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Long-term effects of azithromycin in patients with cystic fibrosis

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Running title: Prolonged azithromycin in cystic fibrosis

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Abstract

Background

Low-dose azithromycin has beneficial effects on severity of the lung disease in cystic fibrosis (CF) patients for a period of 6 to 12 months after initiation of the treatment. Although its impact in the longer term is uncertain, this treatment is frequently used chronically. The aim of this retrospective study was to investigate the effects of low-dose azithromycin treatment on the progression of CF lung disease in patients treated for more than 12 months.

Methods

All of the CF patients followed in our pediatric center and who had been on low-dose azithromycin for more than 12 sequential months were included. The clinical data were collected for one year before and three years after the initiation of the azithromycin treatment. These data comprised lung function analyses, rates of exacerbations and of antibiotic courses, and changes in the airways' bacterial colonization.

Results

A total of 68 patients were included (mean age: 9.95 yrs (3.61)). After 12 months, significant reductions in the numbers of pulmonary exacerbations and antibiotic courses were present. However, this effect was not maintained in the subsequent periods, during which increased rates of both pulmonary exacerbations and antibiotic courses were observed. The lung function decline was not modified during the treatment, and a decreasing time-dependent trend typical of CF was observed for the various parameters. No differences in the airway colonization by pathogens such as *P. aeruginosa* and methicillin-sensitive and/or -resistant *Staphyloccocus aureus* were observed during the treatment. However, isolated *Staphyloccocus aureus* strains became resistant to macrolides after 6 months of azithromycin and remained resistant thereafter.

Conclusions

No clinical benefits of low-doses azithromycin were present after one year of treatment in young CF patients. Selection for macrolide-resistant strains of bacteria occurred, which should lead to a reconsideration of the duration of azithromycin treatment in CF.

Keywords*:* children; cystic fibrosis; azithromycin; lung function; respiratory exacerbation; bacteria resistance

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Introduction

Cystic fibrosis (CF) is the most common severe autosomal recessive genetic disease in Caucasians. CF is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (*CFTR*), a chloride channel expressed in the epithelial cells throughout the body (1). The disease affects many organs including the pancreas, the liver, the intestine, and, most critically, the lungs. CF lung disease still remains the major cause of morbidity and mortality in CF, with a progressive decline of the lung function due to a vicious cycle of airway infection and inflammation (2, 3). The inflammation in the lungs of CF patients is persistently neutrophilic and is associated with an up-regulation of neutrophil chemotactic mediators (4, 5). The accumulation of activated neutrophils in the airways and the resulting release of toxic products impair the host defense and contribute to infection and subsequent chronic colonization by microorganisms such as *Pseudomonas aeruginosa* (*P. aeruginosa*).

Because inflammation is a central contributor to the pathogenesis of CF pulmonary disease, limiting the excessive production of inflammatory mediators represents a major therapeutic strategy for slowing the decline in lung function and improving the overall survival. Worldwide, one of the most frequently prescribed anti-inflammatory drugs in CF is azithromycin, despite the fact that this is an off-label use of the drug. Azithromycin is a macrolide antibiotic that is recognized as having not only antimicrobial but also anti-inflammatory and immunomodulatory properties (6, 7). It is an erythromycin-derived 15-membered ring azalide, structurally modified to increase its half-life and enhance its intracellular accumulation with greater tissue penetration as well as to increase its intra-cellular and extra-cellular antimicrobial activity (8). Therefore, low doses and infrequent dosing schedules (such as three times a week) are possible, making it attractive as a long-term oral therapy. Azithromycin has also been reported to inhibit the release of pro-inflammatory mediators, to limit the pulmonary influx of neutrophils, to regulate mucus secretion, and to alter the formation of the *P. aeruginosa* biofilm matrix (7). In CF, it has also been shown *in vitro* to restore the chloride efflux (9).

The first success of macrolides in lung diseases was observed in patients with diffuse panbronchiolitis. With long-term treatment using macrolides, the survival of patients with diffuse panbronchiolitis increased remarkably from 25 to 95% at 5 years (10-13). Because diffuse panbronchiolitis and CF share many similarities including neutrophil airway inflammation and *P. aeruginosa* infections, macrolides, and more specifically azithromycin, have since been evaluated in several trials in CF patients. A Cochrane Database systematic review has further ascertained that administration of low-dose azithromycin for 6 to 12 months has beneficial effects on the lung function, the occurrence of exacerbations, the need for other antibiotics, and on the weight gain in CF patients (14). Thus, azithromycin, despite its off-label use, is frequently prescribed to CF patients aged more than 6 years throughout the world, but the treatment is rarely interrupted, leading to very long treatment durations, *i.e.,* > 12 months, without any evidence of maintained beneficial effects. We therefore conducted a retrospective study to investigate the effects of prolonged (>12 months) low-dose azithromycin treatment on the progression of CF lung disease.

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Material and methods

Patients

This retrospective study took place in a pediatric CF center in Paris, which cares for 150 CF patients. We queried our Electronic Health Record system for all CF patients under 18 years old who had started azithromycin at anti-inflammatory doses (250 or 500 mg 3 times a week for patients under or above 40kg respectively) between November 1st, 1999 and December 31st, 2013 and had been treated for more than 12 subsequent months. The clinical data were retrospectively collected from the electronic patient records, supplemented when necessary with data from the paper patient records. For each individual patient, the date of the initiation of the azithromycin therapy was designated time T0. The clinical data were subsequently collected 12 months before the start of azithromycin treatment (T-12), at T0, and every 12 months following T0 (T12, T24, T36) or up to discontinuation of treatment. We documented:

- *CFTR* genotypes and pancreatic status, *i.e.,* sufficient or insufficient
- Body Mass Index (BMI) z-score measurements
- Pulmonary function tests, expressed as percent-predicted values, using the modified Knudson equations (15): Forced Expiratory Volume in one second (FEV_1), Forced vital capacity (FVC) and Forced expiratory flow 25% to 75% (FEF_{25-75})
- Arterial blood gas (partial pressure of oxygen, PaO_2, and partial pressure of carbon dioxide, $PaCO_2$);
- Annual rate of acute respiratory exacerbations. A respiratory exacerbation was defined as an acute exacerbation of CF respiratory symptoms that in the opinion of the patient's physician required administration of new oral or intravenous (IV) antibiotics, according to the criteria published by the 1994 CF Foundation Microbiology and Infectious Disease Consensus Conference (16, 17).
- Number of IV and of oral antibiotic courses

- Microbiological analyses of sputum and throat cultures for the common CF pathogens and for nontuberculous mycobacteria as previously described (18-20).

Statistical analysis

The data were expressed as the means ± SD for continuous variables and numbers (%) for categorical variables. Multiple imputation was used for missing pulmonary function data. Analysis of variance for repeated data was performed to compare the pulmonary function data/arterial gases before and after the beginning of the azithromycin treatment and the paired Wilcoxon rank sum test to compare the number of exacerbations and antibiotic cures. The differences were considered significant for P-values less than 0.05.

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Results

Patients

The demographic data for the patients included are described in **table 1**. Briefly, 68 CF patients (33 girls and 35 boys) were included. Of these, 41 (60.3%) were homozygotes for the CFTR F508del mutation, and 66 (97%) were pancreatic insufficient. At T0, the median age was 9.95 ± 3.61 yrs, and the median BMI z-score was -0.3 ±1.8. Among the 68 patients who took azithromycin for more than 12 months, 50 were observed for 2 full years after the initiation of the azithromycin treatment, and 46, for 3 full years.

Lung function and blood gas analyses

Overall, the pulmonary function testing showed typical changes over time, with a slight decreasing trend with time in all parameters (**Figure 1**). From T-12 to T36, the FEV_1 decreased by 2% per year, from 81.9 ± 20.2% to 74.3 ± 23.3% (P=0.01); the FVC by 1.7% per year, from 89.4 ± 15.46% to 82.7 ± 21.0% (P=0.01); and the FEF_{25-75} by 2.9% per year from 73.0 ± 29.7% to 61.7 ± 31.4% (P=0.004). Regarding the blood gas analyses, $PaCO_2$ increased by 0.44 mmHg each year from 35.5 ± 2.7 mmHg to 37.3 ± 2.7 mmHg (P=0.04), and $Pa0_2$ tended to decrease by 0.7 each year from 81.5 ± 7.0 mmHg to 78.3 ± 11.0 mmHg (P=0.07) (**Figure 2**).

Exacerbations and antibiotic courses

We compared the exacerbations and antibiotic courses in patients who were observed for three full years after the initiation of the azithromycin treatment (n=46). The changes in annual rates of respiratory exacerbations and of oral, intravenous, and total (oral plus intravenous) antibiotic courses are summarized in **table 2** and **Figure 3**. In the 12 months before azithromycin was started (T-12 - T0), the patients had 2.2 ± 2.1 exacerbations per year and 2.2 ± 2.1 antibiotic treatments overall, including 1.9 ± 2 oral antibiotic courses and 0.2 ± 0.6 IV antibiotic courses. During the first year of azithromycin treatment (T0 - T12), the annual rate of exacerbations decreased (1.5 ± 1.4; P=0.02), as did the overall number of antibiotic treatments (1.4 ± 1.4,

P=0.03). This last change was due to a decreased number of oral antibiotic courses (1.2 ± 1.4; P=0.007), whereas the number of IV antibiotic courses tended to increase (0.3 ± 0.8; P=0.37). Subsequently, in the 2 years after the first year of azithromycin treatment (T12 - T24 and T24 - T36), the number of exacerbations increased again (P=0.008 in the third year) to reach levels similar to the pre-azithromycin period. The total number of antibiotic treatments also increased back to the original value, mainly due to a large increase in the IV antibiotic treatments, from 0.3 to 0.9 (P=0.02). The number of oral treatment increased as well, although less dramatically. Including patients with only 2 years follow-up after azithromycin treatment or restricting to patients followed for 3 to 4 full years yielded the same qualitative profile of change, with a decrease in the first year followed by an increase back to pre-azithromycin levels (data not shown). Moreover, the qualitative profile of change was also similar between patients chronically infected either with *P. aeruginosa* or *Staphyloccocus aureus* (data not shown).

Bacterial flora evolution

At baseline (T0, table 1), 13 (19%) of the patients were colonized by *P. aeruginosa*; there were no changes in the number of colonized patients after 1 and 2 years, but there was a slight (non-significant) increase after 3 years, at which time 19 patients (28%) were colonized by *P. aeruginosa* (p=0.28). Similarly, no significant changes were observed for the colonization by methicillin-sensitive *Staphyloccocus aureus* (MSSA), with 29 (43%) patients colonized at T0 and 27 (40%) at T36 (p=0.93); nor were there changes in the colonization by methicillin-resistant *Staphyloccocus aureus* (MRSA), with the same number of patients (13; 19%) colonized at T0 and T36. Notably, 100% of the *Staphyloccocus aureus* strains (MSSA and MRSA) became resistant to macrolides (erythromycin being the macrolide tested) after only 6 months of azithromycin and remained resistant during the 3 years thereafter. One patient was infected with a nontuberculous mycobacteria (NTM) at the time the azithromycin treatment was started (*Mycobacterium abcessus*). This infection disappeared during the first year of treatment and did not reappear during the further follow-up. Another patient developed an infection with

Mycobacterium bolleti during the 3rd year of azithromycin treatment. No other patients had NTM infections.

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Discussion

This study is unusual in evaluating the long-term, *i.e.,* up to 3 yrs, effects of low-dose azithromycin in young patients with CF. The results demonstrated that 12 months of treatment with oral azithromycin given 3 days a week led to a significant reduction in the numbers of pulmonary exacerbations and antibiotic courses. However, this beneficial effect disappeared when the azithromycin treatment was prolonged beyond 12 months, after which increasing rates of respiratory exacerbations and antibiotic courses were observed. We did not find any modification in the lung function rate of decline in these young patients, most of whom (80%) were not colonized by *P. aeruginosa.* Interestingly, we did not observe significant differences in airway colonization by pathogens such as *P. aeruginosa,* MSSA and MRSA during azithromycin treatment, but, importantly, the MSSA and MRSA strains all became resistant to erythromycin (which was the macrolide tested in the sputum cultures) after only 6 months of treatment and remained resistant thereafter. Moreover, we did not observe an increasing rate of nontuberculous mycobacterium infections.

The first suggestion that long-term macrolide therapy might be of value in CF was based on the results of low-dose erythromycin treatment in patients with diffuse panbronchiolitis (10-13). On the basis that this disease is also characterized by chronic pulmonary inflammation and *P. aeruginosa* infections, it became of interest to test these molecules in CF patients. The first trials included CF patients colonized by *P. aeruginosa* because it was shown that macrolides were able not only to suppress the inflammation but also to interfere with the bacterial virulence and to decrease the *P. aeruginosa* biofilm formation (7). Following the preliminary report of a pilot study, several randomized placebo-controlled trials were conducted in CF patients chronically infected with *P. aeruginosa* (21-24). The major proof-of-concept study was that of Saiman et al. who conducted a 6-month parallel group trial in 185 adult patients colonized with *P. aeruginosa*, using a dose of 250 or 500 mg of azithromycin given 3 days a week (22). They documented improvements in pulmonary function and decreased rates of pulmonary exacerbations in the azithromycin group. Other trials also

demonstrated that azithromycin benefited the CF patients who were chronically infected with *P. aeruginosa* and, in 2004, a first Cochrane review concluded that azithromycin indeed had a modest but significant effect on the respiratory function in CF patients (25).

The first randomized placebo-controlled trial that also included CF patients who were not chronically infected with *P. aeruginosa* was a multicenter French study coordinated by our team (26). This study lasted one year and included 82 young CF patients, 40 in the azithromycin group and 42 in the placebo group, of whom 80% were not colonized by *P. aeruginosa*. Whereas an FEV_1 improvement was observed at the beginning of the trial in the azithromycin group, the difference between the 2 groups was not sustained beyond 10 months. However, the treatment remained beneficial, as indicated by reduced numbers of respiratory exacerbations and antibiotic courses, independent of the *P. aeruginosa* colonization status. Furthermore, Tramper-Stranders et al. retrospectively analyzed the FEV_1 progression in one hundred young CF patients after 1, 2 and 3 years of azithromycin. Similar to our previous study, they observed that the initial FEV_1 improvement was not maintained during a prolonged treatment. They also observed an important increase in the number of *Staphylococcus aureus* infections resistant to macrolides: up to 83% after 1 year of treatment, 97% after 2 years, and 100% after 3 years. Finally, a large multicenter North-American study was performed in 260 CF children, not infected with *P. aeruginosa,* who were treated for ~5 months by azithromycin or placebo (27). The authors did not find any effects of azithromycin on the lung function but, as in the previous studies, azithromycin reduced the number of exacerbations and the oral antibiotic courses. A meta-analysis of these studies has been performed, with the conclusion that a 6 month treatment with azithromycin led to an improvement of the lung function (~4% of FEV_1 gain), a two-fold lower risk of developing respiratory exacerbations, fewer oral antibiotic courses, and gain of weight (14, 28). These meta-analyses also emphasized that the benefits beyond 6 months were less clear and that the emergence of macrolide-resistant pathogens was a great concern. Despite the lack of evidence of long-term maintained benefits, azithromycin is rarely stopped after 6

months. In addition to this study, two more studies have evaluated longer durations of treatment; one by our team (12 months) and on performed by Tramper-Stranders et al. (3 years) (26, 29). As in the present study, both of these studies observed that the initial improvement in lung function was not maintained during a prolonged treatment. Here, we showed that the FEV_1 rate of decline was slightly lower only during the first year of treatment, and decreased thereafter.

Similar to the other studies, we also observed a significant decrease in the number of pulmonary exacerbations, which paralleled the reduced number of antibiotic courses, during the first year of azithromycin. However, we also showed that both endpoints significantly increased again after the first year. One possible explanation for the lack of efficacy after the first year of treatment could be related to the antibacterial role of azithromycin. Azithromycin is an erythromycin-derived 15-membered ring azalide, structurally modified to permit an enhanced intracellular accumulation with greater tissue penetration, as well as an increased intra-cellular and extra-cellular antimicrobial activity (8). It could be hypothesized that after one year of treatment, azithromycin could lead to changes in the airway patient's microbiota and then to an increase in bacterial exacerbations (30, 31). To confirm this hypothesis, studies of the airway microbiota before and after azithromycin would be required. Interestingly, under azithromycin, we did not observe differences in the airway colonization by relevant CF pathogens, such as *P. aeruginosa,* MSSA and MRSA, but, notably, the MSSA and MRSA strains became resistant to macrolides after only 6 months of treatment and remained resistant thereafter. These disturbing results are similar to those of Tramper-Stranders et al. and should lead to reconsideration of the duration of such treatments (29). The emergence of pathogens resistant to macrolides could become a major public health problem in the near future. Until recently, the prescription of long-term macrolides was reserved to rare respiratory diseases such as panbronchiolitis and CF, and therefore to a limited number of patients. However, increasing numbers of prescriptions for these drugs are being provided for more common respiratory diseases including chronic obstructive diseases

(COPD) and asthma, leading to an increasing number of treated patients worldwide, and thus to possible emergence of resistance in commensal and pathogenic bacteria (32). The risk of resistance emerging from the widespread use of long-term macrolide treatment may be of concern to the entire community. For example, cases of acute articular rheumatism due to macrolide-resistant *Streptococcus* A have been reported in children treated with azithromycin for angina (33). The risk of resistance increases also with the half-life of the molecule, which is particularly long with azithromycin (3 days). This drug is known to remain detectable for approximately 30 days in the blood (34). While the clinical implications of the macrolide resistance of bacterial species such as *Staphylococcus aureus* need to be clarified, the possibility of *Mycoplasma pneumoniae* resistance is disturbing. In Japan, a resurgence of macrolide-resistant *Mycoplasma pneumoniae* has reached 90% of the mycoplasma strains found in children (35). For this reason, new “macrolide-like” drugs are currently under development, with no antibacterial activity, but with similar anti-inflammatory and immunomodulatory properties, that would avoid the risk of the emergence of resistant pathogens (36, 37). In parallel, new macrolides with increased anti-inflammatory activities, such as the solithromycin, are also under development (38-40).

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Conclusions

This study has several limitations, mainly related to its retrospective design and the small size of the cohort studied. However, we observed that the clinical benefits of low-dose azithromycin were not present after 1 year of treatment in our cohort of young CF patients. Moreover, selection for macrolide-resistant S*taphylococcus aureus* strains occurred after 6 months of therapy, which remained resistant during the subsequent three years evaluated in this study. In conclusion, based on the development of macrolide resistance and apparent decline in efficacy of azithromycin in subjects beyond 1 year of therapy, the duration of azithromycin treatment in patients with CF should be reconsidered.

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Conflict of interest

None of the authors have any commercial or other associations that might pose a conflict of interest.

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Tables

Table 1: Baseline characteristics of the patients

Total (n)	**68**
Age (yrs), mean (SD)	9.95 (3.61)
Sex (male/female)	35/33
CFTR genotype	
F508del / F508del	41
F508del / others	20
Others	7
BMI z score (SD)	-0.3 (1.8)
Pancreatic insufficiency (n, %)	66 (97%)
Colonized by *P. aeruginosa*	13 (19%)
Colonized by MSSA	29 (43%)
Colonized by MRSA	13 (19%)
NTM infection	1 (1%)

Abbreviations: *CFTR*: cystic fibrosis transmembrane regulator; BMI: body mass index, *P. aeruginosa*: *Pseudomonas aeruginosa*; MSSA: methicillin-sensitive *Staphyloccocus aureus*; MRSA: methicillin-resistant *Staphyloccocus aureus*; NTM: nontuberculous mycobacteria.

The data are the means (SD) or numbers (%) unless otherwise indicated.

Table 2: Progression of the annual rates of respiratory exacerbations, oral and intravenous antibiotic courses during the year before and up to 3 years after the initiation of azithromycin treatment

Time	Number of patients	Exacerbations (m ± SD)	All antibiotic courses (m ± SD)	Oral antibiotic courses (m ± SD)	Intravenous antibiotic courses (m ± SD)
T-12 - T0	68	2.2 ± 2.1	2.2 ± 2.1	1.9 ± 2.0	0.2 ± 0. 6
T0 - T12	68	1.5 ± 1.4*	1.4 ± 1.4*	1.2 ± 1.4*	0.3 ± 0.8
T12 - T24	50	2.1 ± 2.3$^{£}$	2.1 ± 2.3$^{£}$	1.5 ± 2.0$^{£}$	0.6 ± 1.1$^{£}$
T24 - T36	46	2.6 ± 2.5$^{£}$	2.7 ± 2.6$^{£}$	1.7 ± 2.0$^{£}$	0.9 ± 1.6$^{£}$

*p<0.05: compared to T-12 - T0 (12 months before azithromycin was started); $^{£}$p<0.05: compared to T0 - T12 (first year of azithromycin treatment).

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Figure Legends

Figure 1: Lung function changes under azithromycin

The means (±SD) for the lung function parameters (expressed as % predicted) 12 months before azithromycin was started (T-12) and up to 12, 24, and 36 months under azithromycin (respectively T12, T24 and T36). From T-12 to T36, the FEV_1, FVC and FEF_{25-75} decreased (P=0.01, 0.01 and 0.004, respectively).

Abbreviations: FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; FEF_{25-75}: forced expiratory flow 25% to 75%.

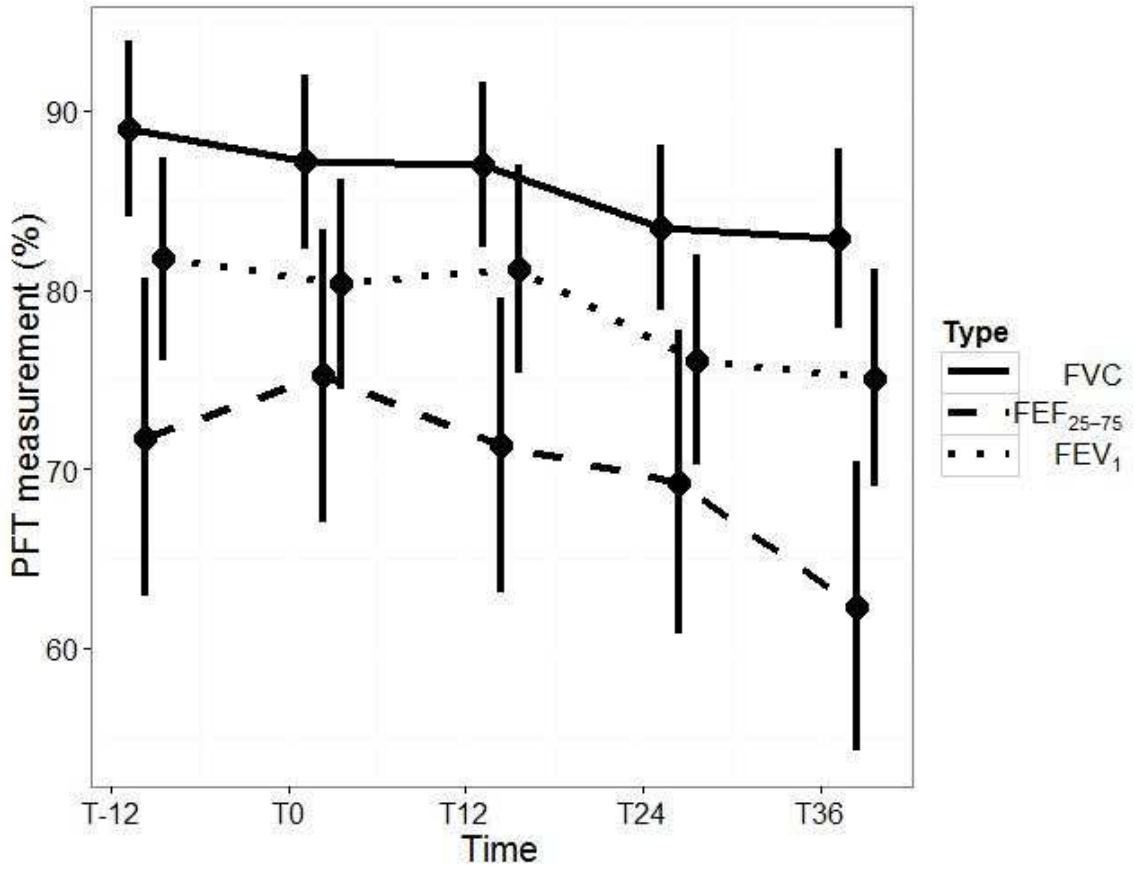
Figure 2: Blood gas changes under azithromycin

The means (±SD) for the PaO_2 and $PaCO_2$ values beginning 12 months before azithromycin was started (T-12) and up to 12, 24, and 36 months under azithromycin (respectively T12, T24 and T36). From T-12 to T36, the $PaCO_2$ increased (P=0.04), whereas the PaO_2 tended to decrease (P=0.07).

Abbreviations: PaO_2: partial pressure of oxygen; $PaCO_2$: partial pressure of carbon dioxide.

Figure 3: Changes in annual rates of respiratory exacerbations and of antibiotic courses (oral and intravenous) during azithromycin treatment

The rates (means ± SD) of the respiratory exacerbations and total (oral plus intravenous) antibiotic courses were compared annually: 12 months before azithromycin was started (T-12 - T0), during the 1st year of treatment (T0 - T12), and during the 2nd and 3rd years of treatment (T12 - T24 and T24 - T36, respectively). Compared to the year before azithromycin was started (T-12 - T0), the annual rates of respiratory exacerbations and antibiotic courses decreased significantly during the first year of azithromycin treatment (T0 - T12; P=0.02 and 0.03, respectively). In the 2 years thereafter (T12 - T24 and T24 - T36), the annual rates of respiratory exacerbations and of antibiotic courses increased again (P=0.008 and 0.02, respectively) to reach levels similar to the pre-azithromycin period.


PFT measurement (%)
90
80
70
60
T-12
T0
T12
T24
T36
Time
Type
FVC
FEF25–75
FEV1

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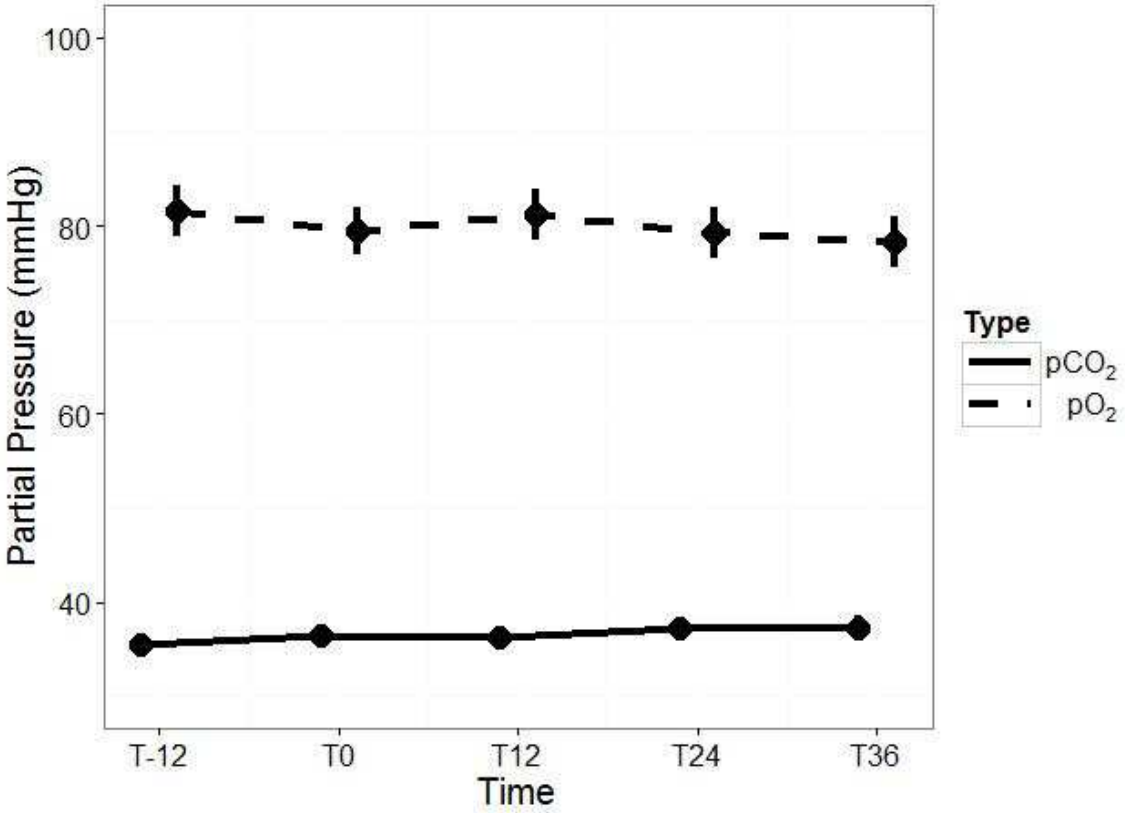

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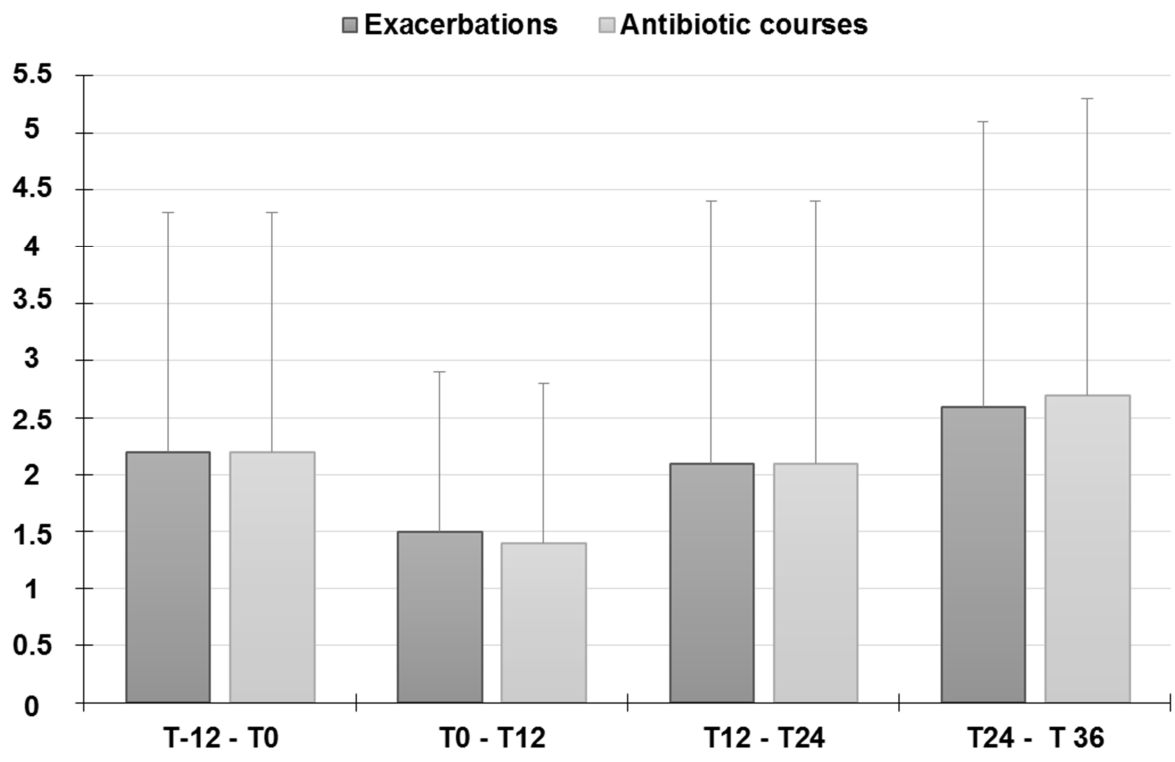

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Highlights

- Low-dose azithromycin treatment is frequently prescribed chronically in cystic fibrosis.
- During the first year of treatment, it leads to a reduction in the number of exacerbations.
- This beneficial effect is not maintained in the following years.
- Selection for macrolide-resistant bacteria occurred after 6 months of azithromycin.

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Point by point response to the reviewer's comments

1. What was the reason to start maintenance Azithromycin Treatment? Frequent exacerbation? Lung function decline? Chronic infection with pathogens? Looking to the data from Non CF bronchiectasis - even this is a different disease - it looks like that the macrolide treatment is much more effective in patients chronically infected with Pseudomonas than with any other pathogen.

We thank the referee for this comment. The reasons to start maintenance azithromycin treatment differed among the patients and were indeed either frequent exacerbations and/or lung function decline. In cystic fibrosis (CF), azithromycin was initially shown to be effective in patients chronically infected with *Pseudomonas aeruginosa* (references 21 to 25 in the manuscript), but was further shown to be as effective in patients not chronically infected with this pathogen (references 26 to 29 in the manuscript). This is detailed in 2 paragraphs of the discussion (second and third paragraphs of the discussion).

The study population is small, but may be the effects are different between Pseudomonas and gram positive pathogens?

As suggested by the reviewer, we looked at the effects between Pseudomonas and gram positive pathogens (MSSA and MRSA) colonized patients. The population is indeed small and lacked of power to detect differences, but we observed a similar change profile with time in both groups for exacerbations (P>0.5 respectively; *see* the table below for the evolution of number of exacerbations between the 2 groups, the first column being reported in the manuscript). We have added a new sentence in the results' section of the manuscript saying that: "the qualitative profile of change was also similar between patients chronically infected either with *P. aeruginosa* or *Staphyloccocus aureus* (data not shown)."

Time	Number of exacerbations in the entire cohort (m ± SD)	Number of exacerbations in patients chronically colonized with *Pseudomonas aeruginosa* (m ± SD)	Number of exacerbations in patients chronically colonized with MSSA + MRSA (m ± SD)
T-12 - T0	2.2 ± 2.1	2.0 ± 2.0	2.9 ± 2.3
T0 - T12	1.5 ± 1.4*	1.4 ± 0.8	1.8 ± 1.4
T12 - T24	2.1 ± 2.3£	2.9 ± 2.5	2.6 ± 2.3

**p<0.05: compared to T-12 - T0 (12 months before azithromycin was started); £p<0.05: compared to T0 - T12 (first year of azithromycin treatment).*

2. The mean Age of the Patient Group is about 10 years at the beginning of the Treatment. However for the older ones, the dosage of 250 three times a week could be too low and this should be discussed.

We thank the reviewer for this comment, and have corrected the manuscript as, indeed, the dosage was different regarding the weight of the patients: 250 mg or 500 mg three times a week for patients under or above 40kg respectively.

3. Even I am aware that this is speculative, you should give a hypothesis why Long term Treatment failed. Is it resistance development? Of pathogens or f.e. of Special genes like the biofilm producin ones?

We thank the reviewer for this comment and, indeed, so far, we could only hypothesize why a long term treatment fails. We discuss this point in the 4th paragraph of the discussion, please *see*: "It could be hypothesized that after one year of treatment, azithromycin could lead to changes in the airway patient's microbiota and then to an increase in bacterial exacerbations (30, 31). To confirm this hypothesis, studies of the airway microbiota before and after azithromycin would be required…etc…"

4. As the manuscript has been in review for a long time, some new publications are present yet. The Phase III study for solithromycin has been published in Lancet Infect Dis, demonstrating that ketolids work also in resistant pathogens, which make them an option for the future of long term treatment.

We thank the referee and have added the corresponding reference at the end of the 4th paragraph of the discussion where solithromycin is discussed: reference 40:
Barrera CM, Mykietiuk A, Metev H, Nitu MF, Karimjee N, Doreski PA, et al. Efficacy and safety of oral solithromycin versus oral moxifloxacin for treatment of community-acquired bacterial pneumonia: a global, double-blind, multicentre, randomised, active-controlled, non-inferiority trial (SOLITAIRE-ORAL). Lancet Infect Dis. 2016 Apr;16(4):421-30.

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