



Efficacy and safety of azithromycin maintenance therapy in primary ciliary dyskinesia (BESTCILIA): a multicentre, double-blind, randomised, placebo-controlled phase 3 trial

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Background Use of maintenance antibiotic therapy with the macrolide azithromycin is increasing in a number of chronic respiratory disorders including primary ciliary dyskinesia (PCD). However, evidence for its efficacy in PCD is lacking. We aimed to determine the efficacy and safety of azithromycin maintenance therapy for 6 months in patients with PCD.

Methods The Better Experimental Screening and Treatment for Primary Ciliary Dyskinesia (BESTCILIA) trial was a multicentre, double-blind, parallel group, randomised, placebo-controlled phase 3 trial done at 6 European PCD clinics (tertiary paediatric care centres and university hospitals in Denmark, Germany, Netherlands, Switzerland, and UK). Patients with a confirmed diagnosis of PCD, aged 7–50 years old, and predicted FEV₁ greater than 40% were recruited. Participants were randomly assigned (1:1), stratified by age and study site, via a web-based randomisation system to azithromycin 250 mg or 500 mg as tablets according to bodyweight ($\leq 40\text{ kg}$) or identical placebo, three times a week for 6 months. The random allocation sequence was a permuted block randomisation, with a block size of four, generated by an external consultancy. Participants, investigators, and care providers were masked to treatment allocation. The primary endpoint was the number of respiratory exacerbations over 6 months. Analysis was by intention to treat. This study is registered in the EU Clinical Trials Register, EudraCT number 2013-004664-58.

Findings Between June 24, 2014, and Aug 23, 2016, 102 patients were screened, of whom 90 were randomly assigned to either azithromycin (n=49) or placebo (n=41). The study was ended without having included the planned number of participants due to recruitment difficulties. The mean number of respiratory exacerbations over 6 months was 0·75 (SD 1·12) in the azithromycin group compared with 1·62 (1·64) in the placebo group, and participants receiving azithromycin had significantly lower rate of exacerbations during the individual treatment periods (rate ratio 0·45 [95% CI 0·26–0·78]; p=0·004). Four serious adverse events were reported, occurring in one (2%) of 47 participants in the azithromycin group and in three (7%) of 41 participants in the placebo group. Loose stools or diarrhoea were more common in the azithromycin group than in the placebo group (11 [23%] vs two [5%]).

Interpretation This first multinational randomised controlled trial on pharmacotherapy in PCD showed that azithromycin maintenance therapy for 6 months was well tolerated and halved the rate of respiratory exacerbations. Azithromycin maintenance therapy is an option for patients with PCD with frequent exacerbations potentially leading to reduced need for additional antibiotic treatments and preventing irreversible lung damage.

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Introduction

Primary ciliary dyskinesia (PCD) is a rare, genetically and clinically heterogeneous disease that manifests from the neonatal period or early childhood and progresses through adulthood.¹ Impaired mucociliary clearance due to abnormal ciliary beating results in excessive accumulation of mucus and bacteria in the upper and lower airways.² PCD is a suppurative disease characterised by chronic rhinosinusitis, recurrent otitis media and conductive hearing impairment, chronic productive cough, and infections in the lower airways.^{1,3,4} Recurrent lower respiratory tract infections lead to chronic infection, bronchiectasis, and decline in lung function.^{1,3,5}

The treatment strategies for PCD have been extrapolated from more common chronic respiratory diseases with different pathophysiologies, notably cystic fibrosis and non-cystic fibrosis bronchiectasis. No drugs have been developed for PCD.^{3,6–8} Only two published randomised controlled studies have investigated the efficacy and safety of pharmacotherapeutics used in the treatment of PCD—inhaled hypertonic saline and salbutamol, respectively—but found no significant change in the primary outcomes, which were quality of life measured by the St George's Respiratory Questionnaire total score in the study with hypertonic saline and FEV₁ and parameters of bronchial responsiveness in the study with

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Research in context

Evidence before this study

A substantial need exists for evidence-based guidelines specifically for the management of primary ciliary dyskinesia (PCD)—given that current treatment strategies must be extrapolated from other chronic respiratory diseases, notably cystic fibrosis and non-cystic fibrosis bronchiectasis. Before undertaking this study, we searched the literature via PubMed in 2013 for clinical trials on pharmacotherapeutics for PCD and randomised controlled trials (RCTs) of azithromycin maintenance therapy for non-cystic fibrosis bronchiectasis. We combined the search terms: “primary ciliary dyskinesia” with “randomized”, and “azithromycin” with “randomized” and “non-cystic fibrosis bronchiectasis”, without restrictions in publication date or language to optimise the search. The search for clinical trials investigating possible pharmacotherapeutics for PCD yielded only three randomised studies, none of which investigated antibiotics. In addition, we were aware of one ongoing trial investigating hypertonic saline in PCD. The search for RCTs with maintenance azithromycin for non-cystic fibrosis bronchiectasis revealed two studies in adult patients lasting 6 months and 12 months and one study in Indigenous children with non-cystic fibrosis bronchiectasis or chronic suppurative lung disease (PCD not excluded as possible aetiology of bronchiectasis). These studies found that azithromycin decreased exacerbations, and in adult patients it also improved lung function and health-related quality of life after 12 months of intervention.

Added value of this study

The Better Experimental Screening and Treatment for Primary Ciliary Dyskinesia study is the first clinical trial in PCD investigating efficacy of maintenance antibiotics. Azithromycin maintenance therapy for 6 months did significantly reduce respiratory exacerbations in PCD, compared with placebo, and was well tolerated. The study also showed that maintenance azithromycin more than halved the rate of detected pathogenic bacterial species in sputum in PCD.

Implications of all the available evidence

This study shows that azithromycin maintenance therapy reduces exacerbations in PCD, which is in complete agreement with previously published results within non-cystic fibrosis bronchiectasis and other chronic respiratory disorders. These study results are the first step toward the development of evidence-based guidelines on pharmacotherapy for PCD. Maintenance azithromycin should now be considered as a treatment option for PCD patients with frequent exacerbations, possibly reducing the need for additional antibiotic treatments and might as a consequence also reduce progression in lung damage. In a next step studies investigating both maintenance azithromycin over a prolonged intervention period and inhaled antibiotic therapy for PCD are warranted.

salbutamol.^{9,10} Evidence-based guidelines for the treatment of PCD have been difficult to develop because of the paucity of clinical studies, and because the scientific evidence for effective treatments is simply lacking.^{3,8} Treatment approaches for PCD have, accordingly, varied widely both within and between European countries.⁶ Evidence-based treatments for PCD are urgently needed to reduce the morbidity of this lifelong disease and its impacts on quality of life.

Maintenance therapy with macrolide antibiotics has been evaluated in several chronic respiratory disorders, following evidence of the effectiveness of long-term treatment with erythromycin in diffuse panbronchiolitis.¹¹ The macrolide azithromycin has, in addition to its bacteriostatic effects, beneficial anti-inflammatory properties and properties regarding quorum sensing inhibition.^{12,13} In cystic fibrosis, azithromycin maintenance therapy improved FEV₁ by 4% over 6 months and led to a reduction in the use of additional oral antibiotics, compared with placebo (odds ratio [OR] 0.28, 95% CI 0.19–0.42). Patients receiving azithromycin were twice as likely to be free of pulmonary exacerbations compared with placebo.¹⁴ Two randomised placebo-controlled trials evaluating azithromycin maintenance therapy for 6 months and for 12 months in adult patients with non-cystic fibrosis bronchiectasis showed decreases in exacerbations (rate ratio [RR] 0.38, 95% CI 0.26–0.54)

over 6 months, 0 exacerbations (IQR 0–1) in the azithromycin group compared with 2 (IQR 1–3) in the placebo group in the 12-month study,^{15,16} and improvement in lung function and health-related quality of life in the 12-month study.^{15,16} A decreased exacerbation rate was also found in Indigenous children with non-cystic fibrosis bronchiectasis or chronic suppurative lung disease.¹⁷ Azithromycin maintenance therapy is increasingly being used in a number of chronic respiratory disorders, including PCD, despite the absence of data on its efficacy and safety.

The Better Experimental Screening and Treatment for Primary Ciliary Dyskinesia (BESTCILIA) trial aimed to determine the efficacy and safety of azithromycin maintenance therapy in patients with PCD.

Methods

Study design and participants

The BESTCILIA study was a multicentre, double-blind, parallel group, randomised, placebo-controlled, phase 3 trial done at six PCD clinics in tertiary paediatric care centres and university hospitals in Europe (Amsterdam, Netherlands; Bern, Switzerland; Copenhagen, Denmark; Muenster, Germany; and London and Southampton, UK). Further details of study design and methodology can be found in the study protocol published previously.¹⁸

Patients were eligible for inclusion in the study if they had a confirmed diagnosis of PCD (appendix p 1); were 7–50 years old; had predicted FEV₁ greater than 40%; had received at least 30 days of antibiotics prescribed for respiratory tract infections or exacerbations within the preceding 2 years; currently received no systemic or inhaled maintenance antibiotics; and had not taken azithromycin within 1 month before screening. Exclusion criteria at screening were current infection with *Achromobacter xylosoxidans* or *Burkholderia cepacia* complex, infection with non-tuberculous mycobacteria within 6 months, or chronic infection with *Pseudomonas aeruginosa* (defined as culture of *Pseudomonas aeruginosa* in 50% or more of the sputum samples within the last year, provided at least three sputum cultures were available). Other exclusions were: allergic reaction to macrolide antibiotics or other ingredients of the study drug; alanine transaminase twice or more the upper limit of normal or history of portal hypertension; serum creatinine concentrations greater than 150 µmol/L or glomerular filtration rate of less than 50 mL/min; prolonged QT interval, cardiac arrhythmia, severe heart failure, or electrolyte disturbances; myasthenia gravis; treatment with medicinal products known to possibly interact with azithromycin or prolong QT interval (appendix p 1); pregnancy, breastfeeding, or fertile women using unreliable contraception; or use of home oxygen or assisted ventilation.

The study was approved by the ethics committees at the participating institutions and the national competent authorities in the participating countries. The study was done in accordance with the Declaration of Helsinki and Good Clinical Practice. All patients and parents on behalf of their children provided written informed consent prior to inclusion.

The study was monitored by regional Good Clinical Practice monitors.

Randomisation and masking

Participants were randomly assigned to azithromycin or placebo (1:1), stratified by age (7–12 years, 13–21 years, and 22–50 years) and study site (the anticipated three least recruiting study sites comprising the sites in Bern, Amsterdam, and Southampton were merged for stratification purposes to ensure overall balance).¹⁸ The random allocation sequence was a permuted block randomisation with block size of four, which had been generated by an external consultancy (DEFACTUM, Central Denmark Region, Aarhus, Denmark). Block size was not masked to study personnel. A web-based data and randomisation system (TrialPartner, DEFACTUM) was used for randomisation by designated study staff. The study drug was prepared by a Good Manufacturing Practice certified hospital pharmacy (Apotheek Haagse Ziekenhuizen, Haag, Netherlands), who bought the azithromycin tablets from a pharmaceutical company (Centrafarm, Etten-Leur, Netherlands) and manufactured

identical placebo tablets. Blinded packaging and labelling of the study drugs were done centrally at the hospital pharmacy before delivery to the study sites. Participants, investigators, study personnel, and care providers were masked to treatment allocation until after the end of the trial. The success of masking has not been assessed.

Procedures

The intervention was oral tablets of azithromycin 250 mg or 500 mg according to bodyweight (</≥ 40 kg) or identical tablets of placebo, self-administered three times a week (Monday, Wednesday, and Friday) for 6 months. Pre-study medication continued unchanged, except for maintenance antibiotics and certain prohibited medications (appendix p 1). Additional systemic and inhaled antibiotic treatment was allowed during clinical exacerbations and in cases where infection with *P aeruginosa*, *A xylosoxidans*, or *B cepacia* complex emerged during the study. Treatment with the study drug continued simultaneously with antibiotics prescribed for exacerbations and infections.

Patients were screened for eligibility and randomised in a clinically stable state. Screening included spirometry, electrocardiogram to exclude arrhythmias and prolonged QT interval, and sputum analysis to exclude non-tuberculous mycobacteria. Screening was followed by a 1-month run-in period to ensure stable lung function (maximal decrease in percent of predicted FEV₁ of 10 percentage points from screening to randomisation) and washout of any prohibited medications. Patients were withdrawn from the study if they received antibiotics to treat an exacerbation or infection for more than 14 days during the run-in period. Study visits were scheduled every second month during the treatment period (at 2 months, 4 months, and 6 months). Patients were instructed to contact the study sites in between their scheduled study visits if they experienced symptoms of an exacerbation, and to complete a weekly diary card on symptoms and antibiotic use. All study visits included assessments of symptoms of exacerbation, adverse events, and concomitant medications, a physical examination including vital signs, completion of the newly developed and validated PCD-specific health-related quality of life questionnaire (QOL-PCD),^{19–22} nitrogen multiple breath washout (N₂ MBW) using identical equipment and software versions across all sites, spirometry, body plethysmography, sputum culture and susceptibility testing, and urine pregnancy test in all sexually active, fertile women. Additional tests done at the randomisation visit and at the 6-month final study visit were audiometry and tympanometry, blood tests (haematology, C-reactive protein, kidney and liver function), and sampling of serum and sputum for later centralised analysis of cytokines (schedule of assessments; appendix p 4). Adherence with the study drug was assessed by count of returned study drugs and participant or parent report. All adverse events, regardless of severity or presumed causality, occurring from the

See Online for appendix

first administration of study drug to the final study visit or withdrawal from the study were recorded. Patients were withdrawn during the study if they met the safety-related exclusion criteria, were lost to follow-up, withdrew consent, had poor compliance, developed infection with non-tuberculous mycobacteria, used prohibited medications, or reported serious or intolerable adverse reactions.

Changes to the eligibility criteria made after initiation of the study were: the age range was increased to 50 years of age from an original maximum age of 40 years owing to recruitment difficulties; participants were allowed to use local maintenance antibiotics (eg, antibiotic cream) except for inhaled antibiotics, since this was not considered to affect the endpoints; and up to 14 days of antibiotic treatment (except macrolide antibiotics) was allowed throughout the run-in period. Originally, no changes in antibiotics or respiratory medicine were allowed during the run-in period but this restriction turned out to be untenable in the PCD population under study. These protocol amendments were approved by the ethics committees and the competent authorities in 2015.

Outcomes

The primary outcome was number of respiratory exacerbations over 6 months. A per-protocol respiratory exacerbation was defined as any respiratory tract symptoms leading to initiation of systemic antibiotics, irrespective of the results of bacterial culture, or decline in percent of predicted FEV₁ of 10 percentage points or greater relative to the average of percent of predicted FEV₁ at screening and randomisation,²³ whether antibiotics were prescribed or not. All exacerbations were reviewed by the coordinating investigator team before database lock and unmasking of the treatment allocations to ensure that they were in accordance with the above definition.

Secondary outcomes were:¹⁸ changes over 6 months in FEV₁, forced vital capacity (FVC), and forced expiratory flow at 25–75% of FVC (FEF_{25–75}) in percent predicted;²³ residual volume, ratio of residual volume to total lung capacity, and airway resistance in percent predicted;^{24,25} lung clearance index and the indices $S_{\text{cond}} \cdot V_T$ and $S_{\text{acin}} \cdot V_T$ (thought to reflect ventilation inhomogeneity of the conducting and intra-acinar airways) in absolute values, derived from N₂ MBW; and the three symptom scales from the QOL-PCD instrument (respiratory symptoms, sinus symptoms, and ear and hearing symptoms); changes from baseline to 6 months in pure tone average and discrimination loss, measured by audiometry; tympanograms; and in inflammatory markers (white blood cells including differential cell counts, C-reactive protein, the cytokines interleukin (IL) 1 β , granulocyte-colony stimulating factor (G-CSF), and IL8 in serum, and the cytokines IL1 β , G-CSF, IL8, IL10, tumour necrosis factor α , growth-regulated oncogene α , and monocyte chemoattractant protein-1 in sputum); sputum

microbiology (number of pathogenic airway bacterial species and resistance to macrolides); and adverse events and serious adverse events (time of assessment of the outcomes; appendix p 5).

After trial commencement the protocol was amended regarding the primary outcome, because the original definition was too narrow to include all the desired cases of this variable. Originally, the primary outcome was defined as “respiratory tract symptoms leading to prescription of antibiotic treatment by either an investigator or another physician consulted by the subject”. However, some participants started antibiotics themselves when experiencing their usual symptoms of exacerbation, before consulting a physician—therefore the requirement of prescription of the antibiotics by a physician was removed from the protocol. No changes were made to the part of the primary outcome concerning decline in FEV₁. Total and differential cell count in sputum was originally part of the secondary outcome on inflammatory markers but was deleted from the protocol because it was considered complicated to do uniformly among the study sites. The protocol amendment was done during the recruitment period of the study and approved by the ethics committees and the competent authorities in 2015.

Statistical analysis

The sample size calculation estimated that 50 patients per treatment group would have to complete the study to have 90% power to detect a 50% reduction in rate of exacerbations in the treatment period in the azithromycin group (corresponding to a RR of 0.50)—assuming a mean of 2.5 exacerbations per year in the placebo group, using the Poisson distribution and a two-sided α level of 0.05. Estimating a 20% drop-out rate, 125 patients would have to be randomly assigned. The power would be 70% to detect a between-group difference of 5 percentage points in the pre-intervention to post-intervention change in percent of predicted FEV₁ (assuming a SD of 10% for intra-participant change)²⁶ and 88% to detect a similar between-group difference in lung clearance index (assuming a SD of 8% for intra-participant change). The assumptions used for the sample size calculations were based on experience and consensus among experts in the BESTCILIA consortium, since no published evidence existed on the yearly rate of exacerbations or trend in spirometric lung function and ventilation inhomogeneity in PCD at the time the study protocol was elaborated. The rationale for the aim of detecting a 50% reduction in exacerbations in the azithromycin group was based on findings in randomised placebo-controlled studies with azithromycin maintenance therapy in non-cystic fibrosis bronchiectasis¹⁵ and cystic fibrosis.^{27,28} Analyses were based on the intention-to-treat population, defined as all patients randomly assigned to treatment, except for analyses of sputum microbiology, hearing outcomes, and inflammatory markers, that were based on a modified intention-to-treat population to include all patients with

relevant follow-up visits. Data from drop-outs were included in the analyses when available. The safety analysis population included all patients who received at least 1 dose of assigned treatment.

The number of exacerbations (primary outcome) and the rate of detected pathogenic bacterial species in sputum (secondary outcome) in the two treatment groups were compared using the negative binomial distribution. The negative binomial distribution provided the best fit to the data in terms of the Akaike information criterion compared to other distributions for count data (the Poisson, zero-inflated Poisson and negative binomial, hurdle Poisson and negative binomial). The number of days from baseline to end of follow-up was used as an offset variable and the analyses were adjusted for the stratification variables (age in groups and study site). For each group, the mean and SD of the primary outcome at 6 months follow-up were determined from the negative binomial distribution using as offset the number of days from baseline to end of follow-up scaled to 6 months. As supplementary analyses, the probability of remaining free of exacerbations and the probability of drop-out were determined using the Kaplan-Meier method. Kaplan-Meier curves were compared by the log-rank test.

Between-group differences in the quantitative secondary outcomes over the 6 months treatment period were assessed using linear mixed models. The fixed part of the models included the interaction between treatment group and visit as qualitative explanatory variable with the constraint that the means in the two treatment groups were assumed equal at baseline due to randomisation. The random part of the model included a random intercept for each patient. In the analyses of the hearing outcomes, an additional random effect of ear within individual was included as measurements were taken on both the left and right ear. All analyses were adjusted for the stratification variables as fixed effects. The linear mixed effects models of the QOL-PCD outcomes were adjusted for the age-appropriate versions of the QOL-PCD instead of the stratifying age variable.

Tympanograms, a secondary outcome, was redefined as a binary variable, where type A curves represented normal and types B and C curves were combined to represent abnormal and analysed using logistic regression model with estimates obtained from generalised estimating equations with an unstructured working correlation matrix. Adverse events and macrolide resistance that emerged during the 6-month treatment period were compared in the two groups by χ^2 test or Fisher's exact test, as appropriate.

Two-sided p-values of less than 0.05 were considered statistically significant. The statistical analyses were done in R version 3.6.1 and in SAS version 9.4.

This study is registered in the EU Clinical Trials Register, EudraCT number 2013-004664-58.

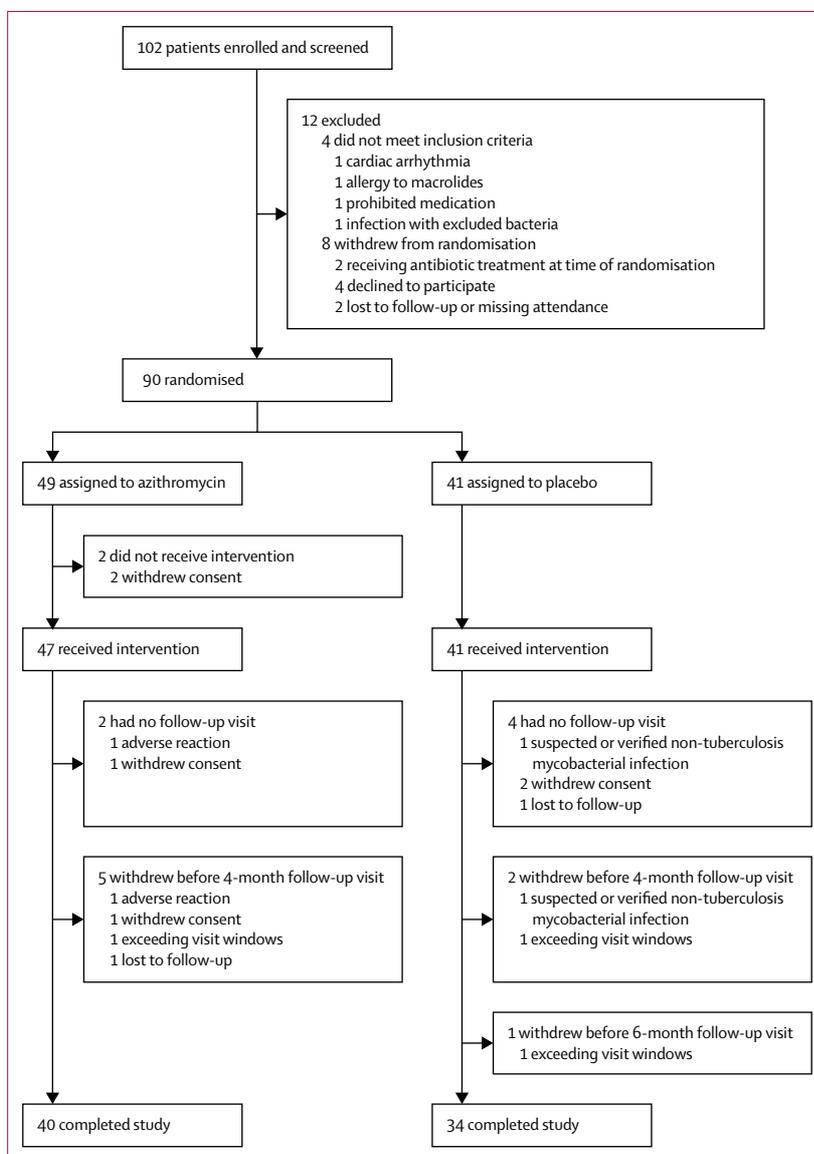


Figure 1: Trial profile

Role of the funding source

The financial sponsors of the study had no role in study design, data collection, analysis, interpretation of the data, or in writing of the report. HEK, FFB, and KGN had access to the raw data. KGN had full access to all the data and the final responsibility to submit for publication.

Results

Between June 24, 2014, and Aug 23, 2016, 102 patients were screened, of whom 90 were randomly assigned to receive either azithromycin (n=49) or placebo (n=41) (figure 1). Follow-up of the last participant ended in May, 2017. The study ended without having included the planned number of participants due to recruitment

	Azithromycin (N=49)	Placebo (N=41)
Female sex	22 (45%)	21 (51%)
Age (years)	18.6 (8.9)	19.7 (10.8)
Age groups		
7-12 years	15 (31%)	11 (27%)
13-21 years	20 (41%)	18 (44%)
22-50 years	14 (29%)	12 (29%)
Pulse oximetric saturation (%)	97.6% (1.6)	97.7% (1.3)
Respiratory rate (breaths per min)	16.7 (3.8)	15.3 (3.1)
Body-mass index (kg/m ²)	19.9 (3.7)	20.5 (4.1)
Spirometric lung function		
FEV ₁ % predicted*	76.9% (15.0)	76.5% (12.7)
FVC % predicted*	89.6% (12.9)	89.3% (13.1)
FEF ₂₅₋₇₅ % predicted*	57.4% (27.0)	53.4% (19.4)
Lung volumes and resistance		
RV % predicted†	164.5% (46.6), n=47	164.1% (50.1), n=37
TLC % predicted†	101.5% (14.0), n=47	99.3% (14.6), n=37
RV/TLC % predicted†	152.9% (35.0), n=47	159.1% (36.1), n=37
Raw % predicted†	126.2% (53.2), n=47	147.6% (71.1), n=35
N ₂ Multiple Breath Washout indices		
LCI (absolute values)	10.7 (2.4), n=45	11.2 (2.6), n=39
S _{cond} *V _T (absolute values)	0.062 (0.025), n=45	0.062 (0.028), n=39
S _{asin} *V _T (absolute values)	0.157 (0.083), n=45	0.164 (0.123), n=39
Hearing		
Pure tone average, air conduction (dB)	21.7 (11.4), n=48	22.2 (17.0)
Discrimination loss (%)	2.2% (3.9), n=35	1.6% (4.6), n=30
Peripheral blood cells		
White blood cells (×10 ⁹ /mL)	8.0 (2.5)	7.9 (2.7)
Neutrophils (×10 ⁹ /mL)	4.8 (2.2), n=47	4.7 (2.3)
Eosinophils (×10 ⁹ /mL)	0.2 (0.2), n=47	0.2 (0.2)
C-reactive protein (mg/L)‡	2.9 (5.9), n=47	2.9 (4.7)
QOL-PCD scale scores§		
Respiratory symptoms	67.31 (16.39), n=47	59.40 (15.69), n=40
Sinus symptoms	61.91 (18.92), n=47	62.33 (22.55), n=40
Ear and hearing symptoms	74.47 (24.10), n=47	75.00 (21.60), n=40
Respiratory symptoms (parent proxy)	65.81 (17.69), n=13	68.52 (19.84), n=9

(Table 1 continues in next column)

difficulties¹⁸ that persisted despite a prolonged recruitment period and due to lack of funding for further extension. The decision to terminate the study prematurely (in regard of the planned number of participants) was made by the coordinating investigator team and the principal investigators. No interim analyses or renewed power

	Azithromycin (N=49)	Placebo (N=41)
(Continued from previous column)		
Sinus symptoms (parent proxy)	56.41 (23.11), n=13	66.67 (26.02), n=9
Ear and hearing symptoms (parent proxy)	73.50 (20.56), n=13	85.19 (13.61), n=9
Respiratory medications		
Any	35 (71%)	28 (68%)
Inhaled β ₂ agonists		
Short-acting	12 (24%)	13 (32%)
Long-acting	5 (10%)	5 (12%)
Inhaled corticosteroids		
Corticosteroid alone	4 (8%)	7 (17%)
Combined with long-acting β ₂ agonists	12 (24%)	8 (20%)
Inhaled anticholinergics	2 (4%)	0
Leukotriene-receptor antagonist	1 (2%)	1 (2%)
Mucolytic agent		
Nebulised isotonic saline	8 (16%)	6 (15%)
Nebulised hypertonic saline	10 (20%)	9 (22%)
Dornase alfa	1 (2%)	1 (2%)
Other (acetylcysteine, bromhexine)	1 (2%)	1 (2%)
Sino-nasal rinse (saline)	15 (31%)	7 (17%)
Nasal corticosteroids	9 (18%)	9 (22%)
Nasal xylometazoline	3 (6%)	0

Data are n (% of the total number of participants) and mean (SD). The number of participants available for specific variables is stated in each cell if different from the total number of participants in the treatment group. FVC=forced vital capacity. FEF₂₅₋₇₅=forced expiratory flow at 25-75% of forced vital capacity. RV=residual volume. RV/TLC=residual volume divided by total lung capacity. Raw=airway resistance. N₂=nitrogen. LCI=lung clearance index. S_{cond}*V_T=regional ventilation inhomogeneity of the conducting airways corrected for tidal volume. S_{asin}*V_T=regional ventilation inhomogeneity of the intra-acinar airways corrected for tidal volume. QOL=quality of life. *Reference values from Quanjer and colleagues.²³ †Reference values from Koopman and colleagues²⁴ for participants aged 7-18 years and from Verbanck and colleagues²⁵ for participants ≥19 years. ‡At some study sites, C-reactive protein <2.5 was not measured more accurately (azithromycin group n=13, placebo group n=12); to calculate mean (SD), C-reactive protein was in these cases set to zero. §Scores range from 0 to 100, with higher scores indicating better health-related QOL.

Table 1: Baseline characteristics of participants by treatment group

calculations had been done at the time the study was terminated. The treatment groups had very similar baseline characteristics (table 1), except lower percent predicted mean airway resistance in the azithromycin group than in the placebo group (126.2 vs 147.6), higher mean respiratory symptom scores on the QOL-PCD in the azithromycin group (67.31 vs 59.40), and lower parent proxy scores on the sinus symptoms and ear and hearing symptoms scales in the azithromycin group (56.41 vs 66.67, and 73.50 vs 85.19). 16 (18%) of 90 participants withdrew from the study over the 6-month treatment period. The reasons for drop-out in the two treatment groups are stated in figure 1. There was no

difference in the cumulative probability of drop-out between the two groups ($p=0.6$).

87 events fulfilled the per-protocol definition of a respiratory exacerbation (31 events in the group receiving azithromycin and 56 in the placebo group). The mean number of respiratory exacerbations over 6 months was 0.75 (SD 1.12) in the azithromycin group compared with 1.62 (1.64) in the placebo group. Patients receiving azithromycin had a significantly lower rate of exacerbations during the individual treatment periods (RR 0.45, 95% CI 0.26–0.78; $p=0.004$), compared with those receiving placebo. The cumulative number of respiratory exacerbations per treatment group from baseline to the last observed visit per individual is shown in figure 2. The probability of remaining free from respiratory exacerbations was 57% (95% CI 44–75) in the azithromycin group compared with 30% (17–51) in the placebo group at 180 days of follow-up. The probability of remaining free from exacerbations during follow-up clearly favoured azithromycin ($p=0.01$; figure 3).

Change in percent of predicted FEV₁ over 6 months did not differ between the groups. At the 6-month visit, a mean between-group difference of 3.09 (95% CI 0.18–6.00; $p=0.0375$) was found; however, no difference were apparent at the 2-month and 4-month visits and the p value should be considered in light of multiple testing. No between-group differences were found in percent of predicted FVC or FEF_{25–75}, or in plethysmographic outcomes or ventilation inhomogeneity indices: residual volume, ratio of residual volume to total lung capacity, and percent of predicted airway resistance, lung clearance index, $S_{\text{cond}} \cdot V_T$, and $S_{\text{acin}} \cdot V_T$ (table 2 and appendix pp 7–8).

No statistically significant between-group differences were seen in changes over 6 months in the three prespecified endpoints from the QOL-PCD instrument: respiratory symptoms, sinus symptoms, and ear and hearing symptoms (table 2 and appendix pp 7–8).

Changes in hearing outcomes and inflammatory markers (peripheral blood cells, C-reactive protein, and cytokines in serum and sputum) from baseline to the 6-month visit did not differ between the treatment groups (table 2 and appendix p 8).

224 sputum cultures were available from the three scheduled follow-up visits, of which 90 (40%) were positive for pathogenic airway bacteria and 75 (83%) of these were tested for susceptibility to macrolides. Occurrence of pathogenic airway bacteria at baseline, at the 6-month visit, and bacteria that emerged during the 6 months treatment period are shown in table 3. The mean number of pathogenic airway bacterial species over the individual treatment periods was 0.93 (SD 1.37) in the azithromycin group versus 2.41 (2.18) in the placebo group, showing a significant difference in favour of the azithromycin group (mean difference 1.47, 95% CI 0.65–2.30; $p=0.0007$). The rate of detected pathogenic bacterial species during the individual treatment periods

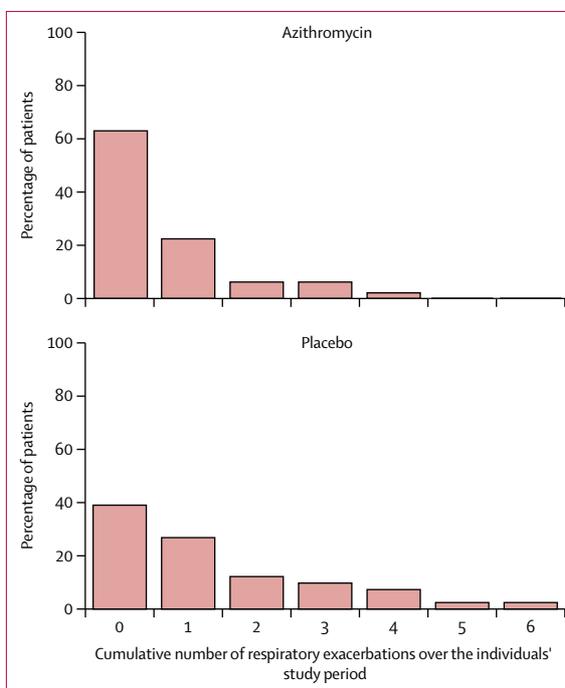


Figure 2: Cumulative number of respiratory exacerbations per treatment group from baseline to the last observed visit per individual

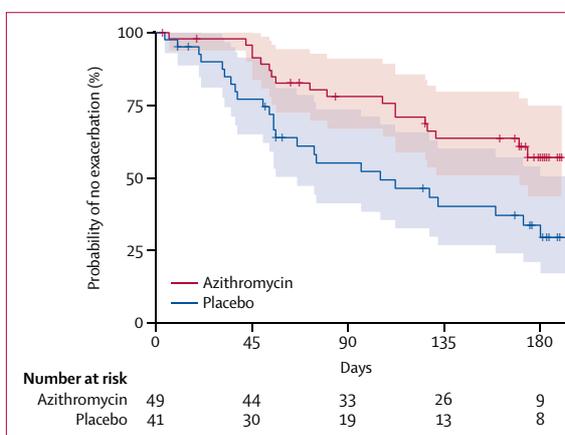


Figure 3: Probability of remaining free from exacerbations during the follow-up

was significantly lower in the azithromycin group compared with the placebo group (RR 0.34 [95% CI 0.21–0.54], $p<0.0001$). 26 (58%) of 45 participants in the azithromycin group had no pathogenic airway bacteria in sputum over the individual treatment periods compared with eight (22%) of 37 participants in the placebo group. Figure 4 shows the cumulative number of pathogenic airway bacteria detected over the treatment period per individual.

Serious adverse events were reported in four participants: two participants (one from each group) were admitted to hospital for a per-protocol respiratory

	Change from baseline to 6 months (95% CI)		Between-group difference (95% CI)	p value
	Azithromycin (n=49)	Placebo (n=41)		
Dynamic lung function				
FEV ₁ % predicted	0.05 (-1.94 to 2.05), n=40	-3.04 (-5.20 to -0.87), n=40	3.09 (0.18 to 6.00)	0.038
FVC % predicted	-0.13 (-2.18 to 1.91), n=40	-1.48 (-3.69 to 0.73), n=34	1.35 (-1.61 to 4.31)	0.37
FEF ₂₅₋₇₅ % predicted*	0% (-7% to 7%), n=40	-9% (-16% to 2%), n=34	10% (-1% to 22%)	0.06
Lung volumes				
RV % predicted	-4.98 (-15.24 to 5.29), n=32	-3.12 (-13.94 to 7.71), n=29	-1.86 (-16.32 to 12.60)	0.80
RV/TLC % predicted	-5.69 (-13.32 to 1.94), n=32	-0.55 (-8.61 to 7.50), n=29	-5.14 (-15.92 to 5.64)	0.35
Airway resistance				
Raw % predicted†	-0.02 (-0.27 to 0.23), n=32	-0.33 (-0.59 to -0.06), n=29	0.31 (-0.05 to 0.67)	0.09
Ventilation inhomogeneity				
LCI	0.06 (-0.43 to 0.55), n=36	-0.40 (-0.91 to 0.11), n=33	0.46 (-0.23 to 1.15)	0.19
S _{cond} *V _T	0.001 (-0.007 to 0.010), n=36	0.003 (-0.006 to 0.011), n=32	-0.001 (-0.013 to 0.010)	0.80
S _{acin} *V _T ‡	-0.028 (-0.054 to -0.002), n=36	0.009 (-0.019 to 0.036), n=32	-0.036 (-0.074 to 0.001)	0.054
QOL-PCD scale scores‡				
Respiratory symptoms	2.13 (-2.56 to 6.83), n=39	3.80 (-1.26 to 8.86), n=33	-1.66 (-8.15 to 4.82)	0.61
Sinus symptoms	3.62 (-1.51 to 8.75), n=39	-0.22 (-5.76 to 5.32), n=33	3.84 (-3.39 to 11.07)	0.30
Ear and hearing symptoms	2.80 (-2.49 to 8.08), n=39	1.60 (-4.12 to 7.31), n=33	1.20 (-6.33 to 8.72)	0.75
Hearing				
Pure tone average (dB)	-0.11 (-0.20 to -0.02), n=39	-0.04 (-0.14 to 0.06), n=32	0.07 (-0.06 to 0.20)	0.30
Discrimination loss (%)	-1.17 (-2.29 to -0.05), n=30	-0.86 (-2.03 to 0.32), n=27	-0.31 (-1.85 to 1.23)	0.69
Tympanograms	n=35	n=30	..	0.18

Data are mean (95% CI) or p value (for the between-group difference). Mean changes from baseline and mean differences between groups are estimated from a linear mixed model with an interaction between treatment group and follow-up visit based on all four study visits. The hearing outcomes were only measured at baseline and the 6-month follow-up visit. The number of participants measured at the 6-month follow-up visit is stated in each cell. A table showing mean changes from baseline to follow-up and between-group differences at all three follow-up visits (2 months, 4 months, and 6 months) is available in the appendix (pp 7–8). FVC=forced vital capacity. FEF₂₅₋₇₅=forced expiratory flow at 25–75% of forced vital capacity. RV=residual volume. RV/TLC=residual volume divided by total lung capacity. Raw=airway resistance. LCI=lung clearance index. S_{cond}*V_T=regional ventilation inhomogeneity of the conducting airways corrected for tidal volume. S_{acin}*V_T=regional ventilation inhomogeneity of the intra-acinar airways corrected for tidal volume. QOL-PCD=PCD-specific health-related quality of life questionnaire. *FEF₂₅₋₇₅ % predicted was log2 transformed for the analysis and has been back-transformed. The data are presented as relative changes. †Raw % predicted and S_{acin}*V_T were square root transformed for the analyses and are presented on square root scale. ‡The QOL-PCD scores are based on data from the QOL-PCD questionnaires completed by the children themselves, adolescents and adult participants. Data from the parent proxy version of the QOL-PCD questionnaire is not included in this table.

Table 2: Difference in secondary outcomes at 6-month follow-up

exacerbations requiring intravenous antibiotics; one participant from the placebo group was admitted to hospital because of upper abdominal pain which resolved without treatment; and another participant from the placebo group was hospitalised for surgical removal of an ovarian cyst, which turned out to be a teratoma. Per-protocol, respiratory exacerbations were not viewed as adverse events, and excluding these, any adverse event was reported by 37 (79%) of 47 participants in the azithromycin group versus 31 (76%) of 41 participants in the placebo group. The most common adverse events are listed in table 4. Loose stools or diarrhoea were reported more frequently in the azithromycin group than the placebo group (11 [23%] of 47 participants vs 2 [5%] of 41 participants; p=0.017). Four participants had self-reported auditory complaints: one from each group reported (increased) hearing impairment as a separate symptom (not part of exacerbation or middle ear inflammation), and one of these participants plus another two participants (azithromycin group) reported tinnitus or worsening of

tinnitus. Six participants (two in the azithromycin group and four in the placebo group) had an increase in hearing threshold of 5 dB or more after the 6-month treatment period, of which two (placebo group) had a hearing threshold above the normal range (>25 dB). Alanine transaminase increased to abnormal values from baseline to the 6-month visit in three participants; however, only one participant (azithromycin group) had a value more than twice the upper limit of normal. Three participants (two from the azithromycin group and one from the placebo group) had mild or moderate eosinophilia after the 6-month treatment period. No participants developed leucopenia, neutropenia, or abnormal increase in creatinine. Two participants in the placebo group were withdrawn due to suspected or verified non-tuberculous mycobacteria and one participant in the placebo group had a positive sputum culture for *Mycobacterium gordonae* within a month after they had ended the study. No significant between-group differences were seen in the emergence of macrolide-resistant pathogenic airway bacteria (table 3).

	Baseline		6-month study visit		Emerged during treatment period	
	Azithromycin (n=49)	Placebo (n=41)	Azithromycin (n=40)	Placebo (n=34)	Azithromycin (n=45)	Placebo (n=37)
Culture of pathogenic bacteria	26 (53%)	22 (54%)	11 (28%)	21 (62%)
Haemophilus influenzae	16 (33%)	14 (34%)	6 (15%)	16 (47%)	4 (11%), n=38	14 (50%), n=28
Macrolide-resistant <i>H influenzae</i>	1 (2%)	0	3 (8%)	1 (3%)	3 (7%), n=44	2 (5%)
Streptococcus pneumoniae	11 (22%)	8 (20%)	2 (5%)	3 (9%)	2 (5%), n=40	5 (15%), n=33
Macrolide-resistant <i>S pneumoniae</i>	2 (4%)	0	1 (3%)	0	4 (9%), n=44	1 (3%)
Moraxella catharrhalis	7 (14%)	5 (12%)	0	4 (12%)	0	11 (31%), n=36
Macrolide-resistant <i>M catharrhalis</i>	0	0	0	0	0	0
Staphylococcus aureus	5 (10%)	3 (7%)	4 (10%)	6 (18%)	6 (14%), n=44	8 (22%), n=36
MRSA	1 (2%)	0	0	0	0	1 (3%)
Macrolide-resistant <i>S aureus</i>	1 (2%)	0	3 (8%)	2 (6%)	5 (11%)	2 (5%)
Pseudomonas aeruginosa	0	3 (7%)	2 (5%)	1 (3%)	6 (13%)	1 (3%), n=36
Stenotrophomonas maltophilia	0	0	0	1 (3%)	1 (2%)	2 (5%)

Data at baseline and at final study visit are number of participants (% of total participants), and for those that emerged during the treatment period are number of newly emerged bacteria/macrolide-resistant bacteria (% of participants with newly emerged bacteria or macrolide-resistant bacteria out of participants with at least one follow-up visit, who were negative for the microorganism or macrolide-resistance at baseline). The number of participants available for specific variables is stated in each cell if different from the total number of participants in the treatment group. No sputum samples were culture positive for *Achromobacter xylosoxidans* or *Burkholderia cepacia* complex.

Table 3: Occurrence of pathogenic airway bacteria in sputum cultures and macrolide resistance

Discussion

This first ever multinational randomised controlled trial (RCT) in PCD evaluated the efficacy and safety of an increasingly prescribed maintenance therapy with the potential to provide evidence-based treatment for this lifelong respiratory disease. Azithromycin maintenance therapy for 6 months in PCD significantly reduced the number of respiratory exacerbations to half, compared with placebo. By decreasing exacerbations azithromycin prevents potentially irreversible declines in lung function and reduces the need for additional antibiotic treatments.²⁹ The only secondary outcome which showed a significant between-group difference, was the rate of detected pathogenic bacterial species in sputum, which was more than halved by maintenance azithromycin. Azithromycin maintenance therapy was safe, but gastrointestinal symptoms were more common in participants receiving azithromycin.

Previous randomised placebo-controlled trials in cystic fibrosis,^{27,28,30} non-cystic fibrosis bronchiectasis,¹⁵⁻¹⁷ and chronic obstructive pulmonary disease (COPD)³¹ have, similarly to our study, shown efficacy of azithromycin maintenance therapy on exacerbations. The efficacy of maintenance azithromycin on exacerbations has now been proven also to apply to patients with PCD.

The present study could not show any significant differences between the treatment groups in lung function. Similarly to our study, two placebo-controlled trials investigating maintenance azithromycin for 6 months in adult patients with non-cystic fibrosis bronchiectasis¹⁵ and in children and adolescents with cystic fibrosis not infected with *P aeruginosa*,²⁸ respectively, found no significant difference between the treatment groups in FEV₁, nor any between-group difference in FVC

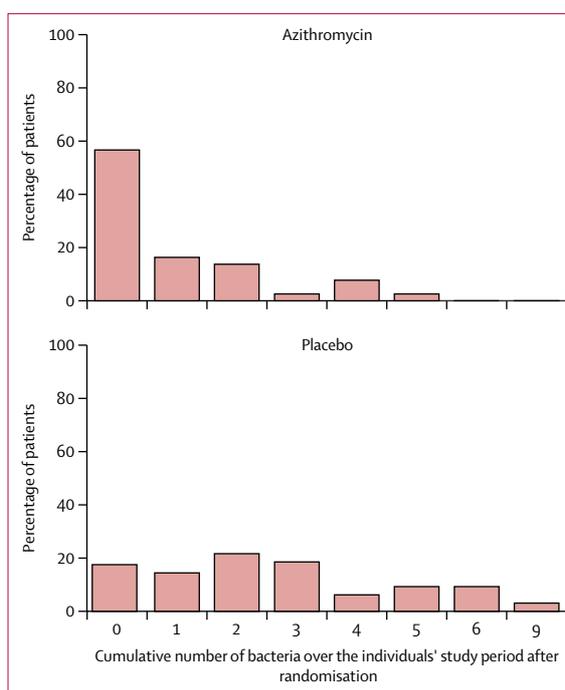


Figure 4: Cumulative number of pathogenic bacteria over the individuals' study period after randomisation

or FEF₂₅₋₇₅ in the latter study.²⁸ In contrast, a 6-month trial in patients with cystic fibrosis chronically infected with *P aeruginosa*³⁰ found a significant relative improvement of 4.4% and 5.0% in FEV₁ and percent of predicted FVC in the azithromycin group compared with the placebo group. Whether maintenance azithromycin will be able to improve lung function in patients with PCD chronically

	Azithromycin (n=47)	Placebo (n=41)	Total (n=88)
Any adverse event	37 (79%)	31 (76%)	68 (77%)
Serious adverse events	1 (2%)	3 (7%)	4 (5%)
Most common adverse events			
Non-per-protocol respiratory tract infections*	12 (26%)	8 (20%)	20 (23%)
Gastrointestinal			
Abdominal pain	11 (23%)	4 (10%)	15 (17%)
Loose stools or diarrhoea	11 (23%) [†]	2 (5%)	13 (15%)
Gastroenteritis	7 (15%)	4 (10%)	11 (13%)
Nausea and/or vomiting	6 (13%)	3 (7%)	9 (10%)
Headache	7 (15%)	6 (15%)	13 (15%)
Fever	6 (13%)	4 (10%)	10 (11%)
Malaise	5 (11%)	5 (12%)	10 (11%)
Fatigue	4 (9%)	4 (10%)	8 (9%)
Pain in extremity	1 (2%) [‡]	7 (17%)	8 (9%)
Middle ear inflammation	2 (4%)	4 (10%)	6 (7%)

Data are number of participants with at least one event (% of total participants). The reported adverse events are those that either developed or increased in severity from receiving the initial dose of study drug until the 6-month visit or withdrawal from the study. The most common adverse events were defined as those that occurred in at least 10% of the participants in either treatment group. *Common cold and other viral respiratory tract infections. [†]p=0.017. [‡]p=0.023.

Table 4: Adverse events

infected with *P aeruginosa* remains to be investigated. We deliberately excluded patients chronically infected with *P aeruginosa* in our study because of huge variation in treatment practises between sites and because it was felt that chronic infection would introduce a high level of treatment activity, with high-dose systemic antibiotic treatment being a critical confounder.¹⁸ The efficacy of a prolonged 12-month azithromycin intervention on lung function has also been evaluated in both cystic fibrosis and non-cystic fibrosis bronchiectasis with different results—a significant relative increase in FEV₁ and percent of predicted FVC of 1.13 and 1.63 per 3 months were seen in adult non-cystic fibrosis bronchiectasis (one patient with PCD included),¹⁶ whereas no between-group difference in FEV₁ and FVC was found in children and adolescents with cystic fibrosis.²⁷ We speculate that a longer intervention period with azithromycin therapy will be required to significantly change lung function in patients with PCD—as was the case in non-cystic fibrosis bronchiectasis—because the annual decline in lung function after diagnosis is on average of limited size.^{5,32}

A secondary focus of this study was evaluation of new outcome measures in PCD. We included MBW indices and symptom scales on the newly developed QOL-PCD instrument as outcome measures for the first time in PCD. Mild to severe ventilation inhomogeneity has been shown in PCD, even in patients with FEV₁ within the normal range,³³ and a minimal, but significant increase in lung clearance index in children and young adults has been observed over a 1-year period.³⁴ Lung clearance index has shown potential as an outcome measure in studies of mild cystic fibrosis.³⁵ However, we could not show any between-group difference in lung clearance

index or the indices of regional ventilation inhomogeneity in this study. No previous trials with maintenance azithromycin have explored MBW indices as outcome measures. As with spirometric lung function, we speculate that a treatment period of 6 months azithromycin might be too short to significantly improve ventilation inhomogeneity.

Contrary to our hypothesis, we could not show efficacy of 6 months azithromycin maintenance therapy on the prespecified endpoints from the QOL-PCD instrument. A possible explanation for why we were not able to show any difference between the treatment groups could be that the participants had high mean scores at baseline, which makes it more difficult to improve the scores significantly. We have analysed the pooled QOL-PCD data from the different age-appropriate versions owing to the small number of participants in each age group, although the versions do not contain the exact same items or same number of items.^{21,22} We consider the pooled analysis approach necessary if the QOL-PCD is to be used as an outcome measure in smaller clinical trials including a wide age range, which will likely also be the case for many future trials in PCD because of the rarity and relatively late diagnosis of the disease. In adult non-cystic fibrosis bronchiectasis patients, a placebo-controlled trial with 6-months azithromycin¹⁵ did not show significant difference in health-related QOL in terms of total score on the St George's respiratory questionnaire, but found a borderline significant improvement in the symptom component of the questionnaire in the azithromycin group, and a similar 12-month study¹⁶ showed improvement in total score on the St George's respiratory questionnaire. The length of the azithromycin intervention might therefore influence change in QOL; however, in children and adolescents with cystic fibrosis, no significant between-group difference in either total score or the physical and psychosocial scores on the cystic fibrosis quality of life questionnaire was seen over 12 months of azithromycin treatment.²⁷ We would expect that an intervention period of 6 months azithromycin should be sufficient to show improvement in the symptom scales on the QOL-PCD.

Similarly to some of the previous clinical trials of maintenance azithromycin in other respiratory disorders, mild gastrointestinal symptoms were overrepresented in the participants receiving azithromycin.^{15,16,30} Gastrointestinal complaints only very rarely led to discontinuation of study drug or withdrawal,^{15,16,30} and thus could be considered as mild and partially expected. No increased frequency of hearing loss was seen in the azithromycin group in our study, though this increase has been found in older patients with COPD;³¹ However, a few participants in our study receiving azithromycin reported tinnitus or worsening of tinnitus. Concern about increased macrolide resistance in common respiratory pathogens has been raised on the basis of previous trials with maintenance azithromycin, in most cases lasting 12 months or

longer.^{16,17,28,31} The present study confirmed that macrolide susceptibility testing of airway bacteria should be done on a regular basis in patients receiving maintenance azithromycin. As our study showed a reduction in the rate of detected pathogenic bacterial species in sputum of more than 50% in the PCD patients receiving azithromycin, and since the macrolide-resistant bacteria can be eradicated with other commonly used antibiotics, we do not consider increased emergence of macrolide-resistant bacteria as a contraindication to maintenance azithromycin. Screening and post-treatment testing for non-tuberculous mycobacteria was done owing to speculation that long-term azithromycin might predispose to infection with non-tuberculous mycobacteria³⁶ and resulted in a few positive tests (all in the placebo group). This finding highlights the importance of screening for non-tuberculous mycobacteria in PCD and of such screening being done before starting long-term azithromycin treatment.

The strengths of the BESTCILIA study include the double-blinded, multicentre, and controlled design and the large sample size for a trial in this relatively rare disease. To our knowledge, this is the first clinical trial in PCD confirming a hypothesis on the treatment of this disease by showing significant change in the primary outcome, and thus the first study in PCD leading to change in disease management and outcome. Reduction in exacerbation rates might potentially prevent progression of lung damage and lung function will probably be better sustained in the long term. Regarding the safety assessment, a strength of this study was the examination of hearing level by audiometry and the high percentage of airway bacteria tested for macrolide susceptibility. The main limitation of the study is that we were not able to include the planned number of participants. Despite this shortcoming, a highly significant reduction in the primary outcome in the azithromycin group compared with the placebo group was still obtained, which might be explained by a higher frequency of exacerbations in the placebo group than assumed in the sample size calculation and by the reduction in exacerbations with azithromycin being a little more than the anticipated 50%. We do not expect that had we obtained the planned number of participants the results of the secondary outcomes would have changed, since no trends towards significant difference in these outcome measures were seen, except for the borderline decline in percent of predicted FEV₁ in the placebo group compared with the azithromycin group after 6-months of treatment. Another limitation of the study is that no definition of exacerbations in PCD existed when this study was initiated. In 2019, an expert consensus definition of pulmonary exacerbations in PCD has been published.³⁷ However, this consensus definition only concerns pulmonary exacerbations. With the definition of 'respiratory exacerbations' used in the present study, we aimed for a definition including exacerbation of both the upper and lower airways, since azithromycin has potential efficacy on both upper and

lower respiratory tract morbidities. In addition, exacerbations in PCD often involve both, and a distinct separation between exacerbations of the upper and lower respiratory tract can be difficult. Doing a post-hoc subgroup analysis based on the newly published expert consensus definition³⁷ would be flawed as the per-protocol exacerbations in the present study do not in all cases include data on the criteria on which the new consensus definition is based. A limitation of the safety assessment of the study is that the statistical tests performed to evaluate between-group differences in adverse events might be considered inadequate since the analyses did not account for the individual treatment periods and repeated adverse events in each patient as we did not have accurate data on the latter. A further challenge of the study was the wide age range including both paediatric and adult patients, necessary due to the rarity of PCD, although a multinational set-up was organised to recruit an adequate number of patients.¹⁸ Finally, since PCD is a congenital disease and children do have early lung injury, we also wanted to include children for the purpose of providing evidence-based treatment for this age group.

In conclusion, this RCT is a first step towards evidence-based pharmacotherapies for PCD. Azithromycin maintenance therapy for 6 months halved exacerbations in patients with PCD and was safe with only a limited tendency for macrolide-resistant bacteria to emerge. Maintenance azithromycin has therefore been shown to be a treatment option to consider for patients with frequent exacerbations, in whom it can reduce the morbidity of exacerbations interrupting everyday life, the need for additional antibiotic treatments, and potentially prevent or reduce the extent of irreversible lung damage. The present study is not capable of answering the question of efficacy and safety of azithromycin maintenance therapy for periods longer than 6 months with certainty or the question of efficacy in patients with PCD and chronic *P aeruginosa* infection and therefore, trials investigating maintenance azithromycin for a prolonged interventions and in patients with chronic *P aeruginosa* are warranted. The finding that both exacerbations and pathogenic bacterial species in sputum were substantially decreased by maintenance azithromycin might give rise to speculation about onset of azithromycin maintenance therapy already in infancy with a view to prevent the development of structural damage and bronchiectasis while carefully observing the likely minimal side-effects.

Contributors

HEK, FFB, EGH, CC, SAC, CH, CEK, JSL, HO, ALQ, CW, CEM, and KGN contributed to the conception and design of the study. HEK, FFB, EGH, CC, SAC, CH, JSL, HO, CW, and KGN participated in data collection. CEM and KT planned and did the analyses of the inflammatory markers. HEK, KGN, SR, and ALS participated in the statistical analysis of the data. All authors participated in the interpretation of the data. HEK, FFB, and KGN drafted the manuscript. EGH, CC, SAC, CH, CEK, JSL, HO, ALQ, CW, CEM, KT, SR, and ALS revised the manuscript. All authors have read and approved the final manuscript.

Declaration of interests

ALQ declares research grants related to chronic respiratory conditions and other rare diseases and consulting income to assist in developing an airway clearance education and training toolkit. All other authors declared no competing interests.

Data sharing

The study protocol is published and available at <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4957315/>. Sharing of data will be considered based on a detailed proposal regarding aim and methods, which can be sent to the email address of the corresponding author: kim.g.nielsen@regionh.dk.

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