

Ischemia-Reperfusion Injury After Lung Transplantation Increases Risk of Late Bronchiolitis Obliterans Syndrome

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Background. Bronchiolitis obliterans syndrome (BOS) is the most common cause of long-term morbidity and mortality after lung transplantation. Our hypothesis was that early ischemia-reperfusion injury after lung transplantation increases the risk of BOS.

Methods. Data on 134 patients who had lung transplantation between January 1, 1990 and January 1, 2000, was used for univariate and multivariate logistic regression analysis.

Results. After lung transplantation, 115 patients (115 of 134, 86%) survived more than 3 months. In that group, 41 patients developed BOS, of which 23 had progressive disease. Univariate analysis revealed that ischemia-reperfusion injury ($p = 0.017$) and two or more acute

rejection episodes ($p = 0.032$) were predictors of BOS onset, whereas ischemia-reperfusion injury ($p = 0.011$) and cytomegalovirus infection ($p = 0.009$) predicted progressive BOS. Multivariate logistic regression analysis showed that ischemia-reperfusion injury was an independent predictor for both BOS development and BOS progression. Two or more acute rejection episodes were also an independent predictor of BOS development, whereas cytomegalovirus infection was an independent predictor of progressive BOS.

Conclusions. Ischemia-reperfusion injury increases the risk of BOS after lung transplantation.

(Ann Thorac Surg 2002;73:1041-8)

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Bronchiolitis obliterans syndrome (BOS) results in a significant decrease in quality of life and is the most common cause of late death after lung transplantation [1, 2]. This pathologic process is thought to be a form of chronic rejection that results in progressive narrowing of respiratory bronchioles [2]. Previous studies have shown that acute rejection, cytomegalovirus (CMV) infection, and possibly pneumonia increase the risk of subsequent BOS [3-5]. The exact etiology of BOS after lung transplantation, however, is still not fully understood.

Acute pulmonary graft dysfunction secondary to ischemia-reperfusion injury continues to be the most common cause of early mortality after lung transplantation [6-8]. Ischemia-reperfusion injury involves neutrophil infiltration into lung tissue followed by release of oxygen radicals and proteases that can damage the surrounding parenchyma [9, 10]. This can result in severe acute pulmonary dysfunction in the early postoperative period [11]. This damage, however, may also result in a permanent lung injury that could predispose patients to late BOS. Our hypothesis was that ischemia-reperfusion injury after lung transplantation increases the risk of late BOS. To test this hypothesis, we performed a multivariate

analysis of our lung transplant population in the early and late postoperative periods.

Material and Methods

Patient Population and Statistics

During the 10-year period from January 1, 1990 to March 1, 2000, 136 lung transplants (132 adult, 4 pediatric) were performed at our institution. Data were collected retrospectively from patients' medical records, clinic charts, and ventilation sheets. Sufficient follow-up data for the study was available for 134 (134 of 136, 99%) patients. The 2 patients for whom follow-up was not available were known to be alive 3 months after transplantation. Both of these patients, however, moved out of the state and were lost to detailed follow-up. Univariate statistics were performed using χ^2 and Student's t tests. Multivariate logistic regression analysis was also performed using all variables that showed at least a trend ($p < 0.20$) toward an increased risk of either BOS development or progressive BOS based on univariate analysis. Data are expressed as mean \pm standard error of the mean with p values less than or equal to 0.05 considered significant.

Perioperative Immunosuppression and Infection Prophylaxis

Induction immunosuppressive therapy consisted of cyclosporin (3 to 6 mg/kg), azathioprine (2.5 mg/kg), anti-

Presented at the Forty-eighth Annual Meeting of the Southern Thoracic Surgical Association, San Antonio, TX, Nov 8-10, 2001.

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thymocyte globulin (ATGAM 15 mg/kg; Upjohn, Kalamazoo, MI), and 500 mg of methylprednisone. The initial dose of methylprednisone was followed by a gradual steroid taper. The initial dose of ATGAM was followed by a 10-mg/kg dose on postoperative day 1 and 7.5 mg/kg doses on postoperative days 2 and 3. Long-term immunosuppression was started with a triple drug regimen of prednisone, cyclosporin, and azathioprine.

Perioperative antibacterial agents consisted of cefazolin for the first 24 hours after transplant. Patients who received lungs having a predominate organism in the donor bronchus were changed to appropriate antibiotic coverage. Recipients who were CMV negative who received CMV positive lungs were considered to be at risk for CMV infection and received appropriate prophylaxis with postoperative ganciclovir. Postoperative and long-term *Pneumocystis carinii* prophylaxis was achieved with either trimethoprim/sulfamethoxazole or aerosolized pentamidine.

Ischemia-Reperfusion Injury

The requirements for the diagnosis of ischemia-reperfusion injury were clinical suspicion, appropriate chest roentgenogram findings, and at least moderate pulmonary dysfunction (oxygenation index, > 7) in the first 24 hours after transplantation. The oxygenation index was determined using the equation: [(mean airway pressure \times percent of inspired oxygen)/partial pressure of arterial oxygen] [12]. Calculation of the oxygenation index started after the patient was transferred from the operating room to the intensive care unit.

Acute Rejection

Treatment strategies for acute rejection have gradually evolved since the early (1990 to 1995) transplant era at our institution. For the majority of patients in the early transplant era, the diagnosis of acute rejection was based on biopsy findings. During more recent years, however, clinical suspicion has been used with increasing frequency to diagnose patients with acute rejection. Thus for this study, acute rejection episodes were defined by one of two methods. Patients with lung biopsies showing least grade 2A perivascular lymphocyte infiltration were considered to have acute rejection. Patients diagnosed with acute rejection based on clinical suspicion followed by an appropriate response to high dose steroids or OKT3 were also included in the study. Patients with two or more acute rejection episodes within the first 6 months after transplantation were considered to have significantly more rejection episodes than their counterparts and were used for statistical analysis.

Cytomegalovirus Infection

Cytomegalovirus infections were diagnosed by a variety of methods. Patients with lung biopsy specimens showing characteristic CMV inclusions in association with an inflammatory infiltrate were considered to have CMV pneumonitis. Cytomegalovirus infections diagnosed by

CMV culture, serologic demonstration of a significant increase in CMV immunoglobulin G, or new onset of CMV antigenemia based on the early antigen fluorescent foci test were also included in the CMV infection group. The presence of CMV infection within the first 6 months after transplantation was considered as a possible risk factor for BOS development. Patients with CMV infection were given at least 6 weeks of intravenous ganciclovir.

Pneumonia

The diagnosis of pneumonia required clinical suspicion, appropriate chest roentgenogram findings, substantiating microbiologic results, and institution of appropriate antibiotic therapy. Isolated cultures not requiring treatment were excluded. Patients that were believed to be colonized with an organism were likewise not considered to have pneumonia. Patients receiving antibiotic treatment for organisms isolated from the donor bronchus at the time of transplantation were also not considered to have pneumonia. Patients diagnosed with bronchitis were similarly excluded.

Bronchiolitis Obliterans Syndrome

Pulmonary function tests were used to identify patients with BOS as outlined by the International Society of Heart and Lung Transplantation guidelines for clinical staging of chronic dysfunction in lung allografts [13]. Briefly, baseline forced expiratory volume in 1 second (FEV₁) was determined by taking the average of the two highest consecutive FEV₁ values after lung transplantation, with such measurements being obtained 3 to 6 weeks apart. Patients with a subsequent unexplained decrease in FEV₁ to 66% to 80% of baseline value for two consecutive measurements, obtained at least 3 weeks apart, met criteria for mild BOS (stage I). Patients with an unexplained decrease in FEV₁ to 51% to 65% and 50% or less of baseline value for two consecutive measurements, obtained at least 3 weeks apart, were considered to have moderate (stage II) and severe (stage III) BOS, respectively. Time until onset of BOS was defined as the time until initial decline in pulmonary function tests (PFT) to the corresponding BOS stage after transplantation.

Patients referred to as having progressive disease are those that deteriorated to stage II or stage III BOS based on PFT data. Patients who did not have adequate PFT criteria for progressive disease but died from BOS ($n = 4$) were also considered to have progressive disease. Patients who had stage I BOS but died secondary to other causes were not considered to have progressive disease.

The BOS staging system used in the study is a reflection of the current status of the lung transplant population. Patients who had an unexplained deterioration to stage II or III BOS but who returned to stage I by the time of the study (March 1, 2000) were considered to have stage I disease. Patients who died of BOS based on autopsy but did not have the PFT data necessary for the diagnosis were considered to have both BOS onset and progressive BOS starting on the day of death.

Table 1. Patient Population

Obstructive lung disease		70
COPD (idiopathic/asthma/tobacco)	54	
Alpha-1 anti-trypsin deficiency	16	
Fibrotic lung disease		32
Idiopathic pulmonary fibrosis	11	
Sarcoidosis	10	
Cystic fibrosis	5	
Asbestosis	2	
Lymphangiomyelitis	1	
Histiocytosis X	1	
Scleroderma	1	
Immunoglobulin deficiency	1	
Bronchiolitis obliterans	1	
Pulmonary hypertension		13
Total		115

COPD = chronic obstructive pulmonary disease.

Results

Patient Population and Demographics

The study population consisted of the 115 (115 of 136, 85%) patients who survived more than 3 months after transplantation. Mean age was 49.4 ± 1.2 years (range, 8 to 68 years) and 56 (49%) were men. Mean graft ischemia time was 215.4 ± 8.0 minutes. Donors and recipients were not prospectively matched for HLA type. Table 1 outlines the indications for lung transplantation. Double lung transplants were performed in 23 patients. The average of the ischemic times for both lungs was used for patients who underwent bilateral lung transplantation. Cardio-pulmonary bypass was used in 20 patients. There were 34 (34 of 115 patients, 30%) deaths during the study period (January 1, 1990 to March 1, 2000). Obliterans bronchiolitis was the most common major cause of death, occurring in 13 of these patients (38%). The next most common cause of death was bacterial or fungal pneumonia, which occurred in 6 patients (18%).

For the 19 patients who did not survive at least 3 months after transplantation, 14 deaths were due to reperfusion injury, 4 were secondary to pneumonia/sepsis, and 1 patient who did not have reperfusion injury died from multisystem organ failure after reoperation for bleeding and bronchial anastomosis complications.

Bronchiolitis Obliterans Syndrome

Of the 115 survivors, 48 (48 of 115, 42%) developed at least stage I BOS. Twenty-three of the patients who had stage I BOS (29 of 48, 50%) developed progressive disease (stage II BOS, stage III BOS, or death from BOS). For the 48 patients who developed at least stage I BOS, mean time to onset of BOS was 804.9 ± 87.9 days (2.3 years; range, 133 to 2,196 days). Patients who eventually had progressive BOS developed stage I disease earlier (601.7 ± 60.2 days; 1.65 years) than those that did not have progressive disease ($1,164.6 \pm 122.3$ days; 3.2 years, $p < 0.001$). For the 29 patients who had progressive disease, mean time to onset was 745.9 ± 80.4 days (2.0 years) after

Table 2. Episodes of Acute Rejection Compared to Bronchiolitis Obliterans Syndrome Onset and Progressive Bronchiolitis Obliterans Syndrome

Number of Rejection Episodes	Number of Patients	BOS Onset	Progressive BOS
1	29	12 (41%)	7 (24%)
2	13	8 (62%)	3 (23%)
3	4	2 (50%)	2 (50%)
4	4	3 (75%)	2 (50%)

BOS = bronchiolitis obliterans syndrome.

transplantation. Of that group, 23 patients were in stage I, 2 patients were in stage II, and no patients were in stage III disease at the time of initial BOS diagnosis based on PFT data. Four other patients without PFT criteria for BOS died of BOS based on findings at autopsy.

Ischemia-Reperfusion Injury

Of the patients who survived more than 3 months, 23 (20%) had previously had clinically significant ischemia-reperfusion injury after lung transplantation. Of the 23 patients who had previous ischemia-reperfusion injury, 14 (60%) developed at least stage I BOS. Ten (43%) of these patients went on to develop progressive BOS. Nine of the 14 patients (64%) with reperfusion injury who went on to have BOS had oxygenation indices more than 10, which is considered to be severe pulmonary dysfunction. Four of the 9 patients (44%) with reperfusion injury who did not develop BOS had oxygenation indices more than 10. There was no statistical difference between these two groups.

Acute Rejection

After lung transplantation, acute rejection was a common occurrence (Table 2). Fifty patients (50 of 115, 43%) had 83 episodes of acute rejection in the first 6 months after lung transplantation. Of these episodes, 44 were diagnosed with lung biopsy and 39 were diagnosed clinically with improvement in symptoms after treatment. Five patients required treatment with OKT3 for refractory rejection. Of the patients with acute rejection, 21 had two or more episodes of acute rejection. Of the 21 patients who had at least two episodes of acute rejection, 13 (62%) developed stage I BOS. Seven (33%) of these patients went on to develop progressive BOS.

Cytomegalovirus Infection

Cytomegalovirus pneumonitis was diagnosed in 16 patients within the first 6 months after transplantation. Five patients who did not have CMV pneumonitis developed CMV antigenemia that was associated with an increase in CMV immunoglobulin titers. One of these patients had also developed CMV gastritis. All of these infections were treated with intravenous ganciclovir. In all, 21 patients developed CMV infection within the first 6 months after transplantation. Of these patients, 10 (48%) had at least stage I BOS. Eight of these patients went on to have progressive BOS (38%).

Table 3. Types of Pneumonia

<i>Pseudomonas</i>	41% (15)
<i>Staphylococcus aureus</i>	14% (5)
<i>Enterobacter cloacae</i>	11% (4)
<i>Mycobacterium avium intracellulare</i>	11% (4)
<i>Aspergillosis</i>	5% (2)
<i>Candida</i>	5% (2)
<i>Haemophilus influenza</i>	5% (2)
<i>Pneumocystis carinii</i>	3% (1)
<i>Klebsiella</i>	3% (1)
<i>Enterococcus</i>	3% (1)

Pneumonia

Bacterial pneumonia was diagnosed in 30 patients who had 37 episodes of pneumonia within the first 6 months after lung transplantation, of whom 6 patients had two or more episodes. Table 3 gives the relative frequency of predominating organisms. Fourteen (43%) of the patients diagnosed with pneumonia developed at least stage I BOS, of which 8 (27%) went on to have progressive disease.

Univariate Statistical Analysis

Univariate statistical analysis demonstrated that the patients with either ischemia-reperfusion injury ($p = 0.017$) or more than two acute rejection episodes ($p = 0.032$) were more likely to have BOS onset (Table 4). There was also a very strong trend toward BOS onset in patients with CMV infection ($p = 0.06$). There was no significant increased risk of BOS onset in patients diagnosed with bacterial/fungal pneumonia. There were no differences in age, gender, or indication for transplantation between the two groups. Ischemia time was not significantly different between those that developed BOS (216.5 ± 9.3 minutes) and those who did not (213.9 ± 15.0 minutes). Patients with double lung transplants, cardiopulmonary bypass during transplantation, or multiple blood transfusions intraoperatively did not have an increased risk for BOS development.

Univariate analysis demonstrated that patients with either ischemia-reperfusion injury ($p = 0.011$) or CMV infection ($p = 0.009$) were more likely to develop progressive BOS (Table 5). There was a trend toward progressive BOS in patients with two or more episodes of acute rejection. Patients with pneumonia were not at increased risk for the development of progressive disease. There were no differences in age, gender, or indication for transplantation between the two groups. Ischemia time was not significantly different between those who had progressive BOS (216.4 ± 17.3 minutes) and those who did not (211.5 ± 21.0 minutes). Patients with double lung transplants, cardiopulmonary bypass during transplantation, or multiple blood transfusions intraoperatively did not have an increased risk for progressive BOS.

Multivariate Linear Regression Model

Multivariate analysis revealed that both ischemia-reperfusion injury and acute rejection were independent

Table 4. Early Predictors of at Least Stage I Bronchiolitis Obliterans Syndrome

Variables	Patients With at Least Stage I BOS (n = 48)	Other Patients (n = 74)	p Value
Demographics			
Age (y)	50.2 ± 1.8	48.9 ± 1.5	
Males	49%	50%	
Lung transplant indications			
Obstructive pulmonary disease	61%	57%	
Fibrotic pulmonary disease	29%	31%	
Pulmonary hypertension	10%	12%	
Perioperative variables			
Double lung transplantation	12%	16%	
Need for cardiopulmonary bypass	12%	19%	
Need for multiple blood transfusions	7%	15%	
Ischemia-reperfusion injury	34%	12%	0.017
Lung ischemia time (minutes)	213.9 ± 15.0	216.5 ± 9.3	
Late postoperative variables			
Acute rejection episodes ≥2	31%	10%	0.032
Bacterial or fungal pneumonia	34%	22%	
CMV infection	24%	14%	0.060

CMV = cytomegalovirus.

predictors for the development of BOS (Appendix). Analysis also showed that both ischemia-reperfusion injury and CMV infection were independent predictors for progressive BOS.

Comment

Obliterans bronchiolitis can be a severely disabling disease and is the leading cause of mortality after lung transplantation [1, 2]. Bronchiolitis obliterans syndrome is considered to be the surrogate marker of this disease process [3]. Clinically, patients with BOS show a serial worsening of lung function on PFTs. Specifically, these patients have a progressive decrease in FEV₁ measurements [13]. Treatment for patients suffering from BOS has included high dose steroid, ATGAM, and OKT3 therapy; however, these options have been largely unsuccessful in controlling the disease [2, 14, 15].

Acute rejection has been consistently marked as one of the prime contributors to the development of BOS. In a large study from Cambridge [3], 230 lung transplant recipients were analyzed. In that series the number of rejection episodes progressively increased the risk of developing BOS per episode of acute rejection. Other investigations have demonstrated that both severity of

Table 5. Early Predictors of Progressive Bronchiolitis Obliterans Syndrome

Variables	Patients With Progressive BOS (n = 29)	Other Patients (n = 92)	p Value
Demographics			
Age (y)	47.5 ± 2.9	49.8 ± 1.2	
Male	52%	49%	
Pulmonary disease process			
Obstructive pulmonary disease	57%	62%	
Fibrotic pulmonary disease	30%	29%	
Pulmonary hypertension	13%	11%	
Perioperative variables			
Double lung transplantation	17%	14%	
Need for cardiopulmonary bypass	13%	17%	
Need for multiple blood transfusions	17%	11%	
Ischemia-reperfusion injury	43%	14%	0.011
Lung ischemia time (minutes)	211.5 ± 21.0	216.4 ± 17.3	
Late postoperative variables			
Acute rejection episodes ≥2	30%	15%	
Bacterial or fungal pneumonia	35%	24%	
CMV infection	35%	14%	0.019

CMV = cytomegalovirus.

acute rejection, based on acute rejection scores, and number of acute rejection episodes are directly associated with a progressive increase in risk of BOS onset [4, 5, 16-18].

Cytomegalovirus infection has been shown to be a risk factor for BOS in the present study and in studies by other researchers, although the data are not as consistent as those found for acute rejection. Kroshus and associates [16] showed a significant correlation between CMV pneumonitis and BOS onset. In the study from Cambridge [3], both positive CMV status and CMV pneumonitis were associated with an increased risk of BOS. Studies by both Cooper [19] and Ettinger [20] and their colleagues, however, showed no increased risk of BOS in patients suffering from CMV infection.

Although not observed in the present series (which may represent a type II error), pulmonary bacterial or fungal infection has also been correlated with an increased risk of developing BOS. Girgis and associates [18] found that the presence of bacterial or fungal pneumonia within the first 6 months of lung transplantation significantly increased the risk of BOS onset. Similarly, in the series from Cambridge [3], patients with episodes of bacterial lung infection within the first 6 months of lung transplantation were more likely to develop BOS.

In addition to acute rejection and CMV infection, the

current series shows a significant correlation between ischemia-reperfusion injury and the development of BOS. Other studies directly correlating ischemia-reperfusion injury with the development of BOS are limited.

Although the exact mechanism of ischemia-reperfusion injury is not fully understood, circulating neutrophils have been strongly implicated, to the extent that lung biopsies showing influx of circulating neutrophils is currently one of the most accurate means for identifying patients with acute ischemia-reperfusion injury [18]. Influx of neutrophils into the lung parenchyma, with the subsequent release of proteases and oxygen radicals, has been shown to be at least partially responsible for the respiratory decline associated with ischemia-reperfusion injury [9, 10]. Activation of the inflammatory system against transplanted lungs may also result in the generation of an allogenic immune response that predisposes patients to long-term complications, such as BOS. Interestingly, we and other investigators have shown that ischemia time (at least less than 8 hours) does not increase the risk of reperfusion injury after lung transplantation [21].

In addition to acute rejection, there may be an increased risk of chronic rejection after lung transplant reperfusion injury. Although formal animal experiments investigating the effects of reperfusion injury on subsequent chronic rejection are lacking, this correlation has been suggested in a limited number of clinical studies. In one study from the University of Pittsburgh [4], the impact of ischemic injury on BOS onset was investigated. In that study, patients underwent early postoperative bronchoscopy for airway assessment. Ischemic injury was defined as the presence of intensely erythematous, friable, edematous airways in lung allografts without any evidence of airway infection. These changes were found to usually resolve within 2 to 3 weeks after transplantation. Patients with evidence of airway ischemic injury were more likely to develop BOS compared to the rest of the transplant population ($p = 0.004$). In another study from Stanford [18], lung biopsies were obtained within the first 30 days of lung transplantation and assessed for ischemia-reperfusion injury, as indicated by the presence of interstitial neutrophil sequestration. A strong trend toward increased risk of late BOS was found ($p = 0.09$). This method, however, may have missed some patients with significant ischemia-reperfusion injury who had recovered from the initial neutrophil-mediated event by the time the biopsy was taken.

The majority of scientific evidence suggests that obliterans bronchiolitis results from an immunologically mediated injury directed against pulmonary vascular endothelium and airway epithelium [2]. One of the important events leading to obliterans bronchiolitis seems to be the upregulation of donor HLA antigens and presentation of these complexes to recipient lymphocytes [22-24]. Activation of lymphocytes by HLA antigens then causes the release of cytokines that facilitate the activation of inflammatory cells and the production of antidonor antibodies by stimulated B-cells. The net effect of these

processes is a direct inflammatory or immune injury to pulmonary endothelial and airway epithelial cells. The repair and remodeling response to this injury results in clinically apparent obliterans bronchiolitis [2]. A few important observations in patients who have developed obliterans bronchiolitis help support this theory. First, there seems to be heightened immune activity in these patients, as assessed by the primed lymphocyte test and cell-mediated cytotoxicity [25, 26]. Second, these patients have enhanced expression of major histocompatibility complex (MHC) class II antigens on bronchial epithelium and pulmonary endothelium [27]. Third, increased numbers and activity of dendritic cells, which bear class II HLA receptor-antigen complexes, are present in patients who have obliterans bronchiolitis [28].

Given this theory, processes that result in the upregulation of donor MHC antigens could increase the risk of bronchiolitis obliterans. Acute rejection, specifically, has been suggested to induce BOS as a result of a direct immunologic lung injury mediated by T lymphocytes. Animal models have shown that acute rejection episodes result in increased expression of class II HLA antigens and increased numbers of antigen-presenting cells in lung grafts [29, 30]. Similarly, CMV infection results in an inflammatory response that is followed by the upregulation of MHC class I and II antigens on endothelial and epithelial cells. The CMV infection also increases the activity and number of antigen-presenting cells, immune responses, and allograft reactivity [27, 31]. Bacterial or fungal pneumonia can also induce an inflammatory response that may result in an increased risk for the development of obliterans bronchiolitis [32]. Certainly pneumonia causes an acute inflammation and it has been previously shown that cytokines and other mediators of inflammation increase the expression of MHC class II antigens in transplanted lungs [2]. Finally, ischemia-reperfusion injury may also result in an immune response that leads to the development of bronchiolitis obliterans.

Studies have shown that ischemia-reperfusion injury after lung transplantation is associated with the upregulation of cytokines, such as interleukin-2, tumor necrosis factor- α , and interferon- γ , which contribute to the induction of an inflammatory response in the lung allograft [4, 15]. In addition to mediating the inflammatory response to reperfusion injury, these upregulated cytokines and recruited cells may also have a role in the expression of HLA antigens. Evidence for this is provided by animal models investigating ischemia-reperfusion injury. Serick and associates [33] demonstrated increased expression of MHC class II antigens after ischemia-reperfusion injury in an experiment using mongrel dogs. Waddell and colleagues [34] also demonstrated that severe ischemia-reperfusion injury leads to a significant increase in MHC class I and II antigen expression using a rat model. Thus, in addition to acute lung dysfunction after reperfusion injury, upregulation of MHC class II antigens in patients with ischemia-reperfusion injury may provide a mechanism for chronic allograft failure.

One drawback of this study is given the relatively small

number of patients in the series, the possibility of a type II error certainly exists. Many studies examining lung transplant populations suffer from this problem. However, we still believe that it is important to examine and try to make comparisons within the population we have to work with.

In summary, the development of BOS can result from several different pathologic processes that seem to lead to a heightened allograft immune response. Several studies have shown the importance of acute rejection and infection in BOS. In addition, however, patients with ischemia-reperfusion injury perioperatively are at increased risk for BOS. Given this, prevention of perioperative pulmonary graft dysfunction may have an impact on both short- and long-term outcomes after lung transplantation.

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Appendix

Logistic Regression Using Acute Rejection, CMV Infection, and Ischemia-Reperfusion Injury to Model BOS Onset and Progressive BOS Independently

Variable	p Value	Lower 95% CI	Upper 95% CI
BOS onset model	0.008		
Acute rejection	0.085	0.129	1.143
CMV	0.387	0.173	1.974
IR injury	0.017	0.111	0.807
Progressive BOS model	0.012		
Acute rejection	0.784	0.258	2.781
CMV	0.049	0.083	0.994
IR injury	0.019	0.109	0.824

BOS = bronchiolitis obliterans syndrome; CMV = cytomegalovirus; IR = ischemia/reperfusion.

DISCUSSION

DR WILLIAM A. BAUMGARTNER (Baltimore, MD): I would like to congratulate Dr Fiser on an excellent presentation and to both him and his coauthors on providing additional information on a most important problem in lung transplantation, bronchiolitis obliterans syndrome (BOS).

The investigators from the University of Virginia are suggesting that perhaps the number one problem may actually be ischemic reperfusion injury, which significantly contributes to the development of bronchiolitis obliterans. We have also been interested in this mechanism as a contributing factor for the development of accelerated atherosclerosis in the transplanted heart.

One of our pathologists, Dr Ralph Hruban, looked retrospectively at the first cardiac biopsy and graded the ischemic injury. We then compared these data with the subsequent development of coronary artery disease in our patients and found a very significant correlation between the degree of ischemic injury and the development of accelerated coronary artery disease. I

noticed in your manuscript, which is, by the way, very well written, that you did routine biopsies on early transplant patients. Have you compared the histological degree of ischemic injury, which is a more objective finding and which has its hallmark as the infiltration of neutrophils, with the subsequent development of bronchiolitis obliterans?

My other question, Dr Fiser, is do you constitute any therapeutic intervention when you suspect a patient is developing bronchiolitis?

You have made an important observation and it should restimulate interest into better preservation strategies for lung transplantation. Thanks again for asking me to review this manuscript.

DR FISER: We have not performed routine biopsies at least in the last six to seven years at our institution. Early on in our transplant experience we performed more biopsies when we suspected acute rejection, however finding consistency in read-

ing the biopsies has been difficult, so we have tended to move away from getting routine biopsies and towards just treating acute rejection based on clinical suspicion.

We have tried to treat BOS with a whole host of immunosuppressive agents including OKT3 (Ortho Biotech, Raritan, NJ), ATGAM (Pharmacia Upjohn, Peapack, NJ), cyclosporine, Tacrolimus, Rapamune (Wyeth-Ayerst, Philadelphia, PA), and total lymphoid irradiation. However, we have not found any treatment that prevents the development of BOS or halts its progression.

DR ROBERT DAVIS (Durham, NC): I very much enjoyed your presentation. This is obviously an extremely important issue of the nonallogeneic effects on allogeneic responses. The problem with this analysis is that you are treating a time-dependent as a non-time-dependent variable. Are the patients who are suffering ischemic reperfusion part of your earlier cohort, or are they at the same duration of risk as the rest of your population?

Question number two I think you may have already answered. I gather you use clinical indications for determining the need for biopsy and therefore to make the diagnosis of acute rejection. The rate of acute rejection is one of the lowest that has been reported. Do you use an induction strategy? What is your immunosuppression protocol, because that type of rate is just not seen. Most reports of acute rejection rate are approximately 60% at six months.

A follow-up comment on this is that compared to other variables, acute rejection scores are much more strongly associated with the development of bronchiolitis in analysis that considers bronchiolitis as a time-dependent variable. That isn't to say that ischemia reperfusion doesn't have a strong role in priming the alloimmune response.

Again, I enjoyed your presentation very much.

DR FISER: Definitely bronchiolitis obliterans is a time-dependent variable. The longer these patients are followed, the more likely they are to develop BOS. What I can tell you, though, is that our incidence of reperfusion injury over the first five years of our transplant program compared to the second five years of our transplant program has been relatively the same. So given that we didn't have more ischemia-reperfusion injury early on compared to later years, I think the observation that ischemia-reperfusion injury increases the risk of BOS is true.

As far as the second question, our induction immunosuppression, for the most part, I guess over the first six to seven years of

our program, it has generally been cyclosporine, ATGAM, and Imuran. Over recent years we have switched to Tacrolimus (Faro Pharmaceuticals, San Diego, CA 92121). Patients are kept on maintenance immunosuppression with prednisone and cyclosporin or Tacrolimus. Additionally, our acute rejection rate is about 43% at six months. This may be lower than other reported values as a result of more aggressive immunosuppression or perhaps differences in definition. In any case, our seemingly lower incidence makes the association between acute rejection and BOS all the more compelling.

DR STEPHEN D. CASSIVI (St. Louis, MO): I appreciated listening to your presentation. At Washington University, we are also investigating ischemia-reperfusion as a cause of late graft dysfunction.

My first question pertains to the time course of your study. Over the 10-year period of your study, I was interested to know if you have made any adjustments in your preservation strategies. There are a number of new solutions being used in different centers. In our center, we have adopted a low potassium dextran solution for our lung preservation, and have been pleased with our results.

The other question I had is in relation to what Dr Davis discussed. Is there a difference in the mean follow-up of your two groups? Was there simply a shorter follow-up period in the group that developed less obliterative bronchiolitis and that given a longer follow-up period, they too would eventually have similar rates of BOS?

DR FISER: For the first question, we still continue to use Euro-Collins preservation solution at our institution. There has not been any clear evidence so far that suggests one solution is better than the rest for ischemic times less than 4 to 6 hours so we have not switched solutions such as University of Wisconsin solution or low potassium dextran.

As far as the difference in mean follow-up, obviously patients that had a transplant during our early transplant era (first five years of our program) are going to be more likely to develop BOS. But the variables we examined (acute rejection episodes, ischemia-reperfusion injury, cytomegalovirus infections) were not particularly increased in our early transplant experience compared to our more recent experience. Thus, I think the observation that these factors increase the risk of BOS holds true.