Table 1: **Demographic, social, and clinical characteristics of participants at enrolment**

	Azithromycin group (n=57)	Placebo group (n=47)	Odds ratio (95% CI) or median difference (95% CI)	p value
Prevalence of bronchiectasis, n (%)	50 (88%)	44 (94%)	OR 0·49 (95% CI 0·12 to 2·00)	0·32
Percentage of airway disease	0·73% (0·23 to 1·36)	0·74% (0·24 to 1·85)	−0·02% (−0·59 to 0·56)	0·96
Extent of bronchiectasis	0·73 (0·22 to 1·30)	0·55 (0·22 to 1·77)	0·18% (−0·30 to 0·66)	0·46
Air trapping extent	0·00 (0·00 to 0·29)	0·00 (0·00 to 2·59)	0·00% (−0·53 to 0·53)	1·00

Data are median (IQR), unless stated otherwise. PRAGMA-CF=Perth-Rotterdam Annotated Grid Morphometric Analysis for Cystic Fibrosis.

Table 2: **Association with structural lung outcomes measured on CT with PRAGMA-CF descriptors at age 36 months**

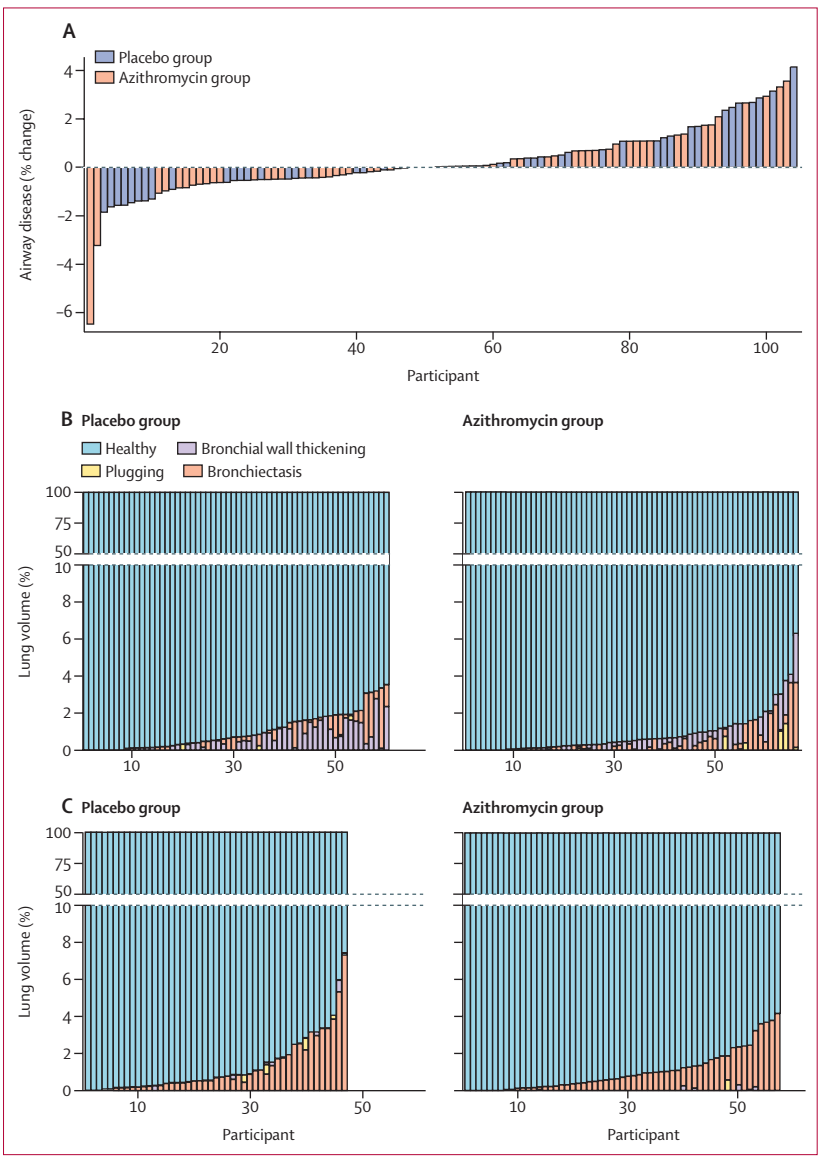

Figure 2: **PRAGMA-CF measurements of airway disease on chest CT scans**
(A) Percentage change in airway disease on chest CT by calculating the absolute difference in PRAGMA-CF scores between age 12 months and 36 months for individual participants. PRAGMA-CF outcomes on chest CT at age 12 months (B) and 36 months (C) for individual participants. PRAGMA-CF=Perth-Rotterdam Annotated Grid Morphometric Analysis for Cystic Fibrosis.

Results

Between June 15, 2012, to July 10, 2017, 281 participants were screened for eligibility (figure 1). During the first 2 years of recruitment, the incidence of cystic fibrosis diagnoses was much lower than expected, and the trial had an overall recruitment rate of 60%, with patients either not meeting the inclusion criteria, or families declining participation. 133 participants were enrolled into the study and randomly assigned. Three participants withdrew before receiving their first dose of study medication; 130 children began the intervention (68 in the azithromycin group *vs* 62 in the placebo group; figure 1). All children with baseline data received at least one dose of their allocated medication. Median enrolment age was 3·3 months (IQR 2·8–4·4). 107 participants completed all 13 trial visits (82%; 59 in the azithromycin group *vs* 48 in the placebo group), and 104 had CT scans acceptable for PRAGMA-CF analysis (80%; 57 in the azithromycin group *vs* 47 in the placebo group; appendix p 14). Adherence to study medications was high (95% in the azithromycin group and 95% in the placebo group; p= 0·81; appendix p 8). Treatment groups did not differ for ages at enrolment (table 1) and genotype distribution and sex for children completing the study (appendix p 14).

Treatment with azithromycin did not affect the primary outcomes for structural lung disease, with PRAGMA-CF scores showing 50 participants (88%) treated with azithromycin versus 44 participants (94%) who received placebo had bronchiectasis at 36 months (odds ratio [OR] 0·49, 95% CI 0·12 to 2·00; p=0·32; table 2). Extent of disease was low and similar in both groups for bronchiectasis (median difference 0·18%, 95% CI −0·30 to 0·66; p=0·46) and airways disease severity (−0·02%, −0·59 to 0·56; p=0·96). Change in CT scores did not differ (median difference 0·04%, 95% CI −0·52 to 0·61; p=0·88) from age 12 months to age 36 months (figure 2), nor did baseline demographics between those participants who completed the study and those who withdrew (appendix p 14).

For secondary outcomes, children who received azithromycin spent less time in hospital for pulmonary exacerbations each year (mean difference −6·3 days, 95% CI −10·5 to −2·1; p=0·0037), had fewer days of intravenous antibiotics each year (0·2 for azithromycin group *vs* 6·7 for placebo group per year; median difference −6·7, 95% CI −12·2 to −1·2; p=0·018), and

fewer courses of inhaled or oral antibiotics each year (incidence rate ratio 0·88, 95% CI 0·81 to 0·97; p=0·0088; appendix p 13). The effect of azithromycin on pulmonary exacerbations was only significant in the first year of life (table 3). Although parents did not report an overall difference in health-related quality-of-life outcomes, such as perceptions of overall health (mean difference 0·4, 95% CI –6·5 to 7·3; p=0·92) and vitality (0·2, –5·4 to 5·8; p=0·94), children allocated to the azithromycin group scored higher in the assessment of physical wellbeing (4·7, 0·6 to 8·9; p=0·026; appendix p 13). Body-mass index did not change over the course of the study in either group (0·4, –0·1 to 0·9; p=0·12; appendix p 13).

The most common organisms isolated at 36 months were *Haemophilus influenzae* (12% azithromycin group *vs* 19% placebo group; OR 0·6, 95% CI 0·2–1·9; p=0·46), *Pseudomonas aeruginosa* (9% azithromycin group *vs* 13% placebo group; 0·6, 0·1–2·7; p=0·71), *Staphylococcus aureus* (10% azithromycin group *vs* 21% placebo group; 0·2, 0·0–2·0; p=0·24), *and Streptococcus* species (17% azithromycin group *vs* 15% placebo group; 1·2, 0·4–4·0; p=0·96; appendix p 15). *Methicillin-resistant S aureus* (MRSA) was identified in five (1%) of 358 samples, four of which were in the placebo group. There was one MRSA-positive result in the azithromycin group at enrolment; however, there was no evidence of MRSA infection in the children treated with azithromycin after commencing treatment, nor selective emergence of fungal pathogens such as *Aspergillus fumigatus* during the 36-month treatment period (appendix p 15). There was no evidence of non-tuberculous mycobacteria in either treatment group during the trial period.

For the preplanned, exploratory anaylsis, at 12 months, both free neutrophil elastase activity and IL-8 did not differ between groups; however, they were lower in the azithromycin group than in the placebo group at 36 months (table 4).

The commonly used interventions—hypertonic saline, dornase alfa, and anti-*Staphylococcus* prophylaxis—had no effects on outcomes in either the azithromycin or placebo groups (appendix p 16), nor were there detectable microbiological effects of chronic use of two antibiotics. Further studies are being explored to understand potential effects at a genomic level. Cystic fibrosis clinic characteristics, including site prophylaxis profiles, are shown in the appendix (p 12).

There were few adverse outcomes with no differences between the treatment groups (appendix p 17). Three participants (two in the azithromycin group and one in the placebo group) discontinued study medication due to elevated liver enzyme concentrations assessed by investigators as being possibly related to the study medication. In the placebo group, one participant discontinued the study medication due to unexplained weight loss and loss of appetite and one participant due to teething pain. These events were assessed by investigators as unrelated to study medication. One participant who received azithromycin discontinued the study medication to receive clinical treatment for a positive bronchoalveolar lavage culture, also assessed as unrelated to treatment with the study medication.

There were three serious adverse events of infection with *P aeruginosa* reported as probably related to study drug in the placebo group. These events resolved with intravenous antibiotic treatment and did not require unblinding of the participants, nor interruption or cessation of study medication administration. One child in the placebo group developed liver failure related to cystic fibrosis.

	Azithromycin group (n=68)	Placebo group (n=62)	Incidence rate ratio* (95% CI)	p value
Overall	1·92 (1·71)	2·55 (1·98)	0·74 (0·54–1·00)	0·052
In the first year of life	1·34 (1·80)	2·49 (2·65)	0·52 (0·34–0·81)	0·0038
In the second year of life	1·86 (1·92)	2·56 (2·83)	0·73 (0·50–1·07)	0·10
In the third year of life	2·38 (2·64)	2·54 (2·38)	0·95 (0·65–1·40)	0·81

Data are mean (SD) pulmonary exacerbations per year. *Calculated with negative binomial regression offset by natural logarithm of time at risk.

Table 3: **Associations with pulmonary exacerbations**

	Azithromycin group		Placebo group		Mean difference (95% CI)	p value
	Participants, n	Mean marker (SD)	Participants, n	Mean marker (SD)		
IL-8 (ln)*						
Enrolment	60	5·5 (1·7)	61	5·8 (1·5)	–0·2 (–0·8 to 0·3)	0·43
12 months	66	5·2 (1·5)	59	5·6 (1·5)	–0·5 (–1·0 to 0·1)	0·10
36 months	56	5·1 (1·8)	47	6·3 (1·7)	–1·2 (–1·9 to –0·5)	0·0012
Neutrophil elastase†						
Enrolment	60	0·4 (1·0)	58	0·6 (1·5)	–0·2 (–0·7 to –0·2)	0·35
12 months	66	0·2 (0·6)	59	0·1 (0·6)	0·0 (–0·2 to 0·2)	0·82
36 months	56	0·3 (0·9)	47	1·0 (1·5)	–0·6 (–1·1 to –0·2)	0·0087

*Measured in pg/mL, then transformed with the natural logarithm. †Measured in µg/mL, then multiplied by 10 and transformed with the natural logarithm.

Table 4: **Associations with inflammatory markers**

Discussion

In our study, for children diagnosed with cystic fibrosis after newborn screening, azithromycin from infancy to 36 months did not reduce the extent of structural lung disease, but was safe, reduced airway inflammation and morbidity including pulmonary exacerbations in the first year of life and hospitalisation, and improved physical wellbeing, but not overall quality of life, as reported by parents.

Our goal was to establish whether azithromycin could alter the trajectory of progressive lung damage observed in infants and young children and was chosen for its anti-inflammatory activity[17] and reported efficacy in older children.[18–22] This treatment, and the use of CT as a clinical endpoint in very young children with

cystic fibrosis, were innovative approaches to address the prevention of progressive lung disease that develops even before diagnosis after newborn screening.[12]

A standardised approach was taken for image acquisition, developed in collaboration with radiation scientists and clinicians at the Erasmus Medical Centre. Lung disease progressed to a similar extent in each group; however, we did not observe any differences between treatment groups with regards to the prevalence of structural lung abnormalities or measures of disease extent. There are many possible explanations. Clearly, treatment with azithromycin might not alter the trajectory of progressive lung damage with the dose used for the trial, or adherence was lower than parents reported. We believe that these explanations are unlikely given the effect of azithromycin on inflammatory markers, in particular neutrophil elastase activity, which is strongly associated with progressive lung damage in this age group.[2,4,6] A more likely explanation is the relative insensitivity of the PRAGMA-CF algorithm to quantify small differences between individuals in lung structure due to a combination of factors in very young children. These include the low number of airways captured due to their small size in relation to the resolution of the CT scans, low resolution for imaging the peripheral airways where early disease is most evident,[23] and the diffuse and heterogenous lung disease in very young children. The increased prevalence of bronchiectasis with age might partly be explained by the fact that more airways can be resolved with current CT technology as lungs grow. The COMBAT CF cohort will be followed to establish whether early intervention with azithromycin has an effect on structural lung disease and lung function in later childhood.

Despite the CT outcomes, a convincing and clinically relevant observation was the effect of treatment with azithromycin on airway inflammation. The considerable interest and extensive research to develop anti-inflammatory therapies for use in cystic fibrosis has been comprehensively reviewed.[11] High-dose ibuprofen is currently the only known effective anti-inflammatory agent used in cystic fibrosis and prevents lung function decline in children.[24] However, it is not generally recommended for infants and preschool children.

In our study, continuous use of azithromycin (10 mg/kg, three times a week) for 36 months reduced neutrophil elastase and IL-8 in bronchoalveolar lavage fluid. Inflammatory markers in bronchoalveolar lavage fluid were considered as exploratory outcomes in this study because bronchoalveolar lavage is used in young children in relatively few centres worldwide. However, the anti-inflammatory activity of azithromycin was a primary rationale for the study. Given the strong association between neutrophilic inflammation and lung disease in all age groups and the particular role of neutrophil elastase in the progression of lung damage in early life,[4,6] azithromycin could be used as a safe and effective anti-inflammatory agent for continuous use in young children.

Whether the antimicrobial, anti-inflammatory, immunomodulatory, or other effects of azithromycin such as, gut prokinesis or epithelial sodium channel inhibition, are responsible for the effects on exacerbations and quality of life that we observed, is unclear. The overall prevalence of lower airway infection was similar to that reported previously from bronchoalveolar lavage fluid samples[25] (appendix p 15). No obvious effect of treatment with azithromycin on key proinflammatory pathogens associated with early lung disease was apparent. Furthermore, azithromycin has beneficial clinical effects regardless of whether children are chronically infected with *P aeruginosa*,[21,22,26] suggesting that the clinical benefits we observed are possibly due to non-antibiotic activities. Multiple inflammatory pathways are activated during exacerbations; however, adjunct therapy with glucocorticoids during an episode, does not appear to effect upon the outcomes in children.[27] We have also reported that inflammatory markers in bronchoalveolar lavage, including neutrophil number, neutrophil elastase, and IL-8, predict future pulmonary exacerbations[28] and it is therefore plausible that modulation of inflammation before an exacerbation attenuates the host response to triggers. Certainly, mucosal priming with neutrophils increases the susceptibility to respiratory syncytial virus in healthy volunteers.[29] Since pulmonary exacerbations in young children are associated with reduced lung function in later childhood,[9] our observations suggest an option for preventing longer term pulmonary decline.

Azithromycin effects pulmonary exacerbations in other chronic respiratory conditions including non-cystic fibrosis bronchiectasis,[30] chronic obstructive pulmonary disease,[31] asthma,[32] preschool wheezing,[33] and primary ciliary dyskinesia.[34] Since exacerbations are often triggered by respiratory viruses in these conditions, antiviral properties of azithromycin might reduce the effect of viral respiratory infections particularly in the first years of life when these infections are most common. The effect of azithromycin on pulmonary exacerbations was only significant in the first 12 months, suggesting a possible modulating effect on viral-induced exacerbations. An alternative explanation is a waning in adherence over the study period; although diary records and the sustained anti-inflammatory effects observed at age 36 months suggest otherwise.

The continuous use of azithromycin from diagnosis to age 36 months appeared to be safe (appendix p 17). Although the development of resistance to azithromycin, particularly of *S aureus*, is well recognised, we did not observe any emerging resistance of *S aureus* to azithromycin in this cohort. This outcome is most probably due to the relatively low numbers of isolates at each site. Furthermore, since azithromycin is not the standard antibiotic therapy for *Staphylococcal* infections, emergence of resistance would not preclude its use as an anti-inflammatory agent.

Most infants and preschool children worldwide do not have access to transformative but expensive cystic fibrosis transmembrane conductance regulator (CFTR) modulator therapies (personal communication, June 3, 2021; Vertex Pharmaceuticals, Boston, MA, USA). One report highlighted the problems health services face when considering the cost–benefit ratio for funding such therapies and these considerations are likely to delay access for many young children in the short-to-medium term.[35] Airway surface liquid depletion, mucous concentration and progressive muco-obstructive inflammation are early pathobiological features of lung disease and its evolution into irreversible structural damage. Early intervention with safe, cost-effective therapies that rehydrate the airways, reduce neutrophilic inflammation, and prevent the release of destructive biochemical mediators is needed for all children with cystic fibrosis unable to access CFTR modulator therapies.

Two studies have shown the benefit of inhaled hypertonic saline in infants younger than 4 months of age[36] and preschool children aged 3–6 years.[37] Both showed beneficial effects on the lung clearance index, a measure of ventilation homogeneity. Since the mechanism of action of hypertonic saline is thought to be through airway rehydration, this widely used therapy fulfils one of the requirements for early lung disease prevention, and is safe and inexpensive. Furthermore, the combination of 7% saline and anti-inflammatory therapy with azithromycin has the potential to improve and stabilise the early airway microenvironment until CFTR modulator therapies can be accessed. Since inpatient hospital costs in patients not receiving CFTR modulator therapy account for about 50% of the total health-care costs for children with cystic fibrosis,[38] the use of hypertonic saline and azithromycin to improve and stabilise the early airway microenvironment could reduce health-care costs for young children. Although the primary uses of interventions, such as azithromycin and hypertonic saline, are likely to be in newly diagnosed and young children who cannot access CFTR modulator therapy, the combined use of these interventions with a CFTR modulator should perhaps be considered in patients with persistent airway inflammation and disease progression despite modulator treatment. These could be addressed with adaptive trial platforms given that CFTR modulators are becoming increasingly available for older children and adults, and the options for discrete randomised controlled studies are becoming fewer.

We acknowledge several limitations to the study. Firstly, the relatively low resolution of the ultralow-dose CT protocol that limits identification of airways to those more than 2 mm in diameter. Although the PRAGMA CF scoring algorithm is suitable for tracking progression of disease in an individual, the relatively low scores in children younger than 36 months and high variability between individuals limits detection of small between-group differences. In addition, our trial did not meet its projected sample size of acquiring CT scan data from 116 participants for the analysis of the primary outcomes, but instead from 104 participants. The primary outcome results were unlikely to have been affected given we did not observe a treatment effect of azithromycin on structural lung disease.

Secondly, when the study was commenced, multiple breath washout was not fully validated in young children—testing equipment and protocols had not been standardised and were not available in every trial centre. In hindsight, and given the reported clinical trial of hypertonic saline in infants and preschool children,[36] the lung clearance index would have been a potentially useful additional outcome measure.

Thirdly, the sample size calculation assumed that 50% of participants in the placebo group would have bronchiectasis at age 36 months; however, due to the improvement in the ability of detection tools to identify bronchiectasis during the course of the study, we identified bronchiectasis in 94% of participants from the placebo group. This high prevalence suggests bronchiectasis might have been pervasive before treatment initiation, although we did not find any significant treatment effect of azithromycin on the severity of bronchiectasis at 36 months. The COMBAT CF cohort will be followed up to establish whether early intervention with azithromycin impacts on structural lung disease outcomes later in childhood (timeline is dependent on obtaining ethic committee approvals).

Finally, we were only able to ascertain adherence from pharmacy records and parental diary records. Although poor adherence might have affected the primary outcome, the effects on important clinical outcomes and biological outcomes suggests that, in general, the azithromycin treatment group adhered to the trial protocol.

In conclusion, azithromycin treatment from diagnosis of cystic fibrosis after newborn screening did not reduce the extent of structural lung disease at age 36 months; however, it did reduce airway inflammation, and improved some clinical outcomes associated with cystic fibrosis lung disease. Although the mechanisms of action of azithromycin in this age group are beyond the scope of this study, the unique and extensive COMBAT CF biobank, database, and image archive are available for further research.

Contributors

Funding for the trial was acquired by SMS. SMS and PDS conceived the trial concept and design and supervised the trial. RSW contributed to the design of the protocol, developed, and implemented the statistical analysis plan, and accessed, verified, and formally analysed the data. HAWMT developed and implemented the CT image acquisition program and is responsible for the scoring and reporting of CT images. AF managed the trial and database and accessed and verified the data. LS contributed to the protocol design and reviewed and interpreted microbiological outcomes. BSC, DSA, HS, AT, PJC, CAB, YB, CW, AJ, and PR contributed to the trial concept and methodology, and the coordination and supervision of the trial at their respective sites. All authors had access to the data; contributed to the interpretation of the data; the writing, review, and editing of the Article; and provided final approval to submit.

Declaration of interests

During the conduct of the trial, SMS and HAWMT had a patent pending for the PRAGMA-CT scoring method used to analyse the CT scans in this trial (PCT/AU2016/000079). CW is on the International Advisory Board for Vertex Pharmaceuticals. HAWMT is Chief Medical Officer for Thirona. All other authors declare no competing interests.

Data sharing

Deidentified trial data and access to the unique COMBAT CF database will be made available to others once sharing has been approved by the relevant human research ethics committees. Enquiries pertaining to data and sample access requests can be sent to combatcf@telethonkids.org.au. Documents including the trial protocol and statistical analysis plan are available in the supplementary appendix.

Acknowledgments

Azithromycin (Zithromax) and placebo were supplied by Pfizer Australia, Sydney, NSW, Australia. The trial was supported by the Cystic Fibrosis Foundation (STICK10K0 and STICK19K0). We thank all participants and their families, and the COMBAT CF Study Team.

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