CARDIOTHORACIC TRANSPLANTATION

SURVEILLANCE TRANSBRONCHIAL LUNG BIOPSIES: IMPLICATION FOR SURVIVAL AFTER LUNG TRANSPLANTATION

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Objectives: **We wished to determine whether early rejection after lung transplantation as assessed by surveillance transbronchial biopsy predicts for survival.** ***Methods:*** **Between 1990 and 1997, 96 consecutive patients had lung transplantation: 89 had a minimum 1-month follow-up. For 71 consecutive patients we have 1-year follow-up and for 69 patients we have the results of the first 3 biopsies. Cytomegalovirus status, bronchiolitis obliterans prevalence, and use of total lymphoid irradiation are noted. Biopsies were done at 1 week and 1, 3, and 6 months. Standard immunosuppression consisted of induction antilymphocyte globulin and high-dose methylprednisolone induction for 1 week and standard maintenance triple therapy. Acute rejection treatment was with pulse methylprednisolone. Bronchiolitis obliterans syndrome was treated with total lymphoid irradiation and a change to tacrolimus and mycophenolate. Blinded grading using International Society for Heart and Lung Transplantation classification was done retrospectively.** ***Results:*** **Survival at 1 month and 1, 2, and 3 years for the 96-patient cohort with 1-year follow-up was 93%, 74%, 62%, and 56%. Survival was not significantly different for subsets with rejection on any combination of the first 3 biopsies (1/3, 2/3, 3/3) or absence of rejection on the first 3 biopsies. Ninety-one positive biopsy results were graded. Eighteen of 71 patients had one or more moderate or severe rejection episodes without survival difference relative to the others. There was no statistically significant association between acute rejection on the first 3 surveillance biopsy results and bronchiolitis obliterans.** ***Conclusions:*** **Intensive induction and maintenance immunotherapy with surveillance transbronchial biopsies and aggressive treatment of acute rejection is associated with a survival similar to that of patients without early acute rejection. This regimen appears to uncouple the association between early acute rejection and bronchiolitis obliterans. Further study may elucidate this mechanism. (J Thorac Cardiovasc Surg 2000;119:27-38)**

Lung transplantation has evolved over the past 15 years to become recognized treatment for various end-stage respiratory conditions including cystic fibrosis, pulmonary hypertension, pulmonary fibrosis, and emphysema.[1] The perioperative issues including lung preservation, airway healing, acute bacterial and viral infections, and immunosuppression have been resolved to the extent that early and 1-year mortality rates are quite reasonable (95% and 76%, respectively).[2] However, long-term survival after lung transplantation

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remains unsatisfactory with a 5-year survival of 40% reported in the most recent United Network for Organ Sharing annual report.[2] The primary reason for attrition is bronchiolitis obliterans, the pathologic hallmark of chronic lung rejection. Once this diffuse fibrotic process has developed, patients become quite dyspneic, and no therapy to date has succeeded in reversing the clinicopathologic condition. Most patients will die of either respiratory insufficiency or superimposed infection. Predictors of bronchiolitis obliterans, and therefore ultimately of patient death, have been identified by several investigators. Bando and colleagues[3] in Pittsburgh noted that airway ischemia, cytomegalovirus disease, and either 1 or more episodes of moderate to severe acute rejection (grade 3/4) or 3 or more episodes of grade 2 (or greater) acute rejection were significant risk factors for the development of bronchiolitis obliterans by univariate analysis. In their multivariate analysis, only the finding of 3 or more episodes of grade 2 (or greater) acute rejection was associated with the development of bronchiolitis obliterans.

The role of transbronchial biopsies in providing histologic confirmation of acute lung rejection is well established.[4-6] The utility of routine surveillance transbronchial biopsies particularly in the late postoperative period has been debated.[6-9] The morbidity of the procedure is relatively low, but whether it provides clinically relevant information is unclear. Certainly the information obtained must be interpreted carefully. Immediately after the transplant, pathologic changes related to the implantation, including the ischemia-reperfusion process, are typically seen. If there are signs of infection, it may be difficult to diagnose rejection. Furthermore, in this era of cost awareness, the expense of the biopsies must be considered.

Our study was designed to look at the potential predictive value for survival of the first 3 surveillance transbronchial biopsies after lung transplantation. This was a retrospective analysis of data collected in our thoracic surgery database.

Methods

We performed 96 consecutive single or double (sequential bilateral) lung transplants between 1990 and 1997. The transplants were done by standard techniques including telescoping and wrapping of the airway anastomosis.[1] Cardiopulmonary bypass was used when necessary to support the patient during implantation.

The routine immunosuppressive regimen at our institution includes induction with either Minnesota antilymphocyte globulin or antithymocyte gamma globulin (ATGAM, Pharmacia Upjohn, Kalamazoo, Mich) for 1 week and high-dose methylprednisolone (1 gm intravenously intraoperatively before opening the circulation to the transplanted lung, then 125 mg intravenously 3 times a day for 1 week). The antithymocyte gamma globulin is administered at a dosage of 15 mg/kg and decreased to 10 mg/kg if the total lymphocyte count falls below 100/mm^3 or the platelet count falls below 50,000/mm^3. Triple-drug maintenance therapy consists of prednisone 60 mg/day beginning at 1 week and tapering to 20 mg/day over 12 weeks, azathioprine 50 mg twice a day adjusted for leukopenia (white blood cell count <4000/mm^3) beginning on postoperative day 7, and cyclosporine A (INN: ciclosporin) begun the night of the operation by intravenous infusion and converted to the oral route with target levels of 300 to 350 ng/mL. Rejection episodes at our institution are treated with pulse methylprednisolone (1 gm intravenously daily for 3 days). Over the past 2 years, maintenance therapy has been changed to mycophenolic acid and tacrolimus after 2 moderately severe or severe episodes. In addition, those patients in whom bronchiolitis obliterans syndrome or bronchiolitis obliterans developed, as noted in Tables I and II, were treated with total lymphoid irradiation (0.4 Gy twice a day 2 times per week for 5 weeks; total dose 8 Gy using inverted Y and pelvic ports).

Transbronchial biopsies during the first year after the transplant were done on a standard schedule as well as when there was a decline in pulmonary function in the absence of diagnosed infection. We took 5 to 6 biopsy samples per procedure (≥100 alveoli)[10] and focused our attention on the lower and middle lobes on the right and the lingula and lower lobe on the left. For the first 24 patients, biopsies were done at 1 week, 1 month, 3 months, 6 months, and 12 months from the time of transplantation. After these first patients, the surveillance transbronchial biopsies were done at 1 month, 3 months, 6 months, and 12 months. The 1-week biopsy was omitted because in most cases the changes observed were related to the implantation.

Cytomegalovirus prophylaxis was used for any patient in whom a donor-recipient mismatch existed or if both the donor and recipient had positive cytomegalovirus status (n = 44, see Tables I and II). In those cases, ganciclovir was given intravenously for 1 year: an induction dose (5 mg/kg twice a day) for the first month followed by maintenance therapy (5 mg/kg daily) for the last 11 months.

Our prospective thoracic database was reviewed in addition to patient charts and pathology and microbiology records to capture morbidity and mortality data and important clinical events such as infection, decline in pulmonary function, and the results of transbronchial biopsies. In a blinded fashion, one of us (L.K.) reviewed the transbronchial biopsy specimens for verification: the data are reported according to the International Society for Heart and Lung Transplantation (ISHLT) revised grading schema.[10]

Bronchiolitis obliterans syndrome was defined according to the ISHLT definition.[11] The presence of bronchiolitis obliterans was determined by transbronchial biopsy.

There were 96 consecutive patients who had lung transplantation and had a potential minimum follow-up of 1 year. This interval was chosen to focus on the association between

Table I. *Patients (n = 32) with evidence of rejection on 1-month biopsy*

Patient	*Status*	*Survival days from TX*	*1-Mo biopsy*	*3-Mo biopsy*	*6-Mo biopsy*	*OB*	*TLI*	*CMV status, recipient/donor, pre-TX*	*CMV disease post-TX*
4	D	714	Pos	Neg	Pos	Pos	No	Neg/Neg	Neg
5	D	591	Pos	Pos	Pos	Neg	No	Pos/Neg	Neg
7	A	3088	Pos	Neg	Neg	Pos	No	Neg/Neg	Neg
8	D	806	Pos	Neg	Neg	Neg	Yes	Neg/Neg	Neg
14	D	1852	Pos	Pos	Neg	Neg	No	Neg/Pos	Pos
18	A	2351	Pos	Pos	Pos	Neg	Yes	Neg/Pos	Neg
21	D	513	Pos	Neg	Neg	Neg	No	Neg/Pos	Neg
24	A	2115	Pos	Pos	Neg	Pos	No	Neg/Neg	Neg
26	D	1056	Pos	Pos	Pos	Neg	Yes	Neg/Pos	Neg
28	A	1879	Pos	Pos	Pos	Neg	Yes	Neg/Neg	Neg
31	A	1641	Pos	Neg	Neg	Pos	No	Neg/Neg	Neg
33	A	1454	Pos	Neg	Neg	Pos	No	Neg/Pos	Neg
34	A	1604	Pos	Pos	Pos	Neg	Yes	Neg/Neg	Neg
37	A	1589	Pos	Neg	Pos	Pos	No	Neg/Pos	Neg
40	A	1548	Pos	Neg	Pos	Pos	Yes	Neg/Pos	Neg
41	D	536	Pos	Pos	Pos	Neg	No	Pos/Pos	Neg
42	A	1518	Pos	Pos	Pos	Pos	Yes	Pos/Pos	Neg
44	A	1473	Pos	Neg	Pos	Pos	No	Neg/Pos	Neg
46	A	1440	Pos	Neg	Neg	Pos	No	Neg/Neg	Neg
47	A	1405	Pos	Pos	Pos	Pos	No	Neg/Pos	Neg
48	A	1404	Pos	Neg	Pos	Neg	No	Pos/Neg	Neg
50	D	444	Pos	Neg	Neg	Neg	No	Neg/Pos	Neg
51	A	1308	Pos	Pos	NA	Neg	Yes	Neg/Pos	Neg
54	D	1249	Pos	Neg	Neg	Neg	No	Neg/Neg	Neg
55	A	1248	Pos	Pos	Pos	Pos	No	Neg/Neg	Neg
59	A	1150	Pos	Neg	Neg	Pos	No	Neg/Neg	Neg
61	A	1121	Pos	Neg	Neg	Pos	No	Neg/Pos	Neg
64	A	882	Pos	Pos	Pos	Pos	No	Neg/Pos	Neg
65	A	831	Pos	Pos	Pos	Pos	No	Neg/Neg	Neg
67	A	781	Pos	Pos	Neg	Pos	No	Neg/Pos	Pos
68	A	750	Pos	Neg	Pos	Pos	No	NA/NA	Neg
69	A	746	Pos	Pos	Neg	Pos	No	NA/NA	Neg

Data as of April 1999. *TX*, Transplantation; *OB*, bronchiolitis obliterans; *TLI*, total lymphoid irradiation; *CMV*, cytomegalovirus; *D*, dead; *Pos*, positive; *Neg*, negative; *A*, alive; *NA*, not available.

early events and long-term outcome: bronchiolitis obliterans and survival. These 96 patients make up the cohort for this report. Seventy-one patients survived 1 year and make up the subset for the bronchiolitis obliterans analysis.

Survival was tabulated by a Kaplan-Meier life table,[12] and statistical analysis was done by log-rank test. The JMP program (SAS Institute, Inc, Cary, NC) was used for the computations and graphic display.

Results

The descriptive profiles, indications, and perioperative results for the 96 consecutive patients who received transplants during the study period, which included an appropriate time interval to allow for at least 1 year of follow-up, are displayed in Table III.

Seventy-one (74%) of the 96 patients survived 1 year and therefore make up the subset for our analysis. The minimum 1-year survival helps to focus the analysis on those patients most likely to have the development of chronic rejection. Analysis of results in the patients who did not survive 1 year demonstrates there was no difference in the incidence of acute rejection in this group (n = 25) versus that in those 71 patients who survived 1 year (P = .40).

Median follow-up was 40 months (range 12-100 months). Sixty-nine patients had 3 early biopsies (≤6 months), and 2 patients only had 1. In 32 patients acute rejection was demonstrated on the 1-month transbronchial biopsy. The results of the first 3 transbronchial biopsies in this group along with the survival and absence or presence of bronchiolitis obliterans and cytomegalovirus disease are shown in Table I. The actuarial survival for those patients with rejection demonstrated on the 1-month biopsy versus those with-

Table II. *Patients (n = 39) without evidence of rejection on 1-month biopsy including 17 with no evidence on first 3 biopsies*

Patient	Status	Survival days from TX	1-Mo biopsy	3-Mo biopsy	6-Mo biopsy	TLI	CMV status, recipient/donor, pre-TX	CMV disease post-TX
1	D	1188	Neg	Neg	Neg	No	Neg/Neg	Neg
2	D	2024	Neg	Neg	Neg	No	Neg/NA	Neg
3	D	1070	Neg	Neg	Neg	No	Neg/Pos	Neg
6	A	3197	Neg	Neg	Pos	No	Neg/Neg	Neg
9	D	1524	Neg	Pos	Neg	No	Neg/Neg	Neg
10	A	2820	Neg	Neg	Pos	Yes	Neg/Neg	Neg
11	D	778	Neg	Pos	Pos	No	Neg/Neg	Neg
12	D	1439	Neg	Pos	Pos	No	Neg/Neg	Pos
13	D	2633	Neg	Neg	Neg	Yes	Neg/Neg	Neg
15	D	1230	Neg	Pos	Neg	Yes	Neg/Pos	Neg
16	D	906	Neg	Neg	Pos	No	Neg/Neg	Neg
17	D	725	Neg	Pos	Pos	No	Neg/Neg	Neg
19	D	339	Neg	Neg	Neg	No	Neg/Pos	Neg
20	A	2327	Neg	Pos	NA	No	Neg/Neg	Neg
22	A	2204	Neg	Neg	Pos	No	Neg/Pos	Neg
23	D	2137	Neg	Pos	Neg	Yes	Neg/Neg	Neg
25	D	1991	Neg	Neg	Pos	Yes	Neg/Neg	Neg
27	D	810	Neg	Neg	Neg	No	Neg/Neg	Neg
29	A	1699	Neg	Neg	Pos	No	Neg/Pos	Neg
30	A	1684	Neg	Neg	Neg	Yes	Neg/NA	Neg
32	D	380	Neg	NA	NA	No	Pos/Neg	Neg
35	D	1006	Neg	Pos	Pos	Yes	Pos/NA	Neg
36	A	1596	Neg	Neg	Neg	No	Neg/Pos	Neg
38	D	445	Neg	Pos	Neg	No	Neg/Neg	Neg
39	A	1576	Neg	Pos	Pos	Yes	Neg/Neg	Neg
43	D	365	Neg	Neg	Neg	No	Neg/Pos	Pos
45	A	1447	Neg	NA	NA	No	Neg/NA	Neg
49	A	1402	Neg	Pos	Pos	Yes	Neg/Pos	Neg
52	A	1280	Neg	Neg	Neg	No	Pos/Neg	Neg
53	A	1252	Neg	Pos	Neg	No	Neg/Pos	Neg
56	A	1220	Neg	Pos	Pos	Yes	Pos/Pos	Neg
57	D	638	Neg	Neg	Neg	No	Neg/Pos	Neg
58	A	1185	Neg	Pos	Pos	No	Neg/Pos	Pos
60	A	1125	Neg	Neg	Pos	No	Neg/Pos	Neg
62	D	943	Neg	Pos	Pos	No	Neg/Pos	Neg
63	A	942	Neg	Pos	Pos	No	Neg/Pos	Neg
66	A	806	Neg	Pos	Neg	No	Pos/Pos	Neg
70	A	739	Neg	Neg	Neg	No	NA/NA	Neg
71	D	416	Neg	Pos	Neg	No	NA/NA	Neg

Data as of April 1999. *TX*, Transplantation; *TLI*, total lymphoid irradiation; *CMV*, cytomegalovirus; *D*, dead; *Pos*, positive; *Neg*, negative; *A*, alive; *NA*, not available.

out (n = 39) is graphed in Fig 1. There was no statistical difference in survival by log-rank test ($P = .15$).

Table II displays the characteristics, including the prevalence of bronchiolitis obliterans, cytomegalovirus disease status, and patient survival, for the 39 patients who did not show rejection on the 1-month biopsy and includes the 17 patients who did not show any evidence of rejection on the first 3 consecutive biopsies. Fig 2 displays a survival graph for those who had no rejection on the 3-month biopsy (n = 35) versus those who did have rejection on the 3-month biopsy (n = 34). Again, there was no statistical difference in survival by log-rank test ($P = .74$). Fig 3 shows the survival of those patients who showed acute rejection on the 6-month biopsy (n = 34) versus those who did not (n = 33). Again, no statistically significant difference was measured by log-rank test ($P = .10$).

Two hundred nine transbronchial biopsy samples were examined by our pathologists. More than 95% of these early biopsies were done for surveillance or to follow up pulse therapy. All biopsy samples were included in the analysis. Of the 209, 91 were interpreted as positive for acute rejection. This represented acute rejection occurring in 52 (73%) of 71 patients

during the first 6 months after lung transplantation. Table IV displays the data on these patients with use of the ISHLT revised grading system. Two of 71 patients had 2 or more moderate (A3 or B3) or severe (A4 or B4) episodes of acute rejection on the first 3 biopsies. Two of 71 patients had severe acute (A4 or B4) rejection on at least 1 of the first 3 biopsies. Moreover, 5 (7%) of 71 patients had 2 or more episodes of mild (A2) rejection.

Analysis looking at 1 or more episodes of A3 rejection or multiple A2 rejections demonstrated that they did not predict the development of bronchiolitis obliterans. The explanation for this other than a β statistical error may be again that we saw a relatively low incidence of A3 rejection events or of 2 or more A2 rejection events. We identified only 7% of our patients in whom multiple A2 rejections developed and 13% of our patients who had a single A3 episode. No patients had more than 1 A3 episode.

Thirty-four patients (34/71, 48%) showed development of either pathologically confirmed bronchiolitis obliterans or clinically defined bronchiolitis obliterans syndrome at a median follow-up of 40 months. Their survival at 5 years was 36% (±9%) versus the 72% (±10%) survival for those patients in whom bronchiolitis obliterans or bronchiolitis obliterans syndrome did not develop (n = 37). This difference in survival was highly significant by log-rank test ($P = .008$). Log-rank analysis demonstrated no significant association between early acute rejection and the incidence of bronchiolitis obliterans for those patients who showed rejection on the 1-month biopsy (Fig 4; n = 32, $P = .31$), on the 3-month biopsy (n = 34, $P = .18$), or on the 6-month biopsy (n = 34, $P = .65$). Also, there was not a significant association between grade of rejection (patients with at least 1 episode of moderate or severe rejection, n = 18) and bronchiolitis obliterans/bronchiolitis obliterans syndrome ($P = .68$).

In 5 of the 71 patients who survived 1 year, cytomegalovirus disease developed (Tables I and II). Twenty-one patients were treated with total lymphoid irradiation for bronchiolitis obliterans syndrome or bronchiolitis obliterans as noted in Tables I and II.

The morbidity related to transbronchial biopsies included 5 cases of pneumothorax necessitating chest tubes on 2 occasions. Bleeding that necessitated observation occurred in fewer than 5% of the 209 biopsies and in one case the bleeding was severe.

Table III. *Transplant cohort (N = 96): perioperative profile*

Sex	
Male	45
Female	51
Age (y)	
Median	46.5
Range	16-68
Mortality*	
Percent	7
No.	7/96
LOS (d)	
Median	22.5
Range	7-162
Transplant	
Single	
Right	37
Left	32
Double	27
Indication	
Emphysema	47
Cystic fibrosis	20
IPF	14
PPH	10
Other	5
Actuarial survival (%)	
One month	93
One year	74
Two years	62
Three years	56
Five years	40
Follow-up (mo)	
Median	40
Range	1-100

LOS, Length of stay; *IPF*, idiopathic pulmonary fibrosis; *PPH*, primary pulmonary hypertension.
*Mortality is 30-day or in-hospital, whichever was longer.

Discussion

Bronchiolitis obliterans remains the major limitation to the long-term success of lung transplantation.[13,14] Experimental work would suggest it is an immune-mediated phenomenon.[15,16] Other investigators have drawn a link between acute rejection and bronchiolitis obliterans and therefore survival.[3,14,17,18]

In this study we did not find an association between acute rejection and bronchiolitis obliterans. Another published study from Washington University in St Louis also did not observe a statistically significant connection between early acute rejection and bronchiolitis obliterans.[19] In that study 94 (84%) of 112 patients survived at least 3 months and formed the basis for their analysis. They found that in 54 of the patients bronchiolitis obliterans did not develop and in 40 patients bronchiolitis obliterans did develop but that there was no statistically significant difference between the number of acute rejection episodes occurring within 90 days of the transplantation for those 2 groups ($P = .43$).

The possible explanations as to why the group from Washington University and our group did not find a link

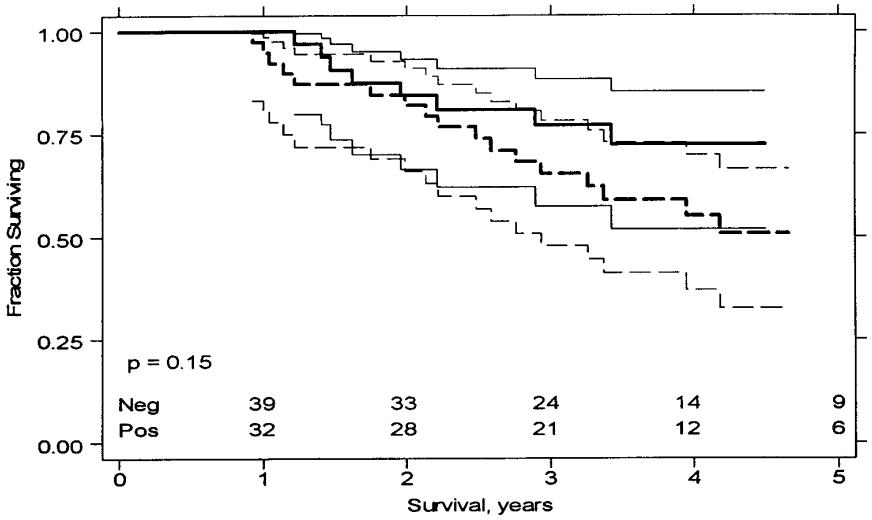

Fig 1. Kaplan-Meier survival curves (95% CI) for 32 patients in whom the 1-month transbronchial biopsy result showed rejection (*solid lines*) versus 39 patients in whom it did not (*dashed lines*).

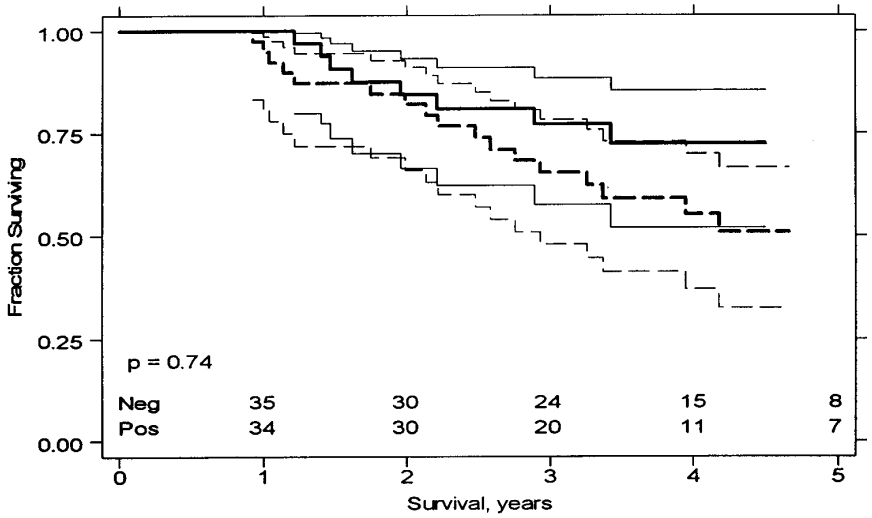

Fig 2. Kaplan-Meier survival curves (95% CI) for 34 patients in whom the 3-month transbronchial biopsy result showed rejection (*solid lines*) versus 35 patients in whom it did not (*dashed lines*).

may lie in part in the immunosuppressive regimen used early after lung transplantation. Bando and colleagues[3] observed high-grade rejection or multiple moderate grade rejections in close to 50% of their patients. Wallwork's group from the United Kingdom did not specify the grade of the biopsy results, but observed that one third of their group showed more than 3 episodes of acute rejection.[14] In our study, we observed high-grade rejection or multiple moderate grade rejection events in less than 3% of our study population. The regimen used by both of the above-mentioned groups was less aggressive than the regimen detailed in the present study. Bando's group only used induction therapy with antilymphocyte globulin for half the study and significantly limited the use of steroids. Prior reports from Wallwork and his colleagues described only a 3-day regimen of antilymphocyte globulin and a very limited regimen of steroids.[4,5,7]

The present study did not demonstrate decreased survival in those patients who were found to have acute rejection on surveillance transbronchial biopsy. An association between acute rejection and bronchiolitis

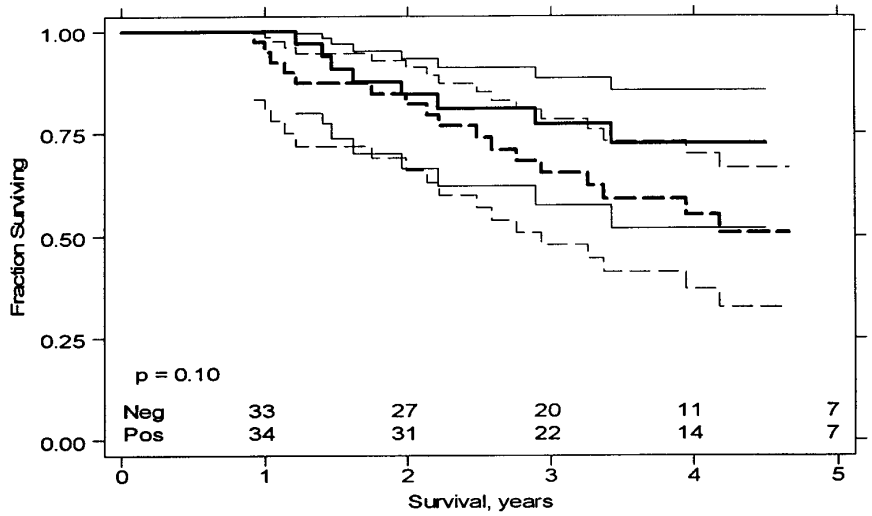

Fig 3. Kaplan-Meier survival curves (95% CI) for 34 patients in whom the 6-month transbronchial biopsy result showed rejection (*solid lines*) versus 33 patients in whom it did not (*dashed lines*).

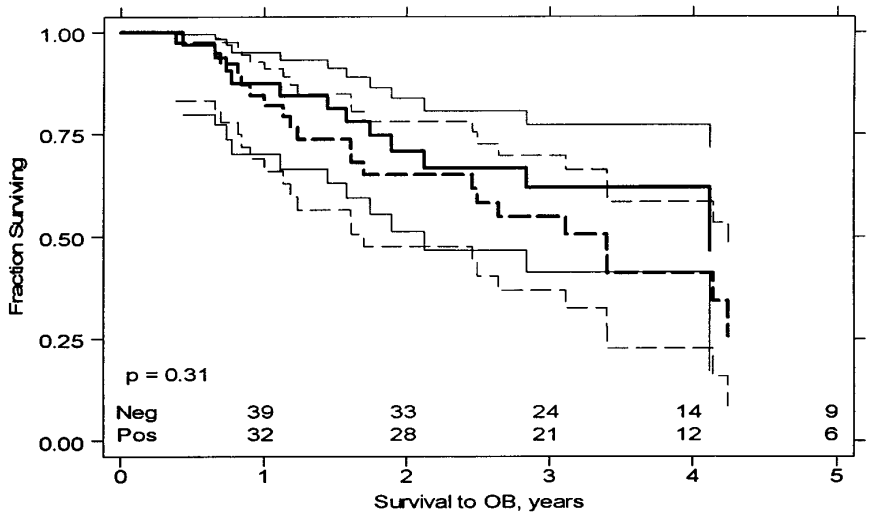

Fig 4. Kaplan-Meier survival curves (95% CI) for the outcome bronchiolitis obliterans (*OB*) for 32 patients in whom the 1-month transbronchial biopsy result showed rejection (*solid lines*) versus 39 patients in whom it did not (*dashed lines*).

obliterans/bronchiolitis obliterans syndrome was not observed. The severity of the acute rejection episodes and the number of persistent cases of acute rejection were found to be quite low with this careful surveillance and treatment strategy. The immunosuppressive regimen detailed in this report is intensive both in terms of the induction scheme and the dosage and duration of steroid administration.

The survival seen in our overall group is similar to that reported by the United Network for Organ Sharing in its most recent publication.[2] This fact coupled with our results suggests several possible explanations. Surveillance transbronchial biopsies, in the patient free of symptoms, do not predict for bronchiolitis obliterans and survival particularly when the acute rejection episode is only mild (grades 1-2). Early transbronchial biopsies in general do not correlate with the chronic process of bronchiolitis obliterans, the cause of the majority of late deaths. Aggressive early immunosuppression eliminates or uncouples the discriminating

Table IV. *ISHLT grade for 53 patients with acute rejection in first 6 months*

Patient	*Survival days from TX*	*Status*	*ISHLT grade*			*At least 1 episode of moderate/severe rejection*
			Biopsy 1	*Biopsy 2*	*Biopsy 3*	
4	715	D	A1B1	Neg	A1B1	No
5	592	D	A1B0	A1B0	A1B1	No
6	3052	A	Neg	Neg	A1B0	No
7	2935	A	Neg	A1B0	Neg	No
8	807	D	A0B1	Neg	Neg	No
9	1525	D	Neg	A1B1	Neg	No
11	779	D	Neg	Neg	A1B0	No
12	1440	D	Neg	A0B1	A1B1	No
14	1853	D	Neg	A1B0	Neg	No
16	891	D	Neg	Neg	A1B0	No
17	726	D	Neg	A0B3	A0B2	Yes
18	2275	A	A0B2	A1B0	A3B2	Yes
21	516	D	A0B1	Neg	Neg	No
23	1767	D	Neg	A0B2	Neg	No
24	2005	A	A1B3	A1B1	Neg	Yes
25	921	D	Neg	Neg	A1B0	No
26	1057	D	A2B0	A2B3	A1B1	Yes
28	1769	A	A0B3	A0B3	A1B0	Yes
29	1589	A	Neg	Neg	A0B2	No
31	1531	A	A2B0	Neg	Neg	No
33	1487	D	A0B3	Neg	Neg	Yes
34	1530	A	Neg	A3B3	A2B2	Yes
35	1005	D	Neg	A3B2	A0B2	Yes
37	445	D	Neg	A0B2	Neg	No
38	1515	A	A1B0	Neg	Neg	No
39	1502	A	Neg	A2B0	A1B0	No
40	1474	A	A3B3	Neg	A2B2	Yes
41	1443	A	A1B0	A1B0	A0B1	No
42	445	D	A3B2	A2B0	A2B0	Yes
44	1398	A	A1B0	Neg	A1B0	No
47	416	D	Neg	A2B1	Neg	No
48	1320	A	A3B2	A1B1	A2B0	Yes
49	1193	D	A1B0	Neg	A1B0	No
50	1317	A	Neg	A2B0	A3B0	Yes
51	444	D	A4B0	Neg	Neg	Yes
52	1223	A	A2B0	A2B2	A0B2	No
54	1167	A	Neg	A0B3	Neg	Yes
55	1164	D	A2B0	Neg	Neg	No
56	1160	A	A1B0	A3B0	A1B0	Yes
57	1135	A	A0B4	A0B1	A3B1	Yes
59	1100	A	Neg	A2B2	A0B2	No
60	1065	A	A2B0	Neg	Neg	No
61	1040	A	Neg	Neg	A2B0	No
62	979	A	A0B2	Neg	Neg	No
63	373	D	Neg	A0B1	A1B1	No
64	861	A	Neg	A2B0	A0B2	No
65	811	A	A2B2	A2B0Cb	A1B0	No
66	751	A	A3B0	A1B0	Neg	Yes
67	726	A	Neg	A1B0	Neg	No
68	701	A	A1B0	A1B0	Neg	No
69	668	A	A1B2	Neg	A0B1Ca	No
70	664	A	A2B0	A0B3	Neg	Yes

Data as of May 1999. *TX,* Transplantation; *D,* dead; *Neg,* negative; *A,* alive.

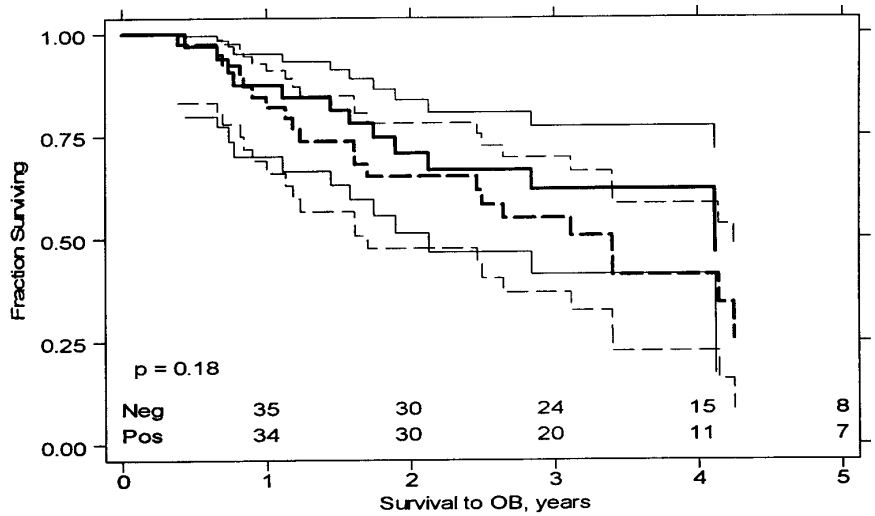

Fig 5. Kaplan-Meier survival curves (95% CI) for the outcome bronchiolitis obliterans (*OB*) for 34 patients in whom the 3-month transbronchial biopsy result showed rejection (*solid lines*) versus 35 patients in whom it did not (*dashed lines*).

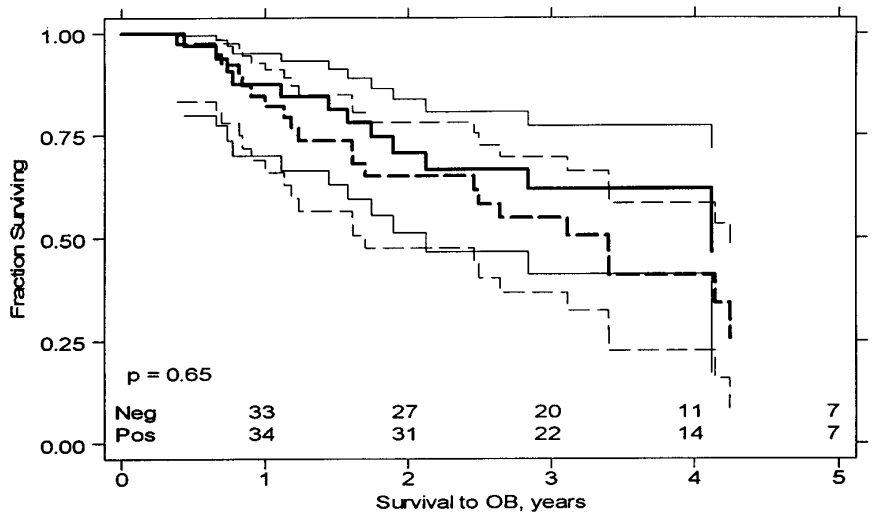

Fig 6. Kaplan-Meier survival curves (95% CI) for the outcome bronchiolitis obliterans (*OB*) for 34 patients in whom the 6-month transbronchial biopsy result showed rejection (*solid lines*) versus 33 patients in whom it did not (*dashed lines*).

ability (variability in severity and persistence) of early transbronchial biopsy to predict later outcome (bronchiolitis obliterans/bronchiolitis obliterans syndrome or survival). Once the effects of the intensive early immunosuppression have waned, as the time from the antilymphocyte globulin treatment becomes greater and the steroids are weaned, the link between the histologic findings on transbronchial biopsy and the later chronic fibrotic process may become more apparent. Whether continued high-dose standard immunosuppression would prevent the emergence of later chronic rejection, as suggested by some animal models,[20] is not a practical question for human beings given the complications of prolonged high-dose immunosuppression, such as infection, and the catabolic effects of high-dose steroid treatment.

The findings of minimal, severe, early, or persistent early rejection documented on surveillance transbronchial biopsies in this study do raise the notion that if a better immunosuppression regimen could be iden-

tified then perhaps one could continue to suppress the immunologic differences that are manifested as rejection. This regimen would need to be intense and certainly more specific. Indeed, specific tolerance would be the ideal.

In the meantime, better discriminators of long-term outcome must be identified. Exhaled nitric oxide levels have been shown to correlate with acute rejection in animal models and human beings after lung transplantation.[21] Interestingly, though, in the human data, exhaled nitric oxide levels did not correlate with bronchiolitis obliterans.[22] Again, there is some hint that the acute and chronic rejection may be related but have different mechanisms. Alternatively E-selectin, an adhesion molecule induced by activated lymphocytes and cytokines, has been reported to be expressed in both acute rejection and bronchiolitis obliterans.[23]

There are obvious limitations to the current method of detecting acute rejection. Although surveillance transbronchial biopsies are quite safe, they may not have the discriminating ability that is necessary to have an impact on the process of bronchiolitis obliterans and, therefore, long-term survival. It may also be that our current immunosuppressive combinations are reasonably good at suppressing the phenomenon of early acute rejection, but that they do not prevent bronchiolitis obliterans, which, although related, may have different mechanisms or only require low-level immunologic activation.

In a manner analogous to the approach to lung cancer of several decades ago, it would seem that we need to continue carefully and completely to collect data that pertain to an apparently insurmountable process if we are going to make strides. It is mandatory that we continue to collect information that will provide insight into the phenomenon of acute and chronic rejection. To make lung transplantation a more broadly successful and applicable technology, we must answer the problem of bronchiolitis obliterans. Aggressive and more sensitive detection methods linked with better immunosuppression are needed.

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Discussion

Dr Thomas M. Egan *(Chapel Hill, NC).* Bronchiolitis obliterans has become the scourge of patients with lung transplants after they have been delivered from a wretched existence of suffocation, and it cuts short lives that transplantation intends to extend. It is the leading cause of death after the first year following transplantation, and some studies suggest that it is the inevitable outcome of transplantation, with actuarial freedom from bronchiolitis obliterans reported near 0% 10 years after lung transplantation.

Several factors have been implicated as etiologic agents or as risk factors for the development of bronchiolitis obliterans, including the number of acute rejection episodes and a variety of infections, including infection with cytomegalovirus. If acute vascular rejection contributes to long-term graft dysfunction, then it is intuitive that attempts to detect this and treat it might have a favorable impact on outcome, both from the point of view of development of bronchiolitis obliterans and ultimately of survival.

To assess the importance of biopsies in the treatment of patients with lung transplants, the authors of this study at the Brigham and Women's Hospital retrospectively analyzed the results of biopsies in 71 consecutive lung transplant recipients in whom 1-year follow-up was available. No matter whether 1, 2, or 3 biopsy results were positive for acute rejection, this had no apparent impact on survival or, by χ^2 analysis, on the presence of bronchiolitis obliterans at the time of this analysis.

The authors surmise that their more aggressive early immunosuppression may have in some way uncoupled acute rejection from the development of bronchiolitis obliterans, but there may be other explanations. My first question for the authors is, did you actually show convincingly that biopsy outcome was not related to the development of bronchiolitis obliterans syndrome? Only 11 patients had 3 positive biopsy results, and while the χ^2 test did not reach significance, do you believe that a long enough time elapsed for this observation to hold?

You state in your manuscript that total lymphoid irradiation was used in 21 patients with "persistent acute rejection." This is a large proportion of your patients and represents an unusually large experience with this treatment modality. Presumably for these patients to have persistent rejection, many of them would have been in the positive biopsy cohort that you studied. Was there an association between positive biopsy results and the subsequent treatment with total lymphoid irradiation? If so, can you speculate as to whether total lymphoid irradiation might have interfered with the development of bronchiolitis obliterans? If you stand by your observation that biopsy outcome has no impact on survival, then should we persist in doing biopsies?

One other unusual feature of your practice is the use of ganciclovir for an entire year. You observed the development of cytomegalovirus after cessation of treatment in 5 patients. Are you not convinced yet that in a mismatch situation cytomegalovirus disease is inevitable and the prolonged use of prophylaxis may aid the emergence of ganciclovir-resistant strains?

When we reported our results in patients with cystic fibrosis at the Southern Thoracic Surgical Association meeting a year and a half ago, we too were unable to demonstrate a relationship between the number of acute rejection episodes and outcome at 2 years or development of bronchiolitis. There are broader questions that one can conjure up from your study and others that should be answered in the future.

Is bronchiolitis obliterans really the pathologic hallmark of chronic rejection as you stated in the introduction of your manuscript or is it the inevitable sequela of lung transplantation? Phil Halloran, an immunologist at the University of Alberta in Edmonton, put forward an interesting hypothesis at the ISHLT last year. He suggested that chronic allograft dysfunction in any transplanted organ could be a form of accelerated senescence, which might be instigated by a variety of stressors to the organ.

Whatever the cause of bronchiolitis obliterans, our ability to prevent it or reduce the incidence will have a substantial impact on the lives of our transplant recipients.

Dr Swanson. You asked whether we think there was enough time between the 3 persistent acute rejection biopsies to determine whether bronchiolitis obliterans was going to develop: the median follow-up period was 40 months, and I think that time should be adequate.

As to the total lymphoid irradiation question, the majority of patients who received this treatment were treated once it was determined that they had bronchiolitis obliterans, and the impetus for the treatment was not persistent acute rejection. This should be clarified, because most of these patients already had bronchiolitis obliterans when they were treated with total lymphoid irradiation. Dr Ingenito presented that data at the American Thoracic Society, I believe 2 years ago, and the treatment did appear to halt the progression and stabilize the condition in more than half that group.

I think your question about whether we should continue to do biopsies is right on. I think that we could not show a differ-

ence, and one way to interpret this result would be that those patients we treated were elevated to do as well as the others. Even if that is not the case, I think we still need to do biopsies because they have low morbidity and do provide us with evidence and data from which we can make further strides. Unless we continue to push, we are not going to make progress.

Most of the reports show a much higher incidence of cytomegalovirus infection than the 6% we saw in our series. I think it is not clear how long prophylactic ganciclovir should be used, but I do think we have a low prevalence of cytomegalovirus disease in our cohort.

Dr David W. Wormuth *(Rochester, NY).* By choosing 1-year survival, did you preselect the patients who were already going to survive an early rejection? Also, did your transbronchial biopsy results predict mortality between 6 months and 1 year?

Dr Swanson. We were mainly trying to look at survival and bronchiolitis obliterans, and that is why we used a group that had 1-year survival. Most of the data now show that survival to a year is pretty good. The problem is that from 1 year to 5 years survival always drops off. To not complicate the interpretation by using patients who died of other causes, we wanted to limit the study to the 1-year survivors.

Dr David M. Follette *(Sacramento, Calif).* I would like to amplify a bit, because, like Dr Egan, the way I interpret the results is that perhaps we do not need to do surveillance biopsies in patients who do not have clinical indications. Should we only do biopsies when there is clinical suspicion of rejection? Do you believe that the routine biopsy in a patient who is otherwise in clinically stable condition is of little or no benefit and perhaps not necessary? I would like to have your thoughts on whether you would interpret your study in the same way.

Dr Swanson. I think that is a good point. I think until we actually suppress bronchiolitis obliterans, it may be that we cannot show the difference that you would like to see, but we did document a much less aggressive form of acute rejection. In the literature, most series show at least a third of the patients have 3 or more episodes of acute rejection that is moderate or severe in each cohort and more than half show severe acute rejection. Thus what we found was a much less significant or much less severe form of acute rejection, and that may well be both from our augmented immunosuppression initially and the treatment of these mild episodes. Until you can completely suppress the situation over the course of several years, you may not see a difference, but I think ultimately we may be able to do that. More important, however, unless we continue to collect information and analyze it in different ways, as I mentioned earlier, I do not think we will make any progress. Thus I do think we still need to pursue this kind of information.