# CARDIAC AND PULMONARY REPLACEMENT

## OBLITERATIVE BRONCHIOLITIS AFTER LUNG AND HEART-LUNG TRANSPLANTATION

### An analysis of risk factors and management

**With a prevalence of 34% (55/162 at-risk recipients) and a mortality of 25% (14/55 affected recipients), obliterative bronchiolitis is the most significant long-term complication after pulmonary transplantation. Because of its importance, we examined donor-recipient characteristics and antecedent clinical events to identify factors associated with development of obliterative bronchiolitis, which might be eliminated or modified to decrease its prevalence. We also compared treatment outcome between recipients whose diagnosis was made early by surveillance transbronchial lung biopsy before symptoms or decline in pulmonary function were present versus recipients whose diagnosis was made later when symptoms or declines in pulmonary function were present. Postoperative airway ischemia, an episode of moderate or severe acute rejection (grade III/IV), three or more episodes of histologic grade II (or greater) acute rejection, and cytomegalovirus disease were risk factors for development of obliterative bronchiolitis. Recipients with obliterative bronchiolitis detected in the preclinical stage were significantly more likely to be in remission than recipients who had clinical disease at the time of diagnosis: 81% (13/15) versus 33% (13/40); $p < 0.05$. These results indicate that acute rejection is the most significant risk factor for development of obliterative bronchiolitis and that obliterative bronchiolitis responds to treatment with augmented immunosuppression when it is detected early by surveillance transbronchial biopsy. (J THORAC CARDIOVASC SURG 1995;110:4-14)**

Ko Bando, MD,[a]* Irvin L. Paradis, MD,[b] Shari Similo, MS,[e]
Hiroaki Konishi, MD,[a] Kanshi Komatsu, MD,[a] Thomas G. Zullo, PhD,[c]
Samuel A. Yousem, MD,[d] John M. Close, PhD,[c] Adriana Zeevi, PhD,[d]
Rene J. Duquesnoy, PhD,[d] Jan Manzetti, RN, PhD,[a] Robert J. Keenan, MD,[a]
John M. Armitage, MD,[a] Robert L. Hardesty, MD,[a] and
Bartley P. Griffith, MD,[a] *Pittsburgh, Pa., and Baltimore, Md.*

The choice of lung transplant procedures to treat patients with end-stage pulmonary vascular or parenchymal disease has evolved since the modern era of lung transplantation began in 1981.[1] Primarily because survival is improving, lung and heart-lung transplantation have become acceptable therapeutic modalities.[2, 3] However, better survival has come principally from improvements in early postoperative care, including improved donor and recipient selection,[4] lung preservation,[5] management of ischemia/reperfusion lung injury,[6] and recognition and treatment of infections caused by bacteria,[7] *Pneumocystis,*[8] and cytomegalovirus (CMV).[9, 10] As a result, survival to hospital discharge after lung transplantation has improved from approximately 50% to 60% 10 years ago to 70% to 90% today.

The primary determinant of long-term survival is obliterative bronchiolitis (OB).[11] With a prevalence of 30% to 40% in long-term survivors, OB has emerged as the most significant long-term compli-

From the Divisions of Cardiothoracic Surgery,[a] Pulmonary Medicine,[b] Learning Resources,[c] and the Department of Pathology,[d] University of Pittsburgh School of Medicine, Pittsburgh, Pa., and the Maryland Medical Research Institute,[e] Baltimore, Md.

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Address for reprints: Ko Bando, MD, Division of Cardiothoracic Surgery, C-700, Presbyterian University Hospital, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213.

*Ko Bando, MD, is the Fortieth Evarts A. Graham Memorial Traveling Fellow of The American Association for Thoracic Surgery.



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**Table I.** *Characteristics of lung recipients at risk for the development of OB*

| | *Early (1982-1987)* | *Late (1988-1992)* | *Overall (1982-1992)* |
|---|---|---|---|
| Gender | | | |
| Male | 12 (39%) | 58 (44%) | 70 |
| Female | 19 (61%) | 73 (56%) | 92 |
| Age (yr) | | | |
| Mean ± standard deviation | 32 ± 10 | 36 ± 15 | 35 ± 14 |
| Range | 4-48 | 1-64 | 1-64 |
| Transplant procedure | | | |
| Single lung | 0 | 45 (34%)* | 45 |
| Bilateral lung | 0 | 57 (44%)* | 57 |
| Heart and lung | 31 (100%) | 29 (22%)* | 60 |
| Pretransplant disease | | | |
| Primary pulmonary hypertension | 14 (45%) | 31 (24%)* | 45 |
| Secondary pulmonary hypertension | 13 (42%) | 22 (17%)* | 35 |
| Emphysema | 0 | 33 (25%)* | 33 |
| Septic lung disease | 3 (10%) | 23 (18%) | 26 |
| Restrictive lung disease | 1 (3%) | 9 (7%) | 10 |
| Other | 0 | 13 (10%) | 13 |
| Immunosuppression | | | |
| Cyclosporine | 31 (100%) | 86 (66%)* | 117 |
| FK 506 | 0 | 38 (29%) | 38 |
| Switch: cyclosporine→FK 506 | 0 | 6 (5%) | 6 |
| Switch: FK 506→cyclosporine | 0 | 1 (1%) | 1 |
| Total number | 31 | 131 | 162 |

* $p < 0.01$, by $\chi^2$ test.

cation after pulmonary transplantation. Although the exact pathogenesis of posttransplantation OB remains unclear, the bulk of the evidence suggests that OB occurs as a result of an immunologic reaction against or by an ischemic injury to the epithelial cells of the airways.[11-17]

Because the pathogenesis of OB after lung transplantation is unclear, one purpose of this study was to elucidate factors that might be important in the pathogenesis of OB. We examined recipient and donor characteristics, as well as clinical events before development of OB, to determine whether these characteristics or events were related to the subsequent development of OB. The second purpose of this study was to determine whether early diagnosis and treatment with augmented immunosuppression affected long-term outcome of recipients with this disorder. We compared survival and functional outcome of recipients whose OB was discovered before symptoms or pulmonary function abnormalities were present with the outcome of recipients whose OB was discovered when symptoms or pulmonary function abnormalities were present.

## Methods

**Patient population.** Because histologic and clinical OB has not been observed in the first 60 days after transplantation, this study included only the 162 pulmonary transplant recipients who survived more than 60 days and were discharged from the hospital after the transplant procedure (Table I). Because significant changes in the management of pulmonary transplant recipients occurred at the end of 1987, we analyzed and compared separately our early (1982 to 1987) and late (1988 to 1992) experience. These management changes included use of surveillance transbronchial lung biopsy (TBBx),[18] prophylactic antibiotics tailored to the results of microbiologic cultures obtained from the airways of the donor and recipient at the time of transplantation,[7] CMV-negative blood and blood products for seronegative donors and recipients, and ganciclovir for treatment and prophylaxis of CMV illness in seropositive recipients or donors or both.[9, 10] Because of the evolution of lung transplant procedures, indications, and immune suppression over time, there were significantly fewer heart-lung procedures, recipients with pulmonary hypertension, and recipients treated with cyclosporine-based immunosuppression in the later as compared with the early period.

**Definitions**

*OB.* OB was defined according to histologic ($n = 52$)[19] or clinical criteria ($n = 3$).[20] Histologically active OB was defined by the presence of dense eosinophilic scar tissue in the airway walls with a mononuclear cell infiltrate. Inactive OB was defined by the presence of scar tissue in the airway wall but without a mononuclear cell infiltrate.[19]

Criteria for the clinical diagnosis of OB included symptoms of dyspnea and/or cough with or without sputum and/or a new obstructive and/or restrictive pulmonary function defect that could not be explained by the pres-

**Table II.** *Severity of the OB based on change in $FEV_1$ from baseline value*

| *Stage* | *Severity* | *$FEV_1$ (L)* |
|---|---|---|
| 0 | None | ≥85% of baseline |
| 1 | Mild | 70%-84% of baseline |
| 2 | Moderate | 55%-69% of baselnie |
| 3 | Severe | <55% of baseline |
| 4 | Dead | Death due to OB |

*$FEV_1$,* Forced expiratory volume in 1 second.

ence of bronchomalacia, stenosis of the anastomosis, or infection in the allograft. The clinical diagnosis and severity of OB were defined by a decline in the expiratory volume in the first second of a forced vital capacity maneuver compared with a baseline value defined as the average of the two previous highest consecutive measurements taken 3 to 6 weeks apart at least 3 months after lung transplantation (Table II).[20]

Recipients with OB were divided into four groups on the basis of clinical status and method of diagnosis (Tables II and III). OB in group 1 recipients was identified only by surveillance TBBx when no symptoms or pulmonary function changes were present. OB in group 2 recipients was identified by TBBx after a new pulmonary function abnormality was present. OB in group 3 recipients was identified by TBBx when symptoms and pulmonary function changes were present. OB in group 4 recipients was identified by methods other than TBBx.

*Acute rejection.* Acute rejection was defined by histologic criteria.[19] Only histologically confirmed episodes that occurred before the development of OB were included in this analysis.

*Bacterial pneumonia.* Bacterial pneumonia was defined by histologic or clinical criteria. Clinical criteria included fever, new or increased radiographic infiltrates, and bacteria recovered from the allograft in a sputum or bronchoalveolar lavage specimen sufficient to warrant a full course of antibiotic therapy. Episodes occurring in the first 2 months after transplantation were analyzed separately from those occurring later.

*CMV illness.* CMV infection was defined by the presence of CMV in a culture obtained from any body site in the absence of symptoms of CMV infection, histologic evidence of CMV disease, rejection, or the isolation of any other infectious pathogen. CMV disease was defined by a positive culture for CMV plus the presence of intracellular inclusions typical of CMV in cells or tissue obtained from any body site. CMV syndrome was defined by the presence of CMV in a culture from any body site plus symptoms typical for CMV infection that were not related to rejection or isolation of any other infectious pathogens. Our five episodes of CMV syndrome have been included in the CMV infection group for the purpose of this analysis.

*Airway ischemia.* Airway ischemia was defined by the presence of intensely erythematous, friable, edematous airways in the allograft as seen bronchoscopically without evidence of airway infection. Such changes usually resolved within 14 to 21 days after transplantation.

**Table III.** *Methods used to detect OB*

| | *Period* | | |
|---|---|---|---|
| | *Early (1982-1987)* | *Late (1988-1992)* | *Total (1982-1992)* |
| Group 1 | 0 | 15 | 15 |
| Group 2 | 3 | 10 | 13 |
| Group 3 | 6 | 11 | 17 |
| Group 4 | 9 | 1 | 10 |
| Clinical | 3 | | |
| OLBx | 4 | 1 | |
| Autopsy | 2 | | |
| Total | 18 | 37 | 55 |

*Group 1,* Surveillance TBBx with no symptoms and stable pulmonary function; *group 2,* TBBx and decreased pulmonary function; *group 3,* TBBx and symptoms with or without decreased pulmonary function; *group 4,* diagnosis made by methods other than TBBx; *OLBx,* open lung biopsy.

*Adult respiratory distress syndrome.* Adult respiratory distress syndrome was defined clinically by the presence of radiographic infiltrates in the absence of volume overload, infection, or rejection or histologically by the presence of diffuse alveolar damage.

*Panel reactive antibody (PRA).* Pretransplantation serum samples were tested for complement-dependent lymphocytotoxic antibody activity against a 45 to 60 cell panel of donors typed by human leukocyte antigen (HLA) who were selected to represent most known HLA antigens.[21] These serum samples were tested with or without dithiotreitol, which inactivates immunoglobulin M antibodies and allows for detection of more significant immunoglobulin G antibodies.[22] A significant response was defined by the detection of lymphocytotoxic antibody activity in 10% or more of the cell panels.

**Immunosuppression.** Postoperative immunosuppression included azathioprine, corticosteroids, and cyclosporine or FK 506, as previously described[23] (Table I). Immediately before transplantation, azathioprine 4 mg/kg and methylprednisolone 5 mg/kg were administered intravenously. Either cyclosporine or FK 506 was begun after the operation by continuous infusion as soon as the recipient was in hemodynamically stable condition. Recipients were switched to oral preparations of these medications after extubation. The dose of cyclosporine was titrated to maintain a blood level of 800 to 1000 ng/ml by the Abbott Laboratories TDx method. The dose of FK 506 was titrated to maintain a blood level of 1.0 to 1.5 ng/ml by an enzyme-linked immunosorbent assay method. The dose of azathioprine was titrated to maintain a white blood cell count of about 5000/$mm^3$. Prednisone 10 to 15 mg/day was begun after the second episode of acute rejection.

The first episode and sometimes the second episode of acute rejection were treated with methylprednisolone 1 gm intravenously each day for 3 days. Intramuscular rabbit antithymocyte globulin 1.5 mg/kg per day for 5 days was administered sometimes for the second and always for any subsequent episodes of acute rejection.

OB was treated with the antilymphocyte agents, rabbit antithymocyte globulin 1.5 mg/kg per day for 5 days or

**Table IV.** *Effect of the method of diagnosis on outcome of OB*

| | *Group 1* | | *Group 2* | | *Group 3* | | *Group 4* | |
|---|---|---|---|---|---|---|---|---|
| *Outcome* | *No.* | *%* | *No.* | *%* | *No.* | *%* | *No.* | *%* |
| Resolved | 12 | 80 | 1 | 7 | 2 | 12 | 2 | 20 |
| Stable | 1 | 7 | 4 | 31 | 2 | 12 | 2 | 20 |
| Decline | 2 | 13 | 4 | 31 | 6 | 35 | 3 | 30 |
| Dead | 0 | 0 | 4 | 31 | 7 | 41 | 3 | 30 |
| Total | 15 | 100 | 13 | 100 | 17 | 100 | 10 | 100 |

*Resolved,* Without histologic active OB, stable forced expiratory volume in 1 second, and no symptoms; *Stable,* current stage is the same as it was at the time of diagnosis; *Decline,* current stage is higher than at the time of diagnosis.

Minnesota antilymphocyte globulin 10 to 15 mg/kg per day for 14 days, or with methylprednisolone 1 gm intravenously each day for 3 days. Response to treatment was assessed by histologic examination, symptoms, and forced expiratory volume in 1 second (Table II). The process was considered "resolved" if no symptoms or pulmonary function changes were present (persistent stage 0) and follow-up TBBx revealed inactive or no OB (Table IV). The process was considered *stable* if symptoms, pulmonary function, or histologic findings remained unchanged after treatment. *Continued decline* was defined as increased symptoms and further deterioration in pulmonary function resulting from histologically active OB despite treatment.

**Assessment of treatment.** The function of the allograft was assessed by history, physical examination, chest radiography, arterial blood gases, spirometry, and fiberoptic bronchoscopy with bronchoalveolar lavage and TBBx every 3 months during the first year after transplantation, every 4 months during the second year, twice a year thereafter, and whenever infection or rejection was suspected. The response to treatment for OB and acute rejection was assessed with the aforementioned studies 4 to 6 weeks after the administration of methylprednisolone and 6 to 8 weeks after the administration of antilymphocytic agents.

**Statistical analysis.** Possible risk factors (Table V) for development of OB were analyzed by univariate ($\chi^2$) and Cox multivariate analysis for our early (1982 to 1987), late (1988 to 1992), and overall experience (1982 to 1992) (Table VI).[24] Computations were performed with SPSS (SPSS Inc., Chicago, Ill.) and BMDPIL programs (BMDP Software, Los Angeles, Calif.). Actuarial freedom from OB for each risk factor was computed by life table analysis and compared by the generalized Wilcoxon test (Figs. 1 to 3).[25] Changes in the severity of OB from the time of diagnosis to the present (April 30, 1993) (Fig. 4) were compared for each diagnostic group as outlined in Table III using the Wilcoxon signed rank test, and comparisons among the four diagnostic groups were made by the Kruskal-Wallis statistic.[26] The impact of treatment on outcome in each diagnostic group was compared by $\chi^2$ analysis (Table IV). A $p$ value of 0.05 or less was considered significant.

**Table V.** *Possible risk factors for the development of OB*

| | |
|---|---|
| *A: Indices analyzed in early (1982-1987), late (1988-1992), and overall (1982-1992) experience* | |
| Recipient | Age |
| | Gender |
| | Date of transplantation |
| | Underlying disease |
| | Type of transplant procedure |
| | Requirement for CPB |
| | ABO blood type |
| | CMV serology |
| | Immunosuppression (cyclosporine or FK 506) |
| Donor | Age |
| | Gender |
| | Ischemic time |
| | ABO blood type |
| | CMV serology |
| Donor-recipient match | Gender |
| | ABO blood type |
| | CMV |
| Complications after transplantation | ARDS/DAD |
| | Airway ischemia |
| | Bacterial pneumonia (early and late) |
| | CMV infection |
| | CMV disease |
| | *Pneumocystis* |
| | PTLD |
| | Acute rejection (frequency) |
| | Acute rejection (severity) |
| *B: Additional indices analyzed in late (1988-1992) experience* | |
| Donor-recipient match | HLA-A, B, DR |
| Recipient | PRA |

*CPB,* cardiopulmonary bypass; *ARDS,* adult respiratory distress syndrome; *DAD,* diffuse alveolar damage; *PTLD,* posttransplantation lymphoproliferative disease.

## Results

**Risk factors for OB.** In our overall experience, airway ischemia, CMV disease, one episode or more of moderate to severe acute rejection (grade III/IV), and three episodes or more of grade II (or greater) acute rejection were identified as significant risk factors for the development of OB by univariate analysis (Table VI). However, by multivariate analysis, only one risk factor—three episodes or more of grade II (or greater) acute rejection—was associated with the development of OB. In our early but not late experience, CMV disease, late bacterial pneumonia, and *Pneumocystis* infection were associated with development of OB by univariate analysis only. For the late experience group, when HLA matching and PRA data were available, fewer HLA-DR ($\leq$1) and HLA-B+DR ($\leq$2) mismatches

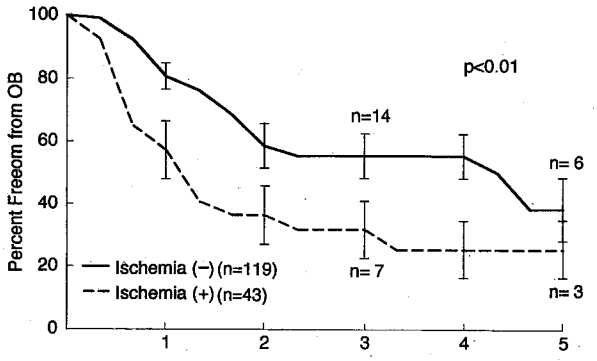


**Fig. 1.** The impact of airway ischemia on freedom from the development of OB between 1982 and 1992. OB was more likely to develop in recipients with airway ischemia than in those without airway ischemia ($p < 0.01$ by generalized Wilcoxon test).

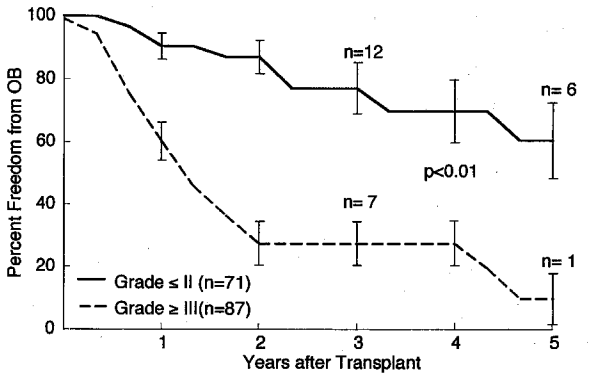


**Fig. 2.** The impact of grade III (or greater) acute rejection on freedom from the development of OB between 1982 and 1992. OB was more likely to develop in recipients with one episode or more of grade III (or greater) acute rejection than in those with less severe (≤ grade II) acute rejection ($p < 0.01$ by generalized Wilcoxon test).

**Table VI.** *Significant risk factors for the development of OB after lung transplantation*

| | *p Value* | | | | | |
|---|---|---|---|---|---|---|
| | *Early period (1982-1987)* | | *Late period (1988-1992)* | | *Entire period (1982-1992)* | |
| *Risk factors for OB* | *Univariate* | *Multivariate* | *Univariate* | *Multivariate* | *Univariate* | *Multivariate* |
| Airway ischemia | 0.007 | NS | 0.003 | NS | 0.004 | NS |
| Late bacterial pneumonia | 0.02 | NS | NS | NS | NS | NS |
| CMV disease | 0.002 | NS | NS | NS | 0.0006 | NS |
| *Pneumocystis* | 0.02 | NS | NS | NS | NS | NS |
| Acute rejection ≥ 3 times | 0.00001 | 0.009 | 0.00001 | 0.00001 | 0.00001 | 0.00001 |
| Acute rejection grade III/IV | 0.0002 | 0.033 | 0.00004 | 0.01 | 0.0001 | NS |
| HLA-DR ≤ 1 mismatch | ND | ND | 0.08 | NS | ND | ND |
| HLA-B + DR ≤ 2 mismatches | ND | ND | 0.05 | NS | ND | ND |
| PRA ≥ 10% | ND | ND | 0.007 | NS | ND | ND |

*NS,* Not significant; *ND,* no data.

and a PRA activity of 10% or more were identified as risk factors for OB in the univariate analysis alone. None of the other indices listed on Table V correlated with the subsequent development of OB.

**Time-related risks for development of OB.** The risk of OB developing was greatest in the first 2 years after transplantation (Fig. 5). By 2 years after transplantation the likelihood of a recipient having OB was 47%. Thereafter, the risk of OB developing diminished but did not cease and the likelihood of the patient having OB at 5 years after transplantation increased to 63%. By 5 years after transplantation, 75% of recipients with early posttransplantation airway ischemia had OB whereas only 60% of recipients without airway ischemia had OB ($p < 0.01$) (Fig. 1). By 5 years after transplantation, 90% of recipients with one episode or more of grade III (or greater) acute rejection had OB, whereas only 40% of recipients with grade II (or less) acute rejection had OB ($p < 0.01$) (Fig. 2). Similarly, 95% of recipients who had three or more episodes of grade II (or greater) acute rejection had OB, whereas only 18% of recipients with two or fewer episodes had OB during 5 years of follow-up (Fig. 3).

**Impact of surveillance TBBx on outcome of OB.** The overall prevalence of OB was 34% (55/162 at risk recipients) (Table II). Concomitant grade II (or greater) acute rejection was present in 11 recipients (20%) when OB was first detected. All episodes of acute rejection resolved with the pulse of augmented immunosuppression used to treat the OB.

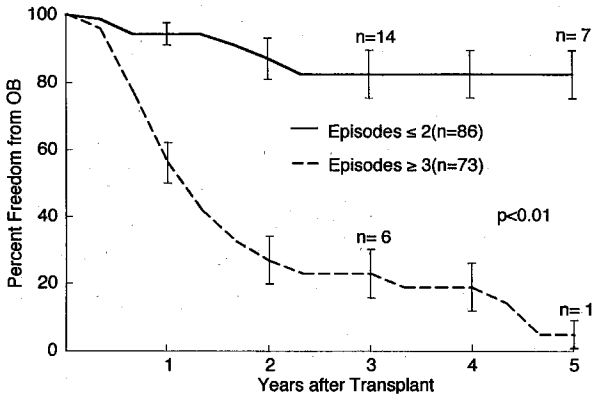


**Fig. 3.** The impact of three episodes or more of grade II acute rejection on development of OB between 1982 and 1992. OB was more likely to develop in recipients with more frequent episodes of acute rejection than in those with fewer episodes of acute rejection ($p < 0.01$ by generalized Wilcoxon test).

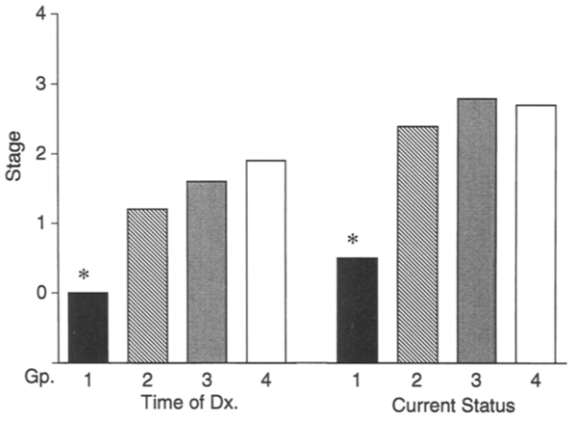


**Fig. 4.** The severity of OB as assessed by the staging categories in Table II at the time of diagnosis and at the time of this analysis (April 30, 1993). *Dx,* Diagnosis; The mean stage for group 1 recipients was significantly lower than that for recipients in groups 2 to 4 (* $p < 0.05$ by Kruskal-Wallis statistic.)

In our early experience, only 50% (9/18) of our recipients with OB were identified by TBBx (groups 2 and 3) and none were identified by surveillance TBBx (group 1) (Table III). In our recent experience, 97% (36/37) of recipients with OB were identified by TBBx and 15 were identified only by surveillance TBBx (group 1).

In the 15 group 1 recipients, which included two recipients with concomitant acute rejection, the OB resolved or stabilized in 87% (13/15) of the affected recipients after treatment with augmented immunosuppression. Stabilization or resolution of the OB, however, occurred in only 38% (5/13) of group 2 (3 with concomitant acute rejection), 24% (4/17) of group 3 (4 with concomitant acute rejection), and 40% (4/10) of group 4 (2 with concomitant acute rejection) recipients. Although 30% to 40% of the affected recipients in groups 2 to 4 have died of OB, none of the affected recipients in group 1 have died of this process ($p < 0.05$ by $\chi^2$ analysis) (Table IV).

By definition, recipients in group 1 were in stage 0 (Table II) at the time of diagnosis, whereas most recipients in groups 2 to 4 had experienced a decline in pulmonary function by the time OB was identified (Fig. 4). Thus the stage or severity of OB in group 1 recipients was significantly lower than in recipients in groups 2 to 4 at the time of diagnosis. More important, although the stage of all affected recipients increased despite treatment, the stage for group 1 recipients remained significantly lower than that of recipients in groups 2 to 4 at the time of this analysis.

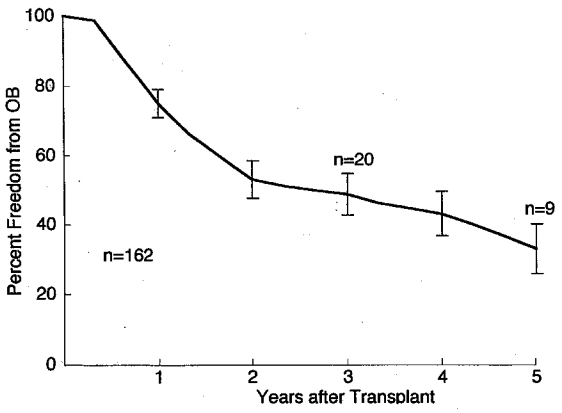


**Fig. 5.** Freedom from the development of OB between 1982 and 1992.

## Discussion

With a prevalence of 34% (55/162 at risk recipients) and a mortality rate of 25% (14/55 affected recipients), OB is the most significant long-term complication after pulmonary transplantation among our patients. Factors predictive of the development of OB by univariate analysis for the entire period (1982 to 1992) included more frequent and more severe acute rejection, CMV disease, and an airway ischemic injury early after transplantation (Table VI). By multivariate analysis, only three or more episodes of grade II (or greater) acute rejection correlated with the subsequent development of OB. Since HLA and PRA data became available in 1988,

fewer HLA-DR or HLA-B+DR mismatches and greater PRA activity were also associated with the development of OB by univariate but not by multivariate analysis. From our early experience when these infections were more prevalent, late bacterial pneumonia, CMV disease, and *Pneumocystis* infection also correlated with the subsequent development of OB by univariate analysis only. The factors that did not correlate with the development of OB were age at the time of operation, gender of donors and recipients and their mismatches, type of pretransplantation lung disease, type of transplant procedure, time of transplantation, need for cardiopulmonary bypass, ABO blood group, CMV serologic status and their mismatches, and postoperative complications of adult respiratory distress syndrome/diffuse alveolar damage, early bacterial pneumonia, CMV infection, and lymphoproliferative disease induced by Epstein-Barr virus.

These observations reinforce concepts regarding the pathogenesis of OB. The strong association between frequency and severity of acute rejection and the development of OB supports the concept that OB occurs as the result of immunologic injury directed against the epithelial cells of the airways. The association between airway ischemia and the subsequent development of OB supports the concept that OB can also result from an ischemic injury that may directly or indirectly alter expression of HLA antigens on airway epithelial cells, which then leads to immunologic attack.

An understanding of pathogenesis is the first step toward influencing natural history of a disease. The longer term goal of this analysis is to try to eliminate or modify risk factors for posttransplantation OB. Because acute rejection appears to be the most important predictor of OB in this study and another study,[14] perhaps more potent and sustained immunosuppression should be given in the early postoperative period to reduce the frequency and severity of acute rejection episodes. However, this might lead to more problems with infections. Because the improvement in early survival has come largely from a reduction in the prevalence of early infections, which is partly due to the administration of less immunosuppression, increasing immunosuppression could result in more infections and hence lower early survival. Nevertheless, this option might be warranted in combination with more aggressive monitoring and prophylaxis for infection. What we really need, however, are better immunosuppression agents—agents that selectively suppress allogenic influences while allowing intact humoral and cellular immunity to deal with invading microorganisms. Efforts to achieve organ tolerance via induction of a chimeric state between donor and recipient are encouraging steps in that direction.[27]

Airway ischemia has not previously been described as a risk factor for OB.[14] Because of the occlusive vascular lesions seen in the late rejection of heart,[28] liver,[29] kidney,[30] and lung[31, 32] allografts, it is not surprising that an ischemic airway injury might be associated with the development of OB. This ischemic injury in lung recipients most likely occurs from interruption of bronchial artery circulation at the time of transplantation. Perhaps it might be prevented by reanastomosis of these vessels to the recipient's intrathoracic artery[33] or ascending or descending thoracic aorta.[34]

Contrary to a previous study,[14] this and our previous studies[35, 36] indicate that there is an association between CMV disease and the subsequent development of OB. The common thread between these two events may be the immunomodulating effect of CMV in altering expression of HLA antigens on epithelial or endothelial cells rather than a direct effect of the virus itself.[37, 38] Gene products of CMV infection appear to block the ability of cyclosporine to inhibit interleukin-2 transcription.[39] Restoration of interleukin-2 production despite the presence of cyclosporine could result in normal T-cell function with resultant allograft rejection. Before the advent of strategies to prevent or treat CMV illness, the prevalence of CMV illness was 76% and the mortality was 27%.[40] Use of CMV-negative blood products for seronegative donors and recipients and of ganciclovir to prevent and treat CMV infection and disease in seropositive donors and recipients have decreased the prevalence of CMV illness to 54% and the mortality to 1%. In our more recent experience, since 1988, the relationship between CMV and OB has disappeared (Table VI). This change may be due to the beneficial effects of the aforementioned measures to prevent or modify CMV infection. Other infectious complications such as late bacterial pneumonia and *Pneumocystis* infection, which were significant risk factors for OB before 1988, were no longer associated with the development of OB in our more recent experience. This is also probably because the prevalence of these infections has decreased dramatically as a

result of the prophylactic use of antibiotics.[7, 8] These findings suggest that eliminating or modifying risk factors does indeed reduce the prevalence of OB.

Interestingly, fewer HLA-DR and HLA-B+DR mismatches were also associated with OB (Table VI). A similar association has also been observed in liver transplant recipients, in whom the relationship may be due to the permissive effect of HLA matching on CMV infection.[41, 42] Among lung recipients, HLA-DR matching between the donor and recipient does increase the prevalence of CMV disease ($p = 0.04$ by $\chi^2$ analysis, unpublished data). Thus perhaps HLA-DR–restricted immunologic mechanisms toward antigens including CMV somehow set the stage for a subsequent immunologic injury against the epithelial cells of the airway or bile duct.[43] If CMV is the "middle man" between HLA matching and OB, efforts to eliminate CMV infection should favorably affect the prevalence of OB.

Although recipients with a PRA activity of 10% or more would be expected to have more problems with early humoral rejection, they also appear to be at risk for OB. This has also been our experience with heart transplant recipients, in whom a PRA activity of 10% or more was associated with a greater risk of acute and chronic rejection.[21]

This study supports the concept that early diagnosis of OB by surveillance TBBx improves the response to treatment. Among recipients whose OB was detected by surveillance TBBx in the absence of symptoms or change in pulmonary function (group 1, Stage 0) (Tables III and II, respectively), 88% (13/15) are currently in remission with no symptoms, pulmonary function changes, or active OB by TBBx. When pulmonary function declined (group 2) or symptoms were present (group 3) before the diagnosis by TBBx, the likelihood of remission was much lower at 39% (5/13) and 24% (4/17), respectively (Table IV). Additionally, although none of the group 1 recipients have died of OB, four recipients (31%) in group 2 and seven recipients (41%) in group 3 have died of OB (Table IV). Because approximately equal numbers of recipients in each of the four groups had concomitant acute rejection at the time of the diagnosis of OB, the presence of concomitant acute rejection is not likely to be the reason for the better outcome of group 1 recipients.

Because all recipients with preclinical disease were treated with augmented immunosuppression, it is not possible to ascertain whether treatment affected long-term outcome. Perhaps clinical disease would never have developed in some or most of these recipients and treatment had no effect on long-term outcome. Nevertheless, because untreated OB is most likely a progressive process,[11, 44] our findings suggest that diagnosis and treatment of OB in the preclinical stage does improve long-term outcome.

Use of TBBx to detect OB remains controversial primarily because it appears to be underdiagnosed by this technique. Although we have successfully used TBBx to identify 82% (45/55) of our recipients with OB,[45, 46] others have been less successful.[47] There are several reasons why the diagnosis by TBBx may be difficult. First, it may be difficult to obtain adequate pieces of lung tissue when OB is well established and a lot of subepithelial and peribronchial fibrosis is present. Our success with TBBx may be the result of our trying to detect the process at its earliest stage, presumably before much fibrosis and scarring is present. Underdiagnosis may also occur because the lesions are patchy and TBBx may acquire too few pieces of lung tissue containing bronchioles. In this situation, only normal-appearing airways might be sampled whereas other un sampled affected airways might be nearby. On the basis of data to accurately detect acute rejection in dogs,[48] we consider six adequate-sized pieces of lung tissue to be the minimal sample required for a TBBx procedure. To reduce sampling error, we obtain each piece from a different segment of the lung. To ensure that tissue samples contain bronchioles, we obtain each piece under fluoroscopic localization between 1 to 2 cm from the pleura. Finally, the pathologist who examines TBBx specimens from three to four recipients per day is more likely to recognize OB than the pathologist who examines this tissue less frequently.

In summary, significant risk factors for the development of OB include airway ischemia, CMV disease, and especially more severe and frequent episodes of acute rejection. Inasmuch as this disorder is most likely immunologic in origin, advances in transplant immunology leading to tolerance between donor and recipient,[27] as well as efforts to prevent CMV illness[9, 10, 49] and airway ischemic injury,[34] will likely be useful preventative measures. Surveillance TBBx offers the opportunity to recognize OB in the preclinical stage, and this early recognition appears to improve response to treatment with augmented immunosuppression.

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## Discussion

**Dr. Bruce A. Reitz** *(Stanford, Calif.).* Dr. Bando and his colleagues bring to our attention an important topic, because OB is the leading factor limiting the late results of lung transplantation, just as coronary artery disease limits the late results of heart transplantation.

This is the largest series of cases of lung transplantation to be presented to date, with a careful analysis of the risk factor for OB. Its findings are consistent with smaller reported series from the groups at Papworth and Stanford.

At Stanford, in 137 patients with various types of lung transplants since 1981, OB has been detected with an almost identical incidence and has resulted in death in 10 patients. We also have noted a decline in the incidence of OB with the introduction of triple-drug therapy for immunosuppression and with more frequent use of TBBx for detection and earlier treatment.

I have several questions. The immunosuppressive regimen at Pittsburgh in the most recent years has included FK 506. Were there any trends suggesting that OB was less prevalent in these patients?

**Dr. Bando.** Regarding the impact of FK 506 on the incidence of OB, we recently started a randomized clinical trial comparing cyclosporine and FK 506 for pulmonary transplantation. The preliminary results suggested that the FK 506 resulted in less acute rejection during the first 6 months after pulmonary transplantation (Griffith BP, Bando K, Hardesty RL, et al. Prospective randomized

trial of FK 506 versus cyclosporine after human pulmonary transplantation. Transplantation 1994;57:848-51). Because the frequency and severity of acute rejection are the most significant risk factors for the development of OB, FK 506 use might result in less OB. However, the duration of follow-up is still too short to make any definite statement. We will continue to monitor the results of this randomized trial and hope to be able to present the impact of FK 506 on the development of OB in the future.

**Dr. Reitz.** The Pittsburgh team has performed a number of studies of the cells recovered at bronchioalveolar lavage. Have there been any patterns that might confirm the rejection etiology for OB?

**Dr. Bando.** Our transplant pathology group has extensively studied the significance of donor-specific alloreactivity as assessed by the primed lymphocyte test of cells obtained from the allograft by bronchoalveolar lavage. Although the presence of significant donor-specific alloreactivity does correlate with the presence of acute and chronic rejection, the assay is not sufficiently sensitive and specific for it to be clinically useful.

**Dr. Reitz.** The unexpected findings of a greater incidence of OB in those patients with fewer mismatches is contrary to what might be anticipated. Could you comment further on this?

**Dr. Bando.** I was also puzzled by these results and I discussed these results with liver transplant surgeons and immunologists. Surprisingly, a similar association has also been observed in liver transplant recipients where the relationship may be due to the permissive effect of HLA matching on CMV infection. Among lung recipients, HLA-DR matching between the donor and recipient increases the incidence of CMV disease. Thus perhaps HLA-DR–restricted immunologic mechanisms toward antigens including CMV somehow set the stage for a subsequent immunologic injury against the epithelial cells of the airway. Thus "CMV" might play a role as a "middleman" between HLA matching and OB.

**Dr. Reitz.** Looking ahead, are you considering other measures that might influence the late development of this complication?

**Dr. Bando.** Inasmuch as OB is most likely immunologic in origin, advances in transplant immunology leading to tolerance between donor and recipient, such as mixed chimerism, which is currently being extensively investigated by Dr. Starzl's group, may have a major impact on decreasing the prevalence of this serious late complication. Efforts to prevent CMV illness and disease and airway ischemic injury will also with be useful preventive measures. Our observation that CMV disease is no longer a risk factor for the development of OB in parallel with a decreasing prevalence of CMV illness and disease suggest that elimination or modifying risk factors can decrease the likelihood of OB developing.