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Journal of Cystic Fibrosis 4 (2005) 35–40

Journal of Cystic Fibrosis

www.elsevier.com/locate/jcf

Long-term azitromycin treatment of cystic fibrosis patients with chronic *Pseudomonas aeruginosa* infection; an observational cohort study☆

Christine Rønne Hansen[a,*], Tacjana Pressler[a], Christian Koch✠, Niels Høiby[b]

[a]*Cystic Fibrosis Center, 5003, Department of Pediatrics, Juliane Marie Center, Rigshospitalet, Blegdamsvej 9, DK-2100 København Ø, Denmark*
[b]*Department of Clinical Microbiology, 9301, Diagnostic center, Rigshospitalet, Juliane Maries Vej 22, DK-2100 København Ø, Denmark*

Received 17 March 2004; accepted 8 September 2004

Abstract

Background: In cystic fibrosis (CF), chronic endobronchial infection with *Pseudomonas aeruginosa* is a serious complication. Macrolides can increase lung function and weight in patients, and reduce exacerbations.
Methods: In 2001, we introduced long-term, low-dose azithromycin (AZ) treatment as an integral part of our routine treatment of these patients. Our study is an observational cohort study of all CF patients with chronic *P. aeruginosa* infection in our CF center comparing clinical parameters of the patients 12 months prior to treatment with the same values during 12 months of treatment.
Results: 45 patients (27 men, median age 29 years) completed 1-year treatment. Median weight increased from 63.1 kg in the pre-treatment period to 63.9 kg during treatment (p=0.01). Median slope of decline in lung function increased from pre-treatment FEV_1 −4.1% and FVC −3.0% to +0.8% (p<0.001) and +1.6% (p=0.01), respectively. 90% of sputum samples contained mucoid *P. aeruginosa* before treatment, decreasing to 81% during treatment (p=0.003). Median CRP decreased from 6.2 mmol/l to 5.8 mmol/l (ns).
Conclusion: Long-term, low-dose AZ treatment in adult CF patients with chronic *P. aeruginosa* infection is safe and reduces the decline in lung function, increases weight, and reduces the percentage of mucoid strains of *P. aeruginosa* in sputum samples.
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Keywords: Azithromycin; Cystic fibrosis; *Pseudomonas aeruginosa*

1. Background

In cystic fibrosis (CF), the most frequent serious clinical complication today is chronic endobronchial infection with *Pseudomonas aeruginosa*, a microorganism characterized by the ability to produce large amounts of alginate, and to grow as a biofilm where microcolonies of bacteria embedded in a matrix of alginate invade the lower respiratory tract. The biofilm mode of growth is a survival strategy, which protects the bacteria from host immune reactions and antibiotics, and it is therefore the most important virulence factor [1]. The chronic nature of this infection leads to a vigorous and sustained inflammatory response and subsequent tissue damage [2] and, in the past, 95% of patients with CF have succumbed to respiratory failure due to this pathogen.

Diffuse pan-bronchiolitis (DPB) is a rare lung disease of unknown etiology seen in adult Japanese patients, in which chronic endobronchial infection with *P. aeruginosa* leading to respiratory failure is common. Surprisingly, it was found that long-term treatment with macrolide antibiotics resulted in markedly increased long-term survival of patients with DPB. A preliminary study of seven children with CF and *P. aeruginosa* infection revealed a marked increase in lung function during long-term azitromycin (AZ) treatment [3], and two subsequent intermediate-term controlled trials showed improvement in several clinical and paraclinical

☆ Data have been presented at the Sixteenth Annual North American Cystic Fibrosis Conference, New Orleans, October 2002.

* Corresponding author. Hostrups Have 23 St. tv., DK-1954 Frederiksberg C, Denmark. Tel.: +45 35 39 07 35; mobile: +45 51 96 35 07.
E-mail address: christunte@hotmail.com (C.R. Hansen).
✠ Deceased.

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doi:10.1016/j.jcf.2004.09.001

parameters of the lung disease, one in adult patients, the majority of whom had *P. aeruginosa* infection [4], and one in children, half of whom appeared to be chronically infected with *P. aeruginosa* [5]. A recent retrospective study showed an increase in lung function and BMI in a selected group of patients with chronic *P. aeruginosa* infection and rapid progression of the lung disease in those who were treated with macrolides as compared to a group of patients with *P. aeruginosa* infection and stable lung function [6].

Following the very encouraging results in the first two reports [3,4], we decided to incorporate long-term low-dose AZ treatment in our routine protocol for treatment of all patients with CF and chronic *P. aeruginosa* infection. In the present study, we assessed the safety of long-term treatment and the effect of this intervention on lung function, nutrition, and bacteriology.

2. Patients and methods

The diagnosis of CF was established on the basis of abnormal sweat electrolytes, characteristic clinical features, and genotype.

All CF patients are seen on a regular monthly basis. At each visit, the clinical status of the patients is assessed by weight and lung function parameters (forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV_1); Pneumothac, Draeger). Lower respiratory tract secretions are obtained by coughing (sputum producers) or naso-laryngeal suction, and sputum microbiology, including isolation and characterization of mucoid and non-mucoid *P. aeruginosa* phenotypes, is carried out. Infection is considered chronic when *P. aeruginosa* has been cultured from secretions for six consecutive months or less if there simultaneously is an increase in precipitating antibodies against *P. aeruginosa* [7].

All patients are treated according to fixed guidelines, which include aggressive anti-*P. aeruginosa* antibiotic treatment, PEP mask, and daily administration of Pulmozyme. No patients receive prophylactic anti-staphylococcal antibiotics [8]. This treatment was not changed during the observation period. Neither was there any change in nutritional support.

The study is an observational cohort study including all of our adult CF patients with chronic *P. aeruginosa* infection.

Fifty-three adult patients (32 males) started AZ treatment. 45 (27 males) completed 1 year of treatment with low-dose AZ, 250 mg daily. Two patients stopped the treatment because of adverse effects (tinnitus, which was reversible when the treatment stopped) and one because of planning of a pregnancy. Five patients could not be evaluated because of insufficient clinical follow-up. Median age was 29 years (range 17.5–50). Median duration of chronic *P. aeruginosa* infection was 22 years (range 2–31); all patients are chronically infected with both mucoid and non-mucoid phenotypes.

On average, the patients were examined 8.6 times in the pre-treatment period, and 8.0 times in the treatment period.

3. Statistical analysis

The means of the values for each period of time (pre-treatment and during treatment) were calculated and compared using a simple *t* test, paired two-sample for means. If more than one lung function test was performed, or if more than one sputum sample was investigated per month, the mean of the results was calculated and used for statistic analysis.

The slope of decline of lung function during 1 year was calculated, using linear regression, then compared in the same manner as mentioned above. Level of significance was <0.05 (two-tailed).

4. Results

4.1. Weight

The weight increased from a median of 63.1 kg (range 46.9–108.4) in the pre-treatment period to a median of 63.9 kg (46.8–114.3) in the treatment period ($p=0.01$).

Body mass index (BMI) increased from a median of 21.9 (17.6–34.1) to a median of 22.0 (17.7–36.1) ($p=0.03$).

4.2. Lung function

Using linear regression, we calculated the slope of decline, expressed as percent of predicted value, during 1 year. During the pre-treatment period, the median slope of decline was −4.1% for FEV_1 (−21.5% to 12.3%) and −3.0% for FVC (−39.5% to 13.6%).

During the year of treatment, the slope of decline changed significantly. The median slope of decline for FEV_1 changed to a positive value of +0.8% (−10.5% to 31.3%, $p<0.001$) for FVC +1.6% (−14.4% to 22.5%, $p=0.01$). The changes in slope of FEV_1 in individual patients in the pretreatment and treatment periods are illustrated in Fig. 1.

31 of 45 patients (69%) responded well to the treatment, reducing the rate of decline in FEV_1. The remaining 14 patients had an increased rate of decline in FEV_1.

4.3. Prevalence of mucoid P. aeruginosa in sputum

In the pre-treatment period, we found that a median of 90% (range 0–100) of lower respiratory tract (LRT) samples contained mucoid strains. In the treatment period, this number decreased significantly to a median of 81% (range 0–100) ($p=0.003$).

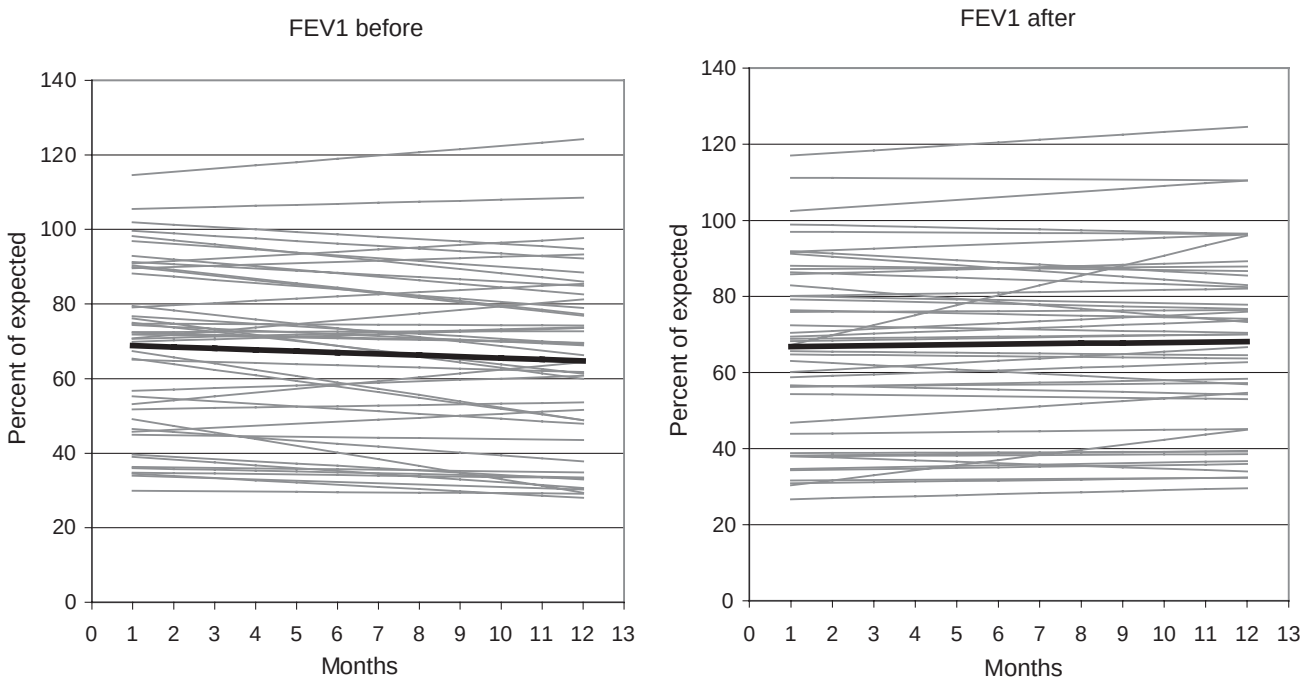

Fig. 1. The slope of decline in lung function in the year before and during treatment. The mean slope of decline of all patients (thick, black line) and the individual values (gray lines).

4.4. Other bacteria

The sputum samples are examined for growth of all common CF relevant microorganisms, and changes in the bacterial flora in the sputum samples during AZ treatment were evaluated. None of the patients was infected with *Burkholderia* species and the percentage of sputum samples containing *Achromobacter xylosoxidans*, *Moraxella catharralis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* did not change during the treatment period. The percentage of cultures containing *Stenotrophomonas maltophilia* increased slightly from a mean value of 3.3% to 5.0% (ns). The percentage of cultures containing *Staphylococcus aureus* decreased significantly, from a mean value of 11.4% to 7.6% (p=0.02).

4.5. C-reactive protein (CRP)

Blood samples were collected at each intravenous course of antibiotics, and at least once a year. In the year prior to treatment, the median CRP value was 6.2 mmol/l (range 3–44.5). In the treatment period, the median CRP was 5.8 mmol/l (p=0.13). One patient had an episode of kidney stone during the treatment period, resulting in urinary tract infection and an outstanding CRP of 132 mmol/l. Omitting that value from the analysis resulted in the drop in CRP becoming statistically significant (p=0.02).

The number of days on intravenous antibiotics did not change, being 51.5 days before treatment and 52.0 days during treatment.

4.6. Safety

With the exception of two patients with tinnitus, the patients reported no adverse effects.

5. Discussion

Several clinically controlled, randomized intervention studies have been published, which shows significant effects of AZ on lung function and other clinical parameters of CF patients with chronic *P. aeruginosa* infection [4,5,9]. It is, however, well-known that the selection criteria for controlled, randomized studies may result in trials that do not properly reflect all CF patients suffering from this infection. Observational cohort studies like the present compensate for this problem [10].

This open observational cohort study of 12-month low-dose AZ treatment, in an unselected group of 45 adult patients with CF and chronic *P. aeruginosa* infection, showed that a negative annual slope of decline of FEV_1 was changed from −4.1% of the predicted value to a positive annual slope with an increase of +0.8% corresponding to a difference of +4.9%. Wolter et al. [4] studied the effect of 250 mg of AZ daily for 3 months in a parallel placebo-controlled study of 30 adult patients and found a decline in FEV_1 of −3.6% in controls as opposed to no change in the treated group. Only 83.3% of the patients carried mucoid *P. aeruginosa.* Equi et al. [5] studied the effect of AZ treatment in 41 children with CF (aged 8–18 years) in a 2×6 months cross-over placebo controlled study

and found a median relative difference in FEV_1% of +5.4% between AZ and placebo. It is unclear how many of the patients had chronic *P. aeruginosa* infection but nearly all (38/41) were on inhaled anti-pseudomonal antibiotics. Similar results are reported by Saiman et al. [9] with +6.2% in mean difference in FEV_1 between AZ and placebo group. Baumann et al. [11] found an increase in lung function of 12.8% in patients treated with AZ, 250 mg/day.

All studies thus showed a significant increase in FEV_1% in the AZ-treated patients, which is likely to be due to decreased inflammation in the bronchial lumen. We found a decrease in CRP, which was statistically significant after exclusion of one patient with a CF non-related urinary tract infection. Wolter et al. [4] found a significant reduction in CRP but not ESR in the treated patients, while Equi et al. [5] did not include ESR or CRP in their study. Saiman et al. [9] found no difference in the levels of elastase and IL-8 between the two groups.

Lung infection and inflammation in patients with CF are often reflected in loss of weight and we detected a slight but statistically significant increase of 0.8 kg (p=0.01) and BMI (p=0.03) in the treated patients. A similar positive effect on weight in the treatment group was reported by Saiman et al. [9]. The weight of the patients was not recorded in the study by Equi et al. [5], and Wolter et al. [4] found no statistical significant difference in BMI between placebo and AZ-treated patients, but BMI is a less sensitive marker than weight, the number of patients was smaller, and the treatment period was shorter than in our study and the study by Saiman et al. [9].

Improved lung function and evidence of decreased inflammation in AZ-treated patients could presumably be due to anti-infectious and/or anti-inflammatory effects of AZ. Sputum cultures are obtained on a regular basis and we found a significant decrease in the percentage of cultures positive for *S. aureus*, which may have contributed to decreased lower airway infection and inflammation. Although the decrease was statistically significant (p= 0.02), it was numerically small (from 11.4% to 7.6% of sputum samples) and cannot explain the observed increase in lung function since all patients are cultured once every month and because a positive culture of *S. aureus* from LRT secretions will be treated aggressively irrespective of the clinical condition. In the study by Equi et al. [5], there were no changes in frequency of isolation or colony density of *S. aureus* during the study period as compared to the previous period. In the study by Wolter et al. [4], there were no differences in organisms isolated or bacterial counts between the placebo and AZ-treated groups at baseline or at final assessment and thus no evidence that clinical improvement was due to treatment of bacteria susceptible to macrolides. In the study by Saiman et al. [9], no difference in eradication rate of *S. aureus* were found between the groups. On the other hand, there were less patient in the AZ group with newly acquired *S. aureus* infection at the end of the study, when compared to the placebo group.

Some CF centres prescribe prophylactic anti-staphylococcal antibiotics. We do not recommend that azithromycin be used in this way because of the risk of encouraging bacterial resistance [12].

The interesting microbiological finding in the present study was the small but 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 AZ treatment. Patients were selected if chronically infected with *P. aeruginosa* and the large majority had, for many years prior to study, harbored both mucoid and non-mucoid strains. This indicates that AZ may have had a selective activity against mucoid (i.e., alginate-producing strains, or mucoid strains rendered non-mucoid in accordance with its known inhibitory activity on a key enzyme in alginate biosynthesis) [13]. In the study by Wolter et al. [4], 57 of 60 patients had sputum analysis at entry, where 83.3% carried mucoid strains and 66.6% non-mucoid. The authors found no significant difference in quality or quantity of pathogens at baseline, or at final assessment between the AZ and placebo groups. Since that study included 30 AZ-treated patients followed over 3 months, as compared to the present 45 patients followed over 12 months, it is possible that the number of sputum samples was too small to disclose a change in phenotype in the treated group. In the study of Equi et al. [5], chronic infection with *P. aeruginosa* was not an entry criterion and only half of the patients (21/41) had three positive cultures in the year prior to entry. The authors found no difference in the number of isolates of *P. aeruginosa* or in the colony density between treatment and placebo periods, but it appears that many patients received nebulized anti-pseudomonal prophylaxis (38/41) during the trial. Furthermore, the authors apparently did not distinguish between morphotypes of cultured *P. aeruginosa.* In the study by Saiman et al. [9], only two sputum or throat cultures were done during the study, one at inclusion and one at day 168. There was no difference between the two groups with regard to occurrence of mucoid phenotype.

Probably because of the rigorous aggressive antimicrobial treatment of the present patients, including elective intravenous antibiotic courses every 3–4 months, we could not detect changes in exacerbations of lung disease or in demand for antibiotics. Wolter et al. [4] found a significant reduction in respiratory exacerbations and demand for hospitalization and intravenous antibiotic treatment, as well as improved quality of life scores. In the study by Equi et al. [5], the treated group received fewer additional courses of oral antibiotics than the placebo group but they did not detect differences in the number of pulmonary exacerbations and intravenous courses of antibiotics. The patients in the latter study were younger and the study probably had less patients with chronic *P. aeruginosa* infection than the study by Wolter et al. [4]. In the study by Saiman et al. [9], there

was no statistically significant difference between the two groups concerning days in hospital and days on intravenous treatment.

The overall conclusion from the present and other studies is that long-term AZ treatment of patients with CF and chronic broncho-pulmonary *P. aeruginosa* infection leads to reduced inflammation resulting in substantial improvement in lung function and decreased morbidity. It is yet unclear whether this effect comes from sub-MIC inhibition of the microorganisms, or from modulation of the local inflammatory response to the infection.

Chronic *P. aeruginosa* lung infection in patients with CF and in patients with DPB is a biofilm where the bacteria form complex community structures [14]. To do so, the bacteria depend upon a system of cell-to-cell communication, which is termed "quorum sensing." *P. aeruginosa* synthesize and release small self-generated signal molecules acting as "autoinducers" since each individual cell can synthesize as well as respond to the signal molecule (synthesis and detection of extracellular signals) [15–17]. *P. aeruginosa* strains defective in genes controlling quorum sensing signaling have markedly reduced virulence in an animal model, and a large number of genes are regulated by quorum sensing signals, many of which code for putative virulence factors, among which are genes coding for alginate production.

The important observation that long-term macrolide therapy significantly increased survival of patients with DPB and chronic *P. aeruginosa* infection [13] has led to a number of in vitro and in vivo studies. It has been shown that macrolides including AZ at concentrations far below minimal inhibitory concentrations (MIC) suppress protein synthesis and synthesis of virulence factors including alginate production in *P. aeruginosa*, which may lead to loss of viability with prolonged exposure [18–24], and AZ specifically inhibits quorum sensing [25]. There is evidence suggesting that AZ at a concentration of as little as 2 μg/ml (which is 1/64 of MIC) may be bactericidal to *P. aeruginosa* in stationary growth phase but not in exponential growth phase [20,26].

Another possible way of action in damaging the bacteria has been mentioned by Saiman et al. [27]. AZ has been shown to, in vitro, act synergistically against *P. aeruginosa* and other bacteria when combined with other antibiotics. All our patients receive regular courses of intravenous antibiotics during AZ treatment.

There is, however, increasing evidence that macrolide antibiotics may possess anti-inflammatory properties through interference with different parts of the immune system [28–31]. Of particular interest to CF and DPB is the finding that the number of neutrophils and the amount of neutrophil-derived elastolytic-like activity are reduced in bronchoalveolar lavage (BAL) fluid after treatment of DPB patients with erythromycin [32] and that AZ modulates PMN function and inflammatory markers in healthy human volunteers [33]. Two other macrolides, flurythromycin and erythromycin, have been shown to inhibit human neutrophil elastase in vitro [34].

Our work is the first to show a significant fall in the amount of mucoid strains when AZ is used in vivo against *P. aeruginosa*, consistent with the results of several in vitro studies. The exact mechanism remains to be found.

6. Conclusion

Long-term, low-dose AZ treatment in adult CF patients with chronic *P. aeruginosa* infection has the ability to inhibit the formation of biofilm by the bacteria, shown by a decrease in sputum samples containing mucoid *P. aeruginosa*. We confirmed that long-term AZ treatment is safe and had a significant positive effect on the course of CF lung disease.

References

[1] Hoiby N. New antimicrobials in the management of cystic fibrosis. J Antimicrob Chemother 2002;49(2):235–8.
[2] Hoiby N. *Pseudomonas aeruginosa* infection in cystic fibrosis. Relationship between mucoid strains of *Pseudomonas aeruginosa* and the humoral immune response. Acta Pathol Microbiol Scand, B Microbiol Immunol 1974;82(4):551–8.
[3] Jaffe A, Francis J, Rosenthal M, Bush A. Long-term azithromycin may improve lung function in children with cystic fibrosis. Lancet 1998;351(9100):420.
[4] Wolter J, Seeney S, Bell S, Bowler S, Masel P, McCormack J. Effect of long term treatment with azithromycin on disease parameters in cystic fibrosis: a randomised trial. Thorax 2002;57(3):212–6.
[5] Equi A, Balfour-Lynn IM, Bush A, Rosenthal M. Long term azithromycin in children with cystic fibrosis: a randomised, placebo-controlled crossover trial. Lancet 2002;360(9338):978–84.
[6] Pirzada O. Improved lung function and body mass index associated with long-term use of macrolide antibiotics. J Cystic Fibros 2003;2:69–71.
[7] Høiby NFB. Microbiology of cystic fibrosis. London: Arnold; 2000.
[8] Hoiby N, Koch C. Maintenance treatment of chronic *Pseudomonas aeruginosa* infection in cystic fibrosis. Thorax 2000;55(5):349–50.
[9] Saiman L, Marshall BC, Mayer-Hamblett N, Burns JL, Quittner AL, Cibene DA, et al. Azithromycin in patients with cystic fibrosis chronically infected with *Pseudomonas aeruginosa*: a randomized controlled trial. JAMA 2003;290(13):1749–56.
[10] Grimes DA, Schulz KF. An overview of clinical research: the lay of the land. Lancet 2002;359(9300):57–61.
[11] Baumann U, King M, App EM, Tai S, Konig A, Fischer JJ, et al. Long term azithromycin therapy in cystic fibrosis patients: a study on drug levels and sputum properties. Can Respir J 2004;11(2):151–5.
[12] Phaff SJ, Tiddens HAW, Ott A. Chronic use of azithromycin in CF is associated with a substantial increase in macrolide resistance of *S. aureus* and *Haemophilus species*. In: Heijerman HGM, editor. 27th European Cystic Fibrosis Conference, 2004, Birmingham, UK. Journal of Cystic Fibrosis, p. S40.
[13] Kobayashi H. Biofilm disease: its clinical manifestation and therapeutic possibilities of macrolides. Am J Med 1995;99(6A):26S–30S.
[14] Hoiby N, Krogh Johansen H, Moser C, Song Z, Ciofu O, Kharazmi A. *Pseudomonas aeruginosa* and the in vitro and in vivo biofilm mode of growth. Microbes Infect 2001;3(1):23–35.
[15] de Kievit TR, Iglewski BH. Bacterial quorum sensing in pathogenic relationships. Infect Immun 2000;68(9):4839–49.

[16] Bassler BL. How bacteria talk to each other: regulation of gene expression by quorum sensing. Curr Opin Microbiol 1999;2(6): 582–7.
[17] Singh PK, Schaefer AL, Parsek MR, Moninger TO, Welsh MJ, Greenberg EP. Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. Nature 2000;407(6805): 762–4.
[18] Yasuda H, Ajiki Y, Koga T, Kawada H, Yokota T. Interaction between biofilms formed by *Pseudomonas aeruginosa* and clarithromycin. Antimicrob Agents Chemother 1993;37(9):1749–55.
[19] Molinari G, Guzman CA, Pesce A, Schito GC. Inhibition of *Pseudomonas aeruginosa* virulence factors by subinhibitory concentrations of azithromycin and other macrolide antibiotics. J Antimicrob Chemother 1993;31(5):681–8.
[20] Tateda K, Ishii Y, Matsumoto T, Furuya N, Nagashima M, Matsunaga T, et al. Direct evidence for antipseudomonal activity of macrolides: exposure-dependent bactericidal activity and inhibition of protein synthesis by erythromycin, clarithromycin, and azithromycin. Antimicrob Agents Chemother 1996;40(10):2271–5.
[21] Tateda K, Ishii Y, Matsumoto T, Kobayashi T, Miyazaki S, Yamaguchi K. Potential of macrolide antibiotics to inhibit protein synthesis of *Pseudomonas aeruginosa*: suppression of virulence factors and stress response. J Infect Chemother 2000;6(1):1–7.
[22] Kondoh K, Hashiba M, Baba S. Inhibitory activity of clarithromycin on biofilm synthesis with *Pseudomonas aeruginosa*. Acta Otolaryngol Suppl 1996;525:56–60.
[23] Ichimiya T, Yamasaki T, Nasu M. In-vitro effects of antimicrobial agents on *Pseudomonas aeruginosa* biofilm formation. J Antimicrob Chemother 1994;34(3):331–41.
[24] Ordonez CL, Stulbarg M, Grundland H, Liu JT, Boushey HA. Effect of clarithromycin on airway obstruction and inflammatory markers in induced sputum in cystic fibrosis: a pilot study. Pediatr Pulmonol 2001;32(1):29–37.
[25] Tateda K, Comte R, Pechere JC, Kohler T, Yamaguchi K, Van Delden C. Azithromycin inhibits quorum sensing in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2001;45(6):1930–3.
[26] Iglewski BH. The role of quorum sensing in azithromycin sensitivity of *P. aeruginosa*. Pediatr Pulmonol 2002,Supplement 24, p. 146.
[27] Saiman L, Chen Y, Gabriel PS, Knirsch C. Synergistic activities of macrolide antibiotics against *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, and *Alcaligenes xylosoxidans* isolated from patients with cystic fibrosis. Antimicrob Agents Chemother 2002;46(4):1105–7.
[28] Labro MT. Anti-inflammatory activity of macrolides: a new therapeutic potential? J Antimicrob Chemother 1998;41(Suppl. B):37–46.
[29] Yanagihara K, Tomono K, Sawai T, Hirakata Y, Kadota J, Koga H, et al. Effect of clarithromycin on lymphocytes in chronic respiratory *Pseudomonas aeruginosa* infection. Am J Respir Crit Care Med 1997;155(1):337–42.
[30] Brennan S, Cooper D, Sly PD. Directed neutrophil migration to IL-8 is increased in cystic fibrosis: a study of the effect of erythromycin. Thorax 2001;56(1):62–4.
[31] Saiman L. Do macrolides have an anti-inflammatory effect? Pediatr Pulmonol 2002,Supplement 24, p. 147–9.
[32] Ichikawa Y, Ninomiya H, Koga H, Tanaka M, Kinoshita M, Tokunaga N, et al. Erythromycin reduces neutrophils and neutrophil-derived elastolytic-like activity in the lower respiratory tract of bronchiolitis patients. Am Rev Respir Dis 1992;146(1):196–203.
[33] Culic O, Erakovic V, Cepelak I, Barisic K, Brajsa K, Ferencic Z, et al. Azithromycin modulates neutrophil function and circulating inflammatory mediators in healthy human subjects. Eur J Pharmacol 2002;450(3):277–89.
[34] Gorrini M, Lupi A, Viglio S, Pamparana F, Cetta G, Iadarola P, et al. Inhibition of human neutrophil elastase by erythromycin and flurythromycin, two macrolide antibiotics. Am J Respir Cell Mol Biol 2001;25(4):492–9.