Supplemental Methods

Processing and coding of pathology reports

Pathology reports at our center are assigned A-scores for acute rejection and BR-scores for lymphocytic bronchiolitis according to the 2007 revision of rejection nomenclature[1] and follow the conventional format of:

> SOURCE: A. Lung transplant, biopsy
> B. Bronchus, biopsy
>
> DIAGNOSIS:
>
> A. Lung transplant, transbronchial biopsy:
> No evidence of rejection (Grade A0, B0); see comment.
> B. Lung transplant, endobronchial biopsy:
> No airway inflammation. No significant pathologic abnormality.

We implemented a program in Python (version 2.7.2) to find the diagnosis field in these reports and search for the words "transbronchial" and "endobronchial." Once found, the transbronchial score was extracted from the "Grade A_, B_" field.Biopsy results obtained prior to 2007 were converted to the newer nomenclature such that grade B1 and B2 bronchiolitis became B1R, and B3 and B4 bronchiolitis became B2R. Because endobronchial biopsies were not given a code, the program assigned codes according to the following table. When a text match was not found, the reports were marked for manual review.

tissue insufficient, insufficient tissue, necrotic debris, scant fibrous tissue only, scant superficial bronchial epithelium	X
no airway inflammation, without significant airway inflammation, without significant inflammation, no significant airway inflammation, without inflammation, no significant inflammation	0
no significant pathologic abnormality, no significant abnormality, no evidence of rejection, without airway inflammation	0
minimal acute airway inflammation, minimal acute bronchial, minimal acute inflammation	1A
minimal chronic airway inflammation, minimal chronic airway inflamation, minimal chronic inflammation, minimal non-specific chronic inflammation, minimal airway inflammation, mild submucosal inflammation	1C
acute airway inflammation, acute inflammation	1A
minimal acute and chronic	1A 1C
mild acute airway inflammation, mild acute airway inflammation, mild acute inflammation, mild acute bronchiolitis	2A
mild chronic airway, mild chronic inflammation, mild airway inflammation	2C
mild to moderate airway inflammation, moderate airway inflammation	2C
moderate acute airway inflammation, moderate acute airway, moderate acute inflammation	3A
moderate chronic airway inflammation, moderate chronic inflammation	3C
mild acute and chronic airway, mild chronic and acute airway, mild acute and chronic bronchial, mild increase in submucosal acute and chronic inflammation	2A 2C
extensive inflammation, marked acute inflammation	4A

severe chronic inflammation	4C
moderate acute and chronic	3A 3C

Following this data processing 3743 records were coded and 117 were marked for manual review. These records were coded by hand. Subsequent review of 200 records, representing 5% of the total sample, revealed no errors.
For our primary analysis, we excluded biopsies from patients with concurrent infection, defined as positive bacterial, fungal, or viral studies from bronchoalveolar lavage (BAL) fluid taken at the time of biopsy. These microbiologic studies were also processed using text recognition. Since viral PCR was introduced midway through the study, we only assessed viral infections by DFA. Bacterial cultures were considered positive if qualitative cultures showed moderate or greater growth or quantitative cultures showed ≥ 10,000 CFU/ml, excluding oronasal flora.

GEE models
To evaluate the concordance between biopsies, we employed generalized estimating equation (GEE) models between pairs as in the example below. GEE was used to correct for multiple observations within a single subject.

```
GEE:  GENERALIZED LINEAR MODELS FOR DEPENDENT DATA
gee S-function, version 4.13 modified 98/01/27 (1998)

Model:
 Link:                      Logit
 Variance to Mean Relation: Binomial
 Correlation Structure:     Non-Stationary M-dependent , M = 1

Call:
gee(formula = EC > 0 ~ B > 0, id = subject_id, family = binomial(link = "logit"),
corstr = "non_stat_M_dep")

Summary of Residuals:
       Min         1Q     Median         3Q        Max
-0.5582281 -0.1879883 -0.1879883 -0.1879883  0.8120117


Coefficients:
            Estimate Naive S.E.   Naive z Robust S.E.  Robust z
(Intercept) -1.463135 0.07148532 -20.46763  0.08933651 -16.37779
B > 0TRUE    1.697109 0.14375790  11.80533  0.14075992  12.05676

Estimated Scale Parameter:  0.9997855
Number of Iterations:  2
```

To evaluate for an association between pathologic findings and the stage of BOS at the time of biopsy, we used a multivariate GEE model. Because patient

characteristics could influence the presence of BOS, we adjust for age, gender, transplant indication and type, and CMV status in this model.

```
 GEE:  GENERALIZED LINEAR MODELS FOR DEPENDENT DATA
gee S-function, version 4.13 modified 98/01/27 (1998)

Model:
 Link:                      Logit
 Variance to Mean Relation: Binomial
 Correlation Structure:     Non-Stationary M-dependent , M = 1

Call:
gee(formula = EC > 0 ~ factor(bos.n) + age.at.transplant + gender +
factor(transplant.type) + (indication == 1) + (indication ==
    2) + (indication == 3) + (indication == 4) + (indication ==
    5) + (indication == 6) + (indication == 8) + factor(cmv_status),
id = subject_id, family = binomial(link = "logit"), corstr = "non_stat_M_dep")

Summary of Residuals:
       Min         1Q     Median         3Q        Max
-0.5140640 -0.2750541 -0.2070474  0.5395349  0.9082255
```

Coefficients:

	Estimate	Naive S.E.	Naive z	Robust S.E.	Robust z
(Intercept)	-0.795	0.536	-1.483	0.666	-1.193
BOS Op	0.355	0.149	2.383	0.172	2.067
BOS stage 1-3	0.653	0.155	4.221	0.165	3.950
Age	-0.007	0.007	-1.019	0.008	-0.907
Male gender	0.062	0.132	0.473	0.151	0.414
Right lung	0.275	0.272	1.008	0.319	0.860
Left lung	-0.341	0.233	-1.468	0.248	-1.375
Double lung	-1.353	0.538	-2.512	0.495	-2.732
Heart/Lung	0.677	1.537	0.440	0.523	1.294
Cystic Fibrosis	0.096	0.257	0.373	0.377	0.254
COPD/Emphysema	-0.301	0.234	-1.289	0.332	-0.908
Bronchictasis	0.631	0.340	1.857	0.452	1.398
Pulmonary Hypertension	-0.059	0.315	-0.187	0.381	-0.155
Other Pulmonary Fibrosis	0.266	0.292	0.910	0.389	0.684
IPF/UIP	0.092	0.200	0.461	0.274	0.337
Other	-0.164	0.330	-0.497	0.379	-0.432
CMV D-/R+	-0.197	0.292	-0.674	0.380	-0.517
CMV D+/R+	-0.091	0.254	-0.357	0.332	-0.273
CMV D+/R-	-0.072	0.282	-0.255	0.398	-0.181

CMV unknown	*-0·081*	*0·249*	*-0·327*	*0·329*	*-0·247*

Estimated Scale Parameter: 1·02028
Number of Iterations: 3

Cox proportional hazards model

We used a multivariate-adjusted Cox proportional hazards model that was left-truncated at 90 days with outcomes of either survival or BOS score > 0. This model was adjusted for age, gender, transplant indication, transplant type and CMV status. Age was treated as an ordinal, gender as a binary variable and the others were treated as binary factors. Left truncation ensured that only subjects who were alive and BOS-free at 90 days were included in the analysis. Patient characteristics were included. The "survival" library in R was used.

Variable Name	Variable type	Coef	se(coef)	z	Pr(>|z|)		HR	95% CI	
Age	ordinal	0.01	0.01	0.42	0.67		1.01	0.98	1.03
Gender	Binary	-0.26	0.26	-1.02	0.31		0.77	0.47	1.27
Cystic Fibrosis	Factor								
COPD/Emphysema	Factor	-0.07	0.61	-0.11	0.91		0.94	0.28	3.08
Bronchictasis	Factor	1.46	0.76	1.92	0.05	.	4.32	0.97	19.20
Pulmonary Hypertension	Factor	0.89	0.63	1.41	0.16		2.44	0.71	8.38
Other Pulmonary Fibrosis	Factor	0.13	0.65	0.21	0.84		1.14	0.32	4.08
IPF/UIP	Factor	0.34	0.54	0.63	0.53		1.41	0.49	4.07
Alpha-1	Factor	-0.61	0.90	-0.68	0.50		0.54	0.09	3.17
Other	Factor	0.29	0.55	0.53	0.60		1.34	0.46	3.92
Right lung	Factor								
Left lung	Factor	0.18	0.54	0.34	0.73		1.20	0.41	3.50
Double lung	Factor	-0.22	0.41	-0.55	0.59		0.80	0.36	1.79
Heart/Lung	Factor	0.31	0.61	0.50	0.62		1.36	0.41	4.52
CMV D-/R-	Factor								
CMV D-/R+	Factor	-0.60	0.54	-1.11	0.27		0.55	0.19	1.57
CMV D+/R+	Factor	-0.22	0.41	-0.54	0.59		0.80	0.36	1.80
CMV D+/R-	Factor	-0.86	0.53	-1.60	0.11		0.43	0.15	1.21
CMV unknown	Factor	-0.31	0.42	-0.74	0.46		0.74	0.33	1.66
90 day max E-score	**Ordinal**	**0.56**	**0.23**	**2.41**	**0.02**	*****	**1.76**	**1.11**	**2.78**

Variable Name	Variable type	Coef	se(coef)	z	Pr(>|z|)		HR	95% CI	
Age	ordinal	0.00	0.01	-0.10	0.92		1.00	0.98	1.02
Gender	Binary	-0.16	0.23	-0.70	0.48		0.85	0.55	1.33
Cystic Fibrosis	Factor								
COPD/Emphysema	Factor	0.09	0.54	0.17	0.86		1.10	0.38	3.20
Bronchictasis	Factor	1.31	0.75	1.73	0.08	.	3.69	0.84	16.14
Pulmonary Hypertension	Factor	0.91	0.54	1.69	0.09	.	2.49	0.87	7.15

Other Pulmonary Fibrosis	Factor	0.13	0.60	0.21	0.83		1.14	0.35	3.66
IPF/UIP	Factor	0.30	0.49	0.62	0.54		1.36	0.51	3.57
Alpha-1	Factor	-0.66	0.86	-0.77	0.44		0.52	0.09	2.81
Other	Factor	0.21	0.51	0.42	0.68		1.24	0.45	3.36
Right lung	Factor								
Left lung	Factor	0.06	0.43	0.13	0.89		1.06	0.46	2.46
Double lung	Factor	-0.62	0.34	-1.80	0.07	.	0.54	0.27	1.06
Heart/Lung	Factor	-0.14	0.56	-0.25	0.80		0.87	0.29	2.61
CMV D-/R-	Factor								
CMV D-/R+	Factor	-0.98	0.51	-1.90	0.06	.	0.38	0.14	1.03
CMV D+/R+	Factor	-0.45	0.40	-1.14	0.26		0.63	0.29	1.39
CMV D+/R-	Factor	-1.10	0.49	-2.23	0.03	*	0.33	0.13	0.87
CMV unknown	Factor	-0.58	0.39	-1.47	0.14		0.56	0.26	1.21
90 day max A-score	**Ordinal**	**-0.06**	**0.13**	**-0.45**	**0.66**		**0.94**	**0.74**	**1.21**

Variable Name	Variable type	Coef	se(coef)	z	Pr(>|z|)		HR	95% CI	
Age	ordinal	0.00	0.01	-0.11	0.91		1.00	0.98	1.02
Gender	Binary	-0.16	0.23	-0.70	0.48		0.85	0.54	1.34
Cystic Fibrosis	Factor								
COPD/Emphysema	Factor	0.12	0.54	0.22	0.83		1.13	0.39	3.28
Bronchictasis	Factor	1.27	0.75	1.69	0.09	.	3.54	0.82	15.37
Pulmonary Hypertension	Factor	0.92	0.54	1.71	0.09	.	2.50	0.87	7.19
Other Pulmonary Fibrosis	Factor	0.16	0.59	0.26	0.79		1.17	0.37	3.74
IPF/UIP	Factor	0.32	0.49	0.66	0.51		1.38	0.52	3.64
Alpha-1	Factor	-0.64	0.86	-0.74	0.46		0.53	0.10	2.87
Other	Factor	0.22	0.51	0.43	0.66		1.25	0.46	3.41
Right lung	Factor								
Left lung	Factor	0.07	0.43	0.16	0.87		1.07	0.46	2.49
Double lung	Factor	-0.60	0.34	-1.75	0.08	.	0.55	0.28	1.07
Heart/Lung	Factor	-0.11	0.56	-0.19	0.85		0.90	0.30	2.70
CMV D-/R-	Factor								
CMV D-/R+	Factor	-0.99	0.51	-1.93	0.05	.	0.37	0.14	1.02
CMV D+/R+	Factor	-0.47	0.40	-1.19	0.24		0.62	0.29	1.36
CMV D+/R-	Factor	-1.10	0.49	-2.23	0.03	*	0.33	0.13	0.88
CMV unknown	Factor	-0.59	0.39	-1.50	0.13		0.56	0.26	1.20
90 day max B-score	**Ordinal**	**-0.01**	**0.22**	**-0.05**	**0.96**		**0.99**	**0.65**	**1.51**

Significance codes: '*' P<0.05, '.' P<0.1

Of note, ISHLT registry data strongly support a protective effect against BOS for double lung transplantation, which is an important reason why it was included as an adjustment term in our statistical model. In our multivariate analysis however, the double lung transplant did not have a statistically significant association with

decreased BOS. We did find an association byunivariate analysis in our Cox model. The HR for BOS with double lung transplantation is 0.56 (95% CI 0.36-0.88, *P*=0.01). We do not observe a statistically significant interaction between endobronchial lymphocytic bronchitis and lung transplant type (*P*-value for interaction 0.50). The failure to observe an association in this study is likely a power issue, since 80% of the transplants at our center were double lung transplants.

As an alternative analysis, we looked at the maximum pathology score over the first year as a predictor of the development of BOS after the first year.

Comparison of one-year maximum pathology scores for predicting outcomes after one-year

As an alternative to using the 90-day cutoff described in the main results section, we analyzed the maximum pathology score over the first year for the prediction of BOS and mortality. We adjusted this Cox proportional hazards model for age, gender, transplant indication, transplant type and CMV status. Subjects were excluded who had death or mortality in the first year.

		BOS				Mortality			
	Subjects	Hazard Ratio	95% CI		P-value	Hazard Ratio	95% CI		P-value
E-score (ordinal)	229	1.28	0.80	2.07	0.31	0.93	0.59	1.48	0.76
E0	105	1.00				1.00			
E1	118	0.94	0.56	1.58	0.82	0.75	0.45	1.26	0.28
E2	6	4.95	1.66	14.7	4.0E-03 **	1.81	0.60	5.50	0.29
A-score (ordinal)	243	0.94	0.73	1.20	0.61	1.01	0.80	1.27	0.95
A0	121	1.00				1.00			
A1	61	0.79	0.44	1.40	0.42	0.62	0.34	1.15	0.13
A2	47	0.68	0.36	1.30	0.24	0.83	0.47	1.47	0.53
A3	14	1.32	0.54	3.23	0.55	1.33	0.58	3.05	0.50
BR-score (ordinal)	243	0.88	0.56	1.38	0.57	0.88	0.56	1.37	0.57
BR0	152	1.00				1.00			
BR1	87	0.78	0.48	1.28	0.33	0.82	0.50	1.33	0.42
BR2	4	1.75	0.38	7.99	0.47	1.27	0.27	5.86	0.76

Neutrophilic bronchitis

A table of maximum 90-day neutrophilic and lymphocytic bronchitis scores from endobronchial biopsies is shown below:

		Lymphocytic Bronchitis Score		
		0	**1**	**2**
Neutrophilic Bronchitis Score	**0**	87	47	1
	1	58	57	1
	2	9	4	5

R^2 =0.04, *P*-value for F-statistic 0.001. Chi-squared test for association *P*<0.001.

The log-likelihood analysis on this cox model was performed as shown below:

```
Analysis of Deviance Table
 Cox model: response is m·bos
Terms added sequentially (first to last)

loglikChisqDf Pr(>|Chi|)
NULL                      -374·07
Age·at·transplant-374·04 0·0739  1    0·78569
Gender                   -373·63 0·8114  1    0·36770
Indication         -371·02 5·2270  8    0·73307
Transplant type-369·95 2·1336  3    0·54514
CMV status-366·84 6·2160  4    0·18359
Neutrophilic Bronchitis -363·99 5·7152  2    0·05741 ·
Lymphocytic Bronchitis  -360·33 7·3167  2    0·02578 *
---
Signif· codes:  0 '***' 0·001 '**' 0·01 '*' 0·05 '·' 0·1 ' ' 1'


Analysis of Deviance Table
 Cox model: response is m·bos
Terms added sequentially (first to last)

loglikChisqDf Pr(>|Chi|)
NULL                      -374·07
Age·at·transplant-374·04  0·0739  1   0·785686
Gender                   -373·63  0·8114  1   0·367696
Indication         -371·02  5·2270  8   0·733065
Transplant type-369·95  2·1336  3   0·545139
CMV status-366·84  6·2160  4   0·183589
Lymphocytic Bronchitis -361·15 11·3899  2   0·003363 **
Neutrophilic Bronchitis -360·33  1·6420  2   0·439989
---
Signif· codes:  0 '***' 0·001 '**' 0·01 '*' 0·05 '·' 0·1 ' ' 1
```

We observed an improvement in prediction for BOS from the lymphocytic bronchitis term whether neutrophilic bronchitis was included or not, while neutrophilic bronchitis did not improve a model that included lymphocytic bronchitis.

1. Stewart S, Fishbein MC, Snell GI, Berry GJ, Boehler A, et al. (2007) Revision of the 1996 working formulation for the standardization of nomenclature in the diagnosis of lung rejection. J Heart Lung Transplant 26: 1229-1242.