Once-weekly azithromycin in cystic fibrosis with chronic *Pseudomonas aeruginosa* infection

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**KEYWORDS**
Azithromycin; Cystic fibrosis; Weekly dosage; Inflammation; Quality of life

**Summary**
Background: Data on the effects of long-term treatment with azithromycin (AZM) on inflammatory markers in cystic fibrosis patients chronically infected with *Pseudomonas aeruginosa* are scarce. So far there is no pharmacokinetic and clinical data on once-weekly dosage of AZM in CF patients.

Methods: In a randomised double-blind, placebo-controlled trial, patients received AZM or placebo 1 per week for 8 weeks (AZM dosage – 20–29 kg: 500 mg, 30–39 kg: 750 mg, 40–49 kg: 1000 mg and ≥50 kg: 1250 mg) after a course of intravenous antipseudomonal antibiotics. Pulmonary function tests, the serum markers LPS-binding protein (LBP), interleukin-8 (IL-8), CRP, *P. aeruginosa* alginate in sputum samples and quality of life scores were evaluated.

Results: Thirty-eight patients (21 AZM/17 placebo) (mean age: 23.7 years; mean FEV1: 62% of predicted) were recruited. After treatment (mean dose of 21.2 mg/kg body weight once a week) pulmonary function declined in both groups compared to baseline (i.e. after cessation of IV antibiotics). The AZM group was significantly better for mean changes in serum CRP (AZM: +0.9 mg/l, placebo: +21.6 mg/l, p = 0.019), lipopolysaccharide binding protein in serum, LBP (AZM: +0.9 μg/ml, placebo: +7.0 μg/ml, p = 0.015), serum interleukin-8 (AZM: –3.1 pg/ml, placebo: +2.9 pg/ml, p = 0.001) and alginate in sputum (AZM: +85 μg/ml, placebo: +353 μg/ml, p = 0.048). Quality of life was significantly better after AZM and there was no increase in treatment-related adverse events.

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Introduction

Chronic infection with mucoid variants of *Pseudomonas aeruginosa* affects approximately 80% of adult patients with cystic fibrosis. These pathogens produce alginate, a mucoid exopolysaccharide, which is a major component of biofilms. By growing in microcolonies and biofilms, these pathogens resist both the host’s innate immune mechanism and antibiotic treatments. Thus, even after a clinically successful 2-week course of intravenous antipseudomonal antibiotics, *P. aeruginosa* can be identified in respiratory secretions of the majority of patients, though in decreased numbers and density compared to baseline.

Chronic bacterial infection leads to progressive destruction of airway tissue, respiratory failure, and finally to premature death. Part of the problem is an inflammatory reaction dominated by neutrophils, which are recruited into the bronchial airways where they produce and release proteases and oxidants. Bronchial lavage studies have documented inflammation even in patients with “normal” lung function. Although regular antibiotic treatment with inhaled, oral or intravenous antibiotics, is able to decrease the bacterial load, there is a desire for an effective immunomodulatory drug which could ameliorate the exaggerated immune response.

Macrolides do not seem to have relevant antimicrobial effects against *Pseudomonas* strains, at least under usual laboratory conditions. However, azithromycin and other macrolide drugs possess important anti-inflammatory activities. They appear to suppress the interleukins (IL)-1β, IL-6, IL-8, and tumour necrosis factor-alpha (TNF-α), inhibit neutrophil chemotaxis, and reduce the concentrations of neutrophil elastase. Furthermore, macrolides were able to reduce the integrity of the *P. aeruginosa* biofilms and to interfere with quorum-sensing-dependent virulence factor production in vitro.

Diffuse panbronchiolitis is a lung disease that shares with CF the chronic mucoid *P. aeruginosa* infection. After macrolides had proven to be effective in Japanese patients, physicians in the United Kingdom (UK) used azithromycin in selected patients with cystic fibrosis. A pilot study in children and adolescents showed relevant improvements in lung function. These promising results led to the conduct of several prospective, randomised, double-blind trials comparing azithromycin with placebo. In these studies, azithromycin improved lung function, prolonged the exacerbation-free interval, and was well tolerated. Interestingly, it was recently shown that long-term use of azithromycin in young patients with cystic fibrosis has a beneficial effect on lung disease expression even before *P. aeruginosa* colonization is present. A Cochrane report concluded that there is clear evidence of a small but significant improvement in respiratory function following treatment with azithromycin. As a consequence, many CF centres have prescribed azithromycin as long-term treatment for lung disease in CF.

To elucidate further the clinical and immunomodulatory effects of azithromycin, we planned a trial in patients who had successfully completed a course of intravenous antipseudomonal antibiotics immediately before study medication was commenced. We hypothesized that azithromycin would diminish the decline in lung function usually observed after cessation of intravenous treatment, and that the macrolide would ameliorate inflammatory reactions compared to placebo. In the previous trials, azithromycin had been administered in doses of either 250 or 500 mg per day, or 500 mg administered on Mondays, Wednesdays, and Fridays. This translates into weekly doses of between 1500 and 3500 mg. Considering the pharmacokinetics of azithromycin with effective serum concentrations even after 10 days of administration, we administered the drug only once per week in a dose of 1250 mg (for patients >50 kg). Here we report the clinical and immunomodulatory efficacy, tolerability and impact on quality of life after 8 weeks of treatment with the new therapeutic regimen.

Methods and materials

Study design and eligibility criteria

This was a multicentre, prospective, randomised, double-blind, placebo-controlled clinical study with parallel group design. Patients with cystic fibrosis, chronic *P. aeruginosa* (PA) infection and an acute respiratory exacerbation were eligible for participation if they had successfully completed an IV course of antipseudomonal combination treatment (the majority received a β-lactam antibiotic and an aminoglycoside) within 1 week before the projected start of study medication. Intravenous treatment was considered successful if a clinically relevant improvement of respiratory symptoms and/or lung function was achieved after antibiotic treatment. Chronic *P. aeruginosa* infection was defined as a PA positive sputum/throat swab before the acute episode/exacerbation leading to IV antibiotic treatment, and 2 or more courses of systemic antipseudomonal antibiotics within the 12 months preceding the acute episode (the latter requirement was removed after an amendment to the study protocol). Long-term inhaled antibiotics were allowed if they had been installed at least 4 weeks before the study started and the dosage had not been changed during the study. Further inclusion criteria were: age 8 years or older, body weight ≥20 kg, and forced expiratory volume in 1 s, FEV₁, in a range of 30–80% of the predicted normal value at the end of the initial course of IV antibiotics. Cystic fibrosis had been diagnosed with positive sweat tests or cystic fibrosis transmembrane regulator (CFTR) mutation analyses.

Conclusion: Once-weekly azithromycin ameliorated inflammatory reactions and improved quality of life. A decline of pulmonary function after cessation of IV antibiotics could not be prevented.
Subjects with any of the following conditions were excluded: (a) treatment with any macrolide, azalide or ketolide antibiotic within 10 weeks prior to the baseline visit, (b) concurrent treatment with any systemic antibiotic other than the study medication, (c) regular elective systemic antipseudomonal treatments several times a year in the absence of pulmonary exacerbations, (d) concomitant treatment with systemic corticosteroids, cyclosporine A, ergot alkaloids, or triazolam, (e) acute pneumothorax, (f) bronchial lavage during the hospitalisation preceding visit 1, and (g) colonization with *Burkholderia cepacia* complex.

Further exclusion criteria were clinically relevant renal, cardiac, or hepatic (ALT/AST >3 times of the upper normal limit, portal hypertension) dysfunction, chronic gastrointestinal disease not related to CF, pregnancy, lactation, inadequate contraception in sexually active females, and intolerance or allergy to study medication, macrolides, azalides or ketolides.

Patients were recruited from 11 certified CF centres in Germany (n = 10) and Switzerland (n = 1), which provide both inpatient and outpatient facilities. Ethics committee approval was secured for the protocol from the respective ethics committees.

The duration of the study for the individual patient was 8 weeks. There were 2 study visits: Visit 1 (baseline) was performed at the end of the initial intravenous antibiotic treatment, and Visit 2 (day 56, end of study) took place after 8 weeks of study medication.

**Study medication**

Azithromycin (Zithromax) was provided as 250 mg tablets, and placebo tablets were made to look and taste identical to the antibiotic. Depending on body weight, patients received the following dosages of azithromycin (or matching placebos): 20–29 kg: 500 mg (2 tablets), 30–39 kg: 750 mg (3 tablets), 40–49 kg: 1000 mg (4 tablets), and ≥50 kg: 1250 mg (5 tablets).

Study medication was taken once per week for 8 weeks. The first dose was administered within 1 week after the end of IV treatment. The day of the week when study medication was to be taken was chosen such that it was 3 weekdays before Visit 2 (end of study). Patients were advised to take the tablets after a meal. If the patient forgot to take the study medication, he or she could take it within 3 days of the scheduled administration time point.

**Efficacy endpoints**

**Primary and secondary endpoints**

The primary endpoint was the absolute change in FEV₁ (% predicted) at the end-of-study treatment, compared with the baseline value prior to study medication. Secondary endpoints were changes compared to baseline of clinical signs and symptoms, spirometry, oxygen saturation, bacteriologic assessments, quality of life, inflammatory parameters (C-reactive protein, total IgG, and interleukin-8 (IL-8) in serum) and sputum parameters (alginate production of *P. aeruginosa*, sputum viscoelasticity, chloride secretion, DNA concentrations). Sputum and serum azithromycin concentrations were measured, and the safety and tolerability of azithromycin were documented. Compliance was estimated by calculating the mean number of tablets taken per week minus the number of prescribed tablets per week.

**Clinical symptoms and spirometry**

Cough, dyspnoea, chest pain, and rales/rhonchi were classified as being either absent, mild, moderate or severe, and haemoptysis was categorized as being either absent or present. Sputum volume in the morning was categorized as follows: no sputum, teaspoon, tablespoon, 1/4 cup, or 1/2 cup. Spirometry was performed according to Quanjer et al., and the following parameters were reported: inspiratory vital capacity (IVC), forced vital capacity (FVC), forced expiratory volume at 1 s (FEV₁), and forced expiratory flow between 25 and 75% of expiratory vital capacity (FEF₂₅₋₇₅). Oxygen saturation was determined either by pulse oximetry or by blood gas analysis from arterioles blood from the ear.

**Laboratory methods**

Haematology including differential count, serum concentrations of immunoglobulin G (IgG) and C-reactive protein (CRP), and bacteriological assessments were determined in the local laboratory of each centre using standard laboratory methods.

Alginic production of *P. aeruginosa* was determined at Prof. Gerd Döring’s laboratory (Hygiene Institute, University of Tübingen, Germany) from sputum specimens of at least 3 ml using the carbazole method. *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* in sputum and serum were searched for using both serology (IgG, IgA, IgM) and a polymerase chain reaction (PCR). Specimens were transported to the laboratory of Prof. Marianne Abele-Horn (Institute for Microbiology, University of Würzburg, Germany). Azithromycin concentrations in sputum and serum were measured with HPLC and mass spectrometry at the Department of Pharmacology and Pharmacoepidemiology, University of Heidelberg, Germany (Prof. Walter E. Haefeli). Samples were obtained on day 56, 3 days after the last dose of study medication had been taken. Interleukin-8 (IL-8) and lipopolysaccharide binding protein (LBP) were analyzed using a two-sided chemiluminescent test (Immulite, DPC, Bad Nauheim, Germany). In addition, serum concentrations of tumour necrosis factor receptor types 1 and 2 (TNF-R1/R2), and soluble endotoxin receptor (sCD14) were determined by commercial ELISAs according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN, USA). Sensitivity of the tests were 2.0 pg/ml for IL-8, 0.2 µg/ml for LBP, 0.77 pg/ml for TNF-R1, 0.6 pg/ml for TNF-R2 and 125 pg/ml for sCD14. All cytokine studies were performed in the laboratory of Profs. Zielen (University Children’s Hospital, Frankfurt, Germany).

**Quality of life (QoL)**

Patients reported their QoL using the validated German version of the cystic fibrosis questionnaire (CFQ). If the children were younger than 13 years, the parents responded to the CFQ-E version of the questionnaire. Older patients used the CFQ-14+ version, and adults used the CFQ-18+ form. Each scale has an optimum of 100 and a (theoretical) minimum of 25 points, and higher values indicate an improvement of quality of life.
Determination of sample size
Considering the results reported by Wolter et al., we believed that the differences in FEV₁ between AZM and placebo groups after 8 weeks of treatment would be 3.8% predicted with a standard deviation of 7.3%. Based on 0.80 power to detect a significant difference ($p = 0.05$, two-sided), 65 evaluable patients were required for each study group. To compensate for nonevaluable patients, we planned to enrol 75 patients per group.

Allocation to treatment
Azithromycin or placebo tablets were allocated to patients according to a computer-generated randomization list which had been prepared consistent with the sponsor’s standard operating procedures. The study physician allocated the next available number on entry into the trial. All study personnel and participants were blinded to treatment assignment for the duration of the study.

Definition of analysis sets
The safety population involved all patients who had received at least one dose of the study medication. The intent to treat (ITT) population consisted of all subjects of the safety population for whom at least one on-treatment efficacy measure was available. The per protocol population (PP) analysis set consisted of all patients of the ITT population who did not show any severe protocol deviations.

Statistical analysis
All data analysis was carried out according to a pre-established analysis plan. Statistical testing was performed at a significance level of $\alpha = 5\%$. Demographic variables, other baseline characteristics and all efficacy variables were analyzed in the ITT analysis set. The primary efficacy criterion, FEV₁, was also analyzed in the PP analysis set.

The primary endpoint, absolute change in the FEV₁ (%), was tested using an ANCOVA model, and the following covariates were incorporated in the model: Baseline FEV₁ (as a percentage of the predicted normal value), age, duration of P. aeruginosa infection, number of courses of systemic antipseudomonal antibiotic treatments within the last 12 months, treatment with inhaled antibiotics, and treatment with dornase alfa.

An exploratory analysis was performed for the secondary efficacy variables using a similar ANCOVA model. Categorical variables were analyzed with appropriate linear models.

All safety variables were analyzed in the safety analysis set in accordance with Pfizer’s worldwide safety standards (WSS), release 3.0. Adverse events were coded according to MedDRA®, version 7.1.

Results
Study participants
Of the 40 patients screened in 11 study centres, 38 patients were randomised (21 to AZM and 17 to placebo) and received at least one dose of study medication (Fig. 1). Two patients were not eligible because of (a) hearing loss and (b) a baseline FEV₁ value of only 22% of predicted. Nine participants (23.7%) prematurely discontinued the study, 6 of these (2 in the AZM and 4 in the placebo group) due to an adverse event. The 17 and 12 patients who completed the study in the AZM and placebo groups, respectively, were recruited between November 2001 and June 2004.

Nine protocol deviations ($n = 4$ in the AZM and $n = 5$ in the placebo group) were judged as major events (e.g. concomitant systemic antibiotic treatment ($n = 4$), or visit “day 56” performed between days 4 and 25 after first dose of study drug ($n = 5$)), so that the respective patients were not included in the per protocol group.

Baseline demographic and clinical characteristics
The mean (SD) age of the whole patient cohort was 24.8 (10.0) years, and 16 of 21 participants in the AZM group and 13 of 17 placebo patients were adults (Table 1). The average duration of PA infection was 10.4 (6.6) years. In the year preceding the study, the patients had received 2.4 (1.1) courses of systemic antibiotic treatments on the average. Immediately before the start of the study, patients had experienced a respiratory exacerbation which was treated with systemic combination antipseudomonal antibiotics, predominantly tobramycin ($n = 34$), ceftazidime ($n = 18$) and meropenem ($n = 12$). These antibiotics were administered during a hospital stay in 22 patients and at home in the remaining 16 subjects. Thirty participants used long-term inhaled antibiotics on a routine basis (colistine: $n = 17$, tobramycin: $n = 13$, additional polymyxin B: $n = 1$).

In accordance with the inclusion criteria, most patients had moderate to severe lung disease with both central and peripheral airways obstruction (Table 1). Ten patients complained of moderate or severe cough and produced 1/4 cup or more of sputum per day, despite of the fact that they had received antipseudomonal antibiotics immediately before the start of the trial. Only 6 patients reported no cough and 2 subjects no sputum. P. aeruginosa was identified in respiratory secretions of 17 AZM and 10 placebo patients, and Staphylococcus aureus grew in 5 and 3 specimens from the respective groups.

Regarding quality of life, the worst scores were found in the subscales energy, daily living, and respiratory symptoms.

No statistically significant differences between groups were found with respect to baseline demographic and clinical characteristics.

The first dose of study medication was administered at a mean (SD) of 4.8 (2.6) days after the end of the preceding intravenous antipseudomonal treatment.

Efficacy
The primary analysis of efficacy was intent-to-treat and involved all randomised patients who received at least one dose of study medication.

FEV₁ and spirometry
We expected lung function to decline after cessation of IV antibiotics. Accordingly, mean FEV₁ decreased by 3.7% (SD
13.3%) of predicted in the AZM and by 5.0% (SD 10.1%) of predicted in the placebo groups, respectively. The mean absolute change of FEV₁ (%) adjusted to baseline FEV₁ (%) did not differ significantly between treatment groups (p = 0.708). Analysis of the per protocol groups revealed similar results: FEV₁ decreased by 4.4% (SD 14.1%) in the AZM and by 5.2% (SD 11.2%) in the placebo group (p = 0.826). The change of FEV₁ (%) from baseline was analyzed with an ANCOVA model including, besides treatment, the following covariates: baseline FEV₁ (%), age (years), duration of colonization with *P. aeruginosa* (years), number of courses of systemic antipseudomonal antibiotic treatments within the last 12 months, treatment with inhaled antibiotics (yes, no) and treatment with dornase alfa (yes, no). None of these factors had a statistically significant (α = 0.05) influence on FEV₁. Treatment responses were comparable in subgroups of patients treated with inhaled antibiotics (n = 21) and/or dornase alfa (n = 17).

Other respiratory function parameters (IVC, FVC, MEF₇₅₋₂₅, and oxygen saturation) also declined during the study (Table 2), and AZM had no statistically superior effects compared to placebo.

### Inflammatory parameters

Results for inflammatory parameters are summarised in Table 3. Immediately after intravenous antibiotics (and before study start), CRP was still elevated (>3 mg/l) in 8 of 21 AZM and in 4 of 16 placebo patients, so that mean CRP serum concentrations were increased at baseline (Table 3). After AZM and placebo treatment, CRP was abnormal in 9 and 11 patients, respectively, and the comparison of changes within groups (AZM: 0.9 mg/l (SD 6.6 mg/l), placebo: 21.6 mg/l (SD 60.4 mg/l)) showed a significant difference (p = 0.019, ANCOVA) at day 56 (Fig. 2). ANCOVA

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**Figure 1** Patient flow.
revealed a significant influence of the number of previous antipseudomonal treatment courses on change of CRP. Interleukin-8 serum concentrations decreased after 8 weeks of AZM treatment (ΔC0 3.1 pg/ml) and increased after placebo (ΔC0 2.9 pg/ml) (Fig. 2), with significant differences (p < 0.001) between groups. Lipopolysaccharide binding protein (LBP) in serum also showed significantly better results (p < 0.015) after AZM (increase of only 0.9 mg/ml, Fig. 2) than after placebo (7.0 mg/ml). Patients treated with azithromycin did not differ from placebo patients regarding the development of other inflammatory parameters, such as IgG, tumour necrosis factor receptor types 1 and 2 (TNF-R1/R2) or soluble endotoxin receptor (sCD14).

### Bacteriology and serology

At start of study medication (immediately after intravenous antibiotics), PA was cultured in respiratory secretions of 81% of patients in the AZM group and of 63% of placebo patients. Only 75% of patients were PA positive after 8 weeks of azithromycin treatment, whereas all placebo patients grew PA in their sputa at day 56. S. aureus was found in 19% of patients at baseline, regardless of treatment group. At day 56, 17% of AZM and 50% of placebo subjects were positive for S. aureus. No differences were detected regarding other pathogens.

At start of study medication, 2 of 13 AZM and 1 of 10 placebo patients had antibodies against M. pneumoniae, and serologic evidence of C. pneumoniae infection was found in 5 of 13 AZM and 3 of 10 placebo subjects. No relevant changes were observed after 8 weeks of treatment.

### Alginate in sputum

At baseline, alginate produced by mucoid P. aeruginosa strains was detected in sputum in considerable amounts, with no difference between groups. After 8 weeks of AZM treatment, the mean increase in alginate concentrations was significantly lower than in placebo patients (85 compared to 353 μg, p = 0.048) (Table 3, Fig. 3). ANCOVA revealed that the absolute changes in alginate concentrations between baseline and final values were significantly larger with (a) a longer duration of PA colonization and (b) more courses with systemic antipseudomonal antibiotics within the last 12 months, whereas changes in alginate were smaller in patients with lower baseline alginate concentrations.

### Signs and symptoms

At day 56, mean cough and sputum scores were 1.3 and 1.9 in patients with AZM and 1.5 and 2.6 in placebo patients, respectively. A larger proportion of placebo (10 of 17) than
of AZM (5 of 21) patients produced more than a tablespoon of sputum per day. No relevant differences between groups were present with respect to other signs and symptoms.

Quality of life

The results of the CFQ-14–18+ questionnaires revealed relevant differences between treatment groups. Eight weeks after start of treatment, patients on AZM rated their quality of life significantly better than placebo patients with respect to body weight (p = 0.021), respiratory symptoms (p = 0.037), and eating disorder (p = 0.046). Patients reported considerable improvements in their daily living, with numerically larger benefits in patients treated with azithromycin (Fig. 4). Ten of 14 CFQ subscales showed more favourable mean changes between baseline and visit day 56 after AZM compared to placebo.

The CFQ-E version of the questionnaire was used for n = 3 AZM children and for n = 4 placebo patients. The responses of parents showed no clear trends in favour of one or the other study drug.

AZM serum and sputum concentrations

Azithromycin-treated patients received a body weight adjusted weekly dosage of 21.2 mg/kg body weight on the average (SD 2.0 mg/kg). On day 56, azithromycin concentrations were detected in serum in all but 3 AZM patients, with a mean on-treatment concentration of 57.7 ng/ml (SD 33.3 ng/ml). Patients had taken the last dose of AZM 3 days before the end-of-study visit. In 2 patients blood samples were drawn later than requested (5 and 24 days after the last dose), and in 1 patient AZM was not detected in serum on the appropriate day, so that this patient was excluded from the per protocol analysis. Azithromycin was found in sputum in 11 of 11 treated patients, with a mean concentration of 14.4 μg/g (SD 10.1 μg/g) after 8 weeks of once-weekly azithromycin. No azithromycin was measured in serum or sputum in any placebo patient.

Tolerability and safety

In general, study medication was well tolerated. Thirty adverse events (AEs) occurred in 13 patients (62%) of the azithromycin group, and 40 adverse events occurred in 10 patients (59%) of the placebo group. Of the treatment-related adverse events, 10 occurred in 4 patients (19.0%) on AZM, and 11 developed in 3 patients (17.6%) on placebo. General disorders and administration site conditions (asthenia, decreased exercise tolerance, influenza like illness, pyrexia) as well as gastrointestinal disorders (upper abdominal pain, dyspepsia, nausea) were more

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**Table 3** Inflammatory parameters in serum and sputum before and after treatment (Mean, SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Azithromycin</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Baseline</td>
</tr>
<tr>
<td>Serum CRP (mg/l)</td>
<td>21</td>
<td>7.3 (11.3)</td>
</tr>
<tr>
<td>Serum IgG (mg/dl)</td>
<td>21</td>
<td>1686 (435.4)</td>
</tr>
<tr>
<td>Serum IL-8 (pg/ml)</td>
<td>18</td>
<td>14.1 (14.5)</td>
</tr>
<tr>
<td>Serum LBP (μg/ml)</td>
<td>20</td>
<td>8.0 (3.1)</td>
</tr>
<tr>
<td>Serum TNF-R1 (pg/ml)</td>
<td>20</td>
<td>1027 (224.3)</td>
</tr>
<tr>
<td>Serum TNF-R2 (pg/ml)</td>
<td>20</td>
<td>2137 (497.7)</td>
</tr>
<tr>
<td>Serum sCD14 (ng/ml)</td>
<td>20</td>
<td>1118 (264.8)</td>
</tr>
<tr>
<td>Sputum Alginate (μg/ml)</td>
<td>11</td>
<td>253.2 (307.4)</td>
</tr>
</tbody>
</table>

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**Figure 2** Inflammatory parameters in serum.
frequent in those receiving placebo \((n = 3/3)\) than in patients on AZM \((n = 2/1)\). Respiratory, thoracic and mediastinal disorders (cough) \((n = 2)\), infections and infestations (bronchopneumonia, rhinitis) \((n = 1)\) and a decrease in pulmonary function test \((n = 1)\) were observed with similar frequency in both groups. Nervous system disorders (dizziness, headache) were more frequent in those receiving AZM \((2 vs. 1)\), the same was true for skin and subcutaneous tissue disorders (urticaria) \((1 vs. 0)\). No serious or severe treatment-related AEs occurred in this study. One patient in each treatment group prematurely discontinued the study due to treatment-related AEs. There was no clinically significant deterioration of auditory performance in any patient and no newly diagnosed hearing loss at visit day 56. Moreover, no systematic changes of laboratory parameters and vital signs were detectable in any of the two treatment groups between baseline and visit day 56.

**Discussion**

The present double-blind trial evaluated the once-weekly dosing of azithromycin compared to placebo in patients with cystic fibrosis. All patients had completed a course of intravenous antipseudomonal antibiotics directly before the start of the study medication. Thus, many participants were in their individual optimum clinical condition when the trial commenced. Not surprisingly, we observed a decrease in pulmonary function parameters during the following 8 weeks, which was of comparable extent in the azithromycin and in the placebo groups. Other study endpoints showed that patients experienced a significant benefit from azithromycin. Inflammatory parameters such as CRP, IL-8 and LBP developed more favourably in patients on AZM. Three of the 14 subscales of the CFQ-14+/18+ questionnaire (problems with body weight, eating disorder, and respiratory symptoms) showed significantly better quality of life in participants treated with azithromycin.

To our knowledge, this is one of the first trials in cystic fibrosis patients with once-weekly administration of azithromycin. In the middle of the dosage interval, we reached mean concentrations of 57.7 ng/ml in serum and 14.4 \(\mu g/g\) in sputum. When healthy volunteers received a single dose of 1500 mg AZM, mean serum concentrations between 3 and 10 days after administration were in the range of approximately 60 ng/ml.\(^{20}\) Adults with CF who were on long-term treatment with daily doses of 500 mg AZM, i.e. a threefold weekly dose compared to the present study, showed considerably higher peak serum concentrations (mean \(C_{\text{max}}: 670 \text{ ng/ml}\)) than our study participants.\(^{15}\) Sputum levels ranged from 4 to 27 mg/l \((\sim 27 \mu g/g)\) at 10 days after the last dose and were comparable to the
sputum concentrations in our patients. Thus, with once-weekly dosing we achieved serum and sputum concentrations that did not differ markedly from those reported in the literature as being therapeutically efficient.\(^{21}\)

Once-weekly azithromycin dosing was well tolerated by the patients. No relevant differences in frequency and intensity of adverse events were noted between the AZM and the placebo groups. Saiman et al. reported more frequent gastrointestinal symptoms (nausea and diarrhoea) and wheezing after 500 mg AZM 3 days a week.\(^9\) In the present study, treatment-related gastrointestinal disorders occurred in 11.8% of placebo and 4.8% of AZM patients, and wheezing was not reported as an adverse event in any patient. The application of the drug on a specific day of the week might be easier to remember than the weekdays for three doses per week, and the dose applied is lower than in daily dosing of 500 mg. Thus, this treatment regimen could improve patient compliance and save costs for the health system. A recent trial compared daily vs. weekly azithromycin in cystic fibrosis in 208 CF patients: In patients aged \(< 18\) years the daily group had significantly better improvements in z-scores for height and weight after 6 months.\(^{22}\) Equivalence was demonstrated between the 2 groups with respect to improvements in lung function (FEV\(_1\) and FVC), CRP, days spent in hospital, admission rate and nutrition (body mass index, z-scores).

Another important feature of our study was that only patients who had successfully completed a course of intravenous antipseudomonal antibiotics were enrolled, respectively, were at their individual optimum when entering the trial. This is in sharp contrast to the larger multicentre trials on azithromycin in CF, which required an interval of the trial. This is in sharp contrast to the larger multicentre trials on azithromycin in CF, which required an interval of other bacterial anti-virulent effect of AZM has been shown almost in vivo by AZM concentrations well below the MIC.\(^{28}\) Quorum sensing (QS) controls virulence factor production and biofilm formation.\(^{17}\) A recently published study investigated the impact of AZM on the global transcriptional pattern and the protein expression profile of \(P.\ aeruginosa\) cultures vs. those in untreated controls.\(^{6}\) By downregulation of quorum-sensing-dependent genes, AZM exhibited extensive quorum-sensing antagonistic activities. In the present study, we showed for the first time that mean sputum alginate concentration after treatment was significantly lower in participants receiving AZM compared to the placebo group. Moreover recently Hansen and co-workers\(^{29}\) also reported after 1-year treatment with low dose AZM (250 mg) daily in adult CF patients a highly significant (\(p = 0.003\)) reduction in the number of sputum samples positive for mucoid strains from 90% in the year before treatment to 81% in the year of AZM treatment. Another bacterial anti-virulent effect of AZM has been shown lately in a 6-month clinical trial in American CF patients. Nguyen et al.\(^{30}\) were able to provide evidence that the change of phospholipase C (PLC) was significantly negative correlated with the change in FEV\(_1\) (\(p = 0.05\)) and occurrence and time to pulmonary exacerbation (both \(p = 0.02\)).

In addition we found that the frequency of neither \(P.\ aeruginosa\) nor \(S.\ aureus\) increased in respiratory secretions from participants treated with AZM, in contrast to placebo patients, who had a greater percentage of positive bacteriological results after 8 weeks.

Interestingly, the patients’ quality of life was significantly better after AZM. Using validated German versions of the CFQ questionnaire for three different age groups,\(^{19}\) we found significantly better results for the subscales problems with body weight, eating disorder and respiratory symptoms in adolescents and adults after 8 weeks of azithromycin treatment compared to placebo. In particular, deteriorations of more than 5 points (mean) occurred in 4 of the 14 scales after placebo (and in none after AZM), whereas azithromycin treatment caused improvements by studied in CF airway epithelial cell lines.\(^{25}\) It was shown that AZM downregulates NF-κB and AP-1 DNA binding indicating inhibition of transcription of pro-inflammatory genes as a possible mechanism.

We also found that lipoprotein binding protein (LBP) increased to a significantly larger extent in the placebo than in the azithromycin group. LBP plays an essential role in the immune response to gram-negative bacterial infection. Lipopolysaccharide (LPS) recognition requires LPS-binding protein and CD14. Both LBP and CD14 control ligand presentation to the receptor complex Toll-like receptor (TLR4) and influence the amplitude of LPS responses and LPS-induced type I interferon production. In previous studies, LBP and IL-8 correlated negatively with clinical status and lung function in homozygous F508del cystic fibrosis patients.\(^{26,27}\) In this regard a significantly lower IL-8 and LBP should reflect a clinical benefit for the patient. It is tempting to speculate that reduced levels of CRP, LBP, and IL-8 indicate a lower bacterial load in the airways of patients treated with AZM. This is in keeping with lower concentrations of sputum alginate which could imply less microcolony formation of \(P.\ aeruginosa\) and less bacterial evasion of host defence.\(^2\) Alginate is a major constituent of \(P.\ aeruginosa\) biofilms. Biofilm formation by \(P.\ aeruginosa\) was inhibited in vitro by AZM concentrations well below the MIC.\(^{28}\) Quorum sensing (QS) controls virulence factor production and biofilm formation.\(^{17}\) A recently published study investigated the impact of AZM on the global transcriptional pattern and the protein expression profile of \(P.\ aeruginosa\) cultures vs. those in untreated controls.\(^{6}\) By downregulation of quorum-sensing-dependent genes, AZM exhibited extensive quorum-sensing antagonistic activities. In the present study, we showed for the first time that mean sputum alginate concentration after treatment was significantly lower in participants receiving AZM compared to the placebo group. Moreover recently Hansen and co-workers\(^{29}\) also reported after 1-year treatment with low dose AZM (250 mg) daily in adult CF patients a highly significant (\(p = 0.003\)) reduction in the number of sputum samples positive for mucoid strains from 90% in the year before treatment to 81% in the year of AZM treatment. Another bacterial anti-virulent effect of AZM has been shown lately in a 6-month clinical trial in American CF patients. Nguyen et al.\(^{30}\) were able to provide evidence that the change of phospholipase C (PLC) was significantly negative correlated with the change in FEV\(_1\) (\(p = 0.05\)) and occurrence and time to pulmonary exacerbation (both \(p = 0.02\)).

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5 or more points in 5 subscales (placebo group: 1 subscale only). In the US trial, quality of life improved regarding the scale "physical factor" of the CF quality of life questionnaire, whereas the other three components remained unchanged.

The present study had some limitations. The results from sputum measurements include a subgroup of patients only, since others were unable to expectorate sufficient amounts of sputum after successful intravenous antibiotic treatment.

In conclusion, once-weekly azithromycin ameliorated inflammatory airway response indicating a reduced bacterial load in treated patients. Furthermore, AZM improved quality of life of patients chronically infected with P. aeruginosa who had successfully completed a course of intravenous antibiotics for an exacerbation. Treatment was well tolerated and did not cause an increase of treatment-related adverse events compared to placebo. These results suggest that one dose of azithromycin per week may represent an alternative treatment regimen for patients with cystic fibrosis.

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