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# Azithromycin and early allograft function after lung transplantation: A randomized, controlled trial

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**KEYWORDS:**
lung transplantation;
azithromycin;
early lung allograft function;
chronic lung allograft dysfunction;
survival

**BACKGROUND:** Chronic lung allograft dysfunction (CLAD) is the single most important factor limiting long-term survival after lung transplantation (LTx). Azithromycin has been shown to improve CLAD-free and long-term survival, yet the possible impact on early lung allograft function is unclear.

**METHODS:** A prospective, randomized, double-blind, placebo-controlled trial of pre-transplant and prompt post-transplant azithromycin treatment was performed at the University Hospitals Leuven. In each arm, 34 patients, transplanted between October 2013 and October 2015, were included for analysis. Study drug was added to standard of care and was administered once before LTx (1,000 mg of azithromycin or placebo) and every other day from Day 1 until Day 31 after LTx (250 mg of azithromycin or placebo). Primary outcome was an anticipated 15% improvement of forced expiratory volume in 1 second ($FEV_1$, percent predicted) during the first 3 months post-LTx. Secondary end-points included length of intubation, days on ventilator, duration of intensive care unit and hospital stay, prevalence and severity of primary graft dysfunction, acute rejection, infection, and CLAD-free and overall survival.

**RESULTS:** $FEV_1$ was not significantly different between the 2 groups ($p = 0.41$). Patients treated with azithromycin demonstrated less airway inflammation, with lower bronchoalveolar lavage (BAL) neutrophilia and BAL interleukin-8 protein levels at Day 30 ($p = 0.09$ and $p = 0.04$, respectively) and Day 90 ($p = 0.002$ and $p = 0.08$, respectively) after LTx. Other secondary outcomes were not significantly different between placebo and azithromycin groups.

**CONCLUSIONS:** Pre-transplant and prompt post-transplant azithromycin treatment was not able to improve early lung allograft function. However, the known anti-inflammatory properties of azithromycin were confirmed (NCT01915082).

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Chronic lung allograft dysfunction (CLAD) is the single most important limiting factor of long-term survival after LTx, resulting in a poor 5-year survival rate of 57% worldwide.[1] CLAD is defined as a persistent decrease in forced expiratory volume in 1 second ($FEV_1$, in liters) of at least 20% compared with baseline, in absence of other causes of lung allograft dysfunction.[2,3]

Azithromycin, a neomacrolide antibiotic, was shown to improve late lung allograft function in approximately 35% of patients with established CLAD in several case series,[4–11] which was confirmed in a randomized, placebo-controlled trial.[12] In addition, a randomized, placebo-controlled trial of pre-emptive azithromycin treatment demonstrated better CLAD-free survival and pulmonary function compared with placebo. Moreover, azithromycin was shown to attenuate both airway and systemic inflammation 2 years after lung transplantation (LTx),[13,14] confirming the anti-inflammatory properties of azithromycin.[4–11,15–19]

In contrast, evidence of a possible impact of azithromycin on early lung allograft function is scarce. However, macrolide antibiotics were recently associated with shorter time of mechanical ventilation and lower 6-month mortality in patients admitted with acute lung injury.[20] In addition, donor and recipient serum cytokine levels before and after LTx, representing the allograft inflammatory state, have been linked to early lung allograft dysfunction.[21,22] Interestingly, azithromycin was shown to reduce airway inflammation in a murine ischemia–reperfusion injury model.[23] In addition, azithromycin reduced isolated interleukin (IL)-17–mediated lymphocytic airway inflammation after LTx.[24,25] Moreover, an obstructive pulmonary function pattern early after LTx has been associated with reduced CLAD-free and overall survival. This early pulmonary function deficit may be the result of infection, acute rejection, or ischemia–reperfusion injury in the early post-transplant period, which often presents as primary graft dysfunction (PGD).[26]

Given these findings, we hypothesized that azithromycin could improve early lung allograft function after LTx by reducing early allograft inflammation, possibly by reducing episodes and severity of PGD, infection, and acute A-grade and/or B-grade rejection. Azithromycin may, in this way, be able to improve CLAD-free and long-term survival.

## Methods

### Study design

A prospective, randomized, double-blind, placebo-controlled trial of pre-transplant and prompt post-transplant oral azithromycin therapy (NCT01915082), in 120 patients undergoing double LTx between October 2013 and October 2015, was performed at the University Hospitals Leuven. Patients who did not consent before LTx ($n = 24$), were included in another trial ($n = 13$), underwent retransplantation ($n = 6$) or multi-organ transplant ($n = 2$), were <18 years old ($n = 3$), or had azithromycin allergy ($n = 0$) were excluded (Figure 1). The remaining 72 patients were randomly assigned to placebo ($n = 36$) or azithromycin ($n = 36$). In both groups, 2 patients were excluded for further analysis, as they accidentally did not receive the first post-transplant dose of study medication ($n = 3$) or had normothermic preservation of the lung allograft with the organ care system before transplantation ($n = 1$). Therefore, each group included 34 patients who completed the 31-day course of study drug, all of whom were

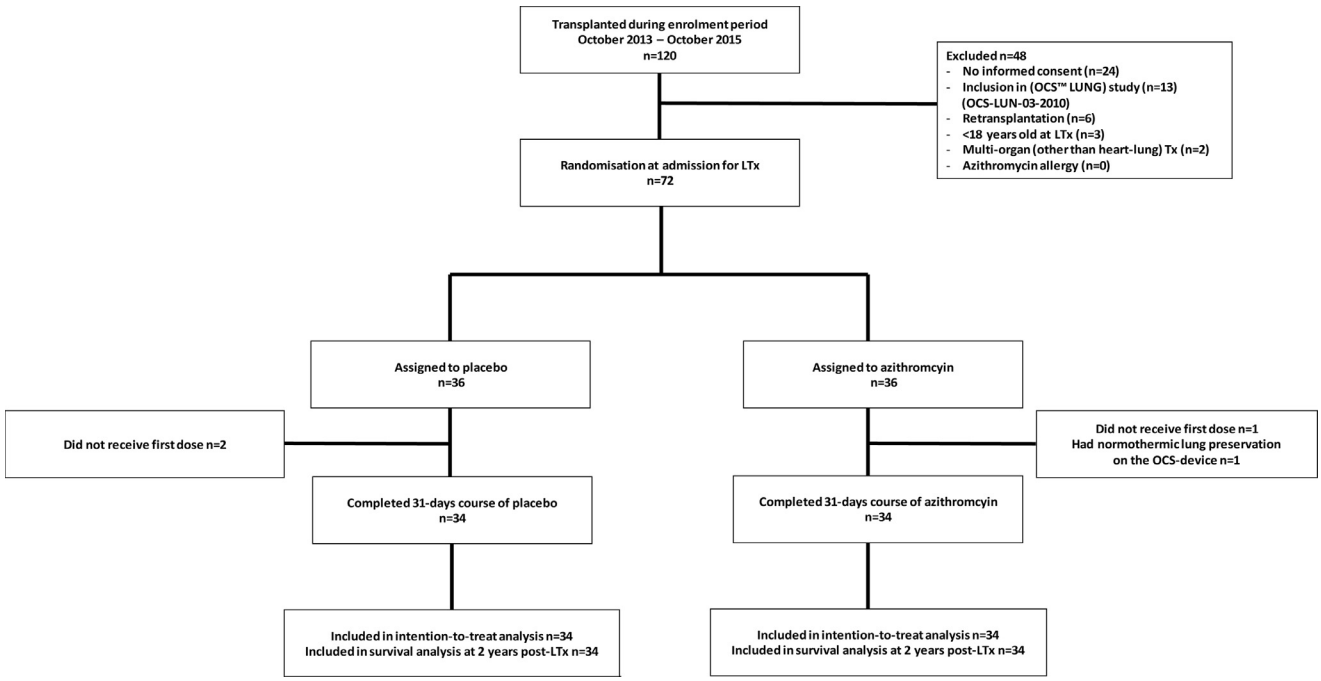


**Figure 1** Flowchart diagram of the study cohort. Patients transplanted between October 16, 2013 and October 7, 2015 were screened ($n = 120$). Patients who did not consent ($n = 24$), were included in the OCS LUNG study (OCS-LUN-03-2010) ($n = 13$), underwent retransplantation ($n = 6$) or multi-organ (other than heart–lung) transplant ($n = 2$), or were <18 years old at LTx ($n = 3$), were excluded. The remaining 72 patients were randomized into the placebo ($n = 36$) or azithromycin ($n = 36$) group. Two patients in both groups were excluded as they accidentally did not receive the first dose of study medication ($n = 3$) or had normothermic lung preservation on the OCS device ($n = 1$). The remaining 34 patients in each group completed the 31-day course of study drug and were included for further analyses.

subsequently included in the intention-to-treat and survival analysis. This trial was approved by the local ethics committee and all included patients provided informed consent when on the waiting list for subsequent LTx.

When admitted for LTx, patients were randomly assigned to oral azithromycin or placebo in a 1:1 ratio according to blinded randomization. In accordance with his/her attributed study number, each patient included was assigned to a pre-numbered bottle containing the study drug. The study drug was added to standard of care and was administered once immediately before LTx (1,000 mg of azithromycin or placebo) and every other day from Day 1 until Day 31 after LTx (250 mg of azithromycin or placebo). At discharge, azithromycin was not routinely started, but liberal use was allowed after Day 90 post-LTx in cases where azithromycin-responsive allograft dysfunction, lymphocytic bronchiolitis, and/or CLAD was diagnosed. The study period was 6 months post-LTx, with thereafter routine, lifelong follow-up.

Group assignment was blinded during entire the study period for participants, nurses, and investigators, and thereafter until the first 2 years after inclusion of the last patient. Later, patients were unblinded and data collection and analyses were performed.

## Study drug

Azithromycin (Zitromax® oral suspension 200 mg/5 mL) was purchased from Pfizer. For placebo, Ora-plus (97% purified water, <1% sodium phosphate monobasic, <1% sodium carboxymethylcellulose, <1% microcrystalline cellulose, <1% xanthan gum, <1% carrageenan) was purchased from Paddock Laboratories (USA). Both were provided in blinded, pre-numbered bottles by the hospital's clinical pharmacy and were administered by nurses of the intensive care unit (ICU) or the LTx ward per os or via (naso)gastric tube.

## Therapeutic management and transplant monitoring

The immunosuppressive regimen and infectious prophylaxis was given to all patients according to standardized protocols, independent of study drug. Standard transplant monitoring, immunosuppressive and prophylactic regimen, bronchoscopic procedures, and processing of specimens have been described elsewhere[13,14] and are summarized in the Supplementary Material (available online at www.jhltonline.org/).

## End-points

The primary end-point was an anticipated 15% improvement in pulmonary function ($FEV_1$, percent predicted) during the first 3 months after LTx. Secondary end-points included length of intubation, days on ventilator, length of ICU and hospital stay, prevalence and severity of PGD, histologic acute rejection (Grade A) and lymphocytic bronchiolitis (Grade B), pulmonary function during the first 6 months after LTx, airway and systemic inflammation, airway colonization and infection, 6-minute walking distance, and overall and CLAD-free survival at 2 years after LTx.

PGD was evaluated according to ISHLT guidelines.[27] CLAD was defined as a persistent decline in $FEV_1$ (liters) of at least 20% compared with baseline, in the absence of other causes of lung allograft dysfunction.[2,3] Quantitative determination of plasma C-reactive protein (CRP) levels was performed at the University Hospitals Clinical Laboratory. IL-6 and IL-8 levels were measured in bronchoalveolar laavage (BAL) supernatant using sandwich enzyme-linked immunoassay (ELISA; Thermo Fisher) according to manufacturer's instructions, as previously described.[22]

Adverse events were monitored by the treating LTx clinicians (blinded for study drug) and were defined as hearing loss, severe cardiac arrhythmias (torsade de pointes), serious allergic reactions including skin reactions (rash, urticaria or Stevens–Johnson syndrome), angioneurotic edema, and anaphylaxis and neurologic disorders (convulsions). In addition, serial daily 12-lead electrocardiographic monitoring was performed per protocol in all included patients during their stay in the ICU and intermediate care unit, on average for the first 9 days after inclusion.

## Statistical analysis

Based on an anticipated 15% improvement in $FEV_1$ during the first 3 months after LTx, a 1:1 inclusion ratio, a dropout rate of 15%, and a two-sided test ($\alpha = 0.05$, $\beta = 0.20$), enrollment of at least 58 patients was needed. The anticipated number of included patients was 70 and the anticipated enrollment period 1.5 years. Actual enrollment of 72 LTx recipients was reached after 2 years, due to a higher exclusion rate than anticipated.

Patients' characteristics are summarized using descriptive statistics. Patient proportions were compared using the chi-square test. Continuous data are presented as mean and standard error of the mean when normally distributed, or as a median with interquartile range when non–normally distributed. Group means were compared using unpaired, two-tailed *t*-test or Mann–Whitney *U*-test for normally or non–normally distributed variables, respectively. Overall and CLAD-free survival after LTx were compared using the Kaplan–Meier method with log-rank testing.

GraphPad Prism 6.0 software (GraphPad) was used for statistical analysis. All reported *p*-values are two-sided, with $p < 0.05$ considered statistically significant.

# Results

## Patients' characteristics

Baseline donor and recipient characteristics (Table 1) and immunosuppressive regimen (Table S1 in the Supplementary Material online) did not differ between placebo and azithromycin. In addition, the number of patients treated with azithromycin before LTx did not differ between the 2 groups ($p = 0.47$). After a median of 372 (164 to 654) days, 29 patients (91%), initially treated with placebo, had been started on open-label azithromycin, whereas, after a median of 203 (131 to 432) days, 33 patients (97%), initially treated with azithromycin, were started on open-label azithromycin ($p = 0.26$ and $p = 0.09$, respectively), based on the treating physician's discretion (Table 1).

## Primary end-point: $FEV_1$ during the first 3 months after LTx

$FEV_1$ was not significantly different between azithromycin and placebo during the first 3 and 6 months after LTx ($p = 0.41$ and $p = 0.11$, respectively). In both groups, patients performed a median of 8 pulmonary function tests

**Table 1** Characteristics of Placebo and Azithromycin Study Groups

| | Placebo ($n = 34$) | Azithromycin ($n = 34$) | $p$-value |
|---|---|---|---|
| Donor | | | |
| Gender male [$n$ (%)] | 20 (51) | 19 (49) | 0.81 |
| Age (years) | 51 ± 3 | 51 ± 3 | 0.93 |
| DBD [$n$ (%)] | 28 (82) | 29 (85) | 0.74 |
| $PaO_2$ (mm Hg) | 437 ± 16 | 472 ± 15 | 0.11 |
| Recipient | | | |
| Gender male [$n$ (%)] | 16 (48) | 17 (52) | 0.81 |
| Age at transplantation (years) | 58 (47-63) | 59 (56-62) | 0.33 |
| BMI ($kg/m^2$) | 22.8 (19.0 to 25.1) | 23.0 (19.8 to 25.7) | 0.69 |
| Underlying disease [$n$ (%)] | | | 0.45 |
| Emphysema | 22 (65) | 23 (68) | |
| Cystic fibrosis | 3 (9) | 1 (3) | |
| ILD | 5 (15) | 8 (24) | |
| PAH | 2 (6) | 0 (0) | |
| Other | 2 (6) | 2 (6) | |
| Time on waiting list (days) | 206 ± 25 | 232 ± 26 | 0.48 |
| Type of transplant (SSLTx) [$n$ (%)] | 34 (100) | 34 (100) | 1.00 |
| Ischemic time first lung (min) | 298 (261 to 369) | 270 (224 to 332) | 0.10 |
| Ischemic time second lung (min) | 477 (406 to 546) | 443 (382 to 505) | 0.19 |
| Use of ECMO [$n$ (%)] | 3 (9) | 2 (6) | 0.64 |
| CMV $D^+/R^-$ [$n$ (%)] | 8 (24) | 4 (12) | 0.20 |
| Time of follow-up (years) | 3.1 (2.5 to 3.6) | 3.2 (2.6 to 3.7) | 0.53 |
| Spirometries per patient = 3 months ($n$) | 8 (6-9) | 8 (7 to 9) | 0.30 |
| Azithromycin before LTx [$n$ (%)] | 16 (47) | 19 (56) | 0.47 |
| Open-label azithromycin after LTx [$n$ (%)] | 29 (91%) | 33 (97%) | 0.09 |
| Time to open-label azithromycin (days) | 372 (164 to 654) | 203 (131 to 432) | 0.26 |

No significant differences could be demonstrated between groups. Patient proportions were compared using the chi-square test. Continuous data are presented as a mean ± standard error of the mean when normally distributed or as a median (interquartile range) when data were of not normally distributed. Group means were compared using unpaired, two-tailed $t$-test or Mann–Whitney $U$-test for normally or non–normally distributed variables respectively. BMI, body mass index; CMV, cytomegalovirus; D, donor; DBD, donation after brain death; ECMO, extracorporeal membrane oxygenation; ILD, interstitial lung disease; PAH, pulmonary artery hypertension; R, recipient; SSLTx, sequential single lung transplantation.

during the first 3 months after LTx ($p = 0.30$). LTx recipients treated with placebo showed an improvement in $FEV_1$ of 6% (2% to 11%) per month during the first 3 months and 5% (2% to 7%) per month during the first 6 months after LTx. Patients treated with azithromycin demonstrated a similar improvement in $FEV_1$ of 5% (1% to 10%) per month during the first 3 months and 2% (1% to 7%) per month during the first 6 months after LTx. At Day 30 after LTx, the azithromycin group showed a trend toward better $FEV_1$ compared with placebo (81 ± 4% vs 71 ± 4%, respectively; $p = 0.07$; Figure 2). At Day 90 and Day 180, $FEV_1$ did not differ between the 2 groups ($p = 0.48$ and $p = 0.75$, respectively).

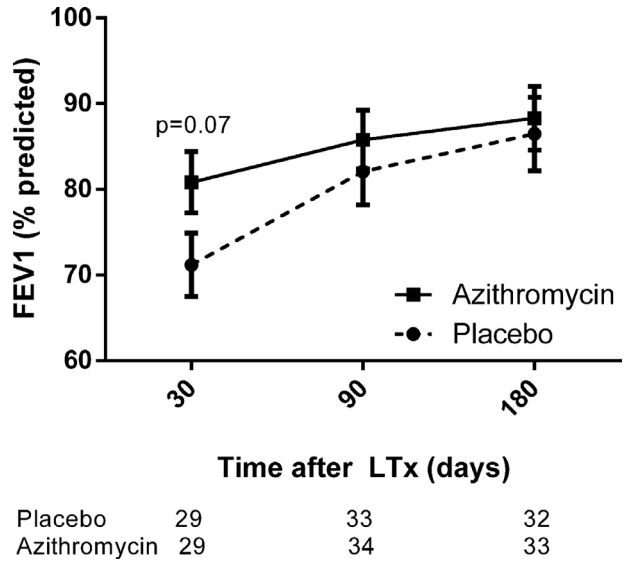


**Figure 2** Change in $FEV_1$ after LTx. $FEV_1$ (percentage predicted) at fixed time-points after LTx, assessed at routine follow-up visits. Data are presented as mean ± SEM. A trend toward better $FEV_1$ was observed at Day 30 after LTx in patients treated with azithromycin ($p = 0.07$). $FEV_1$, forced expiratory volume in 1 second; LTx, lung transplantation; SEM, standard error of the mean.

## Secondary end-points

*Length of intubation and days on ventilator.* Both patients treated with azithromycin or placebo were intubated for a median of 2 days postoperatively ($p = 0.05$). In both groups, a similar proportion of patients were reintubated within 24 hours after extubation ($p = 0.69$), resulting in a non-significantly different total number of days on the ventilator during the first 6 months post-LTx ($p = 0.08$; Table 2).

**Table 2** Secondary End-points of Placebo vs Azithromycin Group

| | Placebo ($n$=34) | Azithromycin ($n$=34) | $p$-value |
|---|---|---|---|
| Intubation period after LTx, days | 2 (1-2) | 2 (1-3) | 0.05 |
| Days on ventilator during hospital stay, days | 2 (1-3) | 2 (2-4) | 0.08 |
| Stay at ICU, days | 5 (3-7) | 5 (4-9) | 0.54 |
| Stay at Medium Care, days | 4 (2-5) | 4 (3-5) | 0.52 |
| Hospital stay, days | 25 (20-31) | 25 (21-30) | 0.79 |
| PGD grade 3 | | | |
| 0 hours after LTx, *n* (%) | 3 (9) | 3 (9) | 1.00 |
| 24 hours after LTx, *n* (%) | 0 (0) | 0 (0) | 1.00 |
| 48 hours after LTx, *n* (%) | 3 (9) | 7 (21) | 0.17 |
| 72 hours after LTx, *n* (%) | 1 (3) | 3 (9) | 0.30 |
| Cumulative score AR (grade A) 6 months per patient | 0.29±0.09 | 0.32±0.08 | 0.79 |
| Cumulative score AR (grade A≥2) 6 months per patient | 0.09±0.05 | 0.12±0.06 | 0.99 |
| Grade A≥2 AR | | | |
| Discharge after LTx, *n* (%) | 1 (3) | 4 (13) | 0.15 |
| 3 months after LTx, *n* (%) | 1 (3) | 0 (0) | 0.33 |
| 6 months after LTx, *n* (%) | 0 (0) | 0 (0) | 1.00 |
| Cumulative score LB (grade B) 6 months per patient | 0.24±0.09 | 0.24±0.09 | 0.99 |
| Cumulative score LB (grade B2R) 6 months per patient | 0.03±0.03 | 0 | 0.99 |
| Grade B2R LB | | | |
| Discharge after LTx, *n* (%) | 2 (6) | 0 (0) | 0.15 |
| 3 months after LTx, *n* (%) | 0 (0) | 1 (3) | 0.31 |
| 6 months after LTx, *n* (%) | 0 (0) | 0 (0) | 1.00 |
| Respiratory culture positive, n(%) | | | |
| Discharge after LTx, *n* (%) | 5 (15) | 6 (18) | 0.74 |
| 3 months after LTx, *n* (%) | 18 (53) | 5 (15) | 0.015[a] |
| 6 months after LTx, *n* (%) | 13 (38) | 7 (21) | 0.11 |
| Respiratory infections <6 months post-LTx, *n* (%) | 7 (21) | 9 (26) | 0.61 |
| 6 MWD after LTx, meters | | | |
| At discharge, meters | 427 (116-519) | 346 (213-537) | 0.90 |
| 1 year after LTx, meters | 504 (461-569) | 486 (442-534) | 0.42 |
| 2 years after LTx, meters | 521 (446-606) | 470 (400-583) | 0.13 |
| CLAD at 2y after LTx, *n* (%) | 5 (15) | 6 (18) | 0.74 |
| Death at 2y after LTx, *n* (%) | 6 (18) | 4 (12) | 0.49 |

Patient proportions were compared using the chi-square test. Continuous data are presented as a mean ± standard error of the mean when normally distributed or as a median (interquartile range) when data were of not normally distributed. Group means were compared using unpaired, two-tailed *t*-test or Mann–Whitney *U*-test for normally or non–normally distributed variables respectively. 6MWD, 6-minute walking distance; AR, acute rejection; CLAD, chronic lung allograft dysfunction; ICU, intensive care unit; LB, lymphocytic bronchiolitis; LTx, lung transplantation; PGD, primary graft dysfunction.

[a]Statistically significant ($p < 0.05$).

*Length of ICU and hospital stay.* There was no difference in ICU, intermediate care, or total hospital stay between the 2 groups ($p = 0.55$, $p = 0.52$, $p = 0.79$; Table 2).

*Primary graft dysfunction.* Grade 3 PGD prevalence did not differ between the groups at 0, 24, 48, and 72 hours after LTx ($p = 1.00$, $p = 1.00$, $p = 0.17$, $p = 0.30$; Table 2).

*Acute rejection and lymphocytic bronchiolitis.* Both total number of acute rejection and Grade ≥A2 episodes were similar between the groups during the first 6 months after LTx ($p = 0.79$, $p = 0.99$). Grade ≥A2 acute rejection prevalence was not significantly different between the groups at discharge, 3 months, and 6 months after LTx ($p = 0.15$, $p = 0.33$, $p = 1.00$; Table 2).

Similarly, both total number of lymphocytic bronchiolitis and Grade B2R lymphocytic bronchiolitis episodes were similar between the 2 groups during the first 6 months after LTx ($p = 0.99$, $p = 0.99$). In addition, Grade B2R lymphocytic bronchiolitis prevalence was not significantly different between the groups at discharge and at 3 months after LTx ($p = 0.15$, $p = 0.31$; Table 2).

*Airway and systemic inflammation.* At Day 30 after LTx, a trend toward lower BAL neutrophilia (4% [2% to 16%] vs 10% [2% to 50%]; $p = 0.09$) and lower BAL IL-8 protein level (58 [16 to 162] pg/ml vs 124 [50 to 357] pg/ml; $p = 0.04$) was observed for azithromycin vs placebo (Figure 3A and B). At Day 90 after LTx, lower BAL neutrophilia (2% [1% to 4%] vs 8% [2% to 23%]; $p = 0.002$) and a trend for lower BAL IL-8 level (29 [7 to 82] pg/ml vs 44 [17 to 190] pg/ml; $p = 0.08$) was seen with azithromycin. No differences in BAL neutrophilia ($p = 0.24$, $p = 0.96$) and BAL IL-8 ($p = 0.20$, $p = 0.52$) were noted on Day 1 and Day 180 after LTx between the 2 groups.

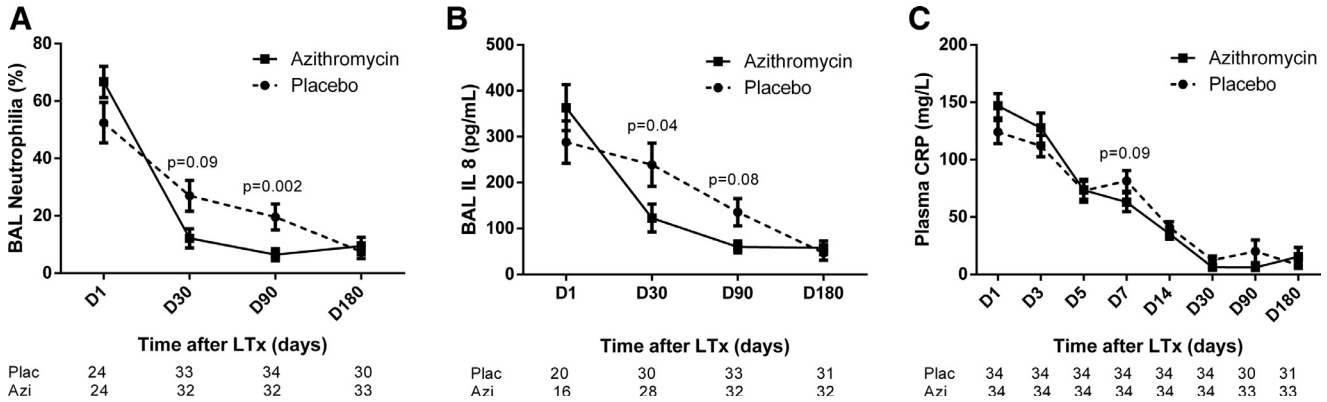


**Figure 3** Change in BAL neutrophilia, BAL IL-8 and plasma C-reactive protein (CRP) after LTx. Change in (A) BAL neutrophilia, (B) BAL IL-8 protein levels, and (C) plasma CRP levels during the first 6 months after LTx in patients treated with placebo or azithromycin, assessed at routine follow-up. Data are presented as mean ± SEM. Group means were compared using unpaired, two-tailed *t*-test, or Mann-Whitney *U*-test for normally or non–normally distributed variables, respectively. BAL, bronchoalveolar lavage; CRP, C-reactive protein; LTx, lung transplantation.

There were no differences in plasma CRP at 1, 3, 5, 7, 14, 30, 90, and 180 days after LTx ($p = 0.12$, $p = 0.61$, $p = 0.69$, $p = 0.09$, $p = 0.48$, $p = 0.99$, $p = 0.50$, $p = 0.44$; Figure 3C).

*Airway colonization and infection.* At 3 months after LTx, more patients in the placebo group had positive respiratory cultures compared with the azithromycin group (41% vs 15%; $p = 0.015$) (Table 2). The placebo group had positive respiratory cultures for *Aspergillus fumigatus* (4, 12%), *Staphloccocus aureus* (3, 9%), *Stenotrophomonas maltophilia* ($n = 2$, 6%), *Pseudomonas aeruginosa* ($n = 1$, 3%), *Scopularis* species ($n = 1$, 3%), *P mirabilis* ($n = 2$, 6%), and *Haemophilus influenzae* ($n = 1$, 3%). The azithromycin group had positive respiratory cultures for *P aeruginosa* ($n = 4$, 12%) or *Klebsiella pneumoniae* ($n = 1$, 3%). At discharge and 6 months after LTx, no differences could be seen between the groups regarding positive respiratory cultures ($p = 0.74$, $p = 0.11$). A similar proportion of patients treated with placebo or azithromycin had a respiratory infection during the first 6 months after LTx ($p = 0.61$; Table 2).

*6-minute walking distance.* The 6-minute walking distance did not differ between the 2 groups at discharge, 1 year, and 2 years after LTx ($p = 0.90$, $p = 0.42$, $p = 0.13$; Table 2).

*Overall and CLAD-free survival.* No patient died in either the placebo or azithromycin group during the first 6 months after LTx. The azithromycin group had a 1-year survival rate of 91% and a 2-year survival rate of 88%, and the placebo group had a 1-year survival rate of 97% and a 2-year survival rate of 85%. The azithromycin group had 1- and 2-year CLAD-free survival rates of 88% and 85%, whereas in the placebo group these rates were 94% and 85%, respectively. Overall and CLAD-free survival did not differ between the 2 groups ($p = 0.56$, $p = 0.93$) (Figure 4).

*Adverse events.* None of the previously defined adverse events were reported in either group. Moreover, electrocardiographic monitoring demonstrated no torsade de pointes in any of the patients. In addition, in the azithromycin group, QTc intervals were not significantly different before and during treatment with azithromycin (428 [415 to 440] ms vs 428 [410 to 452] ms; $p = 0.56$).

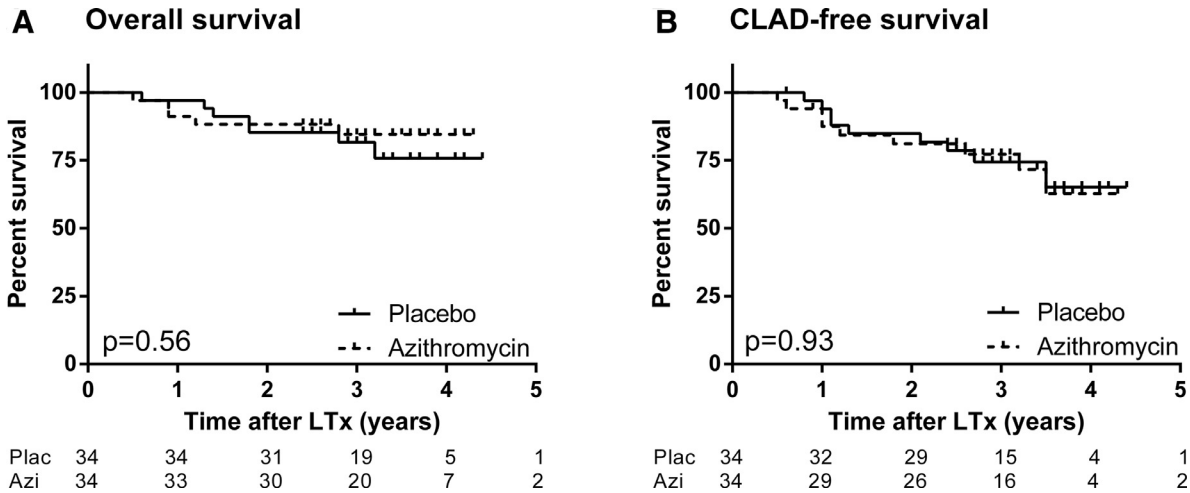


**Figure 4** Overall and CLAD-free survival after LTx. No difference in (A) overall and (B) CLAD-free survival after LTx in patients treated with placebo or azithromycin ($p = 0.56$ and $p = 0.93$, respectively). Overall and CLAD-free survival after LTx were compared with the Kaplan–Meier method with log-rank test. CLAD, chronic lung allograft dysfunction; LTx, lung transplantation.

## Discussion

In this randomized trial, the primary end-point, an anticipated 15% improvement in $FEV_1$ during the first 3 months after LTx in patients treated with pre-transplant and prompt post-transplant azithromycin, was not reached. However, at Day 30 after LTx, the azithromycin group showed a trend toward a higher $FEV_1$ compared with placebo. Clinically relevant secondary end-points were not significantly different between azithromycin or placebo groups. However, known anti-inflammatory effects of azithromycin were confirmed, with lower BAL neutrophilia and IL-8 at 30 and 90 days after LTx in patients treated with azithromycin.[17,27]

In this trial, the first loading dose of study drug was administered just before LTx. Azithromycin is known to reach a maximal serum concentration 2 to 3 hours after administration and to accumulate >70% in tissue (e.g., neutrophils and macrophages).[7,10,28] Because explant lungs are removed during surgery (in most patients >3 hours after administration of azithromycin), we expect ideal exposure to azithromycin when given during the most critical 24 hours post-LTx. Because a trend toward a better $FEV_1$ was seen in patients treated with azithromycin at Day 30 after LTx, it may be more efficacious to prolong azithromycin therapy. This was already shown in our previous trial in which azithromycin was initiated at discharge after LTx (after a median of 36 days) and continued for 2 years.[13,14] Finally, power was calculated based on an anticipated 15% improvement in $FEV_1$ during the first 3 months after LTx, a 1:1 inclusion ratio, a dropout rate of 15%, and a two-sided test ($\alpha = 0.05$, $\beta = 0.20$). The 15% improvement in $FEV_1$ was based on previous research in patients with established CLAD.[4−12] Because our trial was performed in patients without established CLAD, the anticipated 15% improvement may have been too high, and therefore the included number of patients too low to reach a significant difference in primary end-point.

BAL neutrophilia and BAL IL-8 were reduced at Day 30 and Day 90 after LTx in patients treated with azithromycin compared with placebo. These findings are in accordance with literature data, where the anti-inflammatory effects of azithromycin have been described extensively.[13,14,17,18,29] In addition, a significantly lower number of patients treated with azithromycin demonstrated positive respiratory cultures at 3 months after LTx compared with placebo. These findings suggest that the anti-microbial activity of azithromycin may also have contributed to the observed decreased allograft inflammation. However, this effect was not consistent at other time-points, questioning its clinical relevance. Moreover, culture methods are not 100% sensitive, and a presence of some microorganisms in the respiratory tract could have been missed.

We could not show any difference in PGD, acute cellular rejection, or lymphocytic bronchiolitis between patients treated with placebo or azithromycin. However, in the literature, an incidence of 10% to 20% Grade 3 PGD at 72 hours after LTx and an incidence of approximately 30% Grade 3 PGD during the first 72 hours after LTx have been reported.[30] In our study, these rates were remarkably lower. At 72 hours after LTx, 3% of patients treated with placebo and 9% of patients treated with azithromycin demonstrated Grade 3 PGD, whereas 15% of patients treated with placebo and 26% of patients treated with azithromycin had Grade 3 PGD during the first 72 hours after LTx. Similarly, the prevalence of episodes of rejection (A grade and B grade) reported by the ISHLT was remarkably higher than in our cohort.[1] According to the ISHLT, approximately 30% of LTx recipients had an episode of rejection that required treatment or hospitalization, whereas only 18% of patients treated with placebo and 21% treated with azithromycin had severe (Grade ≥2) A- or B-grade rejection in our cohort. Because fewer patients had severe PGD, severe acute perivascular rejection, or lymphocytic bronchiolitis after LTx in our study, it may be possible that the number of patients was not sufficiently large to show a significant effect of treatment with azithromycin.

No adverse events were reported, suggesting low-dose azithromycin is safe in the early post-LTx period. Moreover, in our previous randomized, controlled trial of preventive treatment with azithromycin for 2 years after LTx, no cases of QTc prolongation and no cases of torsades de pointes (or other arrhythmias) were reported.[13,14]

Our study has some limitations. First, it is a single-center trial, necessitating validation of our results in other cohorts. Next, approximately 50% of patients in both groups had already been treated with azithromycin before LTx, possibly influencing our results. However, when patients treated with azithromycin before transplantation were excluded from further analysis, no significant differences in results could be noted. Moreover, later post-LTx initiation of azithromycin occurred in >90% of included patients, likely biasing results on late outcomes, including CLAD. In addition, our study aimed to answer some clinically important questions and not be a mechanistic trial. As a consequence, we were not able to unravel new underlying pathogenic mechanisms.

To conclude, a prospective, randomized, placebo-controlled trial of pre-transplant and prompt post-transplant azithromycin treatment was performed at our center with the aim of improving early lung allograft function and outcome. The primary end-point, an anticipated 15% improvement in $FEV_1$ during the first 3 months after LTx in patients treated with azithromycin, was not reached. Nonetheless, known anti-inflammatory effects of azithromycin were confirmed. Moreover, azithromycin in the early post-LTx period appears to be safe.

## Disclosure statement

The authors have no conflicts of interest to disclose.

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## Supplementary data

Supplementary data associated with this article can be found in the online version at www.jhltonline.org/.

## Supplementary materials

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