Original Article
Effects of azithromycin therapy on bronchiolitis obliterans syndrome after lung transplant: a meta-analysis

Lianjiu Su[1], Cheng Qian[2], Bo Chen[1], Shiqin Zou[3]

Department of [1]Respiratory, Pu Ai Hospital, Departments of [2]Cardiovascular, [3]Social-Health-Development, Wuhan University of Zhongnan Hospital, Wuhan, China

Abstract: Objectives: Azithromycin has been used for the treatment of patients with bronchiolitis obliterans (BOS) syndrome after lung transplant and it has been shown to be effective on improving lung function. The overall effect of azithromycin on the absolute values of FEV_1 has not been compared between reported studies. We studied the effects of azithromycin on lung function in patients with post-lung transplant BOS syndrome. Methods: A meta-analysis was performed using studies identified following an extensive database search. Studies were published without language limitation and explicitly reported lung function in FEV_1 or hazard ratios. Results: A total of 10 studies were eligible for the analysis. Five hundred and sixty-one patients were evaluated after treatment with or without azithromycin for the patients with BOS. The mean percentage increase in FEV_1 (%) was 10.97 (CI 5-16.94, P < 0.001) and the mean absolute values of FEV_1 (L) increase was 0.31 (CI 0.26-0.37, P < 0.001) for a mean follow-up period of seven months. The pooled hazard ratio was 0.96 (CI 0.95-0.98, P < 0.001) for a mean follow-up period of 3.1 year. Conclusions: This study demonstrated a significant improvement in lung function in patients with BOS syndrome after lung transplant for a mean follow-up period of seven months and a trend toward decreased mortality from BOS syndrome on a long-term azithromycin therapy compared with these who were not.

Keywords: Azithromycin, bronchiolitis obliterans syndrome, lung transplantation, lung function, chronic rejection, meta-analysis

Introduction

Chronic lung allograft dysfunction (CLAD) is the main limitation to long-term outcomes following lung transplantation. Bronchiolitis obliterans syndrome is a major feature and the most common form of obstructive CLAD, which is clinically defined as a progressive decline (20% or more) in forced expiratory volume in one's (FEV_1) from best postoperative FEV_1 [1, 2]. It is also said to be a secondary graft deterioration to progressive airway disease because there is no other identified cause [3]. Unfortunately, chronic lung allograft dysfunction, most commonly manifest as bronchiolitis obliterans syndrome, continues to be highly prevalent and remains a major cause of morbidity and mortality in patients post lung transplant [4]. According to the latest ISHLT report, 48% of lung recipients developed BOS in five years and 76% in 10

years [5]. Azithromycin has been shown to improve forced expiratory volume in 1 s (FEV_1) in patients with BOS syndrome post lung transplantation (LTx) [6]. It is an azalide, a subclass of macrolide antibiotics derived from erythromycin [7]. Azithromycin has been used as a major option for the treatment of patients with BOS because of its immunomodulatory and anti-inflammatory properties. Altering signal transduction and gene expression of inflammatory may be the possible pathways for azithromycin to realize its function [8, 9]. Azithromycin has also been shown to inhibit IL-8 and 8-isoprostane releases in airway smooth muscle cells and inhibit nuclear factor-κB activation during lung inflammation [10, 11]. By interacting with MAPK signaling, it attenuates fibroblast growth factor-induced VEGF (vascular endothelial growth factor) production in airway smooth muscle cells [12]. Several studies have demon-
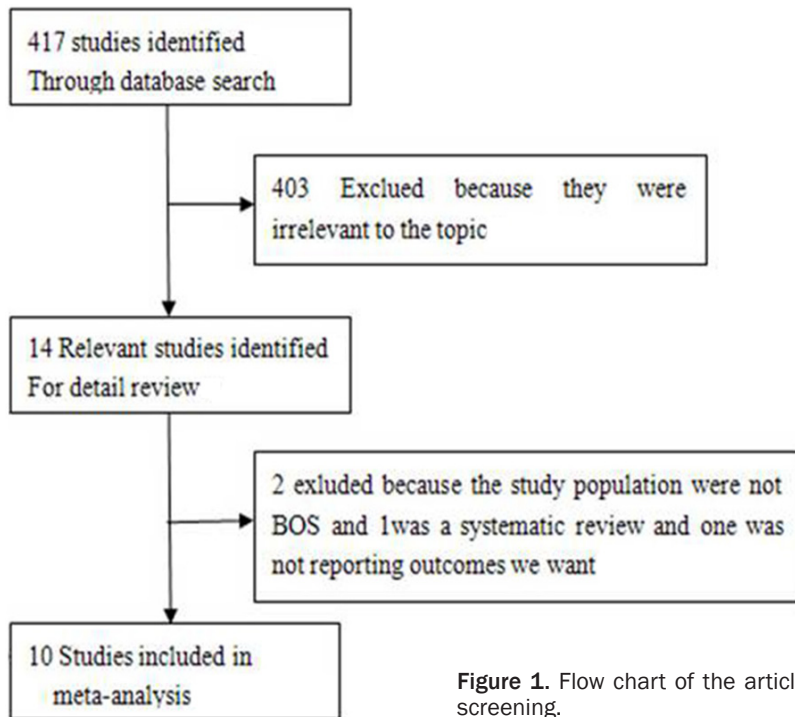
417 studies identified
Through database search

403 Exclued because they were
irrelevant to the topic

14 Relevant studies identified
For detail review

2 exluded because the study population were not
BOS and 1was a systematic review and one was
not reporting outcomes we want

10 Studies included in
meta-analysis

Figure 1. Flow chart of the article screening.

Macrolides" or "Antibiotics and BOS". We limited our search to human studies only. No language restrictions were applied.

Selection criteria

The studies considered in this meta-analysis were those that reported a change in FEV_1 following treatment of BOS with azithromycin over time. Also included were studies that reported hazard ratios for analysis of mortality. Studies were excluded if we were unable to obtain enough details from the article to determine the outcomes we need. We did not assess the quality of the methods of the primary studies.

strated an improvement in FEV_1 by using azithromycin for a long-term therapy on BOS [13-16]. Some studies have shown a stability of FEV_1 following long-term treatment with azithromycin [17, 18]. Same trials have also reported on the survival of patients with BOS following treatment [16, 19-21]. The overall effect of azithromycin on percentage change in FEV_1 and hazard ratios have been analyzed by Kingah et al. between reported studies [22]. Recently, the results of a small, single-center of azithromycin for the treatment of BOS were published by Corris et al. which gave a highlighted data of randomized controlled trial [23].

We added this new data of randomized controlled trial and conducted this meta-analysis to assess whether azithromycin improves lung function on both the absolute values of FEV_1 and percentage change in FEV_1 and survival in patients with post-lung transplantation BOS syndrome.

Methods

We completed a systematic search using MEDLINE, PubMed, Cochrane, Google Scholar and EMBASE published up to 31 July in 2015. Search terms included "Azithromycin and BOS", "Azithromycin and lung transplant", "BOS and

Data extraction

Data were collected using an excel spreadsheet by two reviewers (Qian Chen, Cheng Bo) to determine the eligibility for further review. Data from each study included the following: Publication of first author's last name, Country of the population studied, Year of publication; Study design; Duration of study, Number of participants; Dosing regimen; The outcomes and range; Discrepancies in data collection were resolved by discussion. We also obtained data from primary authors directly in cases where data could not be determined from published articles.

Statistical analysis

We used STATA 12 software for all our statistical analysis by two techniques to calculate the pooled estimates: the Mantel-Haenszel method, which assumes a fixed-effects model, and the DerSimonian-Laird method, which assumes a random effects model if significant heterogeneity was detected. We reported the results of the random-effects model. Dichotomous data were synthesized using risk ratios (HR) and 95% confidence intervals (CI) as the effect measures. The mean difference (MD) and 95% CI were used as the metrics of effect size for

Table 1. Characteristics of the trials included in the analysis

Study and year	country	Study design	Follow-up (months)	samples	Dosing regimen	Outcomes	MD (95% CI)
Federica 2011	Italy	Retrospective	12	62	250 mg three times per week[1]	FEV_1 (%)	0.14 (0.05, 0.25)
Gottieb 2008	Germany	Retrospective	6	81	250 mg three times per week	FEV_1 (%)	16 (14.69, 17.31)
Corris 2015	UK	Prospective	3	33	250 mg three times per week	#FEV_1 (L)	0.278 (0.17, 0.386)
Robin 2010	Belgium	Retrospective	37.2	153	250 mg three times per week[1]	HR	0.71 (0.14, 3.62)
Raksha 2010	USA	Retrospective	39.12	173	250 mg three times per week[2]	HR	0.96 (0.95, 0.98)
Susan 2003	USA	Retrospective	3.35	6	250 mg three times per week	FEV_1 (%)	17.10 (14.92, 19.28)
						#FEV_1 (L)	0.50 (-0.18, 1.36)
Shitrit 2005	Israel	Retrospective	10	11	250 mg three times per week	FEV_1 (%)	-2 (-2.95, -1.05)
Verleden 2004	Belgium	Retrospective	9	8	250 mg three times per week[1]	FEV_1 (%)	18.3 (14.85, 21.75)
						#FEV_1 (L)	0.33 (0.26, 0.39)
Verleden 2006	Belgium	Prospective	3	14	250 mg three times per week[1]	FEV_1 (%)	13 (10, 16)
						#FEV_1 (L)	0.31 (-0.04, 0.667)
Yates 2005	UK	Retrospective	6.25	29	250 mg three times per week	FEV_1 (%)	15.6 (8.3, 22.9)
						#FEV_1 (L)	0.11 (0.26, 0.37)

Week[1], 250 mg daily for five days and then 250 mg three times per week; week[2], 250 mg daily for five daysand then 250 mg three times per week for body weight < 70 Kg and 500 mg three times per week for bodyweight > 70 Kg; #FEV_1, absolute value in FEV_1.
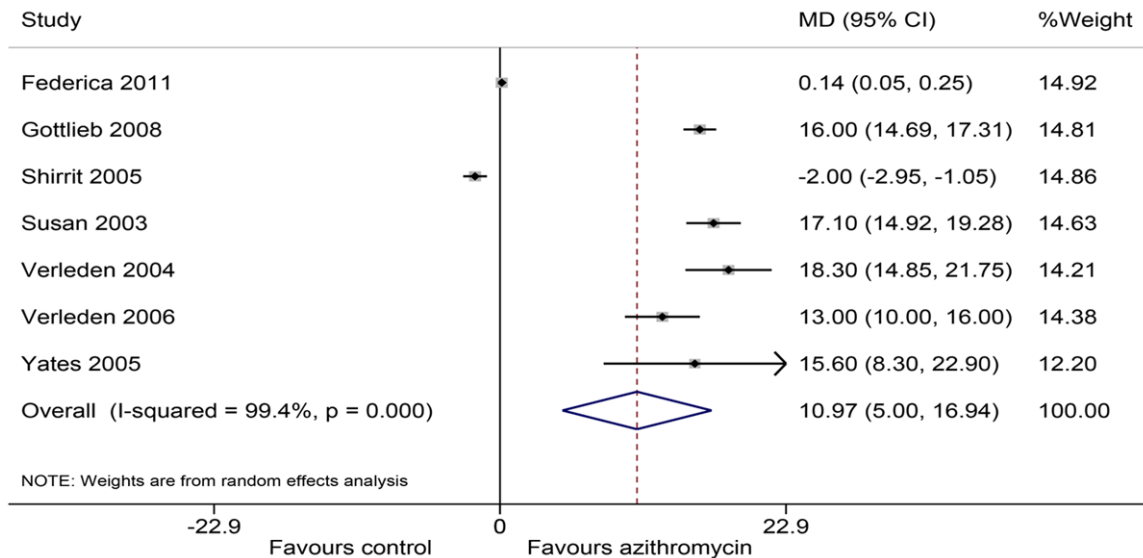
**Figure 2.** Forest plot for effect of percentage changes in FEV_1 using azithromycin in patients with BOS.

continuous outcomes. Publication bias was assessed using the Begg regression asymmetry test. For all tests, a probability level < 0.05 was considered statistically significant and all p-Values were two-tailed.

Results

Following our search, a total of 417 articles were screened. We excluded 403 articles that were not relevant to our topic. Fourteen articles were relevant to our topic, but one was excluded because it did not report the outcomes what we wanted or it could not be computed from the published article [17]. Two articles that was a

systematic review were excluded [6, 24]. We also excluded one article whose study population was not patients with BOS but these after lung transportation [25]. A total of 10 studies were met inclusion criteria and were retained for analysis. The flow diagram (**Figure 1**) shows the details of article selection. Of the 10 studies, seven studies examined the percentage change in FEV_1 and five studies reported the absolute values of FEV_1 and only two studies reported the hazard ratios about the risk of death. Among the seven studies, one was prospective and six were retrospective. Details on the studies are shown in **Table 1**. There
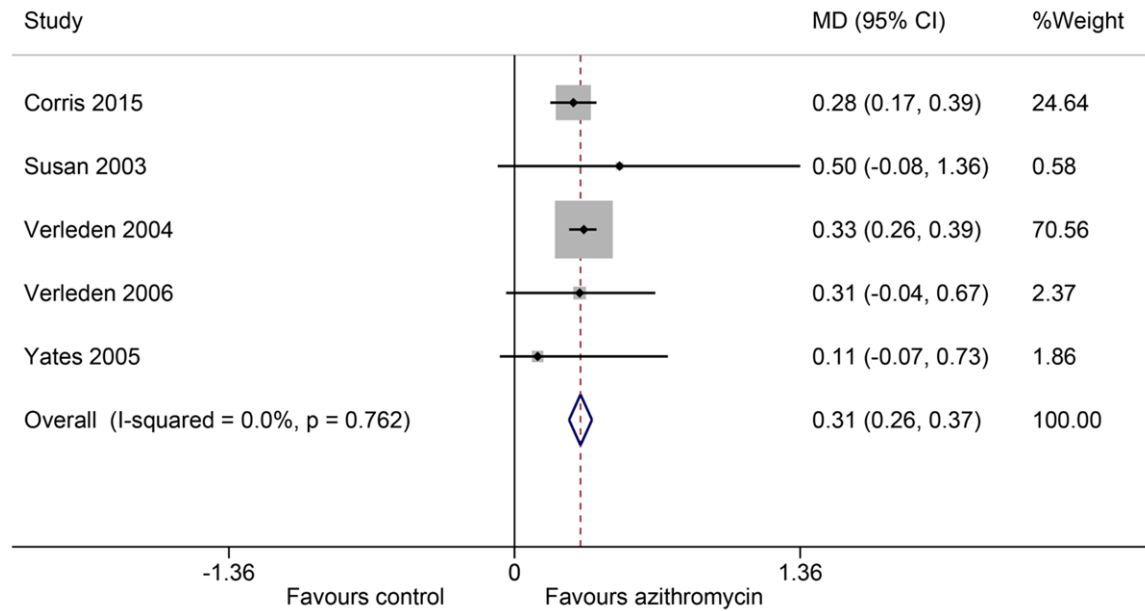
Figure 3. Forest plot for effect of absolute value in FEV$_1$ using azithromycin in patients with BOS.
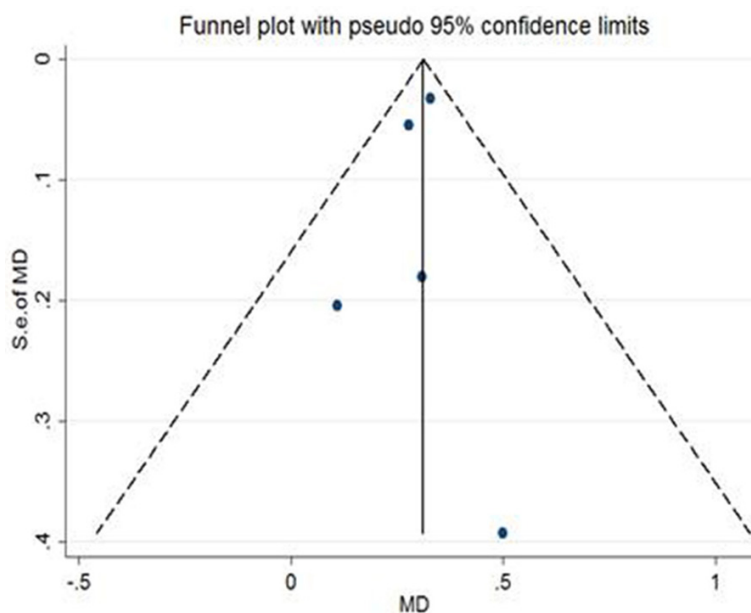
Figure 4. Funnel plot of absolute value in FEV$_1$ for publication bias.

Among the studies that reported hazard ratios, the pooled hazard ratio was 0.96 (CI 0.95-0.98, P = 0.716) for a mean follow-up period of 3.1 yr. The Begg test was used to determine any publication bias between the studies. Among studies that examined on percentage change in FEV$_1$, there was no significant publication bias reported (p$_{begg}$ = 0.764) and among the studies that reported absolute values of FEV$_1$, there was no publication bias detected by the funnel plot (**Figure 4**) (P$_{begg}$ = 0.806).

Discussion

To the best of our knowledge, this is an updated meta-analysis on the topic to address the effects of azithromycin on lung function and mortality in the patients after lung transportation with BOS. A recent study by Corris et al. 2015, which was a placebo-controlled trial of azithromycin therapy in bronchiolitis obliterans syndrome (BOS), reported that Azithromycin therapy improves FEV$_1$ in patients with BOS post lung transplantation and appears superior to placebo [23].

was a significant improvement in FEV$_1$ after using azithromycin over and average of seven months. The overall mean percentage increase in FEV$_1$ (%) was 10.97 (CI 5-16.94, P < 0.001) (**Figure 2**) and the mean absolute values of FEV$_1$ (L) increase was 0.31 (CI 0.26-0.37, P < 0.001) for a mean follow-up period of seven months, no evident of heterogeity was observed across them (I^2 = 0%, P = 0.762) (**Figure 3**).

This study provides strengthened evidence for clinical practice of initiating azithromycin therapy in BOS. We added the new outcome of this RCT trial and reanalysed previous studies with a different data source in our meta-analysis. Seven studies which examined the percentage change in FEV_1 using azithromycin in patients with BOS. There was a significant improvement in FEV_1 (%) after using azithromycin after an average follow-up period of seven months which is similar to Kingah PL's result [2]. Our meta-analysis also demonstrates statistically significant improvement in absolute values of FEV_1 unprecedented when azithromycin was used in the patients with BOS and the no evidence of heterogeneity made our result more convinced than ever before.

In these studies, the diagnosis of BOS was based on International Society for Heart and Lung Transplantation criteria and the low-dose azithromycin was empirically chosen (250 mg daily for five d followed by 250 mg three times per week or 250 mg on alternate days). Patients with BOS may have an iota hope for the treatment by using azithromycin according to the above findings. However, the long-term outcome may be still unclear due to short duration of follow-up.

Azithromycin can inhibit cytokine secreted by alveolar macrophages in BOS [26, 27]. One proposed mechanism of the effect of azithromycin is suppression of airway neutrophilia [14]. Another is suppression of matrix metalloproteinases in the airways of lung transplant recipients [28]. Among the two studies that reported hazard ratios, the overall hazard ratio was protective and there was no publication bias following statistical testing using Begg test. The main limitation of our analysis is due to primary studies that were lack of any control group in the most of studies, apart from Corris's study which was a RCT research, making it difficult to assume that the improvement of lung function was the solely effect by using azithromycin. The studies have no generalizability due to lack of multi-center studies and absence of the long-term effects of azithromycin on BOS are also the limitation. Studies with longer duration of follow-up will be useful. There are no adverse effects of azithromycin being reported.

One other study by Vos et al. at 2011 has been included in the previous analysis [25], but was excluded in our meta-analysis due to the study population which was not patients with BOS but those after lung transportation. The study by Federica M et al. reported on both percentage changes in FEV_1 and hazard ratio but the latter is about freedom from BOS [21]. Gottlieb et al. reported on both percentage changes in FEV_1 and hazard ratio but the latter is survival data in responders and non-responders to azithromycin [16]. So we exclude their data in the pooled hazard ratio analysis about the risk of death which is different from the previous meta-analisis [22]. Four studies made a classification of BOS that divided BOS into four groups from BOS_0 to BOS_3 according to the severity of disease [14, 16, 19, 21]. However, we could not obtain the values in FEV_1 of each group from the authors. We could not make a subgroup analysis in the present meta-analysis because of the reason above. Many more studies will be needed to assess the true effects of azithromycin on these different phenotypes of BOS.

We can conclude from our meta-analysis that patients with BOS after lung transportation treated with azithromycin over seven months duration had a significant improvement in lung function and a trend toward decreased mortality.

Disclosure of conflict of interest

None.

Address correspondence to: Shiqin Zou, Department of Social-Heath-Development, Wuhan University of Zhongnan Hospital, Wuhan 430000, China. Tel: 86-18207154509; E-mail: sqz623@163.com

References

[1] Kapila A, Baz MA, Valentine VG, Bhorade SM; AIRSAC investigators. Reliability of diagnostic criteria for bronchiolitis obliterans syndrome after lung transplantation: a survey. J Heart Lung Transplant 2015; 34: 65-74.

[2] Estenne M, Maurer JR, Boehler A, Egan JJ, Frost A, Hertz M, Mallory GB, Snell GI, Yousem S. Bronchiolitis obliterans syndrome 2001: an update of the diagnostic criteria. J Heart Lung Transplant 2002; 21: 297-310.

[3] Bankier AA, Van Muylem A, Knoop C, Estenne M, Gevenois PA. Bronchiolitis obliterans syndrome in heart-lung transplant recipients: di-

agnosis with expiratory CT. Radiology 2001; 218: 533-9.

[4]   Weigt SS, DerHovanessian A, Wallace WD, Lynch JP 3rd, Belperio JA. Bronchiolitis obliterans syndrome: the Achilles' heel of lung transplantation. Semin Respir Crit Care Med 2013; 34: 336-51.

[5]   Christie JD, Edwards LB, Kucheryavaya AY, Benden C, Dipchand AI, Dobbels F, Kirk R, Rahmel AO, Stehlik J, Hertz MI; International Society of Heart and Lung Transplantation. The Registry of the International Society for Heart and Lung Transplantation: 29th adult lung and heart-lung transplant report-2012. J Heart Lung Transplant 2012; 31: 1073-86.

[6]   Vos R, Vanaudenaerde BM, Verleden SE, Ruttens D, Vaneylen A, Van Raemdonck DE, Dupont LJ, Verleden GM. Anti-inflammatory and immunomodulatory properties of azithromycin involved in treatment and prevention of chronic lung allograft rejection. Transplantation 2012; 94: 101-9.

[7]   Bakheit AH, Al-Hadiya BM, Abd-Elgalil AA. Azithromycin. Profiles Drug Subst Excip Relat Methodol 2014; 39: 1-40.

[8]   Murphy DM, Forrest IA, Corris PA, Johnson GE, Small T, Jones D, Fisher AJ, Egan JJ, Cawston TE, Lordan JL, Ward C. Azithromycin attenuates effects of lipopolysaccharide on lung allograft bronchial epithelial cells. J Heart Lung Transplant 2008; 27: 1210-6.

[9]   Ribeiro CM, Hurd H, Wu Y, Martino ME, Jones L, Brighton B, Boucher RC, O'Neal WK. Azithromycin treatment alters gene expression in inflammatory, lipid metabolism, and cell cycle pathways in well-differentiated human airway epithelia. PLoS One 2009; 4: e5806.

[10]   Stellari FF, Sala A, Donofrio G, Ruscitti F, Caruso P, Topini TM, Francis KP, Li X, Carnini C, Civelli M, Villetti G. Azithromycin inhibits nuclear factor-kappaB activation during lung inflammation: an in vivo imaging study. Pharmacol Res Perspect 2014; 2: e00058

[11]   Vanaudenaerde BM, Wuyts WA, Geudens N, Dupont LJ, Schoofs K, Smeets S, Van Raemdonck DE, Verleden GM. Macrolides inhibit IL17-induced IL8 and 8-isoprostane release from human airway smooth muscle cells. Am J Transplant 2007; 7: 76-82.

[12]   Willems-Widyastuti A, Vanaudenaerde BM, Vos R, Dilisen E, Verleden SE, De Vleeschauwer SI, Vaneylen A, Mooi WJ, de Boer WI, Sharma HS, Verleden GM. Azithromycin attenuates fibroblast growth factors induced vascular endothelial growth factor via p38(MAPK) signaling in human airway smooth muscle cells. Cell Biochem Biophys 2013; 67: 331-9.

[13]   Verleden GM, Dupont LJ. Azithromycin therapy for patients with bronchiolitis obliterans syn-

drome after lung transplantation. Transplantation 2004; 77: 1465-7.

[14]   Verleden GM, Vanaudenaerde BM, Dupont LJ, Van Raemdonck DE. Azithromycin reduces airway neutrophilia and interleukin-8 in patients with bronchiolitis obliterans syndrome. Am J Respir Crit Care Med 2006; 174: 566-70.

[15]   Yates B, Murphy DM, Forrest IA, Ward C, Rutherford RM, Fisher AJ, Lordan JL, Dark JH, Corris PA. Azithromycin reverses airflow obstruction in established bronchiolitis obliterans syndrome. Am J Respir Crit Care Med 2005; 172: 772-5.

[16]   Gottlieb J, Szangolies J, Koehnlein T, Golpon H, Simon A. Long-term azithromycin for bronchiolitis obliterans syndrome after lung transplantation. Transplantation 2008; 85: 36-41.

[17]   Porhownik NR, Batobara W, Kepron W, Unruh HW, Bshouty Z. Effect of maintenance azithromycin on established bronchiolitis obliterans syndrome in lung transplant patients. Can Respir J 2008; 15: 199-202.

[18]   Shitrit D, Bendayan D, Gidon S, Saute M, Bakal I, Kramer MR. Long-term azithromycin use for treatment of bronchiolitis obliterans syndrome in lung transplant recipients. J Heart Lung Transplant 2005; 24: 1440-3.

[19]   Vos R, Vanaudenaerde BM, Ottevaere A, Verleden SE, De Vleeschauwer SI, Willems-Widyastuti A, Wauters S, Van Raemdonck DE, Nawrot TS, Dupont LJ, Verleden GM. Long-term azithromycin therapy for bronchiolitis obliterans syndrome: divide and conquer? J Heart Lung Transplant 2010; 29: 1358-68.

[20]   Jain R, Hachem RR, Morrell MR, Trulock EP, Chakinala MM, Yusen RD, Huang HJ, Mohanakumar T, Patterson GA, Walter MJ. Azithromycin is associated with increased survival in lung transplant recipients with bronchiolitis obliterans syndrome. J Heart Lung Transplant 2010; 29: 531-7.

[21]   Federica M, Nadia S, Monica M, Alessandro C, Tiberio O, Francesco B, Mario V, Maria FA. Clinical and immunological evaluation of 12-month azithromycin therapy in chronic lung allograft rejection. Clin Transplant 2011; 25: E381-9.

[22]   Kingah PL, Muma G, Soubani A. Azithromycin improves lung function in patients with post-lung transplant bronchiolitis obliterans syndrome: a meta-analysis. Clin Transplant 2014; 28: 906-10.

[23]   Corris PA, Ryan VA, Small T, Lordan J, Fisher AJ, Meachery G, Johnson G, Ward C. A randomised controlled trial of azithromycin therapy in bronchiolitis obliterans syndrome (BOS) post lung transplantation. Thorax 2015; 70: 442-50.

[24]   Fietta AM, Meloni F. Lung transplantation: the role of azithromycin in the management of pa-

tients with bronchiolitis obliterans syndrome. Curr Med Chem 2008; 15: 716-23.

[25] Vos R, Vanaudenaerde BM, Verleden SE, De Vleeschauwer SI, Willems-Widyastuti A, Van Raemdonck DE, Schoonis A, Nawrot TS, Dupont LJ, Verleden GM. A randomised controlled trial of azithromycin to prevent chronic rejection after lung transplantation. Eur Respir J 2011; 37: 164-72.

[26] Cai M, Bonella F, Dai H, Sarria R, Guzman J, Costabel U. Macrolides inhibit cytokine production by alveolar macrophages in bronchiolitis obliterans organizing pneumonia. Immunobiology 2013; 218: 930-7.

[27] Kurdowska A, Noble JM, Griffith DE. The effect of azithromycin and clarithromycin on ex vivo interleukin-8 (IL-8) release from whole blood and IL-8 production by human alveolar macrophages. J Antimicrob Chemother 2001; 47: 867-70.

[28] Verleden SE, Vandooren J, Vos R, Willems S, Dupont LJ, Verleden GM, Van Raemdonck DE, Opdenakker G, Vanaudenaerde BM. Azithromycin decreases MMP-9 expression in the airways of lung transplant recipients. Transpl Immunol 2011; 25: 159-62.