

Accepted Manuscript

Double-Blind, Placebo-Controlled Randomized Trial on Low Dose Azithromycin Prophylaxis in Primary Antibody Deficiencies

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PII: S0091-6749(19)30388-4

DOI: <https://doi.org/10.1016/j.jaci.2019.01.051>

Reference: YMAI 13934

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 9 October 2018

Revised Date: 17 January 2019

Accepted Date: 23 January 2019

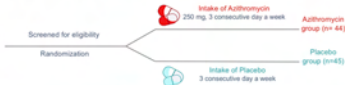
Please cite this article as: Milito C, Pulvirenti F, Cinetto F, Lougaris V, Soresina A, Pecoraro A, Vultaggio A, Carrabba M, Lassandro G, Plebani A, Spadaro G, Matucci A, Fabio G, Dellepiane RM, Martire B, Agostini C, Abeni D, Tabolli S, Quinti I, Double-Blind, Placebo-Controlled Randomized Trial on Low Dose Azithromycin Prophylaxis in Primary Antibody Deficiencies, *Journal of Allergy and Clinical Immunology* (2019), doi: <https://doi.org/10.1016/j.jaci.2019.01.051>.

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DOUBLE-BLIND, PLACEBO-CONTROLLED RANDOMIZED TRIAL ON LOW DOSE AZITHROMYCIN PROPHYLAXIS IN PRIMARY ANTIBODY DEFICIENCIES

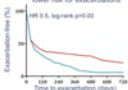
PAD adults with chronic infection-related pulmonary diseases (COPD, bronchiectasis, asthma) receiving treatment with IgRT.



Baseline	Every month	Every 4 month	24-month visit	Analysis
- Sputum sample - HRQoL - FEV1 - Blood analysis	<i>By diary-cards/ clinical interviews:</i> - Exacerbations - Hospitalization - Antibiotics	- Sputum sample - FEV1 - Blood analysis	- Sputum sample - HRQoL - FEV1 - Blood analysis	

Primary outcome

In the **Azithromycin** group lower risk for exacerbations



Secondary outcomes

In the **Azithromycin** group:

- Lower risk for hospitalization
- Lower need for additional antibiotic courses
- No higher rate of macrolides resistant carriage
- No drug-related toxicity
- Improved HRQoL
- No effect on FEV1
- Reduced count in blood of neutrophils

COPD chronic obstructive pulmonary disease
HRQoL Health Related Quality of Life
FEV1 Forced Expiratory Volume 1st sec
IgRT Immunoglobulin Replacement Treatment
PAD Primary Antibody Defect

**DOUBLE-BLIND, PLACEBO-CONTROLLED RANDOMIZED TRIAL ON LOW DOSE
AZITHROMYCIN PROPHYLAXIS IN PRIMARY ANTIBODY DEFICIENCIES**

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Funding. The study (EUDRACT 2011-004351-39) was funded by AIFA, Agenzia Italiana del Farmaco. AIFA is the national authority responsible for drugs regulation in Italy. It is a public body operating autonomously, transparently and according to cost-effectiveness criteria, under the direction of the Ministry of Health and under the vigilance of the Ministry of Health and the Ministry of Economy. As founder, AIFA had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Conflict of interest. IQ and AP receipt of consultation fees and grants by Shire, CSL Behring, Octapharma and Kedrion; CA and AM receipt of consultation fees by Shire, Roche, CSL Behring, Octapharma, Novartis. The remaining authors have no conflict of interest.

ABSTRACT

Background. Lacking protective antibodies, patients with Primary Antibody Deficiencies (PAD) suffer from frequent respiratory infections leading chronic pulmonary damage. Macrolides prophylaxis has been proven effective in chronic respiratory diseases.

Objective: We aimed to test the efficacy and safety of orally administered low-dose azithromycin prophylaxis in PAD patients.

Methods. We designed a three-year, double-blind, placebo-controlled, randomized clinical trial to test whether oral azithromycin (250 mg once daily three-times a week for 2 years) would reduce respiratory exacerbations in PAD with chronic infection-related pulmonary diseases. The primary endpoint was the number of annual respiratory exacerbations. Secondary endpoints included time to first exacerbation, antibiotic additional courses, number of hospitalizations and safety.

Results. Eighty-nine patients received azithromycin (n=44) or placebo (n=45). The number of exacerbations was 3.6 per patient-year (95%CI 2.5-4.7) in the azithromycin arm, and 5.2 (95%CI 4.1-6.4) in the placebo arm (p=0.02). In the azithromycin group the HR for having an acute exacerbation was 0.5 (95%CI 0.3-0.9, p=0.03) and the HR for hospitalization was 0.5 (95%CI 0.2-1.1, p=0.04). The rate of additional antibiotic treatment per patient-year was 2.3 (95%CI 2.1-3.4) in the intervention and 3.6 (95%CI 2.9-4.3) in placebo groups (p=0.004). *H. influenzae* and *S. pneumoniae* were the prevalent isolates and they were non-susceptible to macrolides in 25% of patients of both arms. Azithromycin's safety profile was comparable with placebo.

Conclusion. The study reached the main outcome centered in the reduction of exacerbation episodes per patient-year with a consequent reduction of additional courses of antibiotics, and of risk of hospitalization.

Clinical Trials number: EUDRACT 2011-004351-39.

Clinical Implications: Adding azithromycin prophylaxis to the treatment regimen of PAD with respiratory exacerbations should be considered as a valuable option.

Capsule summary.

- This is the first double-blind randomized trial demonstrating the efficacy and safety of long-term prophylactic treatment with azithromycin in primary antibody deficiencies.
- Long-term azithromycin prophylaxis reduced respiratory exacerbations, need of additional antibiotic courses and hospitalizations.

Keywords: Primary Antibody Defects, Azithromycin, Antibiotic Prophylaxis, Respiratory exacerbation, COPD

Abbreviations

85		
86		
87	AEs	Adverse Events
88	AIFA	Agenzia Italiana del Farmaco
89	CI	Confidence Interval
90	COPD	Chronic Obstructive Pulmonary Disease
91	CF	Cystic Fibrosis
92	CRF	Case Report Form
93	CVID	Common Variable Immunodeficiency
94	ESID	European Society for Immunodeficiencies
95	EUDRACT	European Clinical Trials Database
96	FEV1	Forced Expiratory Volume 1 st sec
97	HR	Hazard Risk
98	HRQoL	Health Related Quality of Life
99	Ig	Immunoglobulin
100	OR	Odds Ratio
101	PAD	Primary Antibody Deficiencies
102	PID	Primary Immunodeficiency Diseases
103	QoL	Quality of Life
104	SF-36	Short form 36
105	SGRQ	Saint George Respiratory Questionnaire
106	XLA	X-linked Agammaglobulinemia

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INTRODUCTION

Primary Antibody Deficiencies (PADs) account for most diagnosed Primary Immune Deficiencies (PID), particularly in adulthood. Although PID are considered as 'rare diseases', the International Union of Immunological Societies estimated that only one per cent of subjects living with a PID all over the world was diagnosed,¹ highlighting the need for a higher index of suspicion in the clinical practice. The spectrum of PADs includes several entities characterized by impairment in antibody production related to B-cell intrinsic or extrinsic defects, including conditions with Mendelian inheritance such as X-linked Agammaglobulinemia (XLA),² and diseases with predominant polygenic inheritance, such as Common Variable Immunodeficiency (CVID).³ Due to the lack of protective antibodies, the respiratory tract is the major target for acute infections, requiring immunoglobulin replacement therapy and frequent courses of antibiotics.⁴ Respiratory exacerbations in PAD are mainly caused by encapsulated bacteria, resulting in frequent visits to physicians' offices and emergency rooms, numerous hospitalizations and work-days lost.⁵ IgG replacement therapy enhances survival and reduces risk of pneumonia and invasive infections.⁶ However, despite appropriate immunoglobulin therapy, patients might develop chronic infection-related pulmonary diseases, including bronchiectasis, Chronic Obstructive Pulmonary Disease (COPD), and asthma.^{7,8}

Under diagnoses, diagnostic delay, severity of the infectious respiratory phenotype, and difficulty to define appropriate treatment strategies account for the high cumulative incidence of chronic lung diseases reaching 80% after a 17-year follow-up in XLA.⁹ In CVID, the incidence of bronchiectasis increased over time for almost all age groups leading to reduced Health-Related Quality of Life (HRQoL), and increased risk of death.^{7,8;10,11} The recurrence of acute infections over these underlying chronic lung conditions has been proposed to be defined as respiratory exacerbations of PAD patients, using the definition already validated for COPD¹⁰. Noteworthy, recent reports underlined that frequently exacerbating COPD, and severe uncontrolled asthmatic patients might mask an under-diagnosis of PADs.¹²

PID patients might take advantage of antibiotics prophylaxis². Macrolide antibiotics have proven to be effective to successfully manage Cystic Fibrosis (CF), non-CF associated

bronchiectasis, COPD, and asthma.¹³⁻¹⁶ In addition to antimicrobial effects, macrolides have immunomodulatory and anti-inflammatory properties, acting on the vicious circle infection-inflammation that leads to airway hyper-reactivity and remodeling.¹⁷

Based on these observations, we conducted a 36-month Phase II, randomized, double-blind, multi-center clinical trial to test the hypothesis that the long-term prophylactic treatment with azithromycin may decrease the frequency of respiratory exacerbations, when added to the usual care of PAD patients suffering from respiratory exacerbations.

METHODS

Trial design and participants. A 3-year, prospective, multicenter, double-blind, parallel-group, placebo-controlled design with equal randomization (1:1) was conducted in seven Italian immunology units (see **Appendix E1** in this article's Online Repository at www.jacionline.org for lists of sites, investigators, and recruitment and follow-up periods). Patients were recruited in in-patient and day-care settings by their immunology physicians if they fulfilled the inclusion/exclusion criteria. Eligible participants were aged 18 to 74 years, had a diagnosis of CVID or XLA according to the revised ESID registry criteria (<http://esid.org/Working-Parties/Registry/Diagnosis-criteria>), and a clinical diagnosis of chronic infection-related pulmonary diseases, including bronchiectasis, COPD, and asthma. Entry and exclusion criteria are listed in Table I. All participants who agreed to participate signed the written informed consent at enrollment. Patients were regularly treated with substitutive treatment by polyvalent IgG ≥ 400 mg/kg/month. IgG replacement monthly dosage was individualized according to clinical judgement before the enrollment. Concomitant medications were administered according to the usual, consolidated clinical approach. The use of co-interventions for respiratory lung conditions and exacerbations was permitted during the study, including antibiotics on-demand, except macrolides. We followed International guidelines for COPD recommending antibiotic therapy if two of the three clinical criteria for acute exacerbation were present: 1) increased dyspnea, 2) increased sputum production, 3) increased purulence of sputum (www.goldcopd.org).

The study protocol (EUDRACT: 2011-004351-39) was reviewed and approved by the Ethical Review Committee of Sapienza, University of Rome, Italy. The description of the full study design, methods and procedures medications are listed in **Appendix E2** in this article's Online Repository. Report of this trial was conforming to CONSORT 2010 guidelines.

Intervention. Study assessments were performed at enrollment (T0), after randomization (T1) and monthly after randomization up to month 12 (T2) and 24 (T3) and after 4 months since intervention discontinuation (T4). Patients received azithromycin, 250 mg once daily, three times a week, for three consecutive days, or an identical-appearing placebo, for 24 months. After 24 months, patients underwent a 5-month run-out period in which they discontinued the study drug. Adherence was monitored by investigator count of empty blisters of study medication at each monthly study visit. At T1, we collected medical history, physical examination, HRQoL questionnaires, routine blood testing, sputum sample, and measures of lung function expressed as Forced Expiratory Volume (FEV1). All patients were trained to report exacerbations to our research teams. Prior and concomitant therapies and their outcomes were reported in the Case Report Form. Every month, we collected recent medical history, physical examination, basic laboratory investigations, diary card, sputum sample for microbiological assessment, report of adverse events (AEs). At each clinic visit, all patients provided diary cards for changes in respiratory symptoms. Physicians determined whether an acute exacerbation had occurred in the prior month. The date of each acute exacerbation was taken as the date treatment was prescribed. Every four months, patients underwent blood tests, FEV1 measurement, and microbiological assessment of sputum samples. Sputum samples were collected at time points defined by study protocol, regardless exacerbation. Every 12 months participants completed HRQoL questionnaires (Short Form 36 questionnaire, and Saint George Respiratory Questionnaire). At the end of the study period we collected physical examination, recent medical history, diary cards, basic laboratory investigations, sputum sample and FEV1 (see **Appendix E2** in this article's Online Repository at www.jacionline.org).

Randomization and masking.

Patients were allocated in a 1:1 intervention-to-control ratio according to a randomization scheme prepared by the epidemiology center with a block size of 4 and kept by personnel not involved in selection of patients, interventions, and collection of relevant information. The randomization was stratified by center (see **Appendix E2** in this article's Online Repository at www.jacionline.org). Patients were sequentially assigned a subject identification code with double-blinded allocation to either azithromycin or an identical-appearing placebo. Placebo tablets were manufactured by a licensed trial pharmacy and were indistinguishable from azithromycin with respect to appearance, feel, and taste.

Outcomes.

The primary outcome measure was the number of annual episodes of respiratory exacerbations. Acute exacerbation was defined as "a complex of respiratory symptoms (increased or new onset) of at least three of the following respiratory symptoms with a duration of at least 3 days: cough; change in sputum production (consistency, color, or volume); fever $>38^{\circ}\text{C}$; wheezing; dyspnea; decreased exercise tolerance.

Secondary endpoints included: time to first exacerbation, number of additional courses of antibiotics, number of hospitalizations, lung function, HRQoL, microbiological analysis of sputum samples and evaluation of AEs. Lung function was measured by FEV1 at enrollment and every 4 months thereafter. Sputum samples were obtained at the time of enrollment and every 4 months thereafter and processed by standard culture for respiratory pathogens. Selected respiratory pathogens were assessed for resistance to macrolides. Sputum samples were also tested by bacterioscopy for acid resistant bacteria. (see **Appendix E3** in this article's Online Repository at www.jacionline.org). HRQoL was assessed by The Short Form-36 (SF-36) questionnaire and the Saint George Respiratory Questionnaire (SGRQ) at enrollment and every 12 months. The SF-36 measures health on 8 multi-item dimensions: physical functioning (PF), role-physical (RF), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role-emotional (RE), and mental health (MH) and two summary measures, the physical component summary (PCS) and mental component summary (MCS).¹⁸ Scores for each dimension range from 0 to 100, with higher scores indicating better health. The SGRQ is a specific questionnaire validated for use in patients with respiratory diseases.¹⁹ It is partitioned into three sections (symptoms, activity, impact), which

are scored separately and can be added to provide a total score ranging from 0 to 100, with 0 indicating no impairment of HRQoL. IgG pre-infusion levels and WBC blood peripheral count were assessed every 4 months to avoid bias due to low IgG serum level or leucopenia in recurrence of respiratory exacerbation. Serious AEs and Serious Unexpected Suspected AEs were monitored using clinical records and reported to the human research ethics committee at each site. Definitions of AEs are shown in the Appendix E, Table E1 in this article's Online Repository at www.jacionline.org.

Statistical Methods. The primary hypothesis was that prolonged treatment with azithromycin would reduce the proportion of patients with respiratory exacerbations from 75% to 50%. We calculated that a sample size of 56 patients per treatment arm would yield a power of 80% with a one-sided $\alpha = 0.025$ and we planned to include 130 patients, assuming a 10% drop-out. The hypothesis of superiority justified the choice of the one-sided test. Eighty-nine patients were enrolled. Keeping the two proportions originally chosen (i.e., 75% and 50%), with 44 patients per group, and α one-sided = 0.05, we calculated a power = 77%. The 77% sub-optimal power remained close to the commonly accepted standard of 80%. The sample-size reduction was due to low prevalence of PADs, leading to the request for a substantial protocol amendment to the Ethical Committee, who approved it. The groups were compared by intention-to-treat survival analysis. Patients who never took one of the study medications were not included in the analysis. Comparisons of continuous parameters between treatment groups were calculated with a t-test if normally distributed and with a Mann-Whitney U test if not normally distributed; differences in frequencies between groups were calculated by Chi-square exact test. There were no patients with missing information on exacerbations during intervention. Rates of respiratory exacerbations were determined by dividing the number of acute exacerbations by person-years. Time to first exacerbation during the treatment period, as well as during the 5 months of run-out, and time to first additional course of antibiotics and hospitalization was assessed using the Kaplan-Meier product-limit estimates and was based on a Log-rank and Gehan-Breslow-Wilcoxon test of the difference between the two treatment groups at the time of first exacerbation, with no adjustment for baseline covariates. A Cox proportional-hazards model was used to adjust for differences in

pre-specified, pre-randomization factors that might predict the risk of acute exacerbations. Change of FEV1 and laboratory parameters attributable to treatment were calculated with a linear mixed-model analysis. HRQoL measures at T1, T2 and T3 were compared using Mann-Whitney U test. The data were analyzed with the use of group sequential testing that allowed “spending” a little of the alpha at each interim analysis such that, at the end of the study, the total type I error did not exceed 0.05. Statistical analyses were performed using the statistical package SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). This trial is registered with the *Agenzia Italiana del Farmaco* (AIFA), Clinical Trials number: EUDRACT 2011-004351-39.

RESULTS

Between November 2012, and December 2016, 90 patients were enrolled. One patient withdrew consent after randomization and 89 were randomized at T0 and included in the analysis; 45 and 44 participants were respectively allocated to the placebo and to the azithromycin arm (Fig 1). The randomization yielded two comparable groups for variables considered as potential confounders (Table II). The mean cumulative period of observation was 25.8 months (95%CI 23.4-28.0) in the azithromycin group and 25.5 months (95%CI 23.5-27.6, $p=0.429$) in the placebo group. At one year (T2), 40 patients were on the placebo group and 35 on the azithromycin group; at two years (T3), 35 patients were on the placebo group and 33 on the azithromycin group. At the end of the study (T4), 34 patients were on the placebo group and 32 were on the azithromycin group. During the study time, groups remained comparable for IgG trough serum levels ($F=1.486$, $p=0.231$), PAD diagnosis, and age between patient groups (Appendix, Fig E1 and Table E2).

A total of 677 respiratory exacerbations occurred during the T1-T3 period: 262 among the participants allocated in the treatment group and 415 among those in the placebo group (Table III). The incidence rate of exacerbations in the azithromycin group was 3.6 episodes per patient-year (95%CI 2.5-4.7) and 5.2 episodes per patient-year (95%CI 4.1-6.4) in the placebo group ($p=0.020$). A post-hoc analysis showed that the risk of respiratory exacerbations was reduced among

participants receiving azithromycin (HR 0.5 [95%CI 0.3-0.9], log-rank $p=0.033$, Fig 2A). The difference remained significant after adjustment by Cox regression in a model including sex, FEV1, age, and study center. The number needed to prevent one respiratory exacerbation was 7.0 [95%CI 3.3-59.1]. The HR of having a respiratory exacerbation did not differ in the run-out period in the two study arms (HR 1.0 [95%CI 0.6-1.8], log-rank $p=0.895$, Fig 2B). Three out of 45 participants receiving placebo and 10 out of 44 receiving azithromycin were free from exacerbations during the study period ($p=0.039$), yielding an absolute risk reduction of 16.1% [95%CI 1.7-30.4%]. Time to first exacerbation did not differ in the two study arms (134.0 days [95%CI 61.5-207.5] vs 104.3 days [95%CI 67.3-141.3], $p=0.236$).

A total of 45 hospitalizations for any cause occurred during the study time, 32 of which (71%) occurred in the placebo group. The rate of hospitalization per patient-year was 0.1 episodes (95%CI 0.1-0.2) in the intervention group and 0.3 episodes (95%CI 0.2-0.5) in the placebo group ($p=0.014$) (Table III). The HR of having a hospitalization in the azithromycin group was 0.5 (95%CI, 0.2-1.1, Gehan-Breslow, $p=0.040$, Fig 2C).

The number of additional courses of antibiotics to treat respiratory exacerbations was lower in the intervention group than in the placebo group (2.3 [95%CI 2.1-3.4] vs 3.6 [95%CI 2.9-4.3], $p=0.004$, HR 0.6, [95%CI 0.4-1.0]; log-rank $p=0.020$, Fig 2D). This effect was lost during the run-out period (HR: 1.01 [95%CI, 0.6-2.1], log-rank $p=0.746$). The mean time to the first antibiotic course was shorter in the placebo group (intervention: 181.5 days, [95%CI 23.5-239.5] vs placebo: 122.4 days, [95%CI 123.5-239.5], log-rank $p=0.046$) (Table III). Nine patients (21%) in the azithromycin arm and two (5%) patients in the placebo arm did not take additional antibiotic course during the study time ($p=0.030$). Changes in per cent of predicted FEV1 over time was not different for patients receiving placebo compared with azithromycin ($F=1.486$, $p=0.231$).

After starting azithromycin, decreased counts of absolute number of peripheral blood WBC and neutrophils were observed in intervention arm than in placebo ($F=4.55$, $p=0.0367$ and $F=4.64$; $p=0.035$, respectively). No changes were observed in lymphocyte and eosinophils counts (Fig 3).

In the azithromycin and placebo arms, bacteria were identified at baseline in 33.3% and 37.5% of samples, respectively ($p=1.000$). During the study time, a cumulative number of 139

sputum samples from 27 participants receiving azithromycin and 165 sputum samples from 30 patients receiving placebo were collected. Bacteria were identified in 35.2% and 42.4% of samples, respectively ($p=0.124$, Table III). *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were detected in 10%, 7%, 10% and 2% of sputum obtained by patients in the azithromycin group (Fig 4A) and in 16%, 11%, 7 %, and 3% of sputum obtained by patients in the placebo group (Fig 4B), whereas no acid resistant bacteria were identified.

Not-susceptible strains accounted for 86% and 79% of *Streptococcus pneumoniae* and *Haemophilus influenzae* isolates in the intervention and in the placebo group, respectively. These not-susceptible strains were obtained from 6/27 patients receiving azithromycin and from 6/30 patients receiving placebo ($p=0.221$, Fig 4C). The risk of developing *Streptococcus pneumoniae* and *Haemophilus influenzae* strains not-susceptible to macrolides was similar in the two study arms (HR 0.9 [95%CI 0.3-2.8], log-rank $p=0.897$, Fig 4D). The difference in the risk for exacerbation remained significant after adjustment for macrolide resistance by Cox regression.

Improvement in mean scores of SF-36 mental-related scales was seen only at T2 in the azithromycin group (MCS, T1: 39.5 ± 14.9 , T2: 42.9 ± 11.7 , $p=0.021$; MH, T1: 58.4 ± 23.8 , T2: 65.0 ± 20.1 , $p=0.020$) (**Appendix, Table E3**). Improvement in mean score of SGRQ Impact scale was found in both study arms (azithromycin: T1 48.8 ± 27.1 ; T3: 27.2 ± 19.7 , $p<0.0001$; placebo T1: 43.0 ± 31.1 ; T3: 24.1 ± 18.3 , $p<0.0001$). Mean Symptom score improved only in the azithromycin group (T1: 47.0 ± 23.1 ; T3: 39.6 ± 24.0 , $p=0.040$) while did not change in the control group (T1: 46.3 ± 23.1 , T3: 42.2 ± 22.0 , $p = 0.463$) and mean Activity score did not change in both study arms (azithromycin T1: 39.9 ± 26.1 , T3: 38.3 ± 30.4 ; $p= 0.828$; placebo T1: 33.4 ± 25.0 ; T3: 31.6 ± 27.1 , $p=0.780$) (**Appendix, Table E4**).

No serious AEs drug-related or drug-related causes of discontinuation were reported in the intervention group (Table III). The rate of death from any cause was 6.8% in the azithromycin group and 4.4% in the placebo group ($p=0.489$). The rate of death from respiratory causes was 5% and 2.2% in the two groups, respectively ($p=0.616$). Even though the study was not designed to analyse the effect of azithromycin on other concomitant conditions, we observed a reduced

frequency of participants reporting diarrhoea (13% vs 53%, $p=0.001$) and acute rhino-sinusitis (4% vs 27%, $p=0.020$) in the azithromycin group.

DISCUSSION

The study on the efficacy and safety of long-term oral azithromycin prophylaxis in patients affected by primary antibody deficiencies and chronic infection-related pulmonary diseases reached the main outcome centered in the reduction of episodes per patient-year. This paralleled the decrease in consumption of antibiotics, the reduced risk of hospitalization and the improved quality of life. Moreover, azithromycin prophylaxis did not increase the rate of macrolide-resistant organisms, and the rate of AEs. Our results on efficacy of azithromycin were comparable with those already proved by other similar randomized studies done in COPD,¹⁶ in CF patients¹⁴ and in non-CF patients with bronchiectasis¹⁵ although we used a lower azithromycin dosage. Moreover, in our study, the exacerbation rate per patient-year was 5.2 in untreated patients. This rate was higher than that described in the other studies: 2.73 (non-CF bronchiectasis¹⁵), 1.83 (COPD¹⁶), 0.81 (asthma²⁰). Under prophylaxis, in PAD patients the rate significantly decreased to 3.60, but it was still higher than those reported in non-CF bronchiectasis (1.58), COPD (1.48) and asthma (0.75). However, as shown in other similar studies,^{15,16,20} the clinical effect did not result in a significant improvement of FEV1. These data confirmed the severity of respiratory disease in PAD¹⁰ where long-life respiratory symptoms tend to peak in early decades of life, leading to chronic pulmonary damage⁷ as in other chronic lung diseases.²¹

The efficacy of a low-dose macrolide therapy on PAD respiratory exacerbation might be explained by antimicrobial and anti-inflammatory action of the new macrolides, especially on the neutrophil chemotaxis, neutrophil-derived elastolytic-like activity and concentrations of IL-8 and LTB4.^{22,23} Our data showed a decrease of neutrophils blood counts during the study time in the intervention group.

Because of lack of a standard treatment regimen, the azithromycin dosage of 250 mg/day three times a week was chosen to increase patient adherence and to minimize adverse effects. It has been previously demonstrated that daily intake of azithromycin 250 mg resulted in

development of colonization by azithromycin-resistant pathogens,¹⁶ while the same dose given three times a week did not increase bacterial resistance.²⁴ Furthermore, our choice of low azithromycin dosage was supported by *in vitro* data on macrolide ability to reduced alginate production, flagellin-induced inflammation and to decrease biomass and maximal thickness biofilms also at sub-inhibitory concentrations.²⁵

Bacteria were isolated from the sputum in one third of patients of the two study arms, with a predominance of *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, as already described in patients with COPD.²⁶ A high rate of *Haemophilus influenzae* and *Streptococcus pneumoniae* isolates non-susceptible to macrolides was detected in a quarter of patients of the two study arms. This data was comparable to our recent data on upper respiratory tract carriage in patients with primary antibody deficiencies.²⁷ The possibility to clarify the clinical relevance of the issue of non-susceptible strains was difficult since the EUCAST breakpoint tables (Version 8, 2018)²⁸ no longer reports clinical breakpoints of macrolide resistance in respiratory infections at least for what concerns *Haemophilus influenzae* strains. However, physicians should take into account the evidence that macrolides resistance is increasing.²⁹ The risk of driving a bacterial resistance because of long-term macrolide prophylaxis could be balanced by benefits on the overall bacterial resistance due to the reduction of number of antibiotic courses.

Azithromycin monotherapy can select resistant non-tuberculous mycobacteria if mycobacteria infection is present, especially in bronchiectasis.³⁰ In this study infections by resistant mycobacteria were not recorded. However, pulmonary non-tuberculous mycobacteria infection should be ruled out before starting long-term azithromycin prophylaxis.³¹

Neither serious side effects, nor reasons for treatment discontinuation related to the drug intake were reported. The high rate of death in the 29-months study period further demonstrated the severity of PAD.^{3,11} No cardiovascular mortality was observed. However, clinicians should have caution with the use of multiple QTc prolonging medications, including fluoroquinolones or antifungal azoles, due to the risk of ventricular arrhythmias in macrolides recipients.³²

In the intervention group, the generic tool SF-36 documented improvement over time related to Mental Dimensions and the lung diseases-specific tool SGRQ documented improvement in the Symptoms scale. SGRQ Impact score improved also in the placebo group, possibly because all patients, regardless of treatment, have been followed up strictly with monthly clinical controls. This further emphasizes the role of patient-reported outcome measures in clinical practice to monitor disease or treatment.³³

The main limitation of the study is that the number of patients enrolled was lower than the initial sample size calculation. It is worth considering the difficulty of recruiting populations of patients with rare diseases such as PADs with chronic infection-related pulmonary diseases. However, the 77% sub-optimal power remained close to the commonly accepted standard of 80%, and in fact even though the reduction of sample size, the differences observed between the two study arms often reached statistical significance and primary and secondary main outcomes were achieved.

In conclusion, given the deleterious effects of respiratory diseases, especially on the risk of death, quality of life, loss of lung function, and cost of care, adding azithromycin to the treatment regimen could be considered as a valuable option for patients with PAD and respiratory exacerbations.

Acknowledgments. We thank the patients, the nurses and the doctors who participated in the study. The Clinical Trials number EUDRACT 2011-004351-39 was founded by the Agenzia Italiana del Farmaco (AIFA). The Rome PID Clinical Research Center is supported by the Jeffrey Modell Foundation.

408 **References**

- 409 1. Picard C, Bobby Gaspar H, Al-Herz W, Bousfiha A, Casanova JL, Chatila T et al. 2017
410 Primary Immunodeficiency Diseases Committee Report on Inborn Errors of Immunity. *J*
411 *Clin Immunol* 2018; **38**: 96-128.
- 412 2. Durandy A, Kracker S, Fischer A. Primary antibody deficiencies. *Nat Rev Immunol* 2013;
413 **13**: 519-33.
- 414 3. Bonilla FA, Barlan I, Chapel H, Costa-Carvalho BT, Cunningham-Rundles C, de la Morena
415 MT et al. International Consensus Document (ICON): Common Variable Immunodeficiency
416 Disorders. *J Allergy Clin Immunol Pract* 2016; **4**: 38-59.
- 417 4. Freeman AF, Holland SM. Antimicrobial prophylaxis for primary immunodeficiencies. *Curr*
418 *Opin Allergy Clin Immunol* 2009; **9**: 525-30.
- 419 5. Thickett KM, Kumararatne DS, Banerjee AK, Dudley R, Stableforth DE. Common variable
420 immune deficiency: respiratory manifestations, pulmonary function and high-resolution CT
421 scan findings. *QJM* 2002; **95**: 655-6.
- 422 6. Quinti I, Soresina A, Spadaro G, Martino S, Donnanno S, Agostini C et al. Italian Primary
423 Immunodeficiency Network. Long-term follow-up and outcome of a large cohort of patients
424 with common variable immunodeficiency. *J Clin Immunol* 2007; **27**: 308-16.
- 425 7. Verma N, Grimbacher B, Hurst JR. Lung disease in primary antibody deficiency. *Lancet*
426 *Respir Med* 2015; **3**: 651-60.
- 427 8. Quinti I, Soresina A, Guerra A, Rondelli R, Spadaro G, Agostini C et al. Effectiveness of
428 immunoglobulin replacement therapy on clinical outcome in patients with primary antibody
429 deficiencies: results from a multicenter prospective cohort study. *J Clin Immunol* 2011; **31**:
430 315-22.
- 431 9. Plebani A, Soresina A, Rondelli R, Amato GM, Azzari C, Cardinale F et al. Italian Pediatric
432 group for XLA-AIEOP. Clinical, immunological, and molecular analysis in a large cohort of
433 patients with X-linked agammaglobulinemia: an Italian multicenter study. *Clin Immunol*
434 2002; **104**: 221-30.
- 435 10. Sperlich JM, Grimbacher B, Workman S, Haque T, Seneviratne SL, Burns SO et al.
436 Respiratory Infections and Antibiotic Usage in Common Variable Immunodeficiency. *J*
437 *Allergy Clin Immunol Pract* 2018; **6**: 159-168.
- 438 11. Lucas M, Lee M, Lortan J, Lopez-Granados E, Misbah S, Chapel H. Infection outcomes in
439 patients with common variable immunodeficiency disorders: relationship to immunoglobulin
440 therapy over 22 years. *J Allergy Clin Immunol* 2010; **125**: 1354-1360.
- 441 12. Berger M, Geng B, Cameron DW, Murphy LM, Schulman ES. Primary immune deficiency
442 diseases as unrecognized causes of chronic respiratory disease. *Respir Med* 2017; **132**:
443 181-8.
- 444 13. Saiman L, Anstead M, Mayer-Hamblett N, Lands LC, Kloster M, Hocevar-Trnka J et al.
445 AZ0004 Azithromycin Study Group. Effect of azithromycin on pulmonary function in patients
446 with cystic fibrosis uninfected with *Pseudomonas aeruginosa*: a randomized controlled trial.
447 *JAMA* 2010; **303**: 1707-15.
- 448 14. Equi A, Balfour-Lynn IM, Bush A, Rosenthal M. Long term azithromycin in children with
449 cystic fibrosis: a randomised, placebo-controlled crossover trial. *Lancet* 2002; **360**: 978-84.
- 450 15. Wong C, Jayaram L, Karalus N, Eaton T, Tong C, Hockey H et al. Azithromycin for
451 prevention of exacerbations in non-cystic fibrosis bronchiectasis (EMBRACE): a
452 randomised, double-blind, placebo-controlled trial. *Lancet* 2012; **380**: 660-7.
- 453 16. Albert RK, Connett J, Bailey WC, Casaburi R, Cooper JA Jr, Criner GJ et al. COPD Clinical
454 Research Network. Azithromycin for prevention of exacerbations of COPD. *N Engl J Med*
455 2011; **365**: 689-98.

- 456 17. Spagnolo P, Fabbri LM, Bush A. Long-term macrolide treatment for chronic respiratory
457 disease. *Eur Respir J* 2013; **42**: 239-51.
- 458 18. Ware JE Jr, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I.
459 Conceptual framework and item selection. *Med Care* 1992; **30**: 473–8310.
- 460 19. Hurst JR1, Workman S, Garcha DS, Seneviratne SL, Haddock JA, Grimbacher B. Activity,
461 severity and impact of respiratory disease in primary antibody deficiency syndromes *J Clin*
462 *Immunol* 2014; **34**: 68-75.
- 463 20. Brusselle GG, Vanderstichele C, Jordens P, Deman R, Slabbynck H, Ringoet V et al.
464 Azithromycin for prevention of exacerbations in severe asthma (AZISAST): a multicentre
465 randomised double-blind placebo-controlled trial. *Thorax* 2013; **68**: 322-9.
- 466 21. Agusti A, Faner R, Celli B, Rodriguez-Roisin R. Precision medicine in COPD exacerbations.
467 *Lancet Respir Med*. 2018; **18**: 30296-0.
- 468 22. Beigelman A, Isaacson-Schmid M, Sajol G, Baty J, Rodriguez OM, Legee E, Lyons K,
469 Schweiger TL, Zheng J, Schechtman KB, Castro M, Bacharier LB. Randomized trial to
470 evaluate azithromycin's effects on serum and upper airway IL-8 levels and recurrent
471 wheezing in infants with respiratory syncytial virus bronchiolitis. *J Allergy Clin Immunol*.
472 2015;**135**:1171-8.
- 473 23. Zhou Y, Bacharier LB, Isaacson-Schmid M, Baty J, Schechtman KB, Sajol G, Wylie K,
474 Storch GA, Castro M, Beigelman A. Azithromycin therapy during respiratory syncytial virus
475 bronchiolitis: Upper airway microbiome alterations and subsequent recurrent wheeze. *J*
476 *Allergy Clin Immunol*. 2016;**138**:1215-1219.e5.
- 477 24. Berkhof FF, Doornewaard-ten Hertog NE, Uil SM, Kerstjens HA, van den Berg JW.
478 Azithromycin and cough-specific health status in patients with chronic obstructive
479 pulmonary disease and chronic cough: a randomised controlled trial. *Respir Res*.
480 2013;**14**:14-125.
- 481 25. Starner TD, Shrout JD, Parsek MR, Appelbaum PC, Kim G. Subinhibitory concentrations of
482 azithromycin decrease nontypeable *Haemophilus influenzae* biofilm formation and diminish
483 established biofilms. *Antimicrob Agents Chemother* 2008; **52**: 137-45.
- 484 26. Rosell A, Monsó E, Soler N, Torres F, Angrill J, Riise G et al. Microbiologic determinants of
485 exacerbation in chronic obstructive pulmonary disease. *Arch Intern Med*. 2005; **165**: 891-7.
- 486 27. Pulvirenti F, Camilli R, Giufrè M, Milito C, Pimentel de Araujo F, Mancini F et al. Risk
487 factors for *H. influenzae* and pneumococcal respiratory tract colonization in CVID. *J Allergy*
488 *Clin Immunol*. 2018; **142**:1999-2002.
- 489 28. Cameron EJ, McSharry C, Chaudhuri R, Farrow S, Thomson NC. Long term macrolide
490 treatment of chronic inflammatory airway disease: risks, benefits and future developments.
491 *Clin Exp Allergy* 2012; **42**: 1302-12.
- 492 29. Malhotra-Kumar S, Lammens C, Coenen S, Van Herck K, Goossens H. Effect of
493 azithromycin and clarithromycin therapy on pharyngeal carriage of macrolide-resistant
494 streptococci in healthy volunteers: a randomised, double-blind, placebo-controlled study.
495 *Lancet* 2007; **369**: 482-90.
- 496 30. Binder AM, Adjemian J, Olivier KN, Prevots DR Epidemiology of nontuberculous
497 mycobacterial infections and associated chronic macrolide use among persons with cystic
498 fibrosis *Am J Respir Crit Care Med*. 2013;**188**:807-12.
- 499 31. Renna M, Schaffner C, Brown K, Shang S, Tamayo MH, Hegyi K et al. Azithromycin blocks
500 autophagy and may predispose cystic fibrosis patients to mycobacterial infection. *J Clin*
501 *Invest* 2011; **121**: 3554-63.
- 502 32. Lee RA, Guyton A, Kunz D, Cutter GR, Hoesley CJ. Evaluation of baseline corrected QT
503 interval and azithromycin prescriptions in an academic medical center. *J Hosp Med*
504 2016;**11**:15-20.

- 505 33. Tabolli S, Giannantoni P, Pulvirenti F, La Marra F, Granata G, Milito C et al. Longitudinal
506 study on health-related quality of life in a cohort of 96 patients with common variable
507 immune deficiencies. *Front Immunol* 2014; **5**: 605.

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511 **TABLE I. Inclusion and exclusion criteria of the study.**

512

Inclusion criteria
• diagnosis of X-linked Agammaglobulinemia or Common Variable Immunodeficiency according to the revised ESID registry criteria¹ • male or female aged 18 to 74 years • clinical diagnosis of chronic infection-related pulmonary diseases • written informed consent ¹ http://esid.org/Working-Parties/Registry/Diagnosis-criteria
Exclusion criteria
• known allergic reaction to azithromycin • patients taking drugs that could adversely interact with macrolides • lymphoproliferative diseases • creatinine concentration >1.5 times the UNL • ASAT or ALAT concentration >2.5 times the UNL • HIV infection, acute hepatitis or clinically active chronic hepatitis • pregnancy or breast-feeding females planning to become pregnant during the study • any condition that is likely to interfere with the evaluation of the study drug or satisfactory conduct of the trial.

513

514 **TABLE II. Baseline characteristics of the intention-to-treat population.**

	Azithromycin (n=44)	Placebo (n=45)	p
Age (years), mean (SD)	45.0 (14.9)	45.0 (14.0)	0.895
Sex , n. (%)			
Female	23 (52%)	26 (58%)	0.602
Male	21 (48%)	19 (42%)	0.602
Diagnosis , n. (%)			
CVID	38 (86%)	35 (78%)	0.409
XLA	6 (14%)	10 (22%)	0.409
Chronic pulmonary diseases , n. (%)			
COPD (all stages)	22 (50%)	23 (51%)	0.543
- Stage I	6 (14%)	3 (7%)	0.283
- Stage II	9 (20%)	10 (22%)	0.522
- Stage III-IV	7 (16%)	10 (22%)	0.313
Bronchiectasis	36 (82%)	40 (89%)	0.260
Asthma	5 (11%)	6 (13%)	0.516
FEV1 (% predicted), mean (SD)	71 (28)	76 (23)	0.391
Blood data , mean (SD)			
WBC (cell/mm ³)	6758.4 (2595.7)	7429.2 (3262.9)	0.166
Neutrophils (cell/mm ³)	4325.2 (2025.9)	4793.4 (2585.9)	0.195
IgG (mg/dL)	767.1 (298.4)	731.0 (234.9)	0.292
IgA (mg/dL)	32.0 (59.9)	28.9 (106.3)	0.441
IgM (mg/dL)	36.7 (69.0)	63.3 (146.7)	0.165
HRQoL assessment , mean (SD)			
SF36, PCS (%)	40.4 (11.6)	44.1 (11.4)	0.458
SF36, MCS (%)	39.5 (14.9)	43.0 (11.8)	0.310
SGRQ, Total (%)	34.7 (16.9)	30.8 (18.9)	0.165

515
516 Data are expressed as n (%) or mean (SD). CVID=Common Variable Immunodeficiencies, XLA=X-linked
517 Agammaglobulinemia, COPD= Chronic Obstructive Pulmonary Disease; FEV1=Forced Expiratory Volume,
518 WBC= White Blood Cells, Ig= immunoglobulin; HRQoL= Health-Related Quality of Life; SF-36=Short Form-
519 36; SGRQ=Saint George Respiratory Questionnaire.

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TABLE III. Exacerbations, hospitalization, additional courses of antibiotics, colonization and safety profile by study groups.

	Azithromycin (n=44)	Placebo (n=45)	p
Exacerbations			
rate per patient/year, mean (SD)	3.6 (3.8)	5.2 (3.9)	0.020
patients free from exacerbations, n. (%)	10 (26)	3 (7)	0.039
Hospitalization			
rate per patient/year, mean (range)	0.1 (0-0.2)	0.3 (0-0.5)	0.014
days at first hospitalization mean, (SD)	650.0 (167.8)	528.4 (285.8)	0.010
Additional course of antibiotics			
patients who had at least one antibiotics course n. (%)	35 (71%)	43 (95%)	0.026
course per patient/year, mean (SD)	2.3 (1.9)	3.6 (2.5)	0.004
Microbiological assessment			
Sputum samples collected, n.	139	165	-
Participants providing sputum samples, n. (%)	27 (61.3)	30 (66.6)	0.662
Positive isolate n. (%)	49 (35.2)	70 (42.4)	0.096
Patients carrying <i>H. influenzae</i> and/or <i>S. pneumoniae</i> not-susceptible to macrolides	6 (22)	6 (20)	1.000
Serious Non-fatal AEs, n.			
Pneumonia	0	6	0.010
Neoplasm	0	3	0.241
GI tract	0	3	0.241
Cardiovascular	0	0	-
Other	3†	5‡	0.713
Total	3	17	0.002
AEs leading to drug discontinuation, n.			
Pneumonia	0	0	-
Neoplasm	0	2	0.494
GI tract	0	1	1.000
Cardiovascular	0	0	-
Other	0	1¶	1.000
Total	0	4	0.116
Fatal AEs, n.			
Pneumonia	1	1	1.000
Respiratory failure	1	0	0.494
Gastric malignancy	0	1	1.000
Parkinson	1	0	0.494
Total	3	2	0.676

Other: †one participant developed thrombocytopenia, one participant had lymphadenopathy, and one showed iron deficiency anaemia; ‡ one participant developed thrombocytopenia, two participants had urticaria; one patient had hyponatremia, and one patient developed hepatic fibrosis; ¶ one participants discontinued for hepatic fibrosis.

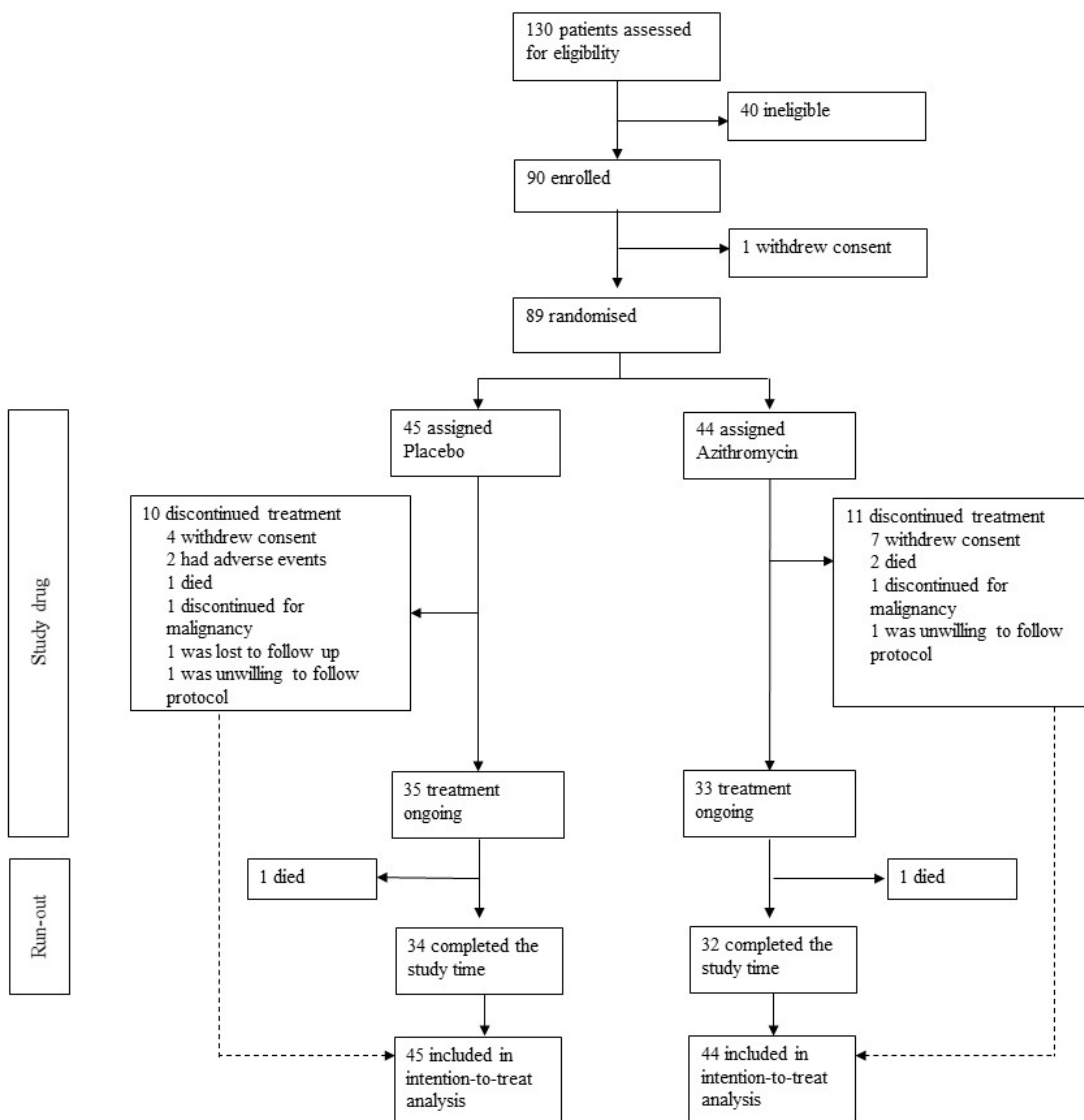
529 **Figure legend**

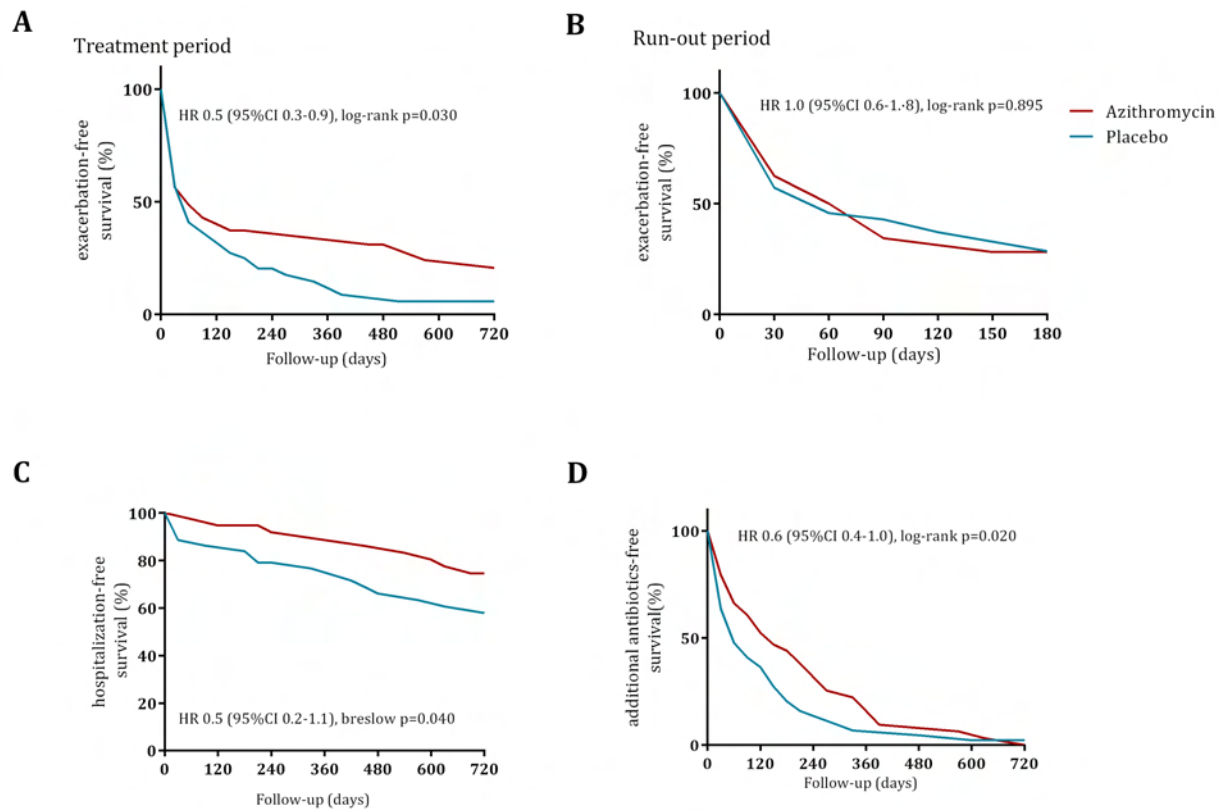
530 **Figure 1.** Trial profile.

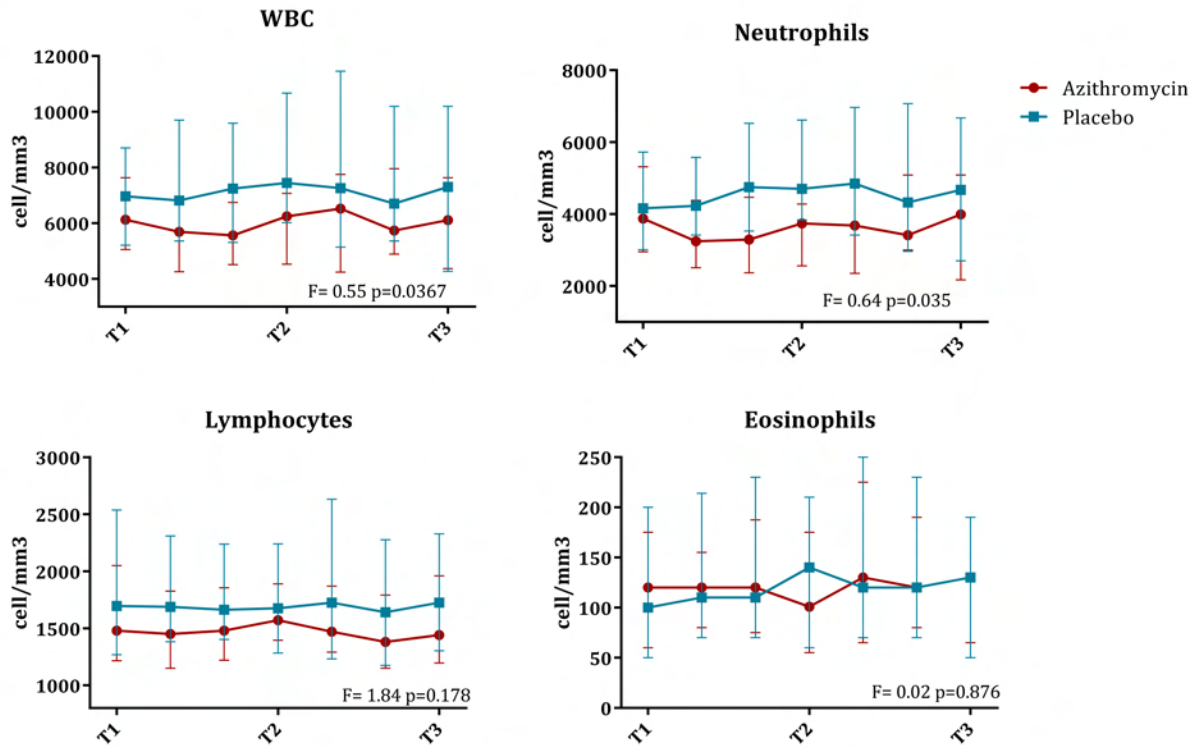
531 **Figure 2.** Kaplan-Meier plot. Proportion of patients remaining exacerbation-free during the study
532 time in the intervention group (red line) and in control group (blue line) in the study period (T1-T3,
533 panel A) and run-out period (T3-T4, panel B). Proportion of participants free from hospital
534 admission (panel C) and additional antibiotic courses (panel D) in the intervention group (red line)
535 and in control group (blue line) during the study time.

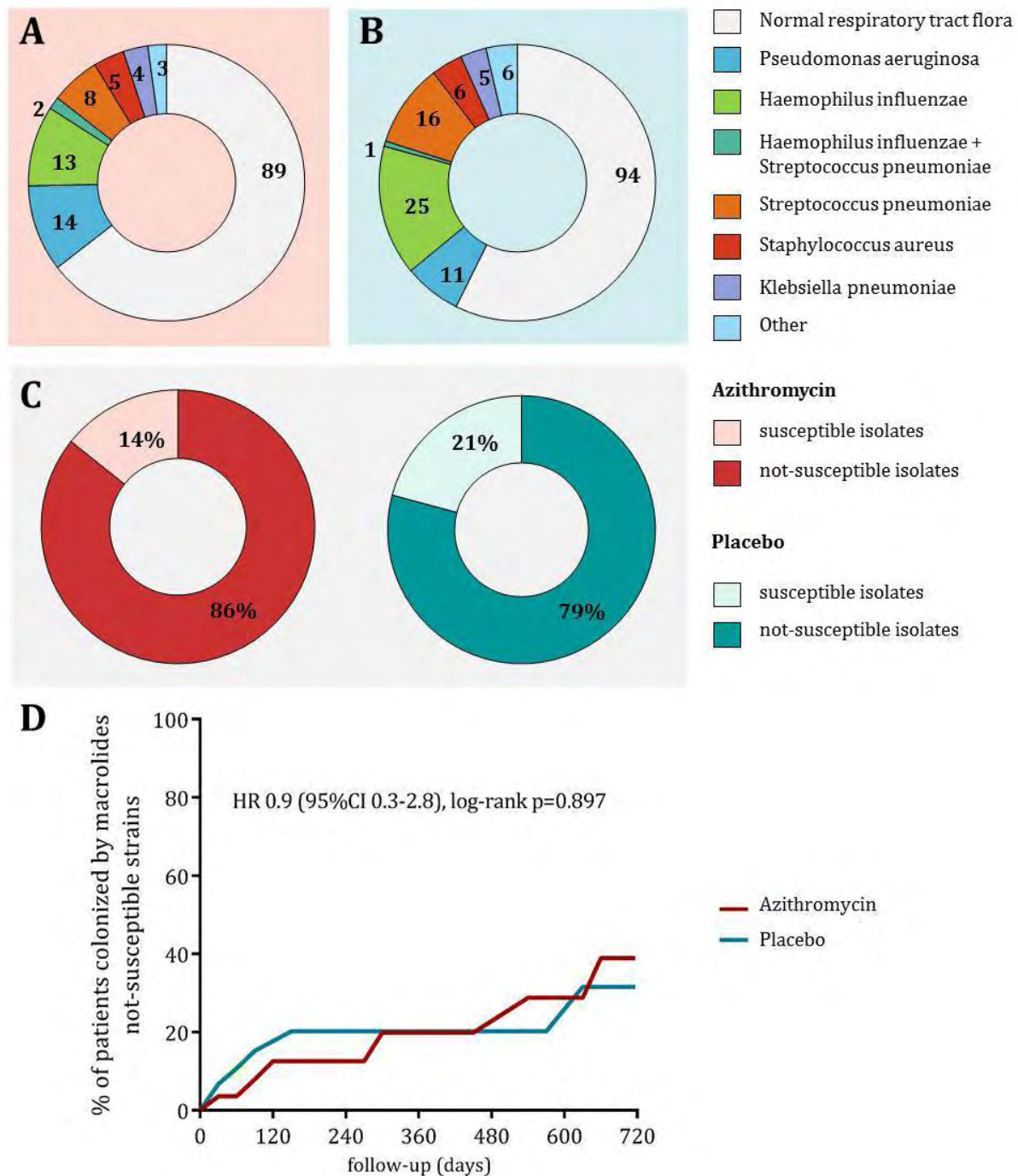
536 **Figure 3.** Absolute blood counts of WBC, Neutrophils, Lymphocytes and Eosinophils over time in
537 the treatment groups.

538 **Figure 4.** Pathogenic bacteria analysis. Number of positive isolates from sputum samples in
539 azithromycin (Panel A) and in placebo (Panel B) group. Proportion of *S. pneumoniae* and *H.*
540 *influenzae* isolates not-susceptible to macrolides (Panel C) in intervention (red pie chart) and
541 placebo (blue pie chart). Time of the appearance of macrolides resistance (Panel D).









APPENDIX E1

PRINCIPAL STUDY INVESTIGATORS AND PERIOD OF RECRUITMENT

Date first subject enrolled: November, 2012

Date last subject completed (DB phase): December, 2016

Open-label extension: July 7, 2016

Report date: August 7, 2017

The following principal investigators participated in the study:

Policlinico Umberto I, Rome, Italy– Isabella Quinti (PI), Cinzia Milito (Coordinator), Federica Pulvirenti (Investigator, Biostatistician)

Ospedale Civile, Padova, Italy - Francesco Cinetto (Investigator), Carlo Agostini (Investigator)

Spedali Civili, Brescia, Italy - Vassilios Lougaris (Investigator), Annarosa Soresina (Investigator), Alessandro Plebani (Investigator).

Policlinico Federico II, Naples, Italy - Antonio Pecoraro (Investigator), Giuseppe Spadaro (Investigator).

Careggi Hospital, Florence, Italy - Alessandra Vultaggio (Investigator), Andrea Matucci (Investigator)

Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy - Maria Carrabba (Investigator) Giovanna Fabio (Investigator), Rosa Maria Dellepiane (Investigator)

Policlinico di Bari, Italy - Giuseppe Lassandro (investigator) Baldassarre Martire (investigator)

Istituto Dermopatico Immacolata, IRCCS, Rome, Italy – Damiano Abeni (Biostatistician), Stefano Tabolli (Biostatistician).

APPENDIX E2

STUDY DESIGN, RANDOMIZATION AND PROCEDURES

Study design. Prospective, parallel-group, placebo-controlled design. Participants were randomly assigned in a 1:1 ratio to receive azithromycin, at a dose of 250 mg once daily orally for three consecutive days/week for 24 months, or an identical-appearing placebo. The study setting were seven clinical immunology Italian units chosen among those with most experience in the area of primary antibody deficiencies. Patients were enrolled in in-patient and day-care settings, if they agreed

to participate and fulfilled the inclusion/exclusion criteria. Written information on these and any other topics relevant to the study participation were provided. Research assistants ascertained that each candidate participant had fully understood the structure of the study and what it implied for him/herself and collected the signed informed consent. The study was approved by the institutional review board at each participating institution. The study was performed in accordance with the Good Clinical Practice guidelines, the International Conference on Harmonization guidelines, and the most recent version of the Declaration of Helsinki. Once defined a patient as eligible, the investigator reported his/her baseline demographic, clinical and laboratory data in the Case Report Form (CRF). At T1 visit, after the protocol procedures (including medical history, physical examination, laboratory examination, sputum exam) a contact with a telephone automatic service was done for randomization (IVRS). The first site started enrolling participants in November 2012 and the last patient ended the follow-up in December 2016. Adherence was monitored by investigator count of empty blisters of study medication at each monthly study visit.

Randomization. Patients were allocated in a 1:1 intervention-to-control ratio according to a randomization scheme prepared by the epidemiology centre and kept by personnel not involved with selection of patients, interventions, and collection of relevant information. The lists were prepared with a manual procedure, starting from random number tables, available at the website: <http://www.morris.umn.edu/~sungurea/introstat/public/instruction/ranbox/randomnumbersII.html>. The randomization was stratified by centre. For all the centre, except for the coordinating one, it was in blocks of four individuals. Four combinations of four blocks were created, originating a list of 16 individuals. If a centre collected more than 16 subjects, it had to contact the coordinating centre. Each combination was assigned a value from 0 to 5. Numbers from 6 to 9 were ignored. Considering A as case and B as control the combinations were: AABB=0; ABAB=1; ABBA=2; BABA=3; BAAB=4; BBAA=5. The starting point for the lists was column 1, line 3. For the coordinating center the randomization was simple, with 0 to 4 corresponding to group A and 5 to 9 to group B. The starting point for the list was column 6, line 20. A list of eighty assignments was created. Randomized patients received azithromycin at the dosage of 250 mg/die administered 3 times a week for 3 consecutive days versus placebo for 24 months following by a 5 months period of follow-up.

Procedures. Study assessments were performed at enrolment (T0), after randomization (T1) and monthly after randomization up to month 12 (T2) and 24 (T3) and after 4 months since intervention discontinuation (T4).

- At T1, we collected medical history, physical examination, HRQoL questionnaires, routine blood testing (Ig serum levels, WBC count, creatinine, AST, ALT and BUN serum levels), sputum sample, FEV1. All patients were trained to report exacerbations to our research teams.

Prior and concomitant therapies and their outcomes were reported in the Case Report Form (CRF).

- Every month, we collected recent medical history, physical examination, basic laboratory investigations, diary card, sputum sample for microbiological assessment, report of adverse events (AEs). At each clinic visit, all patients provided diary cards for changes in respiratory symptoms. Personnel determined whether an acute exacerbation had occurred in the prior month. Acute exacerbation was defined as “a complex of respiratory symptoms (increased or new onset) of at least three of the following respiratory symptoms with a duration of at least 3 days: cough; change in sputum production (consistency, colour, or volume); fever $>38^{\circ}\text{C}$; wheezing; dyspnoea; decreased exercise tolerance; radiographic changes suggesting lung infection and requiring treatment. The date of each acute exacerbation was taken as the date treatment was prescribed. We also collected the number of days of sputum for each episode of acute exacerbation.
- Every four months, patients underwent blood tests, FEV1 measurement, and microbiological assessment of sputum samples.
- Every 12 months participants completed HRQoL questionnaires. The SF-36 (39) measures health on 8 multi-item dimensions: physical functioning (PF), role-physical (RF), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role-emotional (RE), and mental health (MH) and two summary measures, the physical component summary (PCS) and mental component summary (MCS). Scores for each dimension range from 0 to 100, with higher scores indicating better health. The SGRQ is a specific questionnaire validated for use in patients with respiratory diseases (40). It's partitioned into three sections (symptoms, activity, impact), which are scored separately and can be added to provide a total score ranging from 0 to 100, with 0 indicating no impairment of HRQoL.

The project was organized for a period of 36 months. The first period was devoted to the preparation of the materials for the intervention, to train research and administrative/technical personnel involved and to the operational definition and standardization of procedures. The projected recruitment time was 7 months; the total time for each individual was 29 months. Data were collected by each centre's investigators through standardized CRF and self-administered questionnaires. At each centre, trial data including informed consent as well as all follow-up forms were enclosed in the patients' clinical records. A medical doctor was involved in the data entry, fed the forms into an electronic database expressly designed to this purpose. The database was developed to secure controls to protect data confidentiality, integrity, and availability were implemented - including allowing access to database only with a username and password, automatic backup on remote server when closing the database.

The database allowed to enter only pre-set, validated values in order to have a homogeneous dataset. Finally, data were exported in the Microsoft Office Excel 2000 file format to be analysed using statistical software package SPSS.

APPENDIX E3

SPUTUM SAMPLES PROCESSING.

Sputum samples were analysed by bacterioscopy exam and standard culture. Isolates of *S. aureus*, *Haemophilus spp*, *S. pneumoniae*, *P. aeruginosa* and *K. pneumoniae* were identified according to standard guidelines (P. R. Murray, E. J. Baron, J. H. Jorgensen, M.L. Landry, and M. A. Pfaller, (ed.), Manual of clinical microbiology, 9th ed. ASM Press, Washington, DC., 2007). Susceptibility testing was performed according to the methods approved by the Clinical Laboratory Standards Institute (Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard. 9th ed. Document M07-A9. Cockerill, FR: CLSI; 2012). Interpretative criteria followed criteria recommended by EUCAST breakpoints (European Committee on Antimicrobial Susceptibility Testing - EUCAST, Home page at: <http://www.eucast.org>). Bacterioscopy of sputum samples for acid resistant bacteria was performed according to standard guidelines (P. R. Murray, E. J. Baron, J. H. Jorgensen, M.L. Landry, and M. A. Pfaller, (ed.), Manual of clinical microbiology, 9th ed. ASM Press, Washington, DC., 2007).

Figure E legend

Fig.E1 IgG TROUGH LEVELS DURING THE STUDY TIME IN THE AZITHROMYCIN (RED LINE) AND PLACEBO (BLU LINE) GROUP.

TABLE E1. ADVERSE EVENTS, SERIOUS ADVERSE EVENTS AND SERIOUS UNEXPECTED SUSPECTED ADVERSE EVENTS DEFINITION IN THE STUDY PROTOCOL.

Adverse Event
An AE is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Illnesses or injuries were regarded as AEs. Abnormal results of diagnostic procedures were considered to be adverse events if the abnormality: - results in study withdrawal; - was associated with a serious AE; - was associated with clinical signs or symptoms; - lead to additional treatment or to further diagnostic tests; - was considered by the investigator to be of clinical significance. AE were classified as serious or non-serious. AE, including hospitalization, due to the basic diseases were registered in diary cards, but were not reported.
Serious Adverse Events and Serious Unexpected Suspected Adverse Events
A serious adverse event (SAE) was any AE that was: - fatal; - life-threatening; - requires or prolongs hospital stay; - results in persistent or significant disability or incapacity; - a congenital anomaly or birth defect; - an important medical event. Important medical events were those that may not be immediately life threatening but were clearly of major clinical significance. They may jeopardize the subject and may require intervention to prevent one of the other serious outcomes noted above. All AE that did not meet any of the criteria for serious were regarded as non-serious adverse events (mild, moderate).

TABLE E2. DISTRIBUTION OF CONFUNDING VARIABLES AT T2, T3, T4 IN AZITHROMYCIN AND PLACEBO STUDY GROUP.

	T2		T3		T4	
	azithromycin	placebo	azithromycin	placebo	azithromycin	placebo
Age (yrs)						
n. of patients	35	41	33	35	32	34
Median	43.5	45.4	44.5	45.7	43.7	45.7
IQR	34.3-59.5	35.8-58.8	35.3-61.1	36.3-58.8	35.4-60.7	36.6-57.8
<i>p value</i>		ns		ns		ns
CVID						
n. of patients	31	31	29	26	28	25
%	88.5	75.6	87.8	74.2	87.5	73.5
<i>p value</i>		ns		ns		ns
XLA						
n. of patients	4	10	4	9	4	9
%	11.4	24.3	12.1	25.7	12.5	26.5
<i>p value</i>		ns		ns		ns

TABLE E3. SF-36 SCORES AT T1, T2 AND T3. Comparisons were performed for patients in the azithromycin and placebo group between T1 vs T2 and T1 vs T3.

	Azithromycin									
	T1		T2		Delta T2-T1		T3		Delta T3-T1	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PF	72.6	28.9	76.7	22.4	+4.1	6.5	66.8	31.5	-5.8	2.6
RP	47.3	41.6	60.2	44.0	+12.9	2.4	51.2	42.9	+3.9	1.3
BP	65.6	28.0	68.2	28.7	+27.0	0.6	68.8	29.7	+3.3	1.7
GH	32.2	19.0	35.4	26.3	+3.2	7.3	33.2	23.6	+1.0	4.5
VT	52.0	21.3	55.9	17.3	+4.0	4.0	54.3	24.5	+2.4	3.2
SF	65.4	26.0	69.4	27.2	+4.0	1.2	64.2	27.1	-1.2	1.1
RE	65.5	44.0	71.6	38.9	+6.1	5.1	68.3	38.7	+2.8	5.3
MH	58.4	23.8	65.0	20.1*	+6.6	3.7	64.2	21.1	+5.8	2.7
PCS	40.4	11.6	41.8	11.8	+1.4	0.2	38.9	12.2	-1.5	0.6
MCS	39.5	14.9	42.9	11.7*	+3.4	3.3	42.5	11.8	+3.1	3.2

	Placebo									
	T1		T2		Delta T2-T1		T3		Delta T3-T1	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PF	76.7	24.9	73.0	28.3	-3.7	3.4	69.7	26.0	-7.0	1.1
RP	62.9	45.1	51.7	44.5	-11.2	0.6	48.6	44.9	-14.3	0.2
BP	72.7	27.0	66.1	28.9	-6.6	1.8	70.7	28.8	-2.0	1.8
GH	36.3	19.7	32.8	20.7	-3.5	1.1	32.3	20.7	-3.9	1.0
VT	58.6	20.0	56.0	19.5	-2.6	0.4	58.9	18.1	+0.3	1.9
SF	68.6	24.2	66.8	27.1	-1.8	2.8	66.7	25.4	-1.9	1.1
RE	71.1	39.9	62.2	43.5	-8.9	3.7	61.1	44.6	-10.0	4.8
MH	67.6	17.7	62.9	20.2	-4.7	2.5	68.4	18.0	+0.8	0.3
PCS	44.1	11.4	40.9	13.2	-3.2	1.8	39.4	11.2	-4.8	0.2
MCS	43.0	11.8	41.4	11.5	-1.6	0.3	43.3	10.5	+0.3	1.3

(* = p < 0.05)

TABLE E4. SGRQ SCORES AT T1, T2 AND T3. Comparisons were performed for patients in the azithromycin and placebo group between T1 vs T2 and T1 vs T3.

	Azithromycin					Placebo				
	T1 Mean (SD)	T2 Mean (SD)	Delta T2-T1 Mean (SD)	T3 Mean (SD)	Delta T3-T1 Mean (SD)	T1 Mean (SD)	T2 Mean (SD)	Delta T2-T1 Mean (SD)	T3 Mean (SD)	Delta T3-T1 Mean (SD)
Symptoms	47.0 (23.1)	41.1* (22.5)	-5.0 (15.6)	39.6* (24.0)	-6.2 (17.8)	46.3 (23.1)	42.2 (20.9)	-4.1 (12.4)	42.2 (22.0)	-4.6 (16.1)
Activity	39.9 (26.1)	36.6 (27.2)	-4.7 (20.5)	38.3 (30.4)	-2.7 (20.2)	33.4 (25.0)	31.8 (28.8)	-0.9 (17.9)	31.6 (27.1)	-2.3 (15.2)
Impact	48.8 (27.1)	24.3** (19.8)	-26.5 (17.6)	27.2** (19.7)	-23.4 (18.4)	43.0 (31.1)	23.1** (18.6)	-18.7 (21.7)	24.1** (18.3)	-19.3 (21.9)
Total	34.7 (16.9)	30.8* (19.6)	-4.7 (11.9)	32.7 (22.0)	-2.9 (13.7)	30.8 (18.9)	28.9 (20.6)	-1.1 (9.6)	29.4 (19.9)	-2.0 (8.8)

(* p<0.05; **p<0.01)

