Is Transplant Operation Important in Determining Posttransplant Risk of Bronchiolitis Obliterans Syndrome in Lung Transplant Recipients?*

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Study objectives: Lung transplantation continues to be limited by the development of chronic allograft dysfunction in the form of bronchiolitis obliterans syndrome (BOS). The effect of a transplant operation on patients with BOS has not been well-studied, but patients who undergo double-lung transplantation have better long-term survival. We hypothesized that double-lung transplantation leads to decreased rates of BOS.

Methods: A retrospective review of all lung transplant recipients at our institution, surviving for > 6 months after undergoing their transplant operation. Demographic data, information on other factors leading to the development of BOS, survival information, and data on the presence and timing of BOS were collected.

Results: BOS occurred in 41.3% of the recipients (93 of 225 patients) at a median time of 4.2 years. Single-lung transplantation was associated with increased rates of BOS compared to double-lung transplantation (49.3% vs 31.7%, respectively; p = 0.007), at the time of the analysis. Single-lung and double-lung transplant recipients had different baseline characteristics, but after controlling for these factors the type of transplant remained a significant predictor of the length of time to the onset of BOS in a multivariable proportional hazard model.

Conclusions: Double-lung transplantation is associated with a reduced risk for BOS in our study population. A multicenter study with complete BOS information on all patients with a single pretransplant diagnosis would be useful to confirm the above findings. Further research is needed to determine how the type of transplant contributes to the risk for BOS.

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Key words: bronchiolitis obliterans syndrome; chronic rejection; lung transplantation

Abbreviations: ARS = acute rejection score; ARSF = acute rejection score first 6 months; BOS = bronchiolitis obliterans syndrome; CI = confidence interval; CMV = cytomegalovirus; HLA = human leukocyte antigen; MMF = mycophenolate mofetil; OB = obliterative bronchiolitis; OR = odds ratio; PRA = panel reactive antibody

Lung transplantation has become an acceptable treatment option for end-stage lung disease. As of 1999, > 10,000 lung transplants have been performed in North America.¹ Improved surgical techniques have led to 1-year survival of > 75%.² Long-term outcomes continue to be limited, and the 3-year and 5-year survival rates are 54.8% and 42.6%, respectively.² Double-lung transplantation is performed in all patients with cystic fibrosis or other suppurative lung diseases, while single-lung or double-lung transplantation is used for patients with COPD, idiopathic pulmonary fibrosis, and primary pulmonary hypertension.¹ Double-lung transplantation is associated with a slightly better 5-year survival rate.¹

Obliterative bronchiolitis (OB) is the most important cause of limited long-term survival times. OB is considered to be the histologic equivalent of chronic rejection in patients with lung transplantation, and it is characterized by scar formation and fibrosis of the

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small airways. It is often accompanied by intimal thickening and sclerosis of blood vessels. An obstructive pattern with decreased FEV1 is seen on pulmonary function tests. A definite diagnosis can be obtained only by transbronchial or open-lung biopsy. The sensitivity of the former is low, and the invasive nature of the latter makes its use difficult. Since 1993, the term bronchiolitis obliterans syndrome (BOS) has been used to describe a clinical deterioration in graft function that is not due to acute rejection, infection, bronchial stenosis, or other reversible problems. The FEV1 is a reliable indicator of allograft function and is used in the grading of BOS severity.

The occurrence of BOS is very frequent in patients after lung transplantation and has been reported to occur in 40 to 50% of long-term survivors in most series (lowest reported survival rate, 26%; highest reported survival rate, 67%), depending on the length of follow-up after transplantation. The effect of a transplant operation on BOS remains largely unknown. One recent registry study suggested that in patients with emphysema, a transplant operation had no effect on BOS. However, BOS information from the Registry tends to be less complete. In the current study, which includes a large single-center cohort of lung transplant recipients, our aims were to assess the effect of the transplant operation on the time to the development of BOS, while controlling for other BOS risk factors in a multivariable analysis.

**Materials and Methods**

**Transplant Population**

Two hundred eighty-two lung transplant operations were performed at Duke University Medical Center between April 1992 and June 2000. Two hundred twenty-five patients survived for > 6 months after their transplant operation and are included in this report. The 6-month period from the time of transplantation was chosen in order to minimize the effect of events that occurred in the early posttransplant period and avoid the possibility that drops in lung function were due to other causes (like ischemia-reperfusion injury) and not to BOS. Surgical techniques have been described elsewhere. Among all lung transplants performed, 46.2% (104 of 225 transplants) were performed for emphysema, 20.9% (47 of 225 transplants) were performed for cystic fibrosis, 9.8% (22 of 225 transplants) were performed for idiopathic pulmonary fibrosis, 6.7% (15 of 225) were performed for α1-antitrypsin deficiency, 4.4% (10 of 225 transplants) were performed for primary pulmonary hypertension, and 12.0% (27 of 225 transplants) were performed for other diseases. The mean (± SD) age of the patients was 46.6 ± 13.6 years, and 50.7% (114 of 225 patients) were men. The initial immunosuppression regimen included prednisone, azathioprine, and cyclosporine A, in the majority of patients, except for 40 patients who received mycophenolate mofetil (MMF) instead of azathioprine as part of a randomized study. Approximately half of the patients also received perioperative induction therapy with rabbit antithymocyte globulin, daclizumab, or basiliximab. Further information about the immunosuppressive regimens can be found in a different report.

All patients underwent surveillance bronchoscopies at 1, 3, 6, 9, and 12 months after undergoing transplantation. Additional bronchoscopies were performed in the presence of clinical signs such as fever, sputum, dyspnea, radiographic abnormalities, or declining spirometry values. Five to eight biopsy specimens were obtained from different subsegments of the lung. Biopsy specimens were graded according to the criteria of the Lung Rejection Study Group. All mild and moderate rejection episodes were treated with methylprednisolone infusions followed by tapering with prednisone. Repeated rejections were treated with a switch from cyclosporine A to tacrolimus and rabbit antithymocyte globulin infusion.

At our institution, any transbronchial biopsy specimen showing acute allograft rejection was followed up by at least two additional transbronchial biopsy procedures to ensure the resolution of the rejection. As a result, patients with more acute rejections underwent more bronchoscopies and transbronchial biopsies, especially if the episodes occurred late after transplant (ie, beyond the period of surveillance bronchoscopy).

Cytomegalovirus (CMV) status included the following categories: (1) positive match D+a/B+ or D−a/B−; (2) positive mismatch D+a/B−; and (3) negative match D−a/B−. The ischemia time used was the cold ischemia time. In bilateral lung transplants, the ischemia time of the last implanted lung was used. CMV pneumonia was defined by the presence of CMV giant cells or positive results of immunoperoxidase staining on lung biopsy specimens.

**Diagnosis of BOS**

BOS was diagnosed and staged according to the criteria by the International Society for Heart and Lung Transplantation. The diagnosis of OB or BOS required the following: (1) pathologic evidence of OB by biopsy; or (2) a decline in the FEV1 of ≥ 20% compared to the posttransplant baseline maximum that was unexplained by infection, acute rejection, or bronchial stenosis. Transbronchial bronchoscopy was performed to rule out the above causes prior to the diagnosis of BOS.

BOS was graded as follows: stage I, FEV1 values between 66% and 80% of baseline; stage II, FEV1 values between 51% and 65% of baseline; and stage III, FEV1 values ≤ 50% of baseline. The treatment of patients with BOS was similar to that of patients experiencing acute rejection and was followed by the augmentation of immunosuppression.

The posttransplant maximum FEV1 was estimated as the average of two measurements that were taken at least 3 weeks apart, at least 3 months after the transplant operation. Patients also were instructed to monitor their spirometry at home using a portable, handheld spirometer. The first date of a sustained drop in FEV1 that was unexplained by other causes (meeting BOS criteria and occurring at least in two consecutive measurements, unless there was histologic diagnosis) was recorded as the date of onset of BOS. The date of the last follow-up examination was defined as the time of the analysis (December 10, 2000), if the patient had been seen in the clinic the month prior to that date, or as the date of the last clinic visit, if the patient was lost to follow-up (four patients were lost to follow-up at varying times after undergoing transplantation). For patients who had died by the time of the analysis, the date of death was recorded as the date of the last follow-up.
The following information was collected on the study population: recipient age; gender; race; prior transplant; CMV status; human leukocyte antigen (HLA) type; panel reactive antibody (PRA) titer and diagnosis; donor age; HLA type and CMV status; perioperative ischemia time; type of operation; all episodes of biopsy-proven acute rejection; and presence of CMV pneumonia. The acute rejection score (ARS) was calculated by adding the grade of each rejection episode. The ARS for the first 6 months (ARSF) was calculated by adding the grade of each rejection episode in the first 6 months.

Continuous variables were analyzed by using the Student t test (or its nonparametric equivalent), while discrete variables were analyzed by the Fisher exact test. The rates of BOS in single-lung vs double-lung transplant recipients were compared by using the Fisher exact test and also were evaluated by the Kaplan-Meier product-limit estimate. Groups were compared using the log-rank test.

Logistic regression was performed by adding the following to the model: recipient age; prior transplant; CMV status; pretransplant PRA and diagnosis; donor CMV status; perioperative ischemia time; type of operation; all episodes of biopsy-proven acute rejection; the number of HLA mismatches; and CMV pneumonia. The above process was repeated with the Cox proportional hazards method. Variables with p < 0.100 were included in the model.

Analyses by the Kaplan-Meier method and the Cox proportional hazards methods were performed because of the different follow-up in patients with and without BOS (data not shown). Baseline variables or variables that did not vary after the first 6 months were used for the Kaplan-Meier and the Cox proportional hazards method. Data were analyzed with a statistical software package (SAS, version 8.0; SAS Institute; Cary, NC).23,24

Results

Descriptive Characteristics

The median follow-up time for the patients was 2.4 years (interquartile range, 1.5 to 4.0 years). BOS was present in 93 of 225 patients (41.3%) at the time of the analysis. The pathologic diagnosis of OB was available in only 13.9% of patients (13 of 93 patients). All of these patients had met the spirometry criteria for BOS prior to the pathologic diagnosis. The median time to BOS development was 4.2 years (interquartile range, 3.0 to 4.8 years). The rates of freedom from BOS at 1, 2, 3, 4, and 5 years were 93.4%, 67.4%, 58.2%, 50.6%, and 38.1%, respectively (Fig 1). BOS rates were not different by transplant era (as stratified by either pre- or post-1997 or pre- or post-1998).

BOS in Single-Lung vs Double-Lung Transplant Recipients

Patients who had received bilateral lung transplants had lower rates of BOS compared to single-lung transplant recipients (31.7% vs 49.6%, respectively; p = 0.007) at the time of the analysis (median length of follow-up: bilateral transplant, 2.3 years; single-lung transplant, 2.4 years). Patients who had received bilateral transplants were also significantly younger, had CMV pneumonia less frequently, were...
more frequently a CMV-positive mismatch or CMV-negative match, and had longer cold ischemia times. There were fewer patients with COPD who received bilateral lung transplants, while all cystic fibrosis or bronchiectasis patients, and most patients with pulmonary hypertension, received bilateral lung transplants. However, there were no differences in the number of acute rejection episodes, the number of HLA mismatches, or the time of follow-up in the two groups. All variables and their significance are shown in Table 1.

Logistic Regression

Bilateral vs single-lung transplantation remained a significant predictor of BOS after the data were controlled for other factors. The following variables were significant predictors of BOS: (1) ARS (for each increase by 1 point of the ARS: odds ratio [OR] for the development of BOS, 1.31; 95% confidence interval [CI], 1.17 to 1.48; p < 0.001); (2) single-lung transplant (OR for the development of BOS, 2.04; 95% CI, 1.14 to 3.70; p = 0.015); and (3) HLA mismatches (for each additional mismatch: OR for the development of BOS, 1.29; 95% CI, 1.00 to 1.69; p = 0.054). Pretransplant diagnosis, the presence of CMV pneumonia, CMV matching status, and recipient age had no effect on the development of BOS. The association of concordant and discordant pairs was used to assess the quality of the model. The concordant value was 0.717, suggesting a desirable model.

Kaplan-Meier Analysis for Bilateral vs Single-Lung Transplant

The effect of bilateral vs single-lung transplant can be seen in Figure 2. Patients who received bilateral lung transplants were more likely to be free from BOS (p < 0.002). The rates of freedom from BOS at 1, 2, and 3 years were 94.8%, 74.7%, and 68.1%, respectively, for bilateral lung transplant recipients, and 93.1%, 63.0%, and 50.1%, respectively, for single-lung transplant recipients.

Cox Proportional Hazards Analysis

In a model developed by the Cox proportional hazards method, bilateral vs single-lung transplantation retained its significance as a predictor of time to

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**Table 1—Characteristics of Patients Who Have Received Double- vs Single-Lung Transplants***

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Transplant Operation</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Bilateral (n = 104)</td>
</tr>
<tr>
<td>BOS</td>
<td>33 (31.7)</td>
</tr>
<tr>
<td>CMV pneumonia</td>
<td>18 (17.3)</td>
</tr>
<tr>
<td>Ischemia time, min</td>
<td>355 ± 145†</td>
</tr>
<tr>
<td>Donor age, yr</td>
<td>30.8 ± 14.2</td>
</tr>
<tr>
<td>Recipient age, yr</td>
<td>37.3 ± 12.8</td>
</tr>
<tr>
<td>Retransplants</td>
<td>6 (5.8)</td>
</tr>
<tr>
<td>Maximum PRA</td>
<td>2.29 ± 6.71</td>
</tr>
<tr>
<td>HLA mismatches</td>
<td>4.7 ± 1.2</td>
</tr>
<tr>
<td>ARS</td>
<td>2.4 ± 2.7</td>
</tr>
<tr>
<td>ARSF</td>
<td>1.6 ± 1.8</td>
</tr>
<tr>
<td>White patients</td>
<td>93 (89.4)</td>
</tr>
<tr>
<td>Male gender</td>
<td>55 (52.9)</td>
</tr>
<tr>
<td>Time of follow-up, d</td>
<td>1,109 ± 762</td>
</tr>
<tr>
<td>CMV status</td>
<td></td>
</tr>
<tr>
<td>D—/R—</td>
<td>24 (23.1)</td>
</tr>
<tr>
<td>D+ or —/R+</td>
<td>58 (55.8)</td>
</tr>
<tr>
<td>D+/R—</td>
<td>22 (21.1)</td>
</tr>
<tr>
<td>Pretransplant diagnosis</td>
<td></td>
</tr>
<tr>
<td>COPD</td>
<td>16 (15.4)</td>
</tr>
<tr>
<td>CF</td>
<td>47 (45.2)</td>
</tr>
<tr>
<td>α1-antitrypsin</td>
<td>4 (3.8)</td>
</tr>
<tr>
<td>IPF</td>
<td>7 (6.7)</td>
</tr>
<tr>
<td>PPH</td>
<td>8 (7.7)</td>
</tr>
<tr>
<td>Other</td>
<td>22 (21.2)</td>
</tr>
</tbody>
</table>

***Values given as No. of patients (%) or mean ± SD, unless otherwise indicated. CF = cystic fibrosis; PPH = primary pulmonary hypertension; IPF = idiopathic pulmonary fibrosis.
†Of 100 patients.
‡Of 114 patients.
BOS, after controlling for other variables. The following were significant predictors for time to BOS:
(1) ARSF (for every increase in the ARSF by 1 point: hazard ratio for the development of BOS, 1.12; 95% CI, 1.01 to 1.24; p = 0.035); (2) single-lung transplant (hazard ratio for the development of BOS, 2.08; 95% CI, 1.33 to 3.23; p = 0.001); (3) HLA mismatches (for every additional mismatch: hazard ratio for the development of BOS, 1.25; 95% CI, 1.04 to 1.50; p = 0.018); and (4) CMV-positive mismatch status (hazard ratio for the development of BOS, 1.93; 95% CI, 1.07 to 3.50; p = 0.029). Pre-transplant diagnosis, the presence of CMV pneumonia, and recipient age had no effect on the development of BOS. The transplant era had no effect on the development of BOS.

Predictors of BOS in COPD Patients

The appearance of bilateral lung transplantation as a protective factor from BOS necessitated an analysis of a group of patients with the same diagnosis, in order to control for its effect. There were 104 patients with COPD in our cohort. Sixteen of 104 patients (15.4%) received bilateral transplants, and 88 of 104 patients (84.6%) received single-lung transplants. Only 2 of 16 bilateral lung transplant recipients (12.5%) had BOS at the time of the analysis, while 47 of 88 of single-lung transplant recipients (53.4%) had BOS at the time of the analysis (p = 0.009). The small number of patients with BOS among the bilateral lung transplant recipients did not permit any further analysis.

Effect of Bilateral vs Single-Lung Transplant on Survival

At the time of the analysis, 155 of 225 patients (68.9%) were alive. Eighty-two of 104 bilateral lung transplant recipients (78.8%) were alive, while only 73 of 121 single-lung transplant recipients (60.3%) were alive (p = 0.003). The median follow-up periods for patients receiving bilateral and single-lung transplants were 2.3 years (interquartile range, 1.3 to 4.7 years) and 2.4 years (interquartile range, 1.8 to 3.7 years), respectively.

Discussion

This study presents data on the development of BOS in the lung transplant population from a single institution. It found a high incidence of BOS in lung transplant recipients, as has been described in pre-
vious studies.6–19 Approximately 40% of the patients developed BOS by 4 years after transplantation. However, the median time to BOS development was 4.2 years, which is somewhat longer than that previously reported in the literature. Our transplant population received diagnoses mostly by the clinical criteria for BOS, which are probably less sensitive than histologic evidence of OB for the diagnosis of BOS. Alternatively, the rate of BOS might be lower in our lung transplant population, which predominantly consists of patients who have received transplants within the last 5 years. Improvements in immunosuppressive therapy might have led to a delay in the onset of BOS, although our data do not seem to support that. Only larger multicenter studies will clarify whether the incidence of BOS has decreased. Another explanation for these results is the relatively short follow-up period for most of our patients. More patients might have developed BOS if the follow-up was longer.

Bilateral lung transplantation was a protective factor against BOS in our study. The effect was striking using both multivariable logistic regression and Cox proportional hazards models. It remained significant after controlling for native lung disease, recipient age, cold ischemia time, episodes of acute rejection, pretransplant PRA level, HLA mismatches, CMV pneumonia, and CMV mismatch status in a multivariable model. The magnitude of the effect of bilateral lung transplantation on the development of BOS was relatively similar in all analyses. In a separate analysis of patients with COPD (which is the only group with a large number of patients in which both single-lung and bilateral lung transplants were performed), bilateral transplant was protective from BOS, but the small numbers of patients make firm conclusions difficult in this group. Single-lung transplantation, therefore, appears to be a true predictor for the development of BOS in our patient population.

At this time, it is not possible to assess whether this is an effect of the utilization of the BOS system as a surrogate for histologic OB. One would expect single-lung transplants to be more affected by the BOS scoring system, since the presence of OB in the transplanted lung would lead to a larger decrease in the overall FEV₁. However, larger studies with histologically confirmed OB would be needed to definitely answer this question. Another explanation for the higher rates of BOS in single-lung transplant recipients is the presence of the native lung. Previous reports have suggested that hyperinflation of the native lung in COPD patients leads to allograft dysfunction.25 Native lungs also serve as a reservoir for infections, since they are abnormal and are easily colonized by organisms.26 Finally, there might be immunologic mechanisms that predispose single-lung transplant recipients to BOS. The presence of a native lung together with an allograft might activate lymphocytes, macrophages, and other inflammatory cells, leading to the development of OB. Our results are consistent with the improved long-term survival of bilateral lung transplant recipients in the international registry and suggest a possible mechanism by which long-term survival is improved with bilateral transplants.3,27 The decreased rates of BOS in bilateral lung transplant recipients are not related to disproportionate mortality among these patients in our study.

Our results differ from those of a recent study20 from the international registry. In that study, the type of transplant had no effect on BOS in patients with emphysema, even though it led to improved survival in certain groups. The study was large, assessing patients with a uniform diagnosis, but BOS data in the international registry is not complete. Multicenter studies with accurate BOS information on patients with a uniform diagnosis have recently been undertaken by our group in order to define the effect of a transplant operation on the development of BOS.

Acute allograft rejection is a known significant predictor for the development of BOS after lung transplantation. However, the risk of acute rejection episodes and BOS continue to increase with longer follow-up. When the time dependency was taken into account, acute rejection was a less important predictor of BOS than the type of transplant. The number of HLA mismatches was also important in predicting BOS. Other reports6,7,17,28 have identified HLA mismatches as risks for BOS. In all lung transplant recipients, the number of mismatches is usually very high, since it is not possible to make an HLA match prior to transplantation.28 HLA mismatches usually have a small effect on the development of BOS. Early results from a series of patients receiving living donor lobar transplants, in which the number of mismatches tends to be lower, suggest that the rates of BOS are lower.29 This is also consistent with findings in other solid-organ transplant recipients.30 Finally, the presence of a CMV mismatch was a risk factor for the development of BOS. It predisposes to the patient to CMV pneumonia and disease. In our study, CMV mismatch was more significant than CMV pneumonia itself, probably because of the strict criteria used for the definition of the latter, which may have led to an underestimation of its prevalence.

Our study is large but has certain limitations that are inherent to any retrospective study that is performed at a single institution. Regional and temporal variations and changes in practice might account for
some of the findings. Although the data were entered into the database by a dedicated technician and were found to be accurate and complete by each of the coinvestigators, even small errors in data entry could create significant bias. Another limitation of our study is the small number of patients who had histologic evidence of OB. This might have led to the identification of factors that are important in the development of allograft dysfunction (ie, BOS), but not necessarily of OB. Although any form of allograft dysfunction is significant, different factors might be linked more strongly to the development of histologic OB.

Another limitation is that potentially significant additional factors were not included in our analysis. First, we did not specifically control for variations in the initial immunosuppressive regimen. In general, most patients included in this analysis received triple immunosuppression with cyclosporine, azathioprine, and prednisone. Although some patients received MMF as part of a randomized open-label study, the number of patients was relatively small. MMF has not been shown to alter acute or chronic rejection rates in lung recipients, and MMF use was evenly distributed among single-lung and bilateral lung transplant recipients. The effect of induction on BOS is also unknown. Our program began the regular use of induction with daclizumab in 1998. The stratification of patients by era (ie, prior to 1998 and 1998 to the present) demonstrated no difference in BOS rates between the two eras, suggesting that induction is not an important covariate in BOS. Finally, and perhaps most importantly, there may be other important risk factors for BOS that are just being elucidated or have not yet been identified. For example, we have recently demonstrated that gastroesophageal reflux and aspiration represent potentially reversible causes of allograft dysfunction. However, the regression diagnostics suggest that our logistic model is of such high quality and validity that it would be unlikely to be significantly altered by the inclusion of other covariates.

Patients who have undergone bilateral and single-lung transplantation were different in pretransplant diagnosis, recipient age, CMV status, and presence of pneumonia. Even though modeling is very powerful in controlling for these factors, only large studies of patients with a uniform diagnosis could overcome some of these discrepancies between the groups. In addition, the median duration of follow-up for the patients included in the study was short. Further follow-up of these patients is needed in order to confirm the findings of the study. Finally, our retrospective study cannot imply that single-lung transplantation leads to the development of BOS. Only larger prospective studies would be able to overcome these limitations.

In conclusion, our study adds to the literature of risk factors for the development of BOS in lung transplant recipients. In our large cohort, we identified the type of transplant as a novel risk factor for the development of BOS. If confirmed, our results will need to be weighed carefully in decisions regarding the allocation of one or two donor lungs to a single recipient. Additional research also is needed to understand whether single-lung or bilateral lung transplantation leads to differing immunologic responses to the pulmonary allograft and how those responses might contribute to the pathogenesis of OB.

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