Double-blind, placebo-controlled, randomized trial on low-dose azithromycin prophylaxis in patients with primary antibody deficiencies

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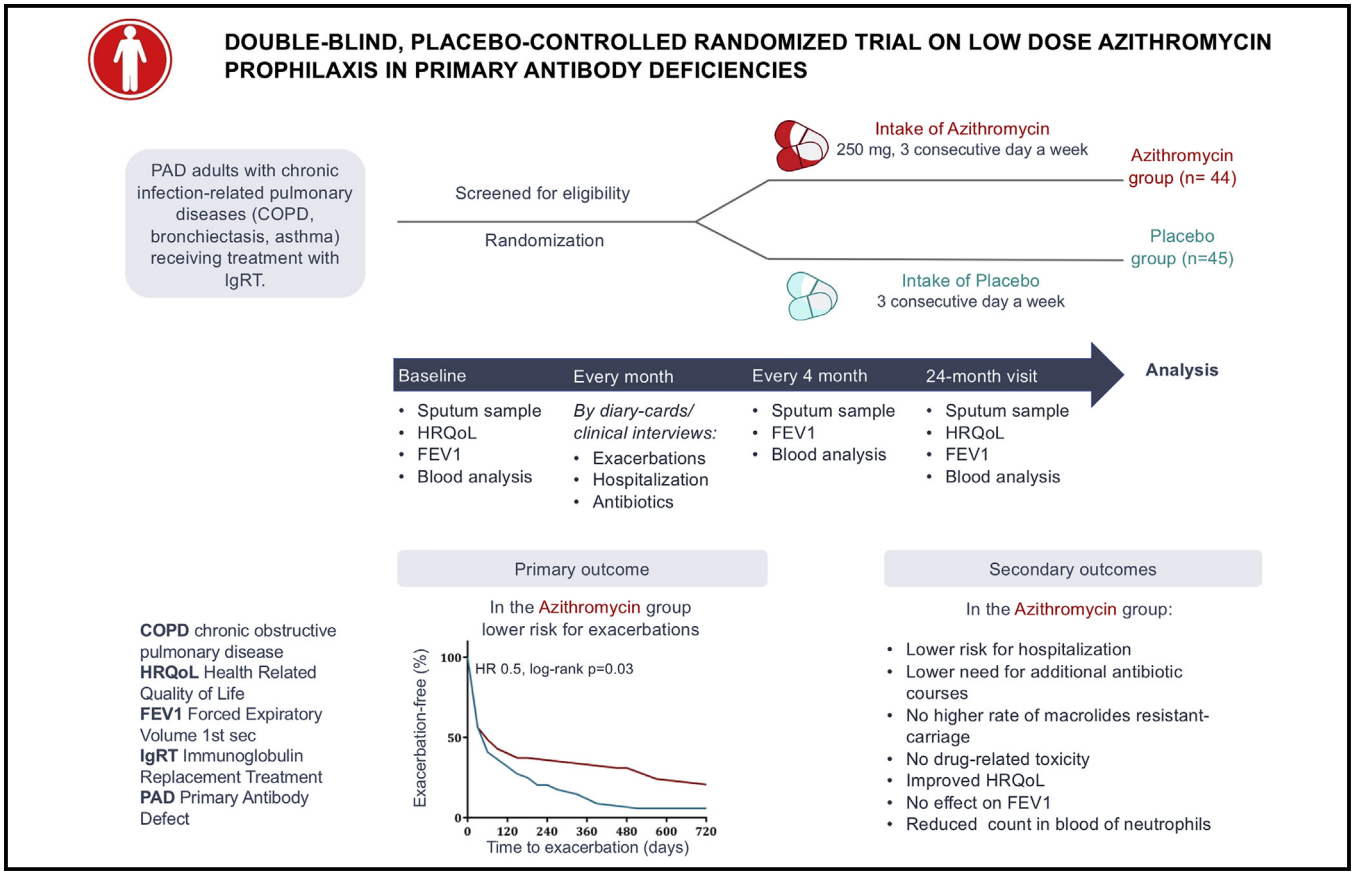
GRAPHICAL ABSTRACT



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Background: Lacking protective antibodies, patients with primary antibody deficiencies (PADs) experience frequent respiratory tract infections, leading to chronic pulmonary damage. Macrolide prophylaxis has proved effective in patients with chronic respiratory diseases.

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

Methods: We designed a 3-year, double-blind, placebo-controlled, randomized clinical trial to test whether oral azithromycin (250 mg administered once daily 3 times a week for 2 years) would reduce respiratory exacerbations in patients with PADs and chronic infection–related pulmonary diseases. The primary end point was the number of annual respiratory exacerbations. Secondary end points included time to first exacerbation, additional antibiotic 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 (95% CI, 2.5-4.7) per patient-year in the azithromycin arm and 5.2 (95% CI, 4.1-6.4) per patient-year in the placebo arm ($P = .02$). In the azithromycin group the hazard risk for having an acute exacerbation was 0.5 (95% CI, 0.3-0.9; $P = .03$), and the hazard risk for hospitalization was 0.5 (95% CI, 0.2-1.1; $P = .04$). The rate of additional antibiotic treatment per patient-year was 2.3 (95% CI, 2.1-3.4) in the intervention group and 3.6 (95% CI, 2.9-4.3) in the placebo group ($P = .004$). *Haemophilus influenzae* and *Streptococcus pneumoniae* were the prevalent isolates, and they were not susceptible to macrolides in 25% of patients of both arms. Azithromycin's safety profile was comparable with that of placebo.

Conclusion: The study reached the main outcome centered on the reduction of exacerbation episodes per patient-year, with a consequent reduction in additional courses of antibiotics and risk of hospitalization. (J Allergy Clin Immunol 2019;■■■:■■■-■■■.)

Key words: *Primary antibody defects, azithromycin, antibiotic prophylaxis, respiratory exacerbation, chronic obstructive pulmonary disease*

Abbreviations used

AE: Adverse event
CF: Cystic fibrosis
COPD: Chronic obstructive pulmonary disease
CVID: Common variable immunodeficiency
HR: Hazard risk
HRQoL: Health-related quality of life
PAD: Primary antibody deficiency
PID: Primary immunodeficiency disease
SF-36: Short Form 36
SGRQ: Saint George Respiratory Questionnaire
XLA: X-linked agammaglobulinemia

Primary antibody deficiencies (PADs) account for most diagnosed primary immunodeficiency diseases (PIDs), particularly in adulthood. Although PIDs are considered rare diseases, the International Union of Immunological Societies estimated that only 1% of subjects living with a PID all over the world received a diagnosis,[1] highlighting the need for a higher index of suspicion in 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]

Because of 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 patients with PADs are mainly caused by encapsulated bacteria, resulting in frequent visits to physicians' offices and emergency department, numerous hospitalizations, and work days lost.[5] IgG replacement therapy enhances survival and reduces the risk of pneumonia and invasive infections.[6] However, despite appropriate immunoglobulin therapy, patients can have chronic infection–related pulmonary diseases, including bronchiectasis, chronic obstructive pulmonary disease (COPD), and asthma.[7,8]

Underdiagnoses, 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 patients with XLA.[9] In patients with 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] Recurrence of acute infections over these underlying chronic lung conditions has been proposed to be defined as respiratory exacerbations of patients with PADs by using the definition already validated for COPD.[10] Of note, recent reports underlined that patients with frequently exacerbating COPD and those with severe uncontrolled asthma might mask an underdiagnosis of PADs.[12]

Patients with PIDs can take advantage of antibiotic prophylaxis.[2] Macrolide antibiotics have proved 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 of infection to inflammation that leads to airway hyperreactivity and remodeling.[17]

Based on these observations, we conducted a 36-month phase II, randomized, double-blind, multicenter clinical trial to test the hypothesis that long-term prophylactic treatment with azithromycin might decrease the frequency of respiratory exacerbations when added to the usual care of patients with PADs experiencing 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 7 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 inpatient and daycare 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 European Society for Immunodeficiencies registry criteria (http://esid.org/Working-Parties/Registry/

TABLE I. Inclusion and exclusion criteria of the study

Inclusion criteria
- Diagnosis of XLA or CVID according to the revised ESID registry criteria*
- Male or female subjects aged 18 to 74 years
- Clinical diagnosis of chronic infection–related pulmonary diseases
- Written informed consent

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
- Pregnant or breast-feeding female subjects planning to become pregnant during the study
- Any condition that is likely to interfere with evaluation of the study drug or satisfactory conduct of the trial

ALAT, Alanine aminotransferase; *ASAT*, aspartate aminotransferase; *ESID*, European Society for Immunodeficiencies; *UNL*, upper limit of normal.
*http://esid.org/Working-Parties/Registry/Diagnosis-criteria.

Diagnosis-criteria), and had 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 form at enrollment.

Patients were regularly treated with substitutive treatment using polyvalent IgG of 400 mg/kg/mo or greater. The IgG replacement monthly dosage was individualized according to clinical judgement before enrollment. Concomitant medications were administered according to the usual consolidated clinical approach. Use of cointerventions 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 2 of the 3 clinical criteria for acute exacerbations were present: (1) increased dyspnea, (2) increased sputum production, and (3) increased purulence of sputum (www.goldcopd.org).

The study protocol (European Clinical Trials Database [EUDRACT]: 2011-004351-39) was reviewed and approved by the Ethical Review Committee of Sapienza, University of Rome, Rome, Italy. The description of the full study design, methods, and procedures medications are listed in the Methods section in this article's Online Repository at www.jacionline.org. Report of this trial conforms to CONSORT 2010 guidelines.

Intervention

Study assessments were performed at enrollment (T0), after randomization (T1), and monthly after randomization up to months 12 (T2) and 24 (T3) and after 4 months since intervention discontinuation (T4). Patients received 250 mg of azithromycin once daily 3 times a week for 3 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 results, HRQoL questionnaires, routine blood test results, sputum samples, and measures of lung function expressed as FEV_1. 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 results, basic laboratory investigation results, diary cards, sputum samples for microbiological assessment, and reports 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 4 months, patients underwent blood tests, FEV_1 measurements, and microbiological assessments of sputum samples. Sputum samples were collected at time points defined by study protocol, regardless of exacerbation. Every 12 months, participants completed HRQoL questionnaires (Short Form 36 [SF-36] questionnaire and Saint George Respiratory Questionnaire [SGRQ]). At the end of the study period, we collected physical examination results, recent medical histories, diary cards, basic laboratory investigation results, sputum samples, and FEV_1 values (see the Methods section in this article's Online Repository).

Randomization and masking

Patients were allocated in a 1:1 intervention/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 the Methods section in this article's Online Repository). 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 3 of the following respiratory symptoms with a duration of at least 3 days: cough, change in sputum production (consistency, color, or volume), temperature of greater than 38°C, wheezing, dyspnea, and decreased exercise tolerance.

Secondary end points 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 based on FEV_1 at enrollment and every 4 months thereafter. Sputum samples were obtained at the time of enrollment and every 4 months thereafter and processed by means of standard culture for respiratory pathogens. Selected respiratory pathogens were assessed for resistance to macrolides. Sputum samples were also tested by using bacterioscopy for acid-resistant bacteria (see the Methods section in this article's Online Repository).

HRQoL was assessed by using the SF-36 questionnaire and the SGRQ at enrollment and every 12 months. The SF-36 measures health on 8 multi-item dimensions (Physical Functioning, Role-Physical, Bodily Pain, General Health, Vitality, Social Functioning, Role-Emotional, and Mental Health) and 2 summary measures (Physical Component Summary and Mental Component Summary).[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 3 sections (symptoms, activity, and 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 preinfusion levels and WBC blood peripheral counts were assessed every 4 months to avoid bias caused by low IgG serum levels or leukopenia in recurrence of respiratory exacerbations. Serious AEs and serious unexpected suspected AEs were monitored by using clinical records and reported to the human research ethics committee at each site. Definitions of AEs are shown in 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 1-sided α value of .025, and we planned to include 130 patients, assuming a 10% dropout. The hypothesis of superiority justified the choice of the 1-sided test. Eighty-nine patients were enrolled. Keeping the 2 proportions originally chosen (ie, 75% and 50%), with 44

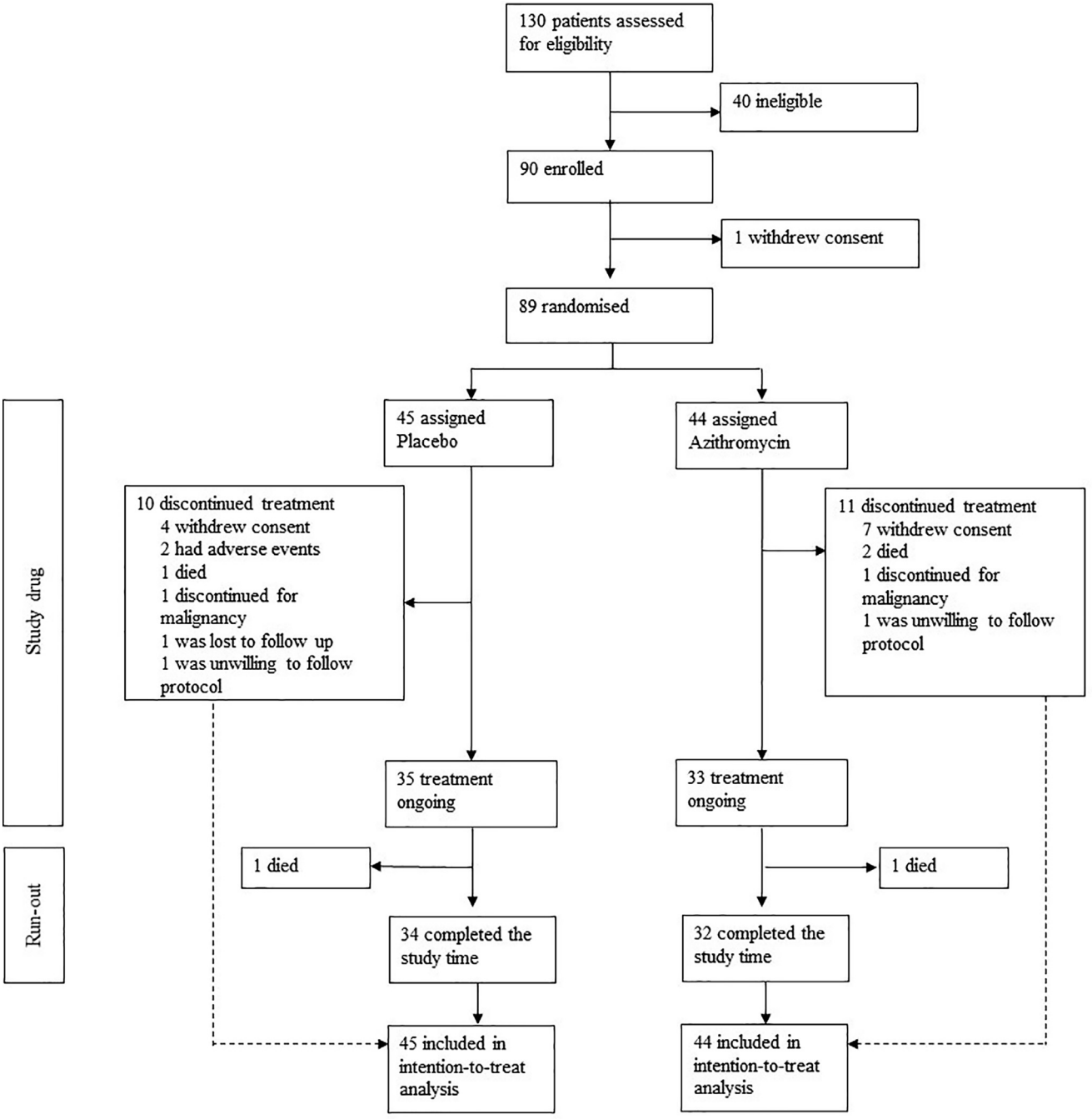

FIG 1. Trial profile.

patients per group and an α 1-sided value of .05, we calculated a power of 77%. The 77% suboptimal power remained close to the commonly accepted standard of 80%. The sample size reduction was due to the 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 using 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 using the χ^2 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 the run-out period, and time to first additional course of antibiotics and hospitalization was assessed by using Kaplan-Meier product-limit estimates and based on a log-rank and Gehan-Breslow-Wilcoxon test of the difference between the 2 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 prespecified prerandomization factors that might predict the risk of acute exacerbation. Changes in FEV_1 and laboratory parameters

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

	Azithromycin (n = 44)	Placebo (n = 45)	*P* value
Age (y), mean (SD)	45.0 (14.9)	45.0 (14.0)	.895
Sex, no. (%)			
Female	23 (52)	26 (58)	.602
Male	21 (48)	19 (42)	.602
Diagnosis, no. (%)			
CVID	38 (86)	35 (78)	.409
XLA	6 (14)	10 (22)	.409
Chronic pulmonary diseases, no. (%)			
COPD (all stages)	22 (50)	23 (51)	.543
Stage I	6 (14)	3 (7)	.283
Stage II	9 (20)	10 (22)	.522
Stage III-IV	7 (16)	10 (22)	.313
Bronchiectasis	36 (82)	40 (89)	.260
Asthma	5 (11)	6 (13)	.516
FEV_1 (% predicted), mean (SD)	71 (28)	76 (23)	.391
Blood data, mean (SD)			
WBC (cell/mm^3)	6758.4 (2595.7)	7429.2 (3262.9)	.166
Neutrophils (cell/mm^3)	4325.2 (2025.9)	4793.4 (2585.9)	.195
IgG (mg/dL)	767.1 (298.4)	731.0 (234.9)	.292
IgA (mg/dL)	32.0 (59.9)	28.9 (106.3)	.441
IgM (mg/dL)	36.7 (69.0)	63.3 (146.7)	.165
HRQoL assessment, mean (SD)			
SF-36, PCS (%)	40.4 (11.6)	44.1 (11.4)	.458
SF-36, MCS (%)	39.5 (14.9)	43.0 (11.8)	.310
SGRQ, total (%)	34.7 (16.9)	30.8 (18.9)	.165

Data are expressed as numbers (percentages) or means (SDs).
MCS, Mental Component Summary; *PCS*, Physical Component Summary.

attributable to treatment were calculated with a linear mixed-model analysis. HRQoL measures at T1, T2, and T3 were compared by using the Mann-Whitney *U* test. Data were analyzed by using group sequential testing that allowed "spending" a little of the α value at each interim analysis such that the total type I error did not exceed .05 at the end of the study.

Statistical analyses were performed with the statistical package SPSS (IBM SPSS Statistics for Windows, version 25.0; IBM, Armonk, NY). This trial is registered with the Agenzia Italiana del Farmaco (Clinical Trials no. European Clinical Trials Database [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 allocated to the placebo and azithromycin arms, respectively (Fig 1). Randomization yielded 2 comparable groups for variables considered potential confounders (Table II). The mean cumulative period of observation was 25.8 months (95% CI, 23.4-28.0 months) in the azithromycin group and 25.5 months (95% CI, 23.5-27.6 months; $P = .429$) in the placebo group. At 1 year (T2), 40 patients were in the placebo group and 35 were in the azithromycin group; at 2 years (T3), 35 patients were in the placebo group and 33 were in the azithromycin group. At the end of the study (T4), 34 patients were in the placebo group, and 32 were in the azithromycin group. During the study period, groups remained comparable for IgG trough serum levels ($F = 1.486$, $P = .231$), PAD diagnosis, and age between patient groups (see Fig E1 and Table E2 in this article's Online Repository at www.jacionline.org).

A total of 677 respiratory exacerbations occurred during the T1 to T3 period: 262 among the participants allocated to 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 episodes per patient-year), and that in the placebo group was 5.2 episodes per patient-year (95% CI, 4.1-6.4 episodes per patient-year; $P = .020$). A *post hoc* analysis showed that the risk of respiratory exacerbations was reduced among participants receiving azithromycin (hazard risk [HR], 0.5; 95% CI, 0.3-0.9; log-rank $P = .033$; Fig 2, *A*). The difference remained significant after adjustment by means of Cox regression in a model, including sex, FEV_1, age, and study center. The number needed to prevent 1 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 2 study arms (HR, 1.0; 95% CI, 0.6-1.8; log-rank $P = .895$; Fig 2, *B*). Three of 45 participants receiving placebo and 10 of 44 participants receiving azithromycin were free from exacerbations during the study period ($P = .039$), yielding an absolute risk reduction of 16.1% (95% CI, 1.7% to 30.4%). Time to first exacerbation did not differ in the 2 study arms (134.0 days [95% CI, 61.5-207.5 days] vs 104.3 days [95% CI, 67.3-141.3 days], $P = .236$).

A total of 45 hospitalizations for any cause occurred during the study period, 32 (71%) of which occurred in the placebo group. The rate of hospitalization per patient-year was 0.1 episodes (95% CI, 0.1-0.2 episodes) in the intervention group and 0.3 episodes (95% CI, 0.2-0.5 episodes) in the placebo group ($P = .014$, Table III). The HR of having a hospitalization in the azithromycin group was 0.5 (95% CI, 0.2-1.1; Gehan-Breslow $P = .040$; Fig 2, *C*).

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]; $P = .004$; HR, 0.6 [95% CI, 0.4-1.0]; log-rank $P = .020$; Fig 2, *D*). This effect was lost during the run-out period (HR, 1.01; 95% CI, 0.6-2.1; log-rank $P = .746$). The mean time to the first antibiotic course was shorter in the placebo group (intervention: 181.5 days [95% CI, 23.5-239.5 days] vs placebo: 122.4 days [95% CI, 123.5-239.5 days], log-rank $P = .046$, Table III). Nine (21%) patients in the azithromycin arm and 2 (5%) patients in the placebo arm did not take additional antibiotic courses during the study period ($P = .030$). Changes in percent predicted FEV_1 over time were not different for patients receiving placebo compared with those receiving azithromycin ($F = 1.486$, $P = .231$).

After starting azithromycin, decreased counts of absolute peripheral blood WBCs and neutrophils were observed in the intervention arm than in the placebo arm ($F = 4.55$, $P = .0367$ and $F = 4.64$, $P = .035$, 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 period, 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 ($P = .124$, Table III). *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* were detected in 10%, 7%, 10%, and 2% of

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

	Azithromycin (n = 44)	Placebo (n = 45)	*P* value
Exacerbations			
Rate per patient-year, mean (SD)	3.6 (3.8)	5.2 (3.9)	.020
Patients free from exacerbations, no. (%)	10 (26)	3 (7)	.039
Hospitalization			
Rate per patient-year, mean (range)	0.1 (0-0.2)	0.3 (0-0.5)	.014
Days at first hospitalization, mean (SD)	650.0 (167.8)	528.4 (285.8)	.010
Additional course of antibiotics			
Patients who had ≥1 antibiotics course, no. (%)	35 (71)	43 (95)	.026
Course per patient-year, mean (SD)	2.3 (1.9)	3.6 (2.5)	.004
Microbiological assessment			
Sputum samples collected, no.	139	165	—
Participants providing sputum samples, no. (%)	27 (61.3)	30 (66.6)	.662
Positive isolate, no. (%)	49 (35.2)	70 (42.4)	.096
Patients carrying *H influenzae* and/or *S pneumoniae* not susceptible to macrolides	6 (22)	6 (20)	1.000
Serious nonfatal AEs, no.			
Pneumonia	0	6	.010
Neoplasm	0	3	.241
Gastrointestinal tract	0	3	.241
Cardiovascular	0	0	—
Other	3*	5†	.713
Total	3	17	.002
AEs leading to drug discontinuation, no.			
Pneumonia	0	0	—
Neoplasm	0	2	.494
Gastrointestinal tract	0	1	1.000
Cardiovascular	0	0	—
Other	0	1‡	1.000
Total	0	4	.116
Fatal AEs, no.			
Pneumonia	1	1	1.000
Respiratory failure	1	0	.494
Gastric malignancy	0	1	1.000
Parkinson	1	0	.494
Total	3	2	.676

*One participant had thrombocytopenia, 1 participant had lymphadenopathy, and 1 participant had iron deficiency anemia.
†One participant had thrombocytopenia, 2 participants had urticaria, 1 patient had hyponatremia, and 1 patient had hepatic fibrosis.
‡One participant discontinued because of hepatic fibrosis.

sputum samples obtained from patients in the azithromycin group (Fig 4, *A*) and in 16%, 11%, 7%, and 3% of sputum samples obtained from patients in the placebo group (Fig 4, *B*), whereas no acid-resistant bacteria were identified.

Nonsusceptible strains accounted for 86% and 79% of *S pneumoniae* and *H influenzae* isolates in the intervention and placebo groups, respectively. These nonsusceptible strains were obtained from 6 of 27 patients receiving azithromycin and 6 of 30 patients receiving placebo ($P = .221$; Fig 4, *C*). The risk of having *S pneumoniae* and *H influenzae* strains that are not susceptible to macrolides was similar in the 2 study arms (HR, 0.9; 95% CI, 0.3-2.8; log-rank $P = .897$; Fig 4, *D*). The difference in the risk for exacerbation remained significant after adjustment for macrolide resistance by using Cox regression.

Improvement in mean scores on SF-36 mental-related scales was seen only at T2 in the azithromycin group (Mental Component Summary T1: 39.5 ± 14.9 vs Mental Component Summary T2: 42.9 ± 11.7, $P = .021$; Mental Health T1: 58.4 ± 23.8 vs Mental Health T2: 65.0 ± 20.1, $P = .020$; see Table E3 in this article's Online Repository at www.jacionline.org). Improvement in mean scores on the SGRQ impact scale was found in both study arms (azithromycin T1: 48.8 ± 27.1 vs azithromycin T3: 27.2 ± 19.7, $P < .0001$; placebo T1: 43.0 ± 31.1 vs placebo T3: 24.1 ± 18.3, $P < .0001$). Mean symptom scores improved only in the azithromycin group (T1: 47.0 ± 23.1 vs T3: 39.6 ± 24.0, $P = .040$), whereas they did not change in the control group (T1: 46.3 ± 23.1 vs T3: 42.2 ± 22.0, $P = .463$), and mean activity scores did not change in either study arm (azithromycin T1: 39.9 ± 26.1 vs azithromycin T3: 38.3 ± 30.4, $P = .828$; placebo T1: 33.4 ± 25.0 vs placebo T3: 31.6 ± 27.1, $P = .780$; see Table E4 in this article's Online Repository at www.jacionline.org).

No serious drug-related AEs 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 ($P = .489$). The rate of death from respiratory causes was 5% and 2.2% in the 2 groups, respectively ($P = .616$). Even though the study was not designed to analyze the effect of azithromycin on other concomitant conditions, we observed a reduced frequency of participants reporting diarrhea (13% vs 53%, $P = .001$) and acute rhinosinusitis (4% vs 27%, $P = .020$) in the azithromycin group.

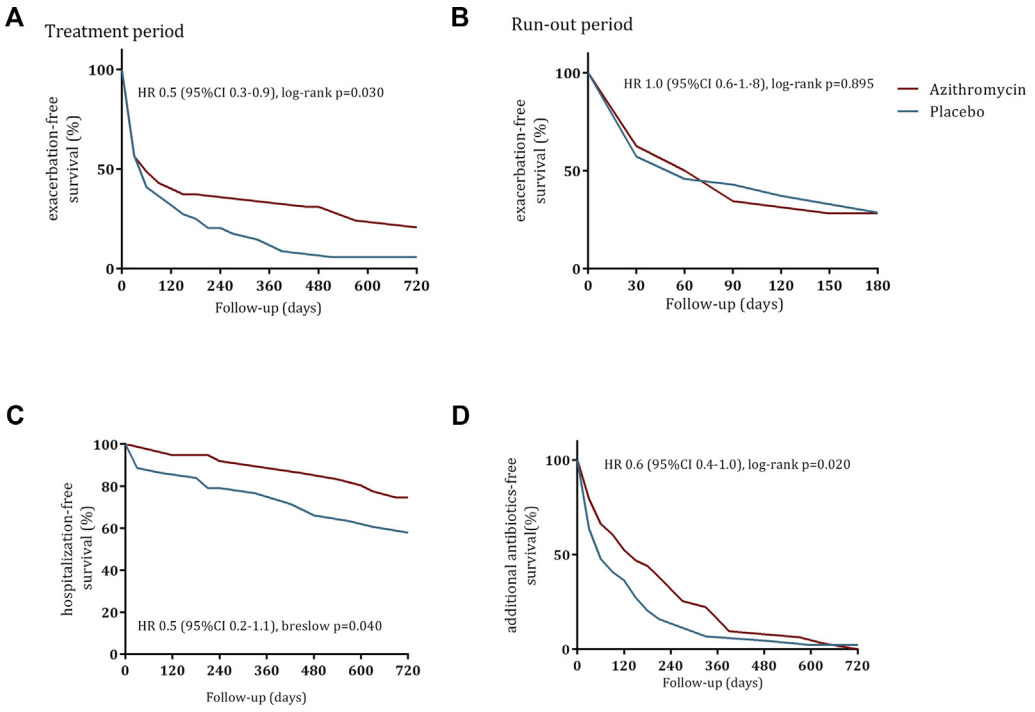

FIG 2. Kaplan-Meier plot. **A** and **B,** Proportion of patients remaining exacerbation free during the study time in the intervention *(red line)* and control *(blue line)* groups in the study period (T1-T3; Fig 2, *A*) and run-out period (T3-T4; Fig 2, *B*). **C** and **D,** Proportion of participants free from hospital admission (Fig 2, *C*) and additional antibiotic courses (Fig 2, *D*) in the intervention *(red line)* and control *(blue line)* groups during the study period.

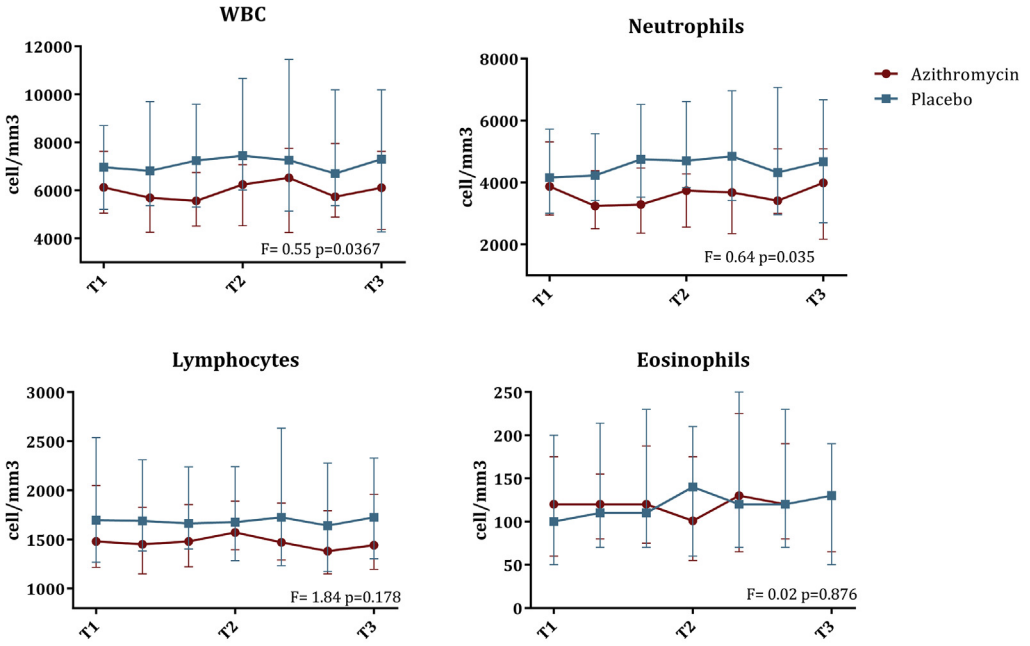

FIG 3. Absolute blood counts of WBCs, neutrophils, lymphocytes, and eosinophils over time in the treatment groups.

DISCUSSION

The study on the efficacy and safety of long-term oral azithromycin prophylaxis in patients affected by PADs 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

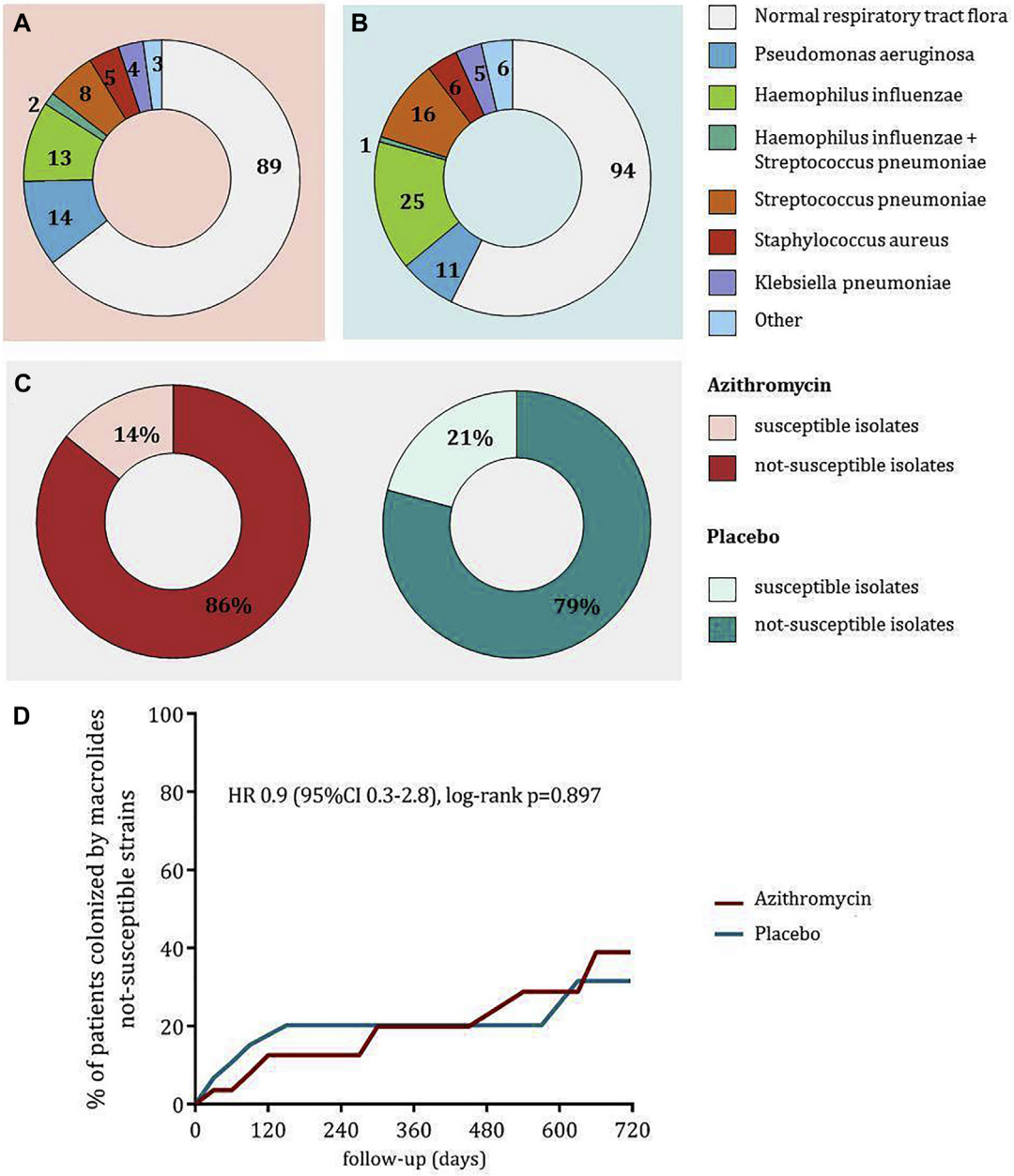

FIG 4. Pathogenic bacteria analysis. **A** and **B,** Number of positive isolates from sputum samples in the azithromycin (Fig 4, *A*) and placebo (Fig 4, *B*) groups. **C,** Proportion of *S pneumoniae* and *H influenzae* isolates not susceptible to macrolides in the intervention *(red pie chart)* and placebo *(blue pie chart)* groups. **D,** Time of the appearance of macrolide resistance.

macrolide-resistant organisms or the rate of AEs. Our results on efficacy of azithromycin were comparable with those already proved by other similar randomized studies done in patients with COPD,[16] patients with CF patients,[14] and patients without CF but with bronchiectasis,[15] 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 greater than that described in the other studies: 2.73 for patients with non-CF bronchiectasis,[15] 1.83 for patients with COPD,[16] and 0.81 for patients with asthma.[20] Under prophylaxis, in patients with PADs, the rate significantly decreased to 3.60, but this was still greater than rates reported in patients with 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 in FEV_1. These data confirmed the severity of respiratory disease in patients with PADs,[10] in whom lifelong respiratory symptoms tend to peak in early decades of life, leading to chronic pulmonary damage,[7] as seen in patients with other chronic lung diseases.[21]

The efficacy of a low-dose macrolide therapy on PAD-related respiratory exacerbation might be explained by the antimicrobial and anti-inflammatory action of the new macrolides, especially on neutrophil chemotaxis, neutrophil-derived elastolytic-like activity, and concentrations of IL-8 and leukotriene B_4.[22,23] Our data

showed a decrease in neutrophil blood counts during the study period in the intervention group.

Because of the lack of a standard treatment regimen, the azithromycin dosage of 250 mg/d 3 times a week was chosen to increase patient adherence and minimize adverse effects. It has been previously demonstrated that daily intake of 250 mg of azithromycin resulted in development of colonization by azithromycin-resistant pathogens,[16] whereas the same dose given 3 times a week did not increase bacterial resistance.[24] Furthermore, our choice of a low azithromycin dosage was supported by *in vitro* data on macrolide ability to reduced alginate production and flagellin-induced inflammation and to decrease biomass and maximal thickness biofilms also at subinhibitory concentrations.[25]

Bacteria were isolated from sputum in one third of patients of the 2 study arms, with a predominance of *H influenzae*, *S pneumoniae*, *P aeruginosa*, and *K pneumoniae*, as already described in patients with COPD.[26] A high rate of *H influenzae* and *S pneumoniae* isolates not susceptible to macrolides was detected in a quarter of patients of the 2 study arms. These data were comparable with our recent data on upper respiratory tract carriage in patients with PADs.[27] The possibility to clarify the clinical relevance of the issue of nonsusceptible strains was difficult because the EUCAST breakpoint tables (version 8, 2018)[28] no longer report clinical breakpoints of macrolide resistance in patient with respiratory tract infections, at least concerning *H influenzae* strains. However, physicians should take into account the evidence that macrolide resistance is increasing.[29] The risk of driving bacterial resistance because of long-term macrolide prophylaxis could be balanced by benefits on overall bacterial resistance caused by reductions in the number of antibiotic courses.

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

Neither serious side effects nor reasons for treatment discontinuation related to drug intake were reported. The high rate of death in the 29-month study period further demonstrated the severity of PADs.[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, because of the risk of ventricular arrhythmias in macrolide recipients.[32]

In the intervention group the generic tool SF-36 documented improvement over time related to Mental Dimensions, and the lung disease–specific tool SGRQ documented improvement in the Symptoms scale. The 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 disease. However, the 77% suboptimal power remained close to the commonly accepted standard of 80%, and in fact, even though there is a reduction in sample size, the differences observed between the 2 study arms often reached statistical significance and the 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 a valuable option for patients with PADs and respiratory exacerbations.

We thank the patients, nurses, and doctors who participated in the study.

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

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METHODS

Study design

This study had a 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 3 consecutive days/week for 24 months or an identical-appearing placebo. The study setting included 7 clinical immunology Italian units chosen among those with the most experience in the area of PADs. Patients were enrolled in inpatient and daycare 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 himself or herself and collected the signed informed consent forms. 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 a patient was defined as eligible, the investigator reported his or her baseline demographic, clinical, and laboratory data in the case report form. At the T1 visit, after the protocol procedures (including medical history, physical examination, laboratory examination, and sputum examination), a contact with an automatic telephone 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/control ratio according to a randomization scheme prepared by the epidemiology center 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 following: Web site http://www.morris.umn.edu/~sungurea/introstat/public/instruction/ranbox/randomnumbersII.html.

The randomization was stratified by center. For all center, except for the coordinating one, it was in blocks of 4 subjects. Four combinations of 4 blocks were created, originating a list of 16 subjects. If a center collected more than 16 subjects, it had to contact the coordinating center. 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 as follows: AABB, 0; ABAB, 1; ABBA, 2; BABA, 3; BAAB, 4; and 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 80 assignments was created. Randomized patients received azithromycin at the dosage of 250 mg/d administered 3 times a week for 3 consecutive days versus placebo for 24 months, followed by a 5-month period of follow-up.

Procedures

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

- At T1, we collected medical histories, physical examination results, HRQoL questionnaires, routine blood test results (immunoglobulin serum levels, WBC counts, and creatinine, aspartate transaminase, alanine transaminase, and blood urea nitrogen serum levels), sputum samples, and FEV_1 measurements. 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 histories, physical examination results, basic laboratory investigation results, diary cards, sputum samples for microbiological assessment, and reports of 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 3 of the following respiratory symptoms with a duration of at least 3 days: cough, change in sputum production (consistency, color, or volume), temperature greater than 38°C, wheezing, dyspnea, decreased exercise tolerance, and 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 4 months, patients underwent blood tests, FEV_1 measurements, and microbiological assessments of sputum samples.
- Every 12 months, participants completed HRQoL questionnaires. The SF-36 measures health on 8 multi-item dimensions (Physical Functioning, Role-Physical, Bodily Pain, General Health, Vitality, Social Functioning, Role-Emotional, and Mental Health) and 2 summary measures (Physical Component Summary and Mental Component Summary). Scores for each dimension range from 0 to 100, with greater scores indicating better health. The SGRQ is a specific questionnaire validated for use in patients with respiratory diseases. It is partitioned into 3 sections (symptoms, activity, and 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 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 subject was 29 months. Data were collected by each center's investigators through a standardized case report form and self-administered questionnaires. At each center, 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 data entry and 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, including allowing access to the database only with a username and password and automatic backup on a remote server when closing the database. The database allowed entry of only preset validated values to have a homogeneous data set. Finally, data were exported in the Microsoft Office Excel 2000 file format to be analyzed by using the statistical software package SPSS.

Sputum sample processing

Sputum samples were analyzed by means of bacterioscopy examination and standard culture. Isolates of *Staphylococcus aureus*, *Haemophilus* species, *S pneumoniae*, *P aeruginosa*, and *K pneumoniae* were identified according to standard guidelines.[E1] Susceptibility testing was performed according to the methods approved by the Clinical Laboratory Standards Institute.[E2] Interpretative criteria followed the criteria recommended by EUCAST breakpoints.[E3] Bacterioscopy of sputum samples for acid resistant bacteria was performed according to standard guidelines.[E1]

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 (Principal Investigator), 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, Bari, Italy—Giuseppe Lassandro (Investigator), Baldassarre Martire (Investigator)

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

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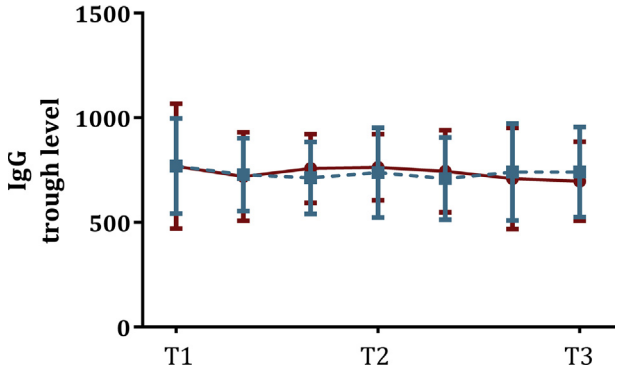

FIG E1. IgG trough levels during the study period in the azithromycin *(red line)* and placebo *(blue line)* groups.

TABLE E1. AE, serious AE, and serious unexpected suspected AE definitions in the study protocol

AE
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 AEs if the abnormality — ● resulted in study withdrawal; ● was associated with a serious AE; ● was associated with clinical signs or symptoms; ● led to additional treatment or further diagnostic tests; or ● was considered by the investigator to be of clinical significance. AEs were classified as serious or nonserious. AEs, including hospitalizations, caused by the basic diseases were registered in diary cards but were not reported.
Serious AEs and serious unexpected suspected AEs
A serious AE was any AE that was — ● fatal; ● life-threatening; ● required or prolonged hospital stay; ● resulted in persistent or significant disability or incapacity; ● a congenital anomaly or birth defect; or ● an important medical event. Important medical events were those that might not be immediately life-threatening but were clearly of major clinical significance. They might jeopardize the subject and might require intervention to prevent one of the other serious outcomes noted above. All AEs that did not meet any of the criteria for being serious AEs were regarded as nonserious AEs (mild or moderate).

TABLE E2. Distribution of confounding variables at T2, T3, and T4 in the azithromycin and placebo study groups

	T2		T3		T4	
	Azithromycin	**Placebo**	**Azithromycin**	**Placebo**	**Azithromycin**	**Placebo**
Age (y)						
No. 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
P value		NS		NS		NS
CVID						
No. of patients	31	31	29	26	28	25
Percentage	88.5	75.6	87.8	74.2	87.5	73.5
P value		NS		NS		NS
XLA						
No. of patients	4	10	4	9	4	9
Percentage	11.4	24.3	12.1	25.7	12.5	26.5
P value		NS		NS		NS

IQR, Interquartile range; *NS*, not significant.

TABLE E3. SF-36 scores at T1, T2, and T3

	T1		T2		Δ T2-T1		T3		Δ T3-T1	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Azithromycin										
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										
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

Comparisons were performed for patients in the azithromycin and placebos group between T1 versus T2 and T1 versus T3.

BP, Bodily Pain; *GH*, General Health; *MCS*, Mental Component Summary; *MH*, Mental Health; *PCS*, Physical Component Summary; *PF*, Physical Functioning; *RE*, Role-Emotional; *RP*, Role-Physical; *SF*, Social Functioning; *VT*, Vitality.

*$P < .05$.

TABLE E4. SGRQ scores at T1, T2, and T3

	Azithromycin					Placebo				
	T1, mean (SD)	**T2, mean (SD)**	**Δ T2-T1, mean (SD)**	**T3, mean (SD)**	**Δ T3-T1, mean (SD)**	**T1, mean (SD)**	**T2, mean (SD)**	**Δ T2-T1, mean (SD)**	**T3, mean (SD)**	**Δ 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)

Comparisons were performed for patients in the azithromycin and placebo groups between T1 versus T2 and T1 versus T3.
*$P < .05$.
†$P < .01$.