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ORIGINAL ARTICLE

Montelukast for bronchiolitis obliterans syndrome after lung transplantation: a pilot study

Geert M. Verleden,[1]* Stijn E. Verleden,[2]* Robin Vos,[2] Stéphanie I. De Vleeschauwer,[2] Lieven J. Dupont,[1] Dirk E. Van Raemdonck[1] and Bart M. Vanaudenaerde[2]

1 Lung Transplantation Unit, University Hospital Gasthuisberg, Leuven, Belgium
2 Laboratory for Pneumology, Katholieke Universiteit Leuven, Belgium

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Correspondence
Prof Geert M. Verleden, Lung Transplantation Unit, University Hospital Gasthuisberg, 49, Herestraat, B-3000 Leuven, Belgium. Tel.: +32 16 346 805; fax: +32 16 346 803; e-mail: geert.verleden@uzleuven.be

Conflicts of Interest
None of the authors have anything to declare.

*Both authors contributed equally to this study.

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Summary

Bronchiolitis obliterans syndrome (BOS) remains the major hurdle to improve long-term survival after lung transplantation, as its treatment remains troublesome. In this pilot study, we investigated the effect of montelukast (a leukotriene receptor antagonist) on the FEV_1 decline after diagnosis of BOS and compared this with a control group. In both groups, 11 patients were included with BOS stage <3 and bronchoalveolar lavage (BAL) neutrophilia <15%, already being treated or concurrently being started on azithromycin. Control patients were selected retrospectively. After adding montelukast (10 mg/day) to the immunosuppressive regimen, the FEV_1 decline significantly decreased from 112 ± 26 ml/month before BOS diagnosis to 13 ± 13 ml/month after 6 months of montelukast therapy ($P = 0.001$). In the control group, there was no significant change in the rate of FEV_1 decline: 103 ± 20 ml/month before BOS diagnosis to 114 ± 27 ml/month ($P = 0.55$). Adding montelukast may be a promising treatment option in patients with low neutrophilic (<15%) BOS after lung transplantation, already or concurrently being treated with azithromycin.

Introduction

The long-term survival after lung transplantation remains inferior compared with other solid organ transplantations [1]. This is mainly because of the development of chronic rejection, clinically known as bronchiolitis obliterans syndrome (BOS) [2].

Bronchiolitis obliterans syndrome is accepted to be an inflammatory process in the airways, leading to final scarring and obliteration of the airways, pathologically characterized as obliterative bronchiolitis (OB) [3]. Several immunological and nonimmunological risk factors for BOS have been identified and recent evidence points to a strong influence of nonimmunological risk factors, at least to explain the inflammatory form of BOS [4]. This latter form is characterized by the presence of increased neutrophils in the bronchoalveolar lavage (BAL) fluid of affected patients. Several groups have now demonstrated that these patients may be responsive to neo-macrolide therapy, such as azithromycin [4–6] or clarithromycin [7], which enables the declined pulmonary function (especially forced expiratory volume in 1 s, FEV_1) to improve and even to normalize in some patients. This specific phenotype was recently connoted as neutrophilic reversible allograft dysfunction (NRAD) [8], characterized by a progressive and obstructive decline in FEV_1, often occurring within the first 2 years after lung transplantation and amenable to macrolide therapy, resulting in an improvement in the FEV_1 with at least 10% [4,8]. We and other researchers could demonstrate that a BAL neutrophilia of >15% to 20% was predictive for the FEV_1 response to azithromycin in these patients [9,10]. Proof of this concept was recently further delivered by a placebo-controlled, double-blind, randomized study in which

azithromycin in addition to classical immunosuppressive treatment, compared with placebo was shown to decrease significantly the development of BOS 2 years after transplantation, with a concomitant lower BAL neutrophilia over the whole 2 years study period [11].

Besides the inflammatory (neutrophilic) obstructive phenotype, a fibroproliferative phenotype was described, leading to a rapid decline in FEV_1, without overt neutrophilic inflammation in the BAL fluid (<15% to 20% neutrophils). This phenotype seems to be largely unresponsive to treatment. The best that can be achieved is a temporary arrest in the FEV_1 decline [12], although in some of these patients, the addition of azithromycin may also seem to stabilize the FEV_1, leading to improved survival [13].

In an attempt to treat these patients, we performed an open-label pilot study with montelukast (MLK), a leukotriene receptor antagonist (LTRA), widely used in the treatment of asthma [14]. MLK has anti-inflammatory effects, especially on eosinophilic inflammation [14], and animals models could also demonstrate its role in treating both hepatic and pulmonary fibrosis [15,16]. Moreover, MLK proved to be of some benefit in improving pulmonary function in patients with graft versus host disease after bone marrow transplantation, a disease very similar to chronic rejection after lung transplantation, which is also pathologically characterized as OB [17].

As a consequence, we hypothesized that adding MLK (10 mg/day) to the immunosuppressive regimen, may arrest the FEV_1 decline in BOS patients with <15% BAL neutrophilia.

Materials and methods

Patients who developed BOS after lung transplantation systematically underwent bronchoscopy with transbronchial biopsies and BAL to exclude acute rejection and infection and to further phenotype BOS (neutrophilic versus non-neutrophilic, with a cut-off value of 15%) [8]. Our methodology to perform biopsies and BAL has been described previously [9]. Around the time BOS was diagnosed, all patients were treated with azithromycin (250 mg/day for 5 days, and thereafter 250 mg three times per week) in addition to their current immunosuppressive regimen (Table 1). Some of these patients were already taking azithromycin for a longer period (>2 months) before the diagnosis of BOS because of a previous NRAD episode. Only patients who were in BOS 1 or 2, according to the definitions of the 'International Society for Heart and Lung Transplantation' (ISHLT) [2], and with a BAL neutrophilia <15% were included in the study and hence, treated with additional MLK (10 mg/day). Furthermore, MLK had to be started as soon as possible after the diagnosis of BOS (i.e. <6 weeks), with a follow-up period of at least 6 months. As a comparison, we included a control group of patients who had been in a comparable situation (BOS stage 1 or 2, BAL neutrophilia at diagnosis <15%, treatment with additional azithromycin initiated at time of BOS diagnosis or already on azithromycin for at least 2 months) but who have not been treated with additional montelukast. They were all included in a sequential retrospective fashion prior to the date that montelukast was first used.

Table 1. Patient characteristics.

	MLK	Control	*P*-value
Age (years)	49.0 ± 3.4	35.7 ± 3.7	0.057
POD (days)	1213 ± 132	851 ± 146	0.10
M/F, *n*	5/6	3/8	0.66
Type (SSLTx/SLTx/HLTx), *n*	10/1/0	7/3/1	0.28
PreLTx diagnosis, *n*			
COPD/emphysema	6	4	0.45
Pulmonary fibrosis	4	4	
Eisenmenger	0	1	
Cystic fibrosis	1	2	
Immunosuppresive therapy, *n*			
Steroids	11	11	0.99
FK/CSA	9/2	8/3	
AZA/MMF/none	7/3/1	8/2/1	
BOS grade 1/2, *n*	5/6	7/4	0.67
BAL neutrophilia (%)	11.1 ± 3.5	9.5 ± 2.3	0.94
BAL eosinophilia (%)	0.8 ± 1.1	0.5 ± 0.2	0.52

MLK, montelukast; BOS, bronchiolitis obliterans syndrome; POD, postoperative day; M, male; F, female; LTx, lung transplantation; SS, sequential single; S, single; H, heart; FK, tacrolimus; CsA, cyclosporine; AZA, azathioprine; MMF, mycophenolate mofetil; BAL, bronchoalveolar lavage.

In both patient groups, we compared the decline in FEV_1 6 months before BOS was diagnosed until 6 months after BOS diagnosis.

This pilot study was approved by the University of Leuven hospital's Ethics Committee and the clinical trial center of the University Hospital (approval number S 52576). All patients gave oral-informed consent.

Statistical analysis

FEV_1 values were used to calculate the decline in FEV_1 (expressed as the mean decline in ml/month) 6 months before and 6 months after the diagnosis of BOS. This value was compared within the control and the montelukast group using the Wilcoxon test. Before and after diagnosis of BOS, the monthly FEV_1 decline in the montelukast group was compared with the FEV_1 decline in the control group using the Mann–Whitney *U* test. All values of FEV_1 decline are given as mean ± SEM. A *P*-value of

<0.05 was considered significant. Contingency tables were evaluated using the Fisher's exact test. Analysis was performed with GRAPHPAD PRISM 4.0 (San Diego, CA, USA).

Results

Eleven patients fulfilled the inclusion criteria for this study and were treated with additional MLK (MLK group) in an open pilot study. The treatment was started at a mean of 21 ± 7 days after the diagnosis of BOS (stage 1 in five and stage 2 in six patients). At this time, six patients were already being treated with azithromycin for at least 2 months, and the other five received additional azithromycin therapy very soon after the diagnosis of BOS (a mean of 8 ± 12 days). The control group consisted of 11 patients who were never treated with MLK (seven patients in BOS stage 1, four in stage 2); four of these 11 patients were already on azithromycin treatment for at least 2 months before BOS diagnosis; the others were started at a mean of 18 ± 3 days after BOS diagnosis. Most of the patients also received an additional oral steroid taper (8/11 in MLK and 10/11 in the control group), whereas two patients in the MLK group were also shifted from cyclosporine to tacrolimus and one patient in the control group received additional treatment with total lymphoid irradiation (TLI). There were no differences in characteristics between these two patient groups, except a trend for age (Table 1).

The individual and mean changes in FEV_1 in both groups are outlined in Fig. 1.

The decline in FEV_1 (ml/month) in the MLK group before the introduction of MLK was 112 ± 26 ml/month and significantly decreased to 13 ± 13 ml/month ($P = 0.001$) during 6 months treatment. In the control group, the decline in FEV_1 6 months before BOS was 103 ± 20 ml/month ($P = 0.89$ compared with the decline in the MLK group) and remained unchanged during the following 6 months at 114 ± 27 ml/month ($P = 0.55$). The decline in FEV_1 during 6 months of montelukast treatment was significantly different compared with the FEV_1 decline of the control group in the same time period ($P = 0.0025$).

When the patients were subdivided into fast FEV_1 decliners (decline >100 ml/month, five in each group) and slow decliners (<100 ml/month), the results remained unchanged. In the MLK fast decliners subgroup, the decline was 187 ± 33 ml/month before and 30 ± 26 ml/month after the addition of MLK ($P = 0.06$). In the control group, the decline in these five patients was 155 ± 27 ml/month before and 139 ± 51 ml/month after the diagnosis of BOS ($P = 1.00$). In the slow decliners receiving MLK, the decline decreased significantly from 51 ± 10 ml/month to 1 ± 6 ml/month ($P = 0.013$), whereas in the control group the decline remained unchanged from 60 ± 14 ml/month before to 94 ± 30 ml/month after BOS diagnosis ($P = 0.19$).

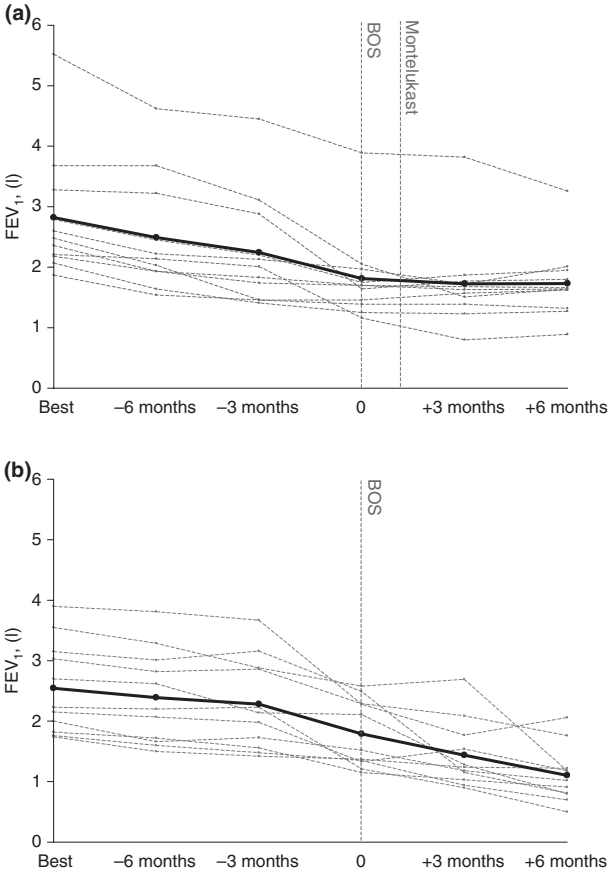

Figure 1 Individual (dashed lines) and mean (full line) changes in FEV_1 in patients who received additional montelukast (a) and in control patients (b). The best postoperative FEV_1 is calculated as the mean of the two best postoperative values obtained at least 3 weeks apart. Time 0 denotes the diagnosis of bronchiolitis obliterans syndrome (BOS) and for group A concurrent addition of montelukast (10 mg/day) is shown (on average started 3 weeks after BOS diagnosis). FEV_1 evolution is depicted from 6 months before this time point to 6 months after time 0. Montelukast leads to a significant slowing of the FEV_1 decline compared to control patients ($P = 0.0025$).

To further extract the potential effect of additional azithromycin, we compared the changes in FEV_1 decline in these patients of both groups, who were either already on azithromycin treatment before BOS diagnosis (azi before) or in whom azithromycin was initiated at BOS diagnosis (azi con). After addition of MLK, the change in FEV_1 decline remained significant irrespective of the timing of initiation of azithromycin [azi before: FEV_1 decline changed from 75 ± 46 to 29 ± 16 ml/month ($P = 0.030$, $n = 6$), whereas in the azi con, the change was almost

Table 2. Decline in FEV_1 in different (sub) groups and time periods.

	MLK			Control		
	Before	After	*P*	Before	After	*P*
All patients (*n* = 11/11)	112 ± 26	13 ± 13	0.001	103 ± 20	114 ± 27	0.55
Fast decliners (*n* = 5/5)	187 ± 33	30 ± 26	0.060	155 ± 27	139 ± 51	1.00
Slow decliners (*n* = 6/6)	51 ± 10	1 ± 6	0.013	60 ± 14	94 ± 30	0.19
AZI before (*n* = 6/4)	75 ± 46	29 ± 16	0.030	66 ± 26	88 ± 45	0.62
AZI con (*n* = 5/7)	157 ± 46	7 ± 17	0.060	125 ± 26	129 ± 35	0.84

All values are mean ± SEM in ml/month.
AZI, azithromycin; con, concomitant treatment; MLK, montelukast.

significantly different from 157 ± 46 to 7 ± 17 ml/month ($P = 0.060$, $n = 5$)]. In the control group, using the same subdivision, the decline in FEV_1 in the azi before group ($n = 4$) changed from 66 ± 26 to 88 ± 45 ml/month ($P = 0.62$) and in the azi con group, the FEV_1 decline was also not significantly changed (from 125 ± 26 to 129 ± 35 ml/month, $P = 0.84$) (Table 2).

Discussion

In this pilot study, we demonstrated that the addition of MLK in patients with BOS and <15% BAL neutrophilia resulted in a slowing of the decline in FEV_1, both in fast ($P = 0.06$) and in slow declining patients ($P = 0.013$), in comparison with those of a retrospective control group who were otherwise in a completely comparable situation regarding BOS status and treatment. BOS was here defined according to the ISHLT criteria [2] and was further characterized as nonresponsive to azithromycin (i.e. <10% FEV_1 improvement after addition of azithromycin) [8]. In fact, the only difference between the MLK and the control group was the additional MLK treatment. Although MLK seemed to slow down the decline in FEV_1, azithromycin did not improve the FEV_1, which is consistent with earlier data [9–11,13].

We deliberately chose to add azithromycin when low neutrophilic BOS was diagnosed as we recently demonstrated that at least in some of these patients it may stabilize the FEV_1 decline and improve the survival [13]. Based on these latter findings, this is now the current practice in our center. On the other hand, some patients were already taking azithromycin because of a previous NRAD [8] and continued its use ever since.

As azithromycin was used under the same circumstances in both groups, and as the changes in FEV_1 decline are also present in the MLK group when we divided the group into azi before and azi con subgroups, we can rule out that the effect we have described in the MLK group is because of azithromycin treatment *per se*. Moreover, azithromycin was started in seven patients in the control group around the time of BOS (azi con) and there was no change in their FEV_1 decline.

We acknowledge that a retrospective control group may be cause for bias in our study, although both patient groups were comparable in terms of transplantation type, BOS grade, immunosuppressive treatment, and %BAL neutrophilia at diagnosis of BOS. There was, however, a trend with difference in age (younger control group), which is because of the fact that the control patients were all included in a sequential retrospective fashion prior to the date that montelukast was first used to exclude selection bias.

The small number of patients may also not be entirely representative of the various evolutions of BOS that can be expected. Nevertheless, in fast as well as slow FEV_1 decliners, we found a (almost) significant decrease in FEV_1 decline after adding MLK, compared with no difference in both subtypes of the control group. As a consequence, the results of this small pilot study suggest that addition of MLK might be helpful to treat a group of patients with an inherent bad prognosis.

We have previously published a comparable experience with the introduction of TLI in a group of BOS patients, who further deteriorated despite azithromycin treatment [18]. After TLI, we demonstrated an arrest in the FEV_1 decline, from 221 to 94 ml/month ($P < 0.05$), a reduction of 57%. In the largest study on TLI so far, the decline in FEV_1 was 123 ml/month pre-TLI and 25 ml/month post-TLI ($P = 0.0004$), a reduction of 80%, although in this study most patients were not treated with azithromycin [19]. In the present study, the FEV_1 decline in the MLK group was reduced by 88% (from 112 ± 26 ml/month to 13 ± 13 ml/month). This is also comparable to the effect of photopheresis, where the FEV_1 decline 6 months before photopheresis significantly decreased from 116 ml/month to 29 ml/month, a 75% reduction [20]. In this latter study, 90% of the patients were also on concurrent azithromycin treatment; however, no control group was involved and furthermore, 58% of the patients were already in BOS stage 3, which means that their decline in FEV_1 was probably already leveling off as it is known that

the most important decline in FEV_1 occurs in the first 6 months after the diagnosis of BOS [21]. That is exactly why in our pilot study we included a control group and only included patients who were not yet in BOS stage 3, meaning that the arrest in decline in FEV_1 in our active treated patient group is probably not a result of the natural course of BOS progression, although this remains speculative.

Total lymphoid irradiation and photopheresis are accepted as relatively safe procedures [18–20]; however, they remain very time consuming and quite expensive treatment options. Moreover, these options are not available in every hospital where lung transplantations are performed. In contrast, the addition of MLK is cheap, safe, and available to everyone. We have encountered no side effects in this small cohort of patients, neither was there any interference with the calcineurin treatment. From large studies in asthmatics, MLK is indeed accepted as a safe drug [22]. Therefore, we believe that further prospective, randomized, placebo-controlled studies with MLK are definitely warranted as it seems a promising agent to treat this very difficult complication after lung transplantation.

How montelukast may interfere with the fibroproliferative process in the small airways remains largely unknown. At least in animal models, LTRA is able to inhibit pulmonary as well as hepatic fibrosis [15,16]. Whether cysteinyl leukotrienes are present in the airways in patients with BOS remains unknown, but cysteinyl leukotrienes are accepted to be involved in airway remodeling, such as basement membrane thickening in asthmatics [23] and in fibroblast proliferation [24], which may offer a possible explanation for the effect of MLK in BOS patients. In the Or *et al.* study, three of five allogeneic stem cell transplantation patients with pulmonary involvement because of chronic graft versus host disease showed an objective improvement when treated with additional MLK [15]. We believe that in this condition the same mechanisms may be operative as in BOS, which provide further evidence for the effect of MLK. Whether the presence of eosinophils in BAL fluid is important for the response to MLK or not remains unknown. At least in the present cohort of patients there was no difference in BAL eosinophilia between the MLK and control group, with both values being <1%.

In conclusion, in patients who develop BOS without overt BAL neutrophilia (<15%) after lung transplantation and who are not responsive to azithromycin treatment [9–11], addition of MLK (10 mg/day) may lead to a significant arrest in the decline in FEV_1. The mechanisms of action, however, remain to be further examined but probably relate to its antiproliferative effects. Further proof of this concept is to be expected with a placebo-controlled randomized study.

Authorship

GMV: participated in research design, writing of the article, and performance of the research. SEV: participated in research design, writing of the article, and data analysis. RV: participated in data analysis. SIDV and BMV: participated in the writing of the article. LJD and DEVR: participated in the performance of the research.

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