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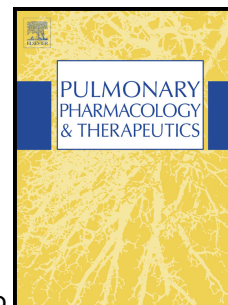
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TITLE

Long term azithromycin use in patients with Chronic Obstructive Pulmonary Disease and Tracheostomy

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ABSTRACT

Patients with Chronic Obstructive Pulmonary Disease (COPD) and tracheostomy are at high risk for exacerbations and hospitalizations. Macrolide treatment has shown to reduce exacerbations in moderate-to-severe COPD. To evaluate the safety and the efficacy of long-term azithromycin use in outpatients with severe COPD and tracheostomy.

A multicenter, randomized, uncontrolled, pilot trial evaluating the safety and the efficacy of azithromycin 500 mg three day-a-week for 6 months (AZI) vs. standard of care (SC) in severe COPD outpatients with tracheostomy. Patients were monitored for six months of treatment *plus* six months of follow up. The primary outcome was the reduction in the number of exacerbations and hospitalizations.

A total of 22 patients was randomized (11 to SC and 11 to AZI). Patients in AZI had a significant lower cumulative number of exacerbations after the first 3 months of treatment when compared to patients in SC ($p=0.001$), as well as hospitalizations ($p=0.02$). Kaplan-Meier survival curves for time to first exacerbation showed a significant reduction in AZI of the rates of first exacerbation when compared to SC (log rank test=12.14, $p<0.001$), as well as to first hospitalization (log-rank= 4.09, $p=0.04$). Azithromycin significantly improved the quality of life in comparison to SC. No serious adverse events in the AZI group were reported.

Long term azithromycin treatment seems to be safe and effective in severe COPD outpatients with tracheostomy in reducing exacerbations, hospitalizations, as well as in improving quality of life.

Key words: macrolide, COPD, acute exacerbation of chronic bronchitis, tracheostomy

Abbreviations: quality of life (QOL); Mageri Respiratory Failure questionnaire (MRF26); C-reactive protein (CRP); erythrocyte sedimentation rate (ESR); Exhaled Breath Condensate (EBC); colony forming units (CFU); minimum inhibitory concentrations (MIC); Tumor necrosis factor- α (TNF- α); enzyme immunoassay kit (EIA); standard care (SC); azithromycin (AZI)

INTRODUCTION

The use of tracheostomy is a well known indication for patients with end-stage chronic obstructive pulmonary disease (COPD) who require airway access for secretion removal, who need to be weaned from mechanical ventilation during an exacerbation or who become chronically ventilator-dependent. The proportion of COPD patients who require long-term tracheostomy is poorly studied. Among long-term tracheostomized patients microbial colonization of the distal airways is extremely frequent, involving up to 50% of patients [1-3]. Bacterial colonization may stimulate secondary host defenses and lead directly to persistent airway inflammation, even in COPD patients in a stable clinical state [4]. Several studies have demonstrated a strong association between an increase in the airway bacterial load and the presence of several inflammatory markers in airway secretions [5]. Microbial colonization in COPD patients has been also proven to accelerate the worsening of lung function and to increase the frequency of exacerbations [6, 7]. In patients affected by chronic bronchitis, exacerbations result in a significant impairment of the quality of life and in an increase of health care utilization and mortality [8, 9].

During the past decade, a growing interest in macrolides has emerged and focused not only on their antibacterial activity but also on their immunomodulating properties, as shown by several *in vitro* and *in vivo* studies [10]. Several studies have shown azithromycin reducing airway neutrophils and interleukin (IL) 8 concentrations in patients with bronchiolitis obliterans syndrome [11] and blocking neutrophil recruitment in *Pseudomonas* endobronchial infection in mice [12]. The anti-inflammatory and antibacterial action of macrolides has been studied in bronchial asthma [13] and it has been suggested in other respiratory diseases including diffuse panbronchiolitis [14], bronchiectasis [15] and cystic fibrosis [16, 17]. Erythromycin therapy has

been also shown to significantly reduce exacerbations in moderate-to-severe COPD patients [18].

In view of these data, azithromycin may be useful in a well-characterized population of COPD patients with tracheostomy who are at highest risk for exacerbations, in decreasing their microbial colonization pattern and reducing the rate of exacerbations and hospitalizations.

The aim of this study was to evaluate the efficacy and safety of long-term azithromycin use in outpatients with severe COPD and tracheostomy in terms of clinical, bacteriological and immunological effects, as well as impact on quality of life.

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MATERIAL AND METHODS

Study design

This was a phase II, multicenter, open-label, randomized, uncontrolled, pilot trial evaluating the efficacy and safety of long term azithromycin use in severe COPD outpatients with tracheostomy.

Patients were recruited between October 2004 and April 2006 at the Istituto Nazionale di Riposo e Cura per Anziani (INRCA), Casatenovo, Lecco, at the Institute of Respiratory Diseases, University of Milan, IRCCS Fondazione Po.Ma.Re., Milan and at the U.O. Pneumologia, Ospedale di Cattinara, Trieste, Italy. The study was coordinated by the Institute of Respiratory Diseases, University of Milan, IRCCS Fondazione Po.Ma.Re., Milan, Italy. Approval from local institutional review board was obtained and a written informed consent was signed by each patient prior to enrolment. The study was conducted in compliance to the Declaration of Helsinki and the Good Clinical Practice Guidelines of the European Union.

Outpatients more than 45 years of age with a history of severe COPD diagnosed with pulmonary function test and tracheostomy were candidates for inclusion in the study. Patients were excluded from the study if any of the following criteria was present: (1) allergy to macrolides; (2) life expectancy less than 1 year.

Patients eligible for the study were randomized to receive standard care (SC group) or standard care *plus* oral Azithromycin (Pfizer Pharmaceutical Company, Italy) at a dose of 500 mg daily three day-a-week (Monday, Tuesday, Wednesday) for 6 months (AZI group). Randomization was carried out with the use of computer-generated block sequences of 4.

Patients were monitored every month over a period of one year after enrolment: six months of treatment *plus* six months of follow up. Every month a phone call was performed and information regarding death, exacerbations, hospitalizations and adverse events was collected. Five visits were also scheduled: at entry and at month 3, 6, 9 and 12. At entry visit, demographic information and a complete history were recorded, and patients were randomized to standard care or standard care *plus* azithromycin. Clinical features were collected and patients were assessed by quality of life (QOL) survey up to the sixth month of protocol. QOL was assessed using the Mageri Respiratory Failure questionnaire (MRF26) that has been previously used in research studies [19]. Tracheal aspirate was obtained and sent to the local microbiology laboratory for quantitative cultures. In addition, a blood gas analysis was performed and a venous blood sample was collected for measurement of albumin, leukocyte count, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). In a subgroup of subjects enrolled at the INRCA, Casatenovo, Lecco, Italy, Exhaled Breath Condensate (EBC) samples were also obtained. At each further study visit, all entry visit procedures were repeated and information regarding exacerbations, hospitalizations and adverse events was collected.

Culture was performed according to standard microbiological methods [20]. Bacterial counts were assessed with the method of serial dilutions with saline (10^4 – 10^7 by serial 10-fold dilutions of the original sample). The medium used was sheep blood agar. Data are reported as colony forming units (CFU)/ml. Antibiotic susceptibility was determined by assessing minimum inhibitory concentrations (MIC) of antimicrobial agents according to National Committee for Clinical Laboratory Standards (NCCLS) recommendations [21].

The EBC sample was collected using a specially designed condensing chamber (Ecoscreen; Jaeger, Hoechberg, Germany). The exhaled air entered and left the chamber through a one-way inlet and outlet valve, in order to keep the chamber closed. The subjects wore noseclips and

breathed tidally for ten minutes through a mouthpiece connected to a condenser. Approximately 1 ml of the sample was collected and transferred to a 2-ml plastic tube and stored at -70°C . Tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) concentrations in the breath condensate were measured using a specific enzyme immunoassay kit (EIA) (Cayman Chemical, Ann Arbor, USA). The assay was directly validated by means of gas chromatography/mass spectrometry in order to obtain a high correlation ($r=0.95$) between the known amounts of TNF- α or IL-6 and the concentration measured by the EIA.

Study definitions and outcomes

Colonization was defined as the presence of a potential pathogenic organism from cultures obtained at baseline without signs or symptoms of acute infection. Exacerbation was defined as a worsening of symptoms requiring both a change in regular respiratory medication or medical assistance, or resulting in hospitalization or in an emergency room treatment. Hospitalization was defined as care at an acute-care hospital lasting for 24 hours or more.

Adverse events were evaluated in all patients that received at least one dose of the study drug. Adverse events were recorded at all phone calls and at all visits. Compliance to azithromycin treatment was evaluated by pills count at each study visit.

The primary outcome of the study was to determine if azithromycin use was associated with a reduction in the number of exacerbations and hospitalizations during the study period. Secondary outcomes included time to first exacerbation and hospitalization, steroid and antibiotic use, evaluation of the inflammatory cytokines values in the EBC, mortality, assessment of the quality of life and safety.

Statistical analysis

Data were analyzed with Stata for Windows, Version 9 (StataCorp 2005). Wilcoxon rank-sum test was used to compare the cumulative number of exacerbations, hospitalizations, steroid treatments and antibiotic treatments experienced in the first 3 months after randomization by patients in the AZI group vs. those in the SC group. Among the sub-sample of survivors who did not experience death or loss to follow-up during the study, attenuation of treatment effect was tested by comparing the number of exacerbations and hospitalizations in the first 6 months of treatment with the cumulative number of the same events occurring in the second 6 months after discontinuation of therapy, by applying a Wilcoxon signed-rank test. In the analysis of the secondary outcomes patients who were lost to follow-up or had not experienced the outcome of interest were censored by the end of the 12-month follow-up period. Kaplan-Meier survival analysis and bivariate Cox regression models were used to assess the times to death, to first exacerbation and to first hospitalization according to treatment status and to obtain quantitative estimates of the effect of azithromycin treatment on the hazard of these secondary outcomes. The Cox regression model requires the assumption of the hazard rate among the categories of the predictor being proportional with time. To test this assumption, we obtained a “log-log” plot by plotting $-\ln[-\ln(\text{Survival probabilities})]$ of each treatment group against $\ln(\text{months})$ and visually confirmed that the plots of the categories were parallel to each other. A t-test for paired data was applied in order to evaluate the effect of the two treatments, azithromycin and standard care over time.

RESULTS

Recruitment and baseline data

A total of 37 patients was recruited between October 2004 and April 2006 and among them, 15 patients met the exclusion criteria. A total of 22 patients were randomized as follows: 11 to standard care (SC group) and 11 to standard care *plus* Azithromycin (AZI group), see Figure 1. There were no appreciable differences in baseline characteristics among the two groups, see Table 1.

Exacerbation and hospitalization frequency

The cumulative number of exacerbations experienced during the one-year study period by both study groups is given in Table 2. Patients in the AZI group had a significant lower cumulative number of exacerbations after the first 3 months of treatment when compared to patients in the SC group (2 vs. 12, respectively, $z=3.237$, $p = 0.001$). Kaplan-Meier survival curves for time to first exacerbation showed a significant reduction in the AZI group of the rates of first exacerbation when compared to SC group (log rank test=12.14, $p<0.001$), as shown in Figure 2. The estimated hazard ratio of having a first exacerbation associated to standard care was 5.41 ($p=0.005$, 95% CI 1.67-17.5). Therefore, on average, patients in the SC group had a more than five-fold significantly higher hazard of experiencing a first exacerbation in comparison to patients in the AZI group.

The mean number of exacerbations after 3, 6, 9 and 12 months among the patients who survived a year without death or loss to follow up is given in Figure 3. No statistical methods were further applied to these data due to the small sample size and the heterogeneity of the two groups.

The number of exacerbations experienced by the patients in the AZI group during the first 6 months of treatment was significantly different from the number of exacerbations experienced in the next 6 months of follow up ($p=0.016$). On the other hand, the number of exacerbations experienced by the patients in the SC group during the first 6 months was not significantly different from the number of exacerbations experienced in the next 6 months of follow up ($p=0.56$).

The cumulative number of hospitalizations experienced during the one-year study period by both study groups is given in Table 2. Comparing the cumulative number of hospitalizations after 3 months of treatment a statistically significant difference was found between patients in the AZI group and those in the SC group (0 vs. 4, respectively, $z=2.275$, $p = 0.02$). Results from Kaplan-Meier survival analysis for time to first hospitalization are shown in figure 4.

A significant lower rate of first hospitalization was found in the AZI group when compared to SC group (log-rank= 4.09, $p=0.04$). On average, patients receiving standard care had a 2.64 times higher risk ($p=0.07$, 95% CI 0.92–7.6) of being hospitalized since entering the study compared to patients receiving azithromycin. The mean number of hospitalizations after 3, 6, 9 and 12 months among the patients who survived a year without death or loss to follow up is given in figure 5. The number of hospitalizations in both AZI and SC group during the 6 months of treatment was not significantly different from the number of hospitalizations experienced in the next 6 months of follow up ($p=0.29$ and $p=0.39$, respectively).

Antibiotic and steroid frequency

The cumulative number of antibiotic and steroid treatments in both study groups during the one-year study period is given in Table 3. Patients in the AZI group had a significantly lower number of antibiotic treatments after 3 months of treatment when compared to patients in the SC group (2

vs. 11, respectively, $z=3.238$, $p = 0.001$). Patients in the AZI group had a significantly lower number of steroid treatments after 3 months of treatment when compared to patients in the SC group (1 vs. 7 respectively, $z=2.801$, $p = 0.005$).

Mortality

Mortality rate observed in the AZI group was lower in comparison to the mortality rate recorded in the SC group, although this finding was not statistically significant (27% vs. 46% respectively, $p=0.33$). Results from a Cox proportional hazards model showed that, on average, patients in the SC group had a hazard of dying that was 2.06 times the hazard of dying among patients in the AZI group ($p= 0.33$). One patient in the SC group was diagnosed with tuberculosis after 7 months and received the appropriate treatment and was lost at follow up at 10 months.

Bacteriology

Microbiology results from tracheal aspirate culture in both study groups are shown in Table 4. A total of 6/11 and 5/11 patients were colonized at baseline with *P. aeruginosa* in the AZI group and the SC group, respectively. In two patients colonized on admission with *P. aeruginosa* in the AZI group colony counts were reduced after 3 months of treatment and in one patient the microorganism was eradicated. *P. aeruginosa* became resistant to ceftazidime after 6 months of treatment in one patient in the AZI group. An erythromycin-resistant *S. pneumoniae* strain was identified in one patient in the AZI group after 6 months of treatment.

Inflammatory markers

Baseline levels of TNF- α and IL-6 were measured on the EBC for 3 patients in the AZI group (9.16 ± 3.24 and 7.55 ± 2.08 pg·L⁻³, respectively) and 2 patients in the SC group (9.76 ± 1.51 and 8.58 ± 1.53 pg·L⁻³, respectively). During azithromycin treatment a decrease in both TNF- α (5.92 ± 1.3) and IL-6 (6.38 ± 2.0) values was registered in the AZI group, while an increase of TNF- α (11.5 ± 0.3) and IL-6 (9.33 ± 1.7) was observed in the SC group. Once azithromycin was discontinued, TNF- α and IL-6 values in the AZI group rose back to baseline (9.86 ± 1.0 and 8.72 ± 2.1 , respectively).

Quality of life

The AZI group significantly improved in terms of quality of life in comparison to the SC group. This improvement was statistically significant for the MRF Total score as well as for the subscale scores (Activity and Disability), see Table 5. In particular, after 3 and 6 months a 8 unit MRF26 Total score improvement was observed versus baseline ($p=0.006$ and $p=0.002$, respectively). Conversely, QoL of patients in the SC group did not improve.

Adverse events profile

No serious adverse events in the AZI group were reported. Mild adverse events occurred in 4 patients: diarrhea (3 patients) and stomachache (3 patients). All these mild adverse events were transient and did not involve discontinuation of therapy, with the exception of one patient with diarrhea and stomachache after 5 months of treatment who interrupted therapy for 7 days.

DISCUSSION

The most important finding of our pilot study is that long-term azithromycin use seems to reduce the rate of exacerbations and hospitalizations in severe COPD outpatients with tracheostomy, as well as the use of antibiotic and parental steroids. We also found that the quality of life of severe COPD patients with tracheostomy treated with azithromycin improved in comparison to those treated with standard of care. Moreover, long-term azithromycin treatment did not show a significant impact on airways bacterial colonization.

We decided to chose a population of COPD patients with tracheostomy due to the high rate of colonization and infection. In this population the possible beneficial effect of a long term antibiotic treatment would be potentially easier to identify. The mechanism by which azithromycin seems to reduce the number of infective exacerbations and chronic symptoms is unknown, but it is likely to be multifactorial. Macrolide prophylaxis has been demonstrated to improve the natural history of cystic fibrosis [16] and diffuse panbronchiolitis [22]. Based on these experiences we decided to perform the present study using long-term macrolide treatment. Particularly, azithromycin has been previously identified as an active agent in terms of anti-inflammatory and immunomodulatory properties [14]. This effect may be due to the down-regulation of the host immune response by azithromycin, leading to a decrease in the host mediated tissue damage, as postulated in the vicious circle hypothesis. It might also benefit patients by reducing bacterial load and therefore the stimulation for neutrophilic inflammation, or by influencing the pathogenic mechanisms of bacteria. The anti-inflammatory action of macrolides seems to be mediated by the inhibition of neutrophil chemotaxis, the reduction of neutrophil elastase, and the change in the release of pro-inflammatory cytokines [23].

Macrolide antibiotics have also been shown to reduce mucus secretion and sputum viscoelasticity and airway adhesion of *P. aeruginosa*, and increase the killing of mucoid *P. aeruginosa*. This main action seems to be determined not only by the disruption of the protective biofilm integrity and the impairment of the transformation of non-mucoid *P. aeruginosa* to a more virulent mucoid phenotype, but also by direct antipseudomonal activity and anti-quorum sensing activities [24-26]. Furthermore, azithromycin is characterized by a favourable profile in terms of pharmacokinetics leading to a high patient compliance.

Because of this, we decided to test azithromycin use in this population. The 6-month length of azithromycin treatment has been based on previous studies performed by Equi et al and Wolter et al on cystic fibrosis patients [16, 17].

From a microbiological point of view, azithromycin treatment was not associated with significant effects in terms of reduction of the bacterial load or bacterial eradication. Moreover, in patients on long-term treatment with azithromycin no resistances were selected except one case. Finally, azithromycin long-term use seems to be a well tolerated and safe treatment.

The present study should be considered as a “proof of concept” that could sustain the development of future placebo-controlled trials in order to improve the reliability of our outcomes. One of the main weakness of our study concerns the high rate of drop out due to unexpected early death rate recorded. This finding is possibly related to inaccurate clinical evaluation of the life expectancy that needs to be acknowledged. In view of this, our main statistical analysis was focused on the first three months of the trial. Results recorded during the rest of the study period are presented as descriptive data. In evaluating the safety profile of azithromycin, we were not able to obtain data on liver function during and after the treatment. Another issue we were not able to evaluate was the azithromycin effect on quorum sensing. Moreover, we were able to evaluate the airways inflammatory levels only in a small subset of our study population.

To our knowledge, this was the first study in the literature evaluating the long-term azithromycin use in a well characterized population composed by severe COPD outpatients with tracheotomy. Recently, Seemungal and colleagues found erythromycin use to reduce exacerbations frequency in moderate-to-severe COPD patients [18]. From a social point of view, an important implication of chronic antimicrobial therapy in COPD patients would be the emergence of bacterial resistance. On the other hand, we suggest long-term azithromycin use in a small proportion of patients with COPD, those with tracheostomy, who are at highest risk for exacerbations. The major strength of our study is represented by the analysis of the azithromycin effect from clinical, microbiological and immunological aspects. Moreover, we found concordant results for all analyzed outcomes. In fact, clinical, immunological and quality of life outcomes showed a consistent trend towards improvement in the severe COPD patients with tracheostomy treated with azithromycin.

The analysis at 3 months showed a concordance in reduction of exacerbations, hospitalizations, antibiotic and steroids use. Although our data involved a small number of patients, we also found a trend in the reduction of cytokines during azithromycin treatment being consistent with the clinical scenario. We also found that after the discontinuation of azithromycin treatment, clinical and immunological parameters returned to baseline. These findings do not appear to be associated with microbiological variations in our patients and we speculate that the effect of azithromycin may be due to its anti-inflammatory properties.

The main implication of our results is an improvement in terms of quality of life in COPD patients undergoing azithromycin treatment. Moreover, the reduction in exacerbations and hospitalizations we observed could significantly reduce health-care costs. If our data will be confirmed in future randomized controlled trials, macrolide treatment could have a role in a long term treatment of patients with COPD and tracheostomy. Furthermore, another interesting

subgroup of patients in whom long term antibiotic treatment would deserve further investigation would be the very severe non tracheostomy COPD patients.

CONCLUSIONS

In conclusion, in our pilot study long term azithromycin treatment seems to be safe and effective in severe COPD outpatients with tracheostomy in reducing exacerbations, hospitalizations, antibiotic and steroids use, as well as in improving quality of life.

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FIGURE LEGENDS

Figure 1. Flow diagram of the study

Footnotes: Pts: patients

Figure 2. Kaplan-Meier survival curve for time to first exacerbation in the standard care (*dashed line*) and azithromycin (*solid line*) groups

Figure 3. Mean number of exacerbations after 3, 6, 9 and 12 months among patients that survived a year without death or loss to follow up for both study groups

Figure 4. Kaplan-Meier survival curve for time to first hospitalization in the standard care (*dashed line*) and azithromycin (*solid line*) groups

Figure 5. Mean number of hospitalizations after 3, 6, 9 and 12 months among the patients that survived a year without death or loss to follow up for both study groups

TABLES

Table 1. Demographics, past medical history, clinical and laboratory findings, bacterial colonization on admission in the two study groups

Variable	Standard care group n = 11	Azithromycin group n =11
Demographics		
Age, years, mean ($\pm$ SD)	73 ($\pm$ 7)	72 ($\pm$ 7)
Men, no. (%)	9 (82)	10 (91)
BMI, mean ($\pm$ SD)	22 ($\pm$ 5)	26 ($\pm$ 6)
Smoking habit		
Current smokers, no. (%)	0 (0)	0 (0)
Former smoker, no. (%)	10 (91)	11 (100)
No smoker, no (%)	1 (9)	0 (0)
Cigarette consumption, mean pack-years ($\pm$ SD)	40 ($\pm$ 30)	32 ($\pm$ 9)
Past medical history, no. (%)		
Essential Hypertension	5 (46)	4 (36)
Coronary Artery Disease	4 (36)	3 (27)
Diabetes	1 (9.1)	2 (18)
Chronic Renal insufficiency	1 (9.1)	0 (0)
Malignancy	2 (18)	3 (27)
Long Term Oxygen Therapy	5 (46)	5 (46)

Mean number of exacerbations in the previous year, mean ($\pm$ SD)	3.1 ($\pm$ 0.9)	3.0 ($\pm$ 1.1)
Mean numbers of antibiotic courses in the previous year, mean ($\pm$ SD)	2.7 ($\pm$ 0.8)	2.6 ($\pm$ 0.7)
MRC, mean ($\pm$ SD)	2.6 ($\pm$ 0.9)	3.0 ($\pm$ 1.0)
MRF26 score		
Total, mean ($\pm$ SD)	70.3 ($\pm$ 15.4)	57.7 ($\pm$ 17.4)
Activity, mean ($\pm$ SD)	70.7 ($\pm$ 26.5)	59.2 ($\pm$ 24.3)
Disability, mean ($\pm$ SD)	65.9 ($\pm$ 20.8)	56.2 ($\pm$ 17.0)
Physical findings, mean ($\pm$SD)		
Systolic BP, mm Hg	141 ($\pm$ 15)	143 ($\pm$ 15)
Diastolic BP, mm Hg	83 ($\pm$ 9)	85 ($\pm$ 7)
Heart rate, beats/min	84 ($\pm$ 9)	81 ($\pm$ 15)
Respiratory rate, breaths/min	22 ($\pm$ 1)	22 ($\pm$ 1)
SpO ₂ , %	92 ($\pm$ 2)	91 ($\pm$ 2)
Blood gas analysis, mean ($\pm$SD)		
pH	7.40 ($\pm$ 0.02)	7.41 ($\pm$ 0.03)
PaO ₂ , mmHg	65 ($\pm$ 7)	63 ($\pm$ 7)
PaCO ₂ , mm Hg	48 ($\pm$ 7)	50 ($\pm$ 11)
Bicarbonates, mmol/L	30 ($\pm$ 3)	31 ($\pm$ 5)
Laboratory values, mean ($\pm$SD)		

Albumin, mg·L ⁻¹	3.7 (±0.4)	3.9 (±0.8)
Leukocyte count , x 10 ⁹ cells·L ⁻¹	7.9 (±1.4)	7.0 (±2.3)
C-Reactive Protein, mg·L ⁻¹ *	1.9 (±1.1)	0.8 (±0.9)
Erythrocyte sedimentation rate, mm·h ⁻¹	42 (±23)	34 (±26)

Microbiology

Patients colonized, no. (%)	10 (91)	8 (73)
Patients colonized, no (%), with:		
<i>Pseudomonas aeruginosa</i>	5 (50)	6 (55)
<i>Haemophilus influenzae</i>	4 (40)	2 (18)
<i>Moraxella catarrhalis</i>	2 (20)	0 (0)
<i>Streptococcus pneumoniae</i>	2 (20)	0 (0)
<i>Candida albicans</i>	2 (20)	1 (9)
<i>Staphylococcus aureus</i>	0 (0)	2 (18)
<i>Serratia marcescens</i>	2 (20)	0 (0)

*: normal value <0.5 BMI: body mass index. MRC: Medical Research Council Dyspnea Scale

CRDQ: Chronic Respiratory Disease Questionnaire. BP: blood pressure. SpO₂: oxygen saturation.

Tables 2. Cumulative number of exacerbations and hospitalizations in both study groups during the one-year study period.

		Treatment period				Follow up period			
		3-mo		6-mo		9-mo		12-mo	
Exacerbations, no (patients)	AZI group	2	(11)	4	(10)	7	(10)	16	(9)
	SC group	12	(10)	10	(6)	11	(5)	17	(5)
Hospitalizations, no (patients)	AZI group	0	(11)	1	(10)	3	(10)	7	(9)
	SC group	4	(10)	1	(6)	3	(5)	5	(5)

SC: standard care AZI: azithromycin.

Tables 3. Cumulative number of antibiotic and steroid treatments in both study groups during the one-year study period.

		Treatment period				Follow up period			
		3-mo		6-mo		9-mo		12-mo	
antibiotic treatments, no (patients)	AZI group	2	(11)	5	(10)	8	(10)	14	(9)
	SC group	11	(10)	11	(6)	11	(5)	17	(5)
steroid treatments, no (patients)	AZI group	1	(11)	3	(10)	5	(10)	9	(9)
	SC group	7	(10)	5	(6)	5	(5)	9	(5)

SC: standard care AZI: azithromycin.

Table 4. Microbiology results from tracheal aspirate culture in the both study groups during the one-year study period, given according to the microorganism isolated and the colony count

Group	Pts	Baseline		Treatment period		Follow up period	
				3 mo	6 mo	9 mo	12 mo
AZI	1	<i>P.aeruginosa</i>	10 ⁵	10 ⁵	10 ⁵	10 ⁵	---
		<i>Klebsiella</i>	10 ⁴	0	0	0	---
	2	<i>P. aeruginosa</i>	10 ⁶	10 ⁵	10 ⁵	10 ⁶	Death
		<i>H. flu</i>	10 ⁵	0	0	0	
	3	<i>P. aeruginosa</i>	10 ⁵	Death			
	4	<i>P. aeruginosa</i>	10 ⁶	10 ⁵	10 ⁵	10 ⁵	---
		<i>H.flu</i>	10 ⁶	0	0	0	---
	5	<i>P. aeruginosa</i>	10 ⁵	---	---	---	<i>P. aeruginosa</i> 10 ⁴
		<i>Candida</i>	10 ⁵	---	---	---	0
	6	<i>P. aeruginosa</i>	10 ⁵	0	0	0	0
							<i>K. pneumonia</i> 10 ⁵
SC	7	<i>S. aureus</i>	10 ⁵	10 ⁴	0	0	---
						<i>P.aeruginosa</i> 10 ⁵	---
	8	<i>S. aureus</i>	10 ⁵	---	0	1	---
		<i>M. chatarralis</i>	10 ⁵	---	0	1	---
	9	0	0	0	<i>S. pneumo</i> 10 ⁵	0	---
	10	0	0	0	0	0	---
	11	0	0	0	0	<i>P.aeruginosa</i> 10 ⁵	Death
	1	<i>P. aeruginosa</i>	10 ⁶	> 10 ⁶	Death		
	2	<i>P. aeruginosa</i>	10 ⁶	>10 ⁶	Death		
	3	<i>P. aeruginosa</i>	10 ⁶	0	---	0	0
	4	<i>P. aeruginosa</i>	10 ⁴	0	0	---	---
		<i>Entero</i>	10 ⁴	10 ⁶	10 ⁶	---	---
		<i>Candida</i>	10 ⁴	10 ⁶	10 ⁶	---	---
		<i>S. epiderm</i>	10 ⁵	0	0	---	---
SC	5	<i>P. aeruginosa</i>	10 ⁶	10 ⁴	0	0	Lost in follow up
		<i>Serratia</i>	10 ⁶	0	0	0	
		<i>Candida</i>	5*10 ⁵	0	0	0	
	6	<i>H.flu</i>	10 ⁵	0	Death		
		<i>Moraxella</i>	10 ⁵	0			
				<i>P. aeruginosa</i> 10 ⁶			
	7	<i>H. flu</i>	10 ⁵	0	Death		

	<i>Moraxella</i>	10 ⁵	0			
				<i>P. aeruginosa</i>		
			10 ⁶			
8	<i>S. pneumonia</i>	10 ⁵	0	0	0	---
	<i>H. flu</i>	10 ⁵	10 ⁵	10 ⁵	10 ⁵	---
					<i>P. aeruginosa</i>	---
9	<i>Proteus</i>	10 ⁴	---	0	---	---
	<i>Serratia</i>	10 ⁴	---	0	---	---
				<i>P. aeruginosa</i> 10 ⁵	---	---
				<i>H. flu</i> 10 ⁵	---	---
10	<i>S. pneumoniae</i>	10 ⁵	0	0	0	
	<i>H. flu</i>	10 ⁵	10 ⁵	10 ⁵	10 ⁵	
					<i>P.aeruginosa</i> 10 ⁴	
11	---	---	Death			

--- = sample not obtained

Table 5. MRF26 changes over time. Negative delta indicates improvement. Positive delta indicates worsening

	Azithromycin group		Standard care group	
	Mean difference	P value	Mean difference	P value
Total score (3 month - baseline)	-8.3	0.006	4.8	NS
Total score (6 month - baseline)	-8.2	0.002	-1.3	NS
Activity score (3 month - baseline)	-7.7	0.02	5.8	NS
Activity score (6 month - baseline)	-6.7	0.02	2.6	NS
Disability score (3 month - baseline)	-8.8	0.03	3.9	NS
Disability score (6 month - baseline)	-9.6	0.005	-5.1	NS

