

Azithromycin Reverses Airflow Obstruction in Established Bronchiolitis Obliterans Syndrome

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Introduction: A recent pilot study noted clinical benefit of macrolide therapy in the management of six lung transplant recipients with bronchiolitis obliterans syndrome (BOS), a condition previously regarded as irreversible. **Objective:** To examine the effect of low-dose macrolides on lung function in lung allograft recipients with established BOS and to assess whether this benefit is sustained. **Methods:** We retrospectively evaluated the effect of azithromycin (250 mg alternate days) on clinical status and lung function in 20 allograft recipients with established BOS, confirmed by decline in FEV₁ or FEF₂₅₋₇₅; consistent high-resolution computed tomography findings; and exclusion of acute rejection, infection, or anastomatic complications. Azithromycin was introduced at mean 82 months after transplantation. BOS staging at initiation of treatment was BOS 3 (10), BOS 2 (2), BOS 1 (6), and BOS0-p (2). All patients were on maintenance immunosuppression comprising cell-cycle inhibitor, oral corticosteroids, and calcineurin inhibitor. **Results:** There was a significant increase in FEV₁ of median 110 ml (range, -70 to 730 ml) between baseline and 3 months of azithromycin therapy ($p = 0.002$). This improvement was sustained beyond 3 months in the majority of patients, who had initially benefited from azithromycin (up to 11 months follow up). **Conclusions:** This case series confirms the benefit of azithromycin in not only halting, but reversing the declining lung function seen in patients with BOS. This benefit appears to be maintained over time. Low-dose macrolides offer a new and exciting therapeutic strategy for the treatment of progressive BOS, and further clinical and translational mechanistic studies are required.

Keywords: lung; macrolide; transplantation

Lung transplantation has evolved to become an accepted strategy in the management of advanced disease in selected patients (1). Unfortunately, although there has been considerable improvement in early outcomes after lung transplantation as a result of advances in surgical techniques and better perioperative management, long-term survival remains limited by the development of bronchiolitis obliterans syndrome (BOS).

The histologic lesion of BOS is obliterative bronchiolitis (OB). This is characterized by epithelial cell activation as a result of alloimmune and nonalloimmune mechanisms. An early feature is peribronchiolar leukocyte infiltration leading to an abnormal, exaggerated repair response, fibroproliferation, and eventual obliteration of the small airways (2-9). This leads to deteriorating graft function that is characterized by the development of progressive, irreversible small airway narrowing, fixed

airflow limitation, progressive dyspnea, and ultimately, premature death (1, 10, 11). International Registry data shows a 50% prevalence of BOS at 5 years after transplant. This is associated with a reduction in quality of life and increased morbidity and mortality, limiting 7-year mean survival to only 31% (12).

The current classification of BOS is based on changes in FEV₁, with the maximum post-transplant FEV₁ being assigned a 100% predicted value (the mean of the two best postoperative FEV₁ values with at least 3 weeks between the measurements). Patients experiencing a persistent decline in FEV₁ (i.e., two consecutive measurements within 3-6 weeks) in the absence of acute rejection, infection, or bronchial anastomosis complications are grouped into stages. Additionally, a reduction in FEF₂₅₋₇₅ is used as an early marker for BOS (13).

Current strategies in the management of BOS have ranged from switching immunosuppressive regimens, augmenting with corticosteroids, and initiating cytolytic therapy. These have had little or no impact on the progression of the condition. Although we have recently demonstrated that total lymphoid irradiation (TLI) significantly reduces the rate of decline in graft function associated with BOS, it fails to halt its progression and may have significant morbidity (14). Significantly, data from *ex vivo* experiments indicate that many of the traditional agents used may upregulate proinflammatory cytokines and growth factors, with a potentially detrimental effect on allografts (15-19).

There has been recent interest in the potential role of macrolide antibiotics in the management of BOS. Macrolides have demonstrated antiinflammatory properties in other respiratory conditions such as asthma, cystic fibrosis, and diffuse panbronchiolitis (15-26). Notably, in a recent pilot study, Gerhardt and co-workers described a significant, short-term improvement (mean follow-up of 14 weeks) in lung function in six lung transplant recipients with BOS who were treated with azithromycin (27). These promising data have been confirmed by Verleden and colleagues in eight further subjects with short-term follow-up (28). In this article we examine the effect of low-dose maintenance azithromycin therapy in 20 patients with BOS, followed for up to 48 weeks. This study has previously been partially presented at The International Society for Heart and Lung Transplantation and The American Thoracic Society annual meetings, with publication in abstract form (29, 30).

METHODS

Study Subjects

Twenty lung transplant recipients with a diagnosis of BOS ($n = 18$) or BOS0-p ($n = 2$) were placed on maintenance azithromycin. A diagnosis of BOS was assigned on the basis of the International Society for Heart and Lung Transplantation criteria (13). Patients displayed no clinical evidence of infection, acute rejection, or other cause for their deterioration in lung function. All patients meeting these criteria for BOS were consecutively treated with azithromycin. The data presented in this paper were retrospectively collected and are presented in a case series format.

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Treatment Regimen

All patients were treated with oral azithromycin at a dose of 250 mg alternate days from the time of BOS diagnosis to preparation of this manuscript. Patients were regularly evaluated at clinic with pulmonary function testing to assess response. Liver function was monitored at regular intervals without any adverse effect of therapy. All patients remained on immunosuppressive therapy comprising a calcineurin inhibitor (cyclosporin or tacrolimus), oral corticosteroids, and a purine antagonist (azathioprine or mycophenolate mofetil). The standard immunosuppression regimen used at our facility is a triple therapy approach of cyclosporin, prednisolone, and azathioprine. In this study two patients were on tacrolimus and one on mycophenolate. Azithromycin was commenced at varying time points. Immunosuppressant levels were not affected by azithromycin therapy in our patient cohort. During the treatment phase, one patient required antibiotic treatment for infection (Patient 15, Table 1) and one patient was switched to mycophenolate (Patient 17, Table 1). Mean follow-up is 6.25 months (range, 3–11 months).

Statistical Analysis

Patients had variable times of follow-up, and spirometry was performed at various time points. Analysis was performed using nonparametric methods (Minitab, release 14 for Windows; State College, PA) and post-treatment data grouped into 3-month blocks and compared with baseline values. Baseline refers to spirometry measurements performed at the initiation of treatment. Spirometry measurements were performed at 3 (2–4 months), 6 (5–7 months), and 9 (8–10) months.

RESULTS

Patient characteristics and responses are shown in Table 1 and Figure 1. Our study population consisted of 10 males and 10 females. The underlying diagnoses were cystic fibrosis ($n = 6$), emphysema ($n = 5$), α_1 -antitrypsin deficiency ($n = 1$), bronchiectasis ($n = 2$), sarcoidosis ($n = 2$), pulmonary hypertension ($n = 2$), and retransplantation for obliterative bronchiolitis ($n = 2$). There were nine bilateral lung transplants, eight single lung

transplants, and three heart-lung transplants. The mean age at transplantation was 38 (range, 17–59 years), with an average postoperative FEV₁ = 2.86 L (range, 1.16–5.01). The average number of acute rejection episodes (Grade A2 or greater) was 1.55, before BOS.

BOS stages at initiation of treatment were BOS 3 ($n = 10$), BOS 2 ($n = 2$), BOS 1 ($n = 6$), and BOS0-p ($n = 2$). Mean follow-up at time of manuscript preparation has been 6.25 months (range, 3–11 months). There was a significant but variable improvement in FEV₁ of mean 110 ml (range, –70 to 730 ml) between baseline and after 3 months of azithromycin therapy (Figure 1, $p = 0.002$). This improvement was sustained beyond 3 months in 12 of 17 patients (up to 11 months follow-up). Figure 1 shows percentage change in FEV₁ from baseline.

DISCUSSION

We have confirmed the recent studies of Gerhardt and coworkers (27) and Verleden and colleagues (28), and have shown that azithromycin therapy can successfully treat BOS, a condition refractory to previous clinical management. We have extended the current literature by performing our study in a bigger patient group, with longer-term follow-up.

These findings are remarkable in that they offer hope in arresting the progression of BOS. There was median gain in lung function of 110 ml for the group, and in one patient a 730-ml increase was observed. There were no side effects of therapy reported by our patients, and no patient has discontinued therapy to date. This benefit profile is unprecedented in our program.

The mechanism of action of macrolides in BOS is not known, but is not likely to be due to a direct microbicidal action, because of the low doses used. Similar dosing regimens demonstrate clinical improvement, efficacy, improved lung function, and anti-inflammatory properties in patients with asthma, cystic fibrosis, and panbronchiolitis (20–26). Several mechanisms have been

TABLE 1. CHARACTERISTICS OF STUDY PATIENTS

Patient No.	Age at Transplant	Diagnosis	Procedure	Maximum FEV ₁	Acute Rejection Episodes	Baseline FEV ₁	BOS Score
1	31	Cystic Fibrosis	BLT*	2.86	0	0.54	3
2	32	Bronchiectasis	HLT†	2.3	0	1.52	1
3	31	Cystic Fibrosis	BLT*	2.2	3	1.7	1
4	59	Emphysema	RSLT‡	1.98	2	1.35	1
5	52	Emphysema	LSLT¶	1.16	1	0.42	3
6	53	Emphysema	BLT*	3.16	0	1.17	3
7	25	Bronchiectasis	BLT*	4.64	1	3.28	1
8	59	Emphysema	LSLT¶	2.37	3	0.72	3
9	17	Cystic Fibrosis	BLT*	2.37	1	1.97	0-p
10	30	Cystic Fibrosis	BLT*	3.16	3	2.24	1
11	58	Sarcoidosis	RSLT‡	1.72	0	0.66	3
12	37	Pulmonary Hypertension	HLT†	4.1	0	0.96	3
13	20	Cystic Fibrosis	BLT*	3.9	4	2.2	2
14	31	Obliterative Bronchiolitis	LSLT¶	1.78	0	1.53	0-p
15	34	Obliterative Bronchiolitis	LSLT¶	1.24	2	0.69	2
16	35	Pulmonary Hypertension	HLT†	3.97	1	1.44	3
17	35	Sarcoidosis	RSLT‡	3.16	3	2.53	1
18	47	α_1 -Antitrypsin deficiency	BLT*	4.02	1	1.97	2
19	58	Emphysema	RSLT‡	2.08	4	1.05	2
20	24	Cystic Fibrosis	BLT*	5.01	2	0.92	3

Definition of abbreviations: BLT = bilateral lung transplant; HLT = heart-lung transplant; RSLT = right single lung transplant; LSLT = left single lung transplant.

Age at transplant refers to the patient's age in years, diagnosis refers to the patients diagnosis/indication for transplantation, maximum FEV₁ refers to the maximum measured post-transplant FEV₁, acute rejection episodes refers to the number of acute rejection episodes of grade A2 or greater before the diagnosis of bronchiolitis obliterans syndrome (BOS), baseline FEV₁ refers to the FEV₁ at the commencement of azithromycin, and BOS score refers to the BOS score assigned according to the International Society of Heart and Lung Transplantation guidelines (37).

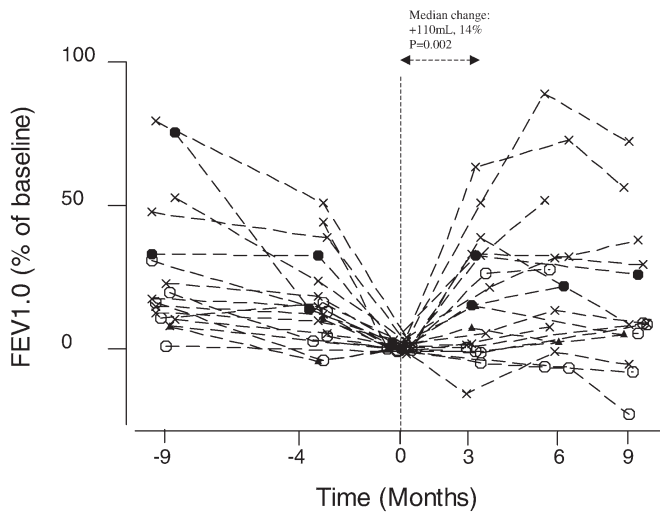


Figure 1. Change in FEV₁ over time. Each symbol represents an individual patient, and the broken line indicates the pattern of change in their FEV₁ over time in months. The change in FEV₁ is represented as a percentage change of FEV₁ at the time azithromycin was commenced. For comparison lung function before treatment is also included. Data at -9 months was measured 9 months before azithromycin therapy. Data at -4 months refers to values measured at -3 to -5 months. Open circles = BOS 1; filled circles = BOS 2; cross = BOS 3; filled triangles = BOS 0-p.

proposed to explain these effects, including suppression of inflammatory mediators and neutrophilic infiltration (31, 32), inhibition of airway remodeling through suppression of matrix metalloproteinases (33), and modulation of the effects of low-grade *Pseudomonas* infection (34, 35).

Long-term low-dose azithromycin therapy offers a novel, safe, and exciting therapy for the treatment of progressive BOS, and our data confirm the beneficial effects in not only preventing a further decline in lung function, but leading to a variable level of improvement in lung function in a majority of patients with established BOS. The fact that airflow limitation regarded as “fixed” has been reversed implies that structural changes have occurred. In preliminary findings from the heterotopic tracheal allograft animal model of lung transplantation, macrolide premedication has been shown to inhibit pathologic airway fibrosis (36).

Our study is limited by the small patient cohort and is a descriptive case series rather than a randomized, placebo-controlled study. Hence, our findings, although extremely promising, need to be interpreted with caution. The magnitude of the treatment effect we observed suggests that a formal randomized placebo-controlled trial may be feasible. Further clinical and translational mechanistic studies of the effects of macrolide therapy in BOS are now required.

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References

1. Arcasoy SM, Kotloff RM. Lung transplantation. *N Engl J Med* 1999; 340:1081-1091.
2. Sharples LD, McNeil K, Stewart S, Wallwork J. Risk factors for bronchiolitis obliterans: a systematic review of recent publications. *J Heart Lung Transplant* 2002;21:271-281.
3. Ross DJ, Marchevsky A, Kramer M, Kass RM. “Refractoriness” of airflow obstruction associated with isolated lymphocytic bronchiolitis/bronchitis in pulmonary allografts. *J Heart Lung Transplant* 1997;16: 832-838.
4. Ward C, Snell GI, Zheng L, Orsida B, Whitford H, Williams TJ, Walters EH. Endobronchial biopsy and bronchoalveolar lavage in stable lung transplant recipients and chronic rejection. *Am J Respir Crit Care Med* 1998;158:84-91.
5. Ward C, Snell GI, Orsida B, Zheng L, Williams TJ, Walters EH. Airway versus transbronchial biopsy and BAL in lung transplant recipients: different but complementary. *Eur Respir J* 1997;10:2876-2880.
6. Ward C, Whitford H, Snell G, Bao H, Zheng L, Reid D, Williams TJ, Walters EH. Bronchoalveolar lavage macrophage and lymphocyte phenotypes in lung transplant recipients. *J Heart Lung Transplant* 2001; 20:1064-1074.
7. Ward C, De Soyza A, Fisher AJ, Pritchard G, Forrest IA, Corris PA. Reticular basement membrane thickening in airways of lung transplant recipients is not affected by inhaled corticosteroids. *Clin Exp Allergy* 2004;34:1905-1909.
8. DiGiovine B, Lynch JP III, Martinez FJ, Flint A, Whyte RI, Iannettoni MD, Arenberg DA, Burdick MD, Glass MC, Wilke CA, Morris SB, Kunkel SI, Strieter RM. Bronchoalveolar lavage neutrophilia is associated with obliterative bronchiolitis after lung transplantation: role of IL-8. *J Immunol* 1996;157:4194-4202.
9. Boehler A, Estenne M. Obliterative bronchiolitis after lung transplantation. *Curr Opin Pulm Med* 2000;6:133-139.
10. Corris PA. Bronchiolitis obliterans syndrome. *Chest Surg Clin N Am* 2003;13:543-557.
11. Ouwens JP, van der Mark TW, Koeter GH, de Boer WJ, Grevink RG, van der Bij W. Bronchiolar airflow impairment after lung transplantation: an early and common manifestation. *J Heart Lung Transplant* 2002;21:1056-1061.
12. Hertz MI, Mohacs PJ, Boucek MM, Taylor DO, Trulock EP, Deng MC, Rowe AW. The Registry of the International Society for Heart and Lung Transplantation: past, present and future. *J Heart Lung Transplant* 2002;21:945-949.
13. 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.
14. Fisher AJRR, Bozzino J, Parry G, Dark JH, Corris PA. The safety and efficacy of total lymphoid irradiation in progressive bronchiolitis obliterans syndrome after lung transplantation. *Am J Transplant* (In press)
15. Borger P, Kauffman HF, Timmerman JA, Scholma J, van den Berg JW, Koeter GH. Cyclosporine, FK506, mycophenolate mofetil, and prednisolone differentially modulate cytokine gene expression in human airway-derived epithelial cells. *Transplantation* 2000;69:1408-1413.
16. Briffa N, Morris RE. New immunosuppressive regimens in lung transplantation. *Eur Respir J* 1997;10:2630-2637.
17. Hojo M, Morimoto T, Maluccio M, Asano T, Morimoto K, Lagman M, Shimbo T, Suthanthiran M. Cyclosporine induces cancer progression by a cell-autonomous mechanism. *Nature* 1999;397:530-534.
18. Muraoka K, Fujimoto K, Sun X, Yoshioka K, Shimizu K, Yagi M, Bose H Jr, Miyazaki I, Yamamoto K. Immunosuppressant FK506 induces interleukin-6 production through the activation of transcription factor nuclear factor (NF)-kappa(B): implications for FK506 nephropathy. *J Clin Invest* 1996;97:2433-2439.
19. Zhang JG, Walmsley MW, Moy JV, Cunningham AC, Talbot D, Dark JH, Kirby JA. Differential effects of cyclosporin A and tacrolimus on the production of TGF-beta: implications for the development of obliterative bronchiolitis after lung transplantation. *Transpl Int* 1998;11: S325-S327.
20. Equi A, Balfour-Lynn IM, Bush A, Rosenthal M. Long term azithromycin in children with cystic fibrosis: a randomised, placebo-controlled cross-over trial. *Lancet* 2002;360:978-984.
21. Everard ML, Sly P, Brennan S, Ryan G. Macrolide antibiotics in diffuse panbronchiolitis and in cystic fibrosis. *Eur Respir J* 1997;10:2926.
22. Gorrini M, Lupi A, Viglio S, Pamparana F, Cetta G, Iadarola P, Powers

- JC, Luisetti M. Inhibition of human neutrophil elastase by erythromycin and flurythromycin, two macrolide antibiotics. *Am J Respir Cell Mol Biol* 2001;25:492-499.
23. Ordonez CL, Stulbarg M, Grundland H, Liu JT, Boushey HA. Effect of clarithromycin on airway obstruction and inflammatory markers in induced sputum in cystic fibrosis: a pilot study. *Pediatr Pulmonol* 2001;32:29-37.
24. Shimane T, Asano K, Suzuki M, Hisamitsu T, Suzaki H. Influence of a macrolide antibiotic, roxithromycin, on mast cell growth and activation in vitro. *Mediators Inflamm* 2001;10:323-332.
25. Spencer D. Macrolide antibiotics in diffuse panbronchiolitis and in cystic fibrosis. *Eur Respir J* 1998;11:1428.
26. Gotfried MH. Macrolides for the treatment of chronic sinusitis, asthma, and COPD. *Chest* 2004;125:52S-60S.
27. Gerhardt SG, McDyer JF, Girgis RE, Conte JV, Yang SC, Orens JB. Maintenance azithromycin therapy for bronchiolitis obliterans syndrome: results of a pilot study. *Am J Respir Crit Care Med* 2003;168:121-125.
28. Verleden GM, Dupont LJ. Azithromycin therapy for patients with bronchiolitis obliterans syndrome after lung transplantation. *Transplantation* 2004;77:1465-1467.
29. Yates B, Ward C, Murphy D, Forrest I, Fisher AJ, Lordan J, Dark JH, Corris PA. Azithromycin reverses airflow obstruction in established Bronchiolitis Obliterans Syndrome (BOS) following lung transplantation. *J Heart Lung Transplant* 2005;24:S102.
30. Yates B, Ward C, Murphy D, Forrest I, Fisher AJ, Lordan J, Dark JH, Corris PA. Azithromycin reverses airflow obstruction in established Bronchiolitis Obliterans Syndrome (BOS) following lung transplantation. *Proc Am Thorac Soc* 2005;2:A893.
31. Takizawa H, Desaki M, Ohtoshi T, Kawasaki S, Kohyama T, Sato M, Tanaka M, Kasama T, Kobayashi K, Nakajima J, Ito K. Erythromycin modulates IL-8 expression in normal and inflamed human bronchial epithelial cells. *Am J Respir Crit Care Med* 1997;156:266-271.
32. Yamasawa H, Oshikawa K, Ohno S, Sugiyama Y. Macrolides inhibit epithelial cell-mediated neutrophil survival by modulating GM-CSF release. *Am J Respir Cell Mol Biol* 2004;30:569-575.
33. Kanai K, Asano K, Hisamitsu T, Suzaki H. Suppression of matrix metalloproteinase production from nasal fibroblasts by macrolide antibiotics in vitro. *Eur Respir J* 2004;23:671-678.
34. Schultz MJ. Macrolide activities beyond their antimicrobial effects: macrolides in diffuse panbronchiolitis and cystic fibrosis. *J Antimicrob Chemother* 2004;54:21-28.
35. Tateda K, Comte R, Pechere JC, Kohler T, Yamaguchi K, Van Delden C. Azithromycin inhibits quorum sensing in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2001;45:1930-1933.
36. Redmund KF GZ, Hofer M, Reichsteiner T, Vogt P, Russi EW, Boehler A. Clarithromycin has a preventative effect on chronic graft rejection [abstract]. *Eur Respir J* 2004;24:465s.
37. Estenne M, Hertz MI. Bronchiolitis obliterans after human lung transplantation. *Am J Respir Crit Care Med* 2002;166:440-444.