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TITLE PAGE

Title:

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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Summary (416 words)

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

Methods: The BESTCILIA (Better Experimental Screening and Treatment for Primary Ciliary Dyskinesia) trial was a multi-centre, double-blind, parallel group, randomised, placebo-controlled phase 3 trial at 6 European PCD clinics at tertiary paediatric level and university hospitals (Denmark, Germany, Netherlands, Switzerland, and United Kingdom). Patients with a confirmed diagnosis of PCD, aged 7-50 years, and FEV₁ >40% predicted, were recruited. Participants were randomly assigned (1:1), stratified by age and study site, via a web-based randomisation system to azithromycin 250 or 500 mg as tablets according to body weight (</≥ 40 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 system exacerbations over 6 months. Analysis was by intention-to-treat. This study is registered in the European Union Clinical Trials Register, number 2013-004664-58.

Findings: Between June 24, 2014, and August 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 (SD) number of respiratory system exacerbations over 6 months was 0.75 (1.12) in the azithromycin group compared to 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 [95% CI]: 0.45 [0.26-0.78], p=0.004). In total, four serious adverse events were reported, occurring in one (2.1%) of 47 participants in the azithromycin group and in three (7.3%) of 41 participants in the placebo group. Loose stools or diarrhoea were more common in the azithromycin group than in the placebo group (11 [23.4%] vs two [4.9%]).

Interpretation: This first multi-national 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 PCD patients with frequent exacerbations potentially leading to reduced need for additional antibiotic treatments and preventing irreversible lung damage.

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Research in context

Evidence before this study

There is a substantial need for evidence-based guidelines specifically for the management of 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 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 optimize 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 randomised controlled trials with maintenance azithromycin for non-cystic fibrosis bronchiectasis revealed two studies in adult patients lasting 6 and 12 months, respectively, 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 BESTCILIA study is the first clinical trial in PCD investigating efficacy of maintenance antibiotics. Azithromycin maintenance therapy for 6 months did significantly reduce respiratory system exacerbations in PCD, compared to placebo, and was well tolerated. The study also demonstrated that maintenance azithromycin more than halved the detection rate of 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 towards 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 logical consequence also reduce progression in lung damage. In a next step studies investigating both maintenance azithromycin over a prolonged intervention period as well as inhaled antibiotic therapy for PCD are warranted.

Introduction (body: 5678 words)

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 rhino-sinusitis, 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 orphan 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 forced expiratory volume in one second (FEV₁) and parameters of bronchial responsiveness in the study with salbutamol.^{9,10} Importantly, it has been difficult to develop evidence-based guidelines for the treatment of PCD because of the paucity of clinical studies, and the scientific evidence for effective treatments is simply lacking in PCD.^{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 effectiveness in long-term treatment with erythromycin for those with 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 with 4% over 6 months and reduced the use of additional oral antibiotics, compared to placebo (odds ratio 0.28, 95% confidence interval [CI] 0.19-0.42). Patients receiving azithromycin were twice as likely to be free of pulmonary exacerbations.¹⁴ Two randomised placebo-controlled trials evaluating azithromycin maintenance therapy for 6 and 12 months, respectively, in adult patients with non-cystic fibrosis bronchiectasis demonstrated decreases in exacerbations, rate ratio 0.38, 95% CI 0.26-0.54 over 6 months and 0 exacerbations (interquartile range [IQR], 0-1), compared with 2 (IQR, 1-3) in the placebo group in the 12 months study,^{15,16} along with improvement in lung function and health-related quality of life.^{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 different chronic respiratory disorders including PCD, despite the lack of data on efficacy and safety.

The BESTCILIA (Better Experimental Screening and Treatment for Primary Ciliary Dyskinesia) trial aimed to determine both the efficacy of azithromycin maintenance therapy for six months on as

primary objective the number of respiratory system exacerbations and as secondary objectives lung function, health-related quality of life, hearing impairment, sputum microbiology, and inflammatory markers in patients with PCD, aged 7-50 years, and concurrently, its safety in PCD.

Methods

Study design and participants

The BESTCILIA study was a multi-centre, double-blind, parallel group, randomised, placebo-controlled, phase 3 trial performed at six European PCD clinics at tertiary paediatric level and university hospitals (Amsterdam, Netherlands; Bern, Switzerland; Copenhagen, Denmark; Muenster, Germany; and London and Southampton, United Kingdom). 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 (see the Appendix for diagnostic criteria); were 7-50 years old; had FEV₁ >40% predicted; had received at least 30 days of antibiotics prescribed for respiratory tract infections or exacerbations within the previous two years; currently received no systemic or inhaled maintenance antibiotics; and had not taken azithromycin within one month prior to 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 $\geq 50\%$ 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 >150 $\mu\text{mol/l}$ or glomerular filtration rate <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); pregnancy, breastfeeding, or fertile women using unreliable contraception; or 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 performed in accordance with the Declaration of Helsinki and Good Clinical Practice. All patients and/or 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 (7·0-12 years; 13·0-21 years; and 22·0-50 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, Central Denmark Region, Aarhus, Denmark) was used for randomisation by delegated 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 and manufactured identical placebo tablets. Blinded packaging and labelling of the study drugs were performed 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.

Procedures

The intervention was oral tablets of azithromycin 250/500 mg according to body weight ($</\geq$ 40 kg) or identical tablets of placebo, self-administered three times a week (Monday-Wednesday-Friday) for six months. Pre-study medication continued unchanged, except for maintenance antibiotics and certain prohibited medications (Appendix). Additional systemic and inhaled antibiotic treatment was allowed during clinical exacerbations and in case of infection with *Pseudomonas aeruginosa*, *Achromobacter xylosoxidans*, or *Burkholderia 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 one-month run-in period to ensure stable lung function (maximal decrease in FEV₁ percent predicted of 10 %-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 exacerbation and 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 performed 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 (see Schedule of assessments – table A2 in the Appendix). Adherence with study drug was assessed by count of returned study drugs and participant/parent report. All adverse events, regardless of severity or presumed causality, occurring from the 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.

The following changes to the eligibility criteria were made after initiation of the study: The age range was increased to 50 years of age from an originally maximum age of 40 years due to recruitment difficulties; participants were allowed to use local maintenance antibiotics e.g. antibiotic cream, except from inhaled antibiotics, since this was not considered to affect the endpoints; and up to 14 days 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 turned out to be untenable in PCD. These protocol amendments were approved by the ethics committees and the competent authorities in 2015.

Outcomes

The primary outcome was number of respiratory system exacerbations over 6 months. A per-protocol respiratory system exacerbation was defined as any respiratory tract symptoms leading to initiation of systemic antibiotics, irrespective of results of bacterial culture, or decline in FEV₁ percent predicted ≥ 10 %-points relative to the average of FEV₁ percent predicted at screening and randomisation,²³ whether antibiotics were prescribed or not. All exacerbations were reviewed by the coordinating investigator team prior to 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 (FEF₂₅₋₇₅) in percent predicted;²³ residual volume (RV), RV/total lung capacity (TLC), and airway resistance (Raw) in percent predicted;^{24,25} lung clearance index (LCI) and the indices $S_{\text{cond}} \cdot V_T$ and $S_{\text{acin}} \cdot V_T$ in absolute values, derived from N₂ MBW; and the three symptom scales from the QOL-PCD instrument (Respiratory Symptoms, Sinus Symptoms, and Ears & Hearing Symptoms); changes from baseline to six 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-1 beta (IL-1 β), granulocyte-colony stimulating factor (G-CSF), and interleukin-8 (IL-8) in serum, and the cytokines IL-1 β , G-CSF, IL-8, interleukin-10 (IL-10), tumor necrosis factor alpha (TNF- α), growth-regulated oncogene alpha (Gro-Alpha), and monocyte chemoattractant protein-1 (MCP-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 is showed in table A3 in the Appendix.

After trial commencement the protocol was amended with regard to the primary outcome, because the original definition was too narrow to include all the desired cases of this variable. Originally, the definition of the primary outcome was “respiratory tract symptoms leading to prescription of antibiotic treatment by either an investigator or another physician consulted by the subject”. However, it turned out that some participants started antibiotics themselves when experiencing their usual

symptoms of exacerbation, before consulting a physician – so therefore the requirement of prescription of the antibiotics by a physician was deleted 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 perform uniformly among the study sites. The protocol amendment was performed 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 rate ratio 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, 125 patients would have to be randomised. The power would be 70% to detect a between-group difference of five percentage points in the pre- to post-intervention change in FEV₁ percent predicted (assuming a SD of 10% for intra-subject change)²⁶ and 88% to detect a similar between-group difference in LCI (assuming a SD of 8% for intra-subject change). The assumptions utilized 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 randomized 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 randomised patients, 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 one dose of assigned treatment.

The rate of exacerbations (primary outcome) and the rate of 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 A-curves represented 'normal' and B- and C-curves were combined to represent 'abnormal' and analysed using logistic regression model with estimates obtained from generalized estimating equations with an unstructured working correlation matrix. Adverse events and macrolide-resistance that emerged during the 6 months treatment period were compared in the two groups by Chi-Square test or Fisher's Exact test, as appropriate.

Two-sided p-values <0.05 were considered statistically significant. The statistical analyses were performed in R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria) and in SAS version 9.4 (SAS Institute Inc., Cary, North Carolina, USA).

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

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. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

Results

Between June 24, 2014, and August 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 was ended without having included the planned number of participants due to recruitment difficulties,¹⁸ despite a prolonged recruitment period, and lack of funding. 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 calculations had been performed at the time the study was terminated. The treatment groups had very similar baseline characteristics (table 1), except lower mean Raw percent predicted 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 & Hearing symptoms scales in the azithromycin group (56.41 vs 66.67, and 73.50 vs 85.19). Sixteen (17.8%) of 90 participants

withdrew from the study over the 6 months 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 ($p=0.6$).

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

Change in FEV₁ percent predicted over 6 months did not differ between the groups (at the 6-month visit, a mean (95% CI) between-group difference of 3.09 (0.18-6.00), $p=0.0375$, was found; however, no trends were apparent at the 2-month and 4-month visits and the p -value should be considered in light of the multiple testing. There were no between-group differences in FVC or FEF₂₅₋₇₅ percent predicted, nor in the plethysmographic outcomes or ventilation inhomogeneity indices: RV, RV/TLC, and Raw percent predicted, LCI, $S_{\text{cond}} \cdot V_T$, and $S_{\text{acin}} \cdot V_T$ (table 2 and Appendix table A5).

There were no statistically significant between-group differences in changes over 6 months in the three pre-specified endpoints from the QOL-PCD instrument: respiratory symptoms, sinus symptoms, and ear & hearing symptoms (table 2 and Appendix table A5).

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 table A6).

In total, 224 sputum cultures were available from the three scheduled follow-up visits, of which 90 (40.2%) were positive for pathogenic airway bacteria and 75 (83.3%) 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 (SD) number of pathogenic airway bacterial species over the individual treatment periods was 0.93 (1.37) in the azithromycin group versus 2.41 (2.18) in the placebo group, demonstrating a significant difference in favour of the azithromycin group [mean (95% CI) difference: 1.47 (0.65-2.30), $p=0.0007$]. The detection rate of pathogenic bacterial species during the individual treatment periods was significantly lower in the azithromycin group compared to the placebo group [rate ratio (95% CI): 0.34 (0.21-0.54), $p<1e-04$]. Twenty-six (57.8%) of 45 participants in the azithromycin group had no pathogenic airway bacteria in sputum over the individual treatment periods compared to eight (21.6%) of 37 participants in the placebo group. Figure 4 shows the cumulative number of pathogenic airway bacteria 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 system exacerbation requiring intravenous antibiotics; one participant from the placebo group was admitted to hospital due to upper abdominal pain which resolved without treatment; and another participant from the placebo group was hospitalized for surgical removal of an ovarian cyst, which turned out to be a teratoma. Excluding per-protocol respiratory system exacerbations, any adverse event was reported by 37 (78.7%) of 47 participants in the azithromycin group versus 31 (75.6%) 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 than placebo group (11 [23.4%] of 47 participants vs 2 [4.9%] 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 azithromycin group and four in placebo group) had an increase in hearing threshold of ≥ 5 dB after the 6-month treatment period, of which two (placebo group) had 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 azithromycin group and one from placebo group) had mild or moderate eosinophilia after the 6 months treatment period. No participants developed leukopenia, neutropenia, or abnormal increase in creatinine. From the placebo group, two participants were withdrawn due to suspected or verified non-tuberculous mycobacteria and one participant had a positive sputum culture for *Mycobacterium gordonae* within a month after he had ended the study. There were no significant between-group differences in the emergence of macrolide-resistant pathogenic airway bacteria (table 3).

Discussion

This first ever multi-national randomised controlled trial 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 rate of respiratory system exacerbations to half, compared to placebo. By decreasing the rate of 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 disease³¹ 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 demonstrate 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 *Pseudomonas aeruginosa*,²⁸ respectively, found no significant difference between the treatment groups in FEV₁, and neither any between-group difference in FVC or FEF₂₅₋₇₅ in the latter study.²⁸ In contrast, a 6-months trial in cystic fibrosis patients chronically infected with *Pseudomonas aeruginosa*³⁰ found a significant relative improvement of 4.4% and 5.0% in FEV₁ and FVC% predicted in the azithromycin group compared to the placebo group. Whether maintenance azithromycin will be able to improve lung function in PCD patients chronically infected with *Pseudomonas aeruginosa* remains to be investigated. We deliberately excluded patients chronically infected with *Pseudomonas 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-months 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 FVC % predicted of 1.13% and 1.63% per 3 months were seen in adult non-cystic fibrosis bronchiectasis (one PCD patient 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 PCD patients - 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 PCD-specific health-related quality of life instrument as outcome measures for the first time in PCD. Mild to severe ventilation inhomogeneity has been demonstrated in PCD, even in patients with FEV₁ within the normal range,³³ and a minimal, but significant increase in LCI in children and young adults has been observed over a 1-year period.³⁴ The LCI is increasingly utilized as outcome in cystic fibrosis, where it has shown potential as outcome measure in mild cystic fibrosis.³⁵ However, we could not demonstrate any between-group difference in LCI or the indices of regional ventilation inhomogeneity in this study. None of the 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 may 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 pre-specified endpoints from the QOL-PCD instrument. A possible explanation why we were not able to demonstrate any difference between the treatment groups, may 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 due to the limited 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 outcome measure in smaller clinical trials including a wide age range, which will likely also be the case for many future trials in PCD due to 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 quality of life 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 therefore could be suspected to influence change in quality of life. 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 azithromycin treatment.²⁷ We would expect that an intervention period of 6 months azithromycin should be sufficient to demonstrate improvement in the symptom scales on the QOL-PCD.

Similarly, to some of the previous clinical trials with maintenance azithromycin, 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 has been found in older patients with chronic obstructive pulmonary disease;³¹ However, a few participants receiving azithromycin reported tinnitus or worsening of tinnitus. Concern about increased macrolide-resistance among common respiratory pathogens has been raised based on 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 performed on a regular basis in patients receiving maintenance azithromycin. As our study showed a reduction in rate of 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 performed due to speculations that long-term azithromycin may predispose to infection with non-tuberculous mycobacteria³⁶ and resulted in a few positive tests (all in the placebo group). This highlights the importance of screening for non-tuberculous mycobacteria in PCD and to perform such screening before starting long-term azithromycin treatment.

The strengths of the BESTCILIA-WP5 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, a highly significant reduction in the primary outcome in the azithromycin group compared to the placebo group was still obtained, which may 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 having obtained the planned number of participants would change the results of the secondary outcomes, since there were no trends towards significant difference in these outcome measures, except for the borderline decline in FEV₁ percent predicted in the placebo group compared to the azithromycin group after 6 months treatment. Another limitation of the study is that there existed no definition of exacerbations in PCD, when this study was initiated. Recently, 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 system exacerbations’ utilized 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 involves both, and a distinct separation between exacerbations of the upper and lower respiratory tract can be difficult. Performing 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 does 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 may 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, even though a multi-national 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 to this age group.

In conclusion, this randomised controlled trial is a first step towards evidence-based pharmacotherapies for PCD. Azithromycin maintenance therapy for 6 months halved exacerbations in PCD patients and was safe with only a limited tendency to emergence of macrolide-resistant bacteria. Maintenance azithromycin has thereby been proven to be an obvious treatment option to consider for patients with frequent exacerbations, where it can reduce the morbidity of exacerbations interrupting everyday life and the need for additional antibiotic treatments and potentially prevent or reduce extent of irreversible lung damage. The present study is not capable of answering the question on efficacy and safety of azithromycin maintenance therapy for longer periods than 6 months with certainty nor the question on efficacy in PCD patients with chronic *Pseudomonas aeruginosa* infection and therefore, trials investigating maintenance azithromycin for a prolonged intervention period and in patients with chronic *Pseudomonas aeruginosa* are warranted. The finding that both exacerbations and pathogenic bacterial species in sputum were substantially decreased by maintenance azithromycin may 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 performed 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.

Conflict of interest

The authors declared no conflicts of interest.

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 send to the email address of the corresponding author: kim.g.nielsen@regionh.dk.

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Table 1: Baseline characteristics of participants by treatment group		
	Azithromycin (n=49)	Placebo (n=41)
Female sex	22 (44.9%)	21 (51.2%)
Age (years)	18.6 (8.9)	19.7 (10.8)
Age groups		
7-12 years	15 (30.6%)	11 (26.8%)
13-21 years	20 (40.8%)	18 (43.9%)
22-50 years	14 (28.6%)	12 (29.3%)
Pulse oximetric saturation (%)	97.6 (1.6)	97.7 (1.3)
Respiratory rate (breaths/min)	16.7 (3.8)	15.3 (3.1)
Body mass index (kg/m²)	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 & resistance		
RV % predicted†	164.5 (46.6) [47]	164.1 (50.1) [37]
TLC % predicted†	101.5 (14.0) [47]	99.3 (14.6) [37]
RV/TLC % predicted†	152.9 (35.0) [47]	159.1 (36.1) [37]
Raw % predicted†	126.2 (53.2) [47]	147.6 (71.1) [35]
N₂ Multiple Breath Washout indices		
LCI (absolute values)	10.7 (2.4) [45]	11.2 (2.6) [39]
S _{cond} *V _T (absolute values)	0.062 (0.025) [45]	0.062 (0.028) [39]
S _{acin} *V _T (absolute values)	0.157 (0.083) [45]	0.164 (0.123) [39]
Hearing		
Pure tone average - air conduction (dB)	21.7 (11.4) [48]	22.2 (17.0)
Discrimination Loss (%)	2.2 (3.9) [35]	1.6 (4.6) [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) [47]	4.7 (2.3)
Eosinophils (×10 ⁹ /mL)	0.2 (0.2) [47]	0.2 (0.2)
C-reactive protein (mg/L)‡	2.9 (5.9) [47]	2.9 (4.7)
QOL-PCD scale scores[§]		
Respiratory symptoms	67.31 (16.39) [47]	59.40 (15.69) [40]
Sinus symptoms	61.91 (18.92) [47]	62.33 (22.55) [40]
Ear & Hearing symptoms	74.47 (24.10) [47]	75.00 (21.60) [40]
Parent proxy - Respiratory symptoms	65.81 (17.69) [13]	68.52 (19.84) [9]
Parent proxy - Sinus symptoms	56.41 (23.11) [13]	66.67 (26.02) [9]
Parent proxy - Ear & Hearing symptoms	73.50 (20.56) [13]	85.19 (13.61) [9]
Respiratory medications		
Any	35 (71.4%)	28 (68.3%)
Inhaled β ₂ agonists		
Short-acting	12 (24.5%)	13 (31.7%)
Long-acting	5 (10.2%)	5 (12.2%)
Inhaled corticosteroids		
Corticosteroid alone	4 (8.2%)	7 (17.1%)
Combined with long-acting β ₂ agonists	12 (24.5%)	8 (19.5%)
Inhaled anticholinergics	2 (4.1%)	0
Leukotriene-receptor antagonist	1 (2.0%)	1 (2.4%)
Mucolytic agent		
Nebulized isotonic saline	8 (16.3%)	6 (14.6%)
Nebulized hypertonic saline	10 (20.4%)	9 (22.0%)
Dornase alfa	1 (2.0%)	1 (2.4%)
Other (acetylcysteine, bromhexine)	1 (2.0%)	1 (2.4%)
Nasal/sinus rinse (saline)	15 (30.6%)	7 (17.1%)
Nasal corticosteroids	9 (18.4%)	9 (22.0%)
Nasal xylometazoline	3 (6.1%)	0

Data are presented as mean (SD) for quantitative variables and counts (% of total) for categorical variables. The number of participants available for the specific variables is stated in square brackets in the table cells if different from the total number of participants in the treatment group.

*Reference values from Quanjer et al. Eur Respir J 2012.²³

†Reference values from Koopman et al. Respir Med 2011 for participants aged 7-18 years and from Verbanck et al. Eur Respir J 2016 for participants ≥19 years.^{24,25}

‡At some study sites, C-reactive protein <2.5 was not measured more accurately (azithromycin group: n=13, placebo group: n=12). In order 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 quality of life.
FEV₁=forced expiratory volume in one second. 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_{acin}*V_T=regional ventilation inhomogeneity of the intra-acinar airways corrected for tidal volume.

	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) [40]	-3.04 (-5.20 to -0.87) [34]	3.09 (0.18 to 6.00)	0.038
FVC % predicted	-0.13 (-2.18 to 1.91) [40]	-1.48 (-3.69 to 0.73) [34]	1.35 (-1.61 to 4.31)	0.37
FEF ₂₅₋₇₅ % predicted*	0% (-7% to 7%) [40]	-9% (-16% to 2%) [34]	10% (-1% to 22%)	0.06
Lung volumes				
RV % predicted	-4.98 (-15.24 to 5.29) [32]	-3.12 (-13.94 to 7.71) [29]	-1.86 (-16.32 to 12.60)	0.80
RV/TLC % predicted	-5.69 (-13.32 to 1.94) [32]	-0.55 (-8.61 to 7.50) [29]	-5.14 (-15.92 to 5.64)	0.35
Airway resistance				
Raw % predicted†	-0.02 (-0.27 to 0.23) [32]	-0.33 (-0.59 to -0.06) [29]	0.31 (-0.05 to 0.67)	0.09
Ventilation inhomogeneity				
LCI	0.06 (-0.43 to 0.55) [36]	-0.40 (-0.91 to 0.11) [33]	0.46 (-0.23 to 1.15)	0.19
S _{cond} *V _T	0.001 (-0.007 to 0.010) [36]	0.003 (-0.006 to 0.011) [32]	-0.001 (-0.013 to 0.010)	0.80
S _{acin} *V _T ‡	-0.028 (-0.054 to -0.002) [36]	0.009 (-0.019 to 0.036) [32]	-0.036 (-0.074 to 7e-04)	0.054
QOL-PCD scale scores‡				
Respiratory Symptoms	2.13 (-2.56 to 6.83) [39]	3.80 (-1.26 to 8.86) [33]	-1.66 (-8.15 to 4.82)	0.61
Sinus Symptoms	3.62 (-1.51 to 8.75) [39]	-0.22 (-5.76 to 5.32) [33]	3.84 (-3.39 to 11.07)	0.30
Ear & Hearing Symptoms	2.80 (-2.49 to 8.08) [39]	1.60 (-4.12 to 7.31) [33]	1.20 (-6.33 to 8.72)	0.75
Hearing				
Pure tone average (dB)	-0.11 (-0.20 to -0.02) [39]	-0.04 (-0.14 to 0.06) [32]	0.07 (-0.06 to 0.20)	0.30
Discrimination Loss (%)	-1.17 (-2.29 to -0.05) [30]	-0.86 (-2.03 to 0.32) [27]	-0.31 (-1.85 to 1.23)	0.69
Tympanograms	[35]	[30]		0.18

The first column shows the mean change from baseline to the 6 months follow-up visit (95% CI) in each treatment group, the second column the mean between-group difference (95% CI) after 6 months follow-up, and the p-value for the between-group difference. The changes and differences 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 months follow-up visit. The number of participants measured at the 6 months follow-up visit is stated in square brackets in the table cells. 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 (table A5).
*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.
FEV₁=forced expiratory volume in one second. 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.

	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 (53.1%)	22 (53.7%)	11 (27.5%)	21 (61.8%)		
<i>Haemophilus influenzae</i>	16 (32.7%)	14 (34.1%)	6 (15.0%)	16 (47.1%)	4 (10.5%) [38]	14 (50.0%) [28]
macrolide-resistant <i>H. Influenzae</i>	1 (2.0%)	0	3 (7.5%)	1 (2.9%)	3 (6.8%) [44]	2 (5.4%)
<i>Streptococcus pneumoniae</i>	11 (22.4%)	8 (19.5%)	2 (5.0%)	3 (8.8%)	2 (5.0%) [40]	5 (15.2%) [33]
macrolide-resistant <i>S. pneumoniae</i>	2 (4.1%)	0	1 (2.5%)	0	4 (9.1%) [44]	1 (2.7%)
<i>Moraxella catharrhalis</i>	7 (14.3%)	5 (12.2%)	0	4 (11.8%)	0	11 (30.6%) [36]
macrolide-resistant <i>M. catharrhalis</i>	0	0	0	0	0	0
<i>Staphylococcus aureus</i>	5 (10.2%)	3 (7.3%)	4 (10.0%)	6 (17.6%)	6 (13.6%) [44]	8 (22.2%) [36]
- MRSA	1 (2.0%)	0	0	0	0	1 (2.7%)
macrolide-resistant <i>S. aureus</i>	1 (2.0%)	0	3 (7.5%)	2 (5.9%)	5 (11.1%)	2 (5.4%)
<i>Pseudomonas aeruginosa</i>	0	3 (7.3%)	2 (5.0%)	1 (2.9%)	6 (13.3%)	1 (2.8%) [36]
<i>Stenotrophomonas maltophilia</i>	0	0	0	1 (2.9%)	1 (2.2%)	2 (5.4%)

Data at baseline and at final study visit are presented as no. of participants (% of total participants).

*Data are presented as no. of newly emerged bacteria/macrolide-resistant bacteria (% of participants with newly emerged bacteria/macrolide-resistant bacteria out of participants with at least one follow-up visit, who were negative for the microorganism/macrolide-resistance at baseline). The number of participants available for the specific variables is stated in square brackets in the table cells 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 4: Adverse events			
	Azithromycin (n=47)	Placebo (n=41)	Total (n=88)
Any adverse event	37 (78.7%)	31 (75.6%)	68 (77.3%)
Serious adverse events	1 (2.1%)	3 (7.3%)	4 (4.5%)
Most common adverse events			
Non-per-protocol respiratory tract infections†	12 (25.5%)	8 (19.5%)	20 (22.7%)
Gastrointestinal			
Abdominal pain	11 (23.4%)	4 (9.8%)	15 (17.0%)
Loose stools or diarrhoea	11 (23.4%)*	2 (4.9%)	13 (14.8%)
Gastroenteritis	7 (14.9%)	4 (9.8%)	11 (12.5%)
Nausea and/or vomiting	6 (12.8%)	3 (7.3%)	9 (10.2%)
Headache	7 (14.9%)	6 (14.6%)	13 (14.8%)
Fever	6 (12.8%)	4 (9.8%)	10 (11.4%)
Malaise	5 (10.6%)	5 (12.2%)	10 (11.4%)
Fatigue	4 (8.5%)	4 (9.8%)	8 (9.1%)
Pain in extremity	1 (2.1%)**	7 (17.1%)	8 (9.1%)
Middle ear inflammation	2 (4.3%)	4 (9.8%)	6 (6.8%)
Data are presented as no. of participants with at least one event (% of total participants). *p=0.017, **p=0.023.			
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.			

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