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LATE PRIMARY GRAFT DYFUNCTION AFTER LUNG TRANSPLANTATION AND BRONCHIOLITIS OBLITERANS SYNDROME

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Abstract

Primary graft dysfunction (PGD) is a common early complication after lung transplantation. We conducted a retrospective cohort study of 334 recipients to evaluate the impact of PGD graded at 24, 48, and 72 hours on the risk of bronchiolitis obliterans syndrome (BOS) development (stage 1) and progression (stages 2 and 3). We constructed multivariable Cox proportional hazards models to determine the risk of BOS attributable to PGD in the context of other potential risk factors including acute rejection, lymphocytic bronchitis, and respiratory viral infections. All grades of PGD at all time points were significant risk factors for BOS development and progression independent of acute rejection, lymphocytic bronchitis, and respiratory viral infections. Specifically, PGD grade 1 at T24 was associated with a relative risk of BOS stage 1 of 1.93, grade 2 with a relative risk of 2.29, and grade 3 with a relative risk of 3.31. Furthermore, this direct relationship between the severity of PGD and the risk of BOS persisted at all time points. We conclude that all grades of PGD at all time points are independent risk factors for BOS development and progression. Future strategies that might attenuate the severity of PGD may mitigate the risk of BOS.

Keywords

lung transplantation; primary graft dysfunction; bronchiolitis obliterans syndrome

INTRODUCTION

Primary graft dysfunction (PGD) is a common early complication after lung transplantation (1-3). It represents an acute lung injury that typically occurs in the first 72 hours after transplantation and has a wide spectrum of severity ranging from an almost asymptomatic radiographic finding to severe hypoxemic respiratory failure. To capture this spectrum of severity and standardize the nomenclature, the International Society for Heart and Lung Transplantation (ISHLT) proposed a definition and grading scheme based on the presence of radiographic infiltrates consistent with edema and the ratio of the partial pressure of arterial

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oxygen to the fraction of inspired oxygen ($\text{PaO}_2/\text{FiO}_2$ ratio) (4). According to this definition, PGD grade 0 is characterized by a clear chest x-ray, grade 1 by radiographic evidence of pulmonary edema and a $\text{PaO}_2/\text{FiO}_2$ ratio > 300 mm Hg, grade 2 by radiographic evidence of pulmonary edema and a $\text{PaO}_2/\text{FiO}_2$ ratio of 200-300 mm Hg, and grade 3 by pulmonary edema and a $\text{PaO}_2/\text{FiO}_2$ ratio < 200 mm Hg (4). In addition, PGD is graded at four time points over the first 72 hours after transplantation (T0, T24, T48, and T72 hours) to capture the change in severity of the acute lung injury over time.

Not surprisingly, PGD has a considerable impact on both short- and long-term outcomes after transplantation (3,5-8). We have previously identified PGD graded immediately after transplantation (T0) as a significant independent risk factor for the development of bronchiolitis obliterans syndrome (BOS) stage 1 (8). In the current study, we hypothesized that PGD graded at later time points (T24, T48, and T72) would be an independent predictor of the development of BOS stage 1 and its progression to stage 2 and stage 3. We also sought to investigate the impact of a change in the PGD grade over the first 72 hours after transplantation on the risk of BOS development and progression.

METHODS

Study Design and Patient Population

We conducted a retrospective cohort study to examine the impact of PGD graded at 24, 48, and 72 hours after transplantation on the risk of BOS development and progression. Between January 1, 1998 and June 30, 2004, 336 adults underwent 337 lung transplant procedures at our center. Three transplant procedures were complicated by hyperacute rejection and were excluded from the cohort; two of these recipients died and the third underwent retransplantation (Figure 1). The post-operative course of the recipient who underwent retransplantation was included in this analysis. Immediately after transplantation (T0), 334 recipients were alive and were included in our previous analysis (8). One recipient died within 24 hours of transplantation ($n = 333$ at T24; Figure 1). Two additional recipients died between 24 and 48 hours after transplantation and two were excluded from the 48-hour and 72-hour analyses because of missing data ($n = 329$ at T48 and T72; Figure 1). We reviewed baseline characteristics, operative variables, and post-operative outcomes for these recipients.

Our program's standard medical management and follow-up protocols have been previously detailed (8,9). Our approach to managing PGD was fairly consistent during the study period. Specifically, we used a lung protective ventilation strategy and targeted plateau airway pressures less than 30 cm H_2O . Similarly, we used the minimum necessary fraction of inspired oxygen and positive end expiratory pressure to achieve adequate oxygenation. In addition, we instituted diuretic therapy to target a negative fluid balance as hemodynamics allowed. Nine recipients required extracorporeal membrane oxygenation despite aggressive conventional therapy. Lastly, we treated all recipients with broad-spectrum antibiotics prophylactically for the first 10-14 days. The study protocol was approved by the Washington University School of Medicine Institutional Review Board for human studies.

Variables

A single investigator assigned PGD grades retrospectively based on the arterial blood gas result, the fraction of inspired oxygen, and the chest x-ray report at 24, 48, and 72 hours after transplantation according to the ISHLT definition (4). We diagnosed and graded acute rejection and lymphocytic bronchitis histologically according to the ISHLT criteria (10,11), and identified community-acquired respiratory viral (CARV) infections using virus-specific

immunofluorescent labeling and culture as previously described (12). Lastly, we diagnosed and staged BOS according to the standard criteria (13,14).

Statistical Analysis

A priori, we hypothesized that all grades of PGD at all time points would be risk factors for BOS stage 1 and its progression to stage 2 and stage 3 independent of acute rejection, lymphocytic bronchitis, and CARV infections. To test these hypotheses, we constructed multivariable Cox proportional hazards models including these four variables with CARV infection as the only time-dependent variable. We also constructed univariable Cox proportional hazards models to identify other potential risk factors for BOS ($p < 0.2$) and forced these into the multivariable models. We removed covariates from the multivariable models if they did not improve the models' -2 log likelihood ratio or if their p value was ≥ 0.05 . Lastly, we evaluated the impact of PGD on long-term survival using univariable and multivariable Cox proportional hazards models with BOS stage 1 as the only time-dependent variable. We confirmed the proportional hazards assumption for all time-independent variables using the log-minus-log method and excluded nonlinearity using partial residual plots. We did not evaluate the impact of PGD on the risk of acute rejection or lymphocytic bronchitis in this study because we found no relationships with these endpoints in our previous analysis (8). We defined statistical significance as $p < 0.05$ and conducted the analysis using SPSS 13.0 (SPSS, Inc., Chicago, IL).

RESULTS

Follow-up was complete through May 1, 2006. The mean duration of observation per patient was 3.7 ± 2.1 years and the study included 1236 person-years of follow-up. The baseline characteristics of the entire cohort are shown in Table 1. As detailed in our previous analysis, 65 (19%) recipients did not have PGD (grade 0), 130 (39%) had PGD grade 1, 69 (21%) had PGD grade 2, and 70 (21%) had PGD grade 3 immediately after transplantation (T0) (8). The distribution of PGD grades over time is shown in Figure 2. Among the 195 recipients who had PGD grade 0 or grade 1 at T0, the PGD grade remained 0 or 1 in 182 (93%) at T72. In contrast, among the 139 recipients who had PGD grade 2 or grade 3 at T0, the PGD grade improved to 0 or 1 in 100 (72%) at T72. Indeed, only 47 recipients had PGD grade 2 or grade 3 at T72.

PGD and the risk of BOS

We excluded 20 recipients from the BOS analysis because they were not evaluable for BOS (13): 14 recipients died in the first 90 days after transplantation, 5 did not have pulmonary function testing because they were ventilator dependent, and 1 had tracheal stenosis requiring permanent tracheostomy. To test our hypotheses, we constructed a series of multivariable Cox proportional hazards models that included acute rejection grade $\geq A2$, lymphocytic bronchitis grade $\geq B2$, CARV infections, and PGD graded at T24, T48, and T72. All grades of PGD at all time points were significant risk factors for the development of BOS stage 1 independent of acute rejection, lymphocytic bronchitis, and CARV infections (Tables 2, 3, 4). Specifically, PGD grade 1 at T24 was associated with a relative risk of BOS stage 1 of 1.93 (95% CI: 1.13 - 3.31, $p = 0.016$), grade 2 with a relative risk of 2.29 (95% CI: 1.24 - 4.24, $p = 0.008$), and grade 3 with a relative risk of 3.31 (95% CI: 1.80 - 6.09, $p < 0.0005$; Table 2). In addition, all grades of PGD at all time points were significant risk factors for the progression of BOS to stage 2 and stage 3 independent of the other covariates (Tables 2, 3, 4). It is noteworthy that there was a consistent direct relationship between the severity of PGD and the risk of BOS development (stage 1) and progression (stages 2 and 3) at all PGD time points. Furthermore, PGD grades at T72 were associated with a higher risk of BOS development and progression than the same PGD grades at T24

and T0 (8) suggesting that persistent graft dysfunction beyond the first 24 hours carries a higher risk of BOS than early or transient PGD. Indeed, 15 of the 20 recipients (75%) who had PGD grade 3 at T72 and were evaluable for BOS developed BOS stage 1 during the study period. In contrast, 49 of the 69 recipients (71%) who had PGD grade 0 at T72 did not develop BOS during the study period. Figure 3 graphically demonstrates freedom from the three BOS stages based on PGD grade using Kaplan-Meier analyses.

To explore other potential risk factors for BOS development, we constructed univariable Cox proportional hazards models and identified an association between the underlying diagnosis, the use of cardiopulmonary bypass, and single lung transplantation with BOS stage 1, although none of these was statistically significant (Table 5). Nevertheless, we forced these variables into the hypotheses-driven multivariable models individually. None of these improved the -2 log likelihood ratios of the overall models, and none was statistically significant. Importantly, these additional covariates did not alter the relative risks or statistical significances of the original covariates, and our final models are the hypotheses-driven original models (Tables 2, 3, 4). Furthermore, because the cohort consisted primarily of bilateral recipients, we repeated the analyses excluding single lung recipients and the results were unchanged; all grades of PGD at all time points were significant risk factors for BOS development (stage 1) and progression (stages 2 and 3; data not shown).

Change in PGD severity and the risk of BOS

To investigate the impact of changes in PGD grade over time on the risk of BOS, we divided recipients who had PGD grade 0 or grade 1 at T0 and were evaluable for BOS ($n = 180$) into two subgroups: those who remained grade 0 or grade 1 at T72 ($n = 175$) and those who worsened to PGD grade 2 or grade 3 at T72 ($n = 5$). We then compared the risk of BOS development (stage 1) and progression (stages 2 and 3) between the two subgroups using Cox proportional hazards models. Recipients whose PGD grade worsened had a higher risk of BOS stage 1 than those whose PGD grade remained 0 or 1, but this was not statistically significant (RR = 2.47, 95% CI: 0.90 - 6.79; $p = 0.08$). Furthermore, those whose PGD grade worsened had a significantly higher risk of BOS stage 2 (RR = 3.61, 95% CI: 1.11 - 11.8; $p = 0.03$) and stage 3 (RR = 5.25, 95% CI: 1.59 - 17.3; $p = 0.006$) than those whose PGD grade remained 0 or 1.

Conversely, we divided recipients who had PGD grade 2 or grade 3 at T0 and were evaluable for BOS ($n = 132$) into two subgroups: those who remained grade 2 or grade 3 at T72 ($n = 34$) and those who improved to PGD grade 0 or grade 1 at T72 ($n = 98$) and compared the risk of BOS development and progression between the two subgroups. There was a trend to a lower risk of BOS stage 1 favoring those whose PGD grade improved but this was not statistically significant (RR = 0.71, 95% CI: 0.44 - 1.14; $p = 0.16$). Likewise, there were trends to lower risks of BOS stage 2 (RR = 0.64, 95% CI: 0.37 - 1.09; $p = 0.09$) and stage 3 (RR = 0.59, 95% CI: 0.31 - 1.12; $p = 0.11$) favoring those whose PGD grade improved but these were not statistically significant.

PGD and long-term survival

To evaluate the impact of PGD on long-term survival, we performed univariable Cox proportional hazards survival analyses conditional on survival to 90 days and identified single lung transplantation (RR = 3.45, 95% CI: 2.08 - 5.73, $p < 0.0005$), BOS stage 1 (RR = 5.67, 95% CI: 3.73 - 8.64, $p < 0.0005$), PGD grade 2 at T48 (RR = 2.43, 95% CI: 1.09 - 5.43, $p = 0.03$) and T72 (RR = 3.27, 95% CI: 1.48 - 7.20, $p = 0.003$), and PGD grade 3 at all time points (at T24, RR = 2.57, 95% CI: 1.30 - 5.08, $p = 0.007$; at T72, RR = 4.09, 95% CI: 1.92 - 8.72, $p < 0.0005$) as significant predictors of death. However, recipient age, gender,

and diagnosis, PGD grade 1 at all time points and PGD grade 2 at T24 were not significant predictors of mortality.

To investigate the impact of PGD on long-term survival in the context of other risk factors, we constructed multivariable Cox proportional hazards models including BOS stage 1, single lung transplantation, and PGD at T24, T48, and T72. Single lung transplantation and BOS stage 1 remained significant predictors of late mortality in all the models (Table 6). However, it should be noted that although single lung transplantation was a significant risk factor for late mortality, recipients were not randomized to receive a single vs. a bilateral transplant and a selection bias may be responsible for the increased mortality. Only PGD grade 2 at T72 was a significant risk factor for late death (RR = 2.60; 95% CI: 1.15 - 5.88, $p = 0.02$; Table 6) among the PGD grades at all time points, although there was a statistically non-significant trend associated with PGD grade 3 at T72 (RR = 1.98; 95% CI: 0.87 - 4.52, $p = 0.10$; Table 6).

DISCUSSION

In this study, we evaluated the impact of PGD graded at 24, 48, and 72 hours after transplantation on the risk of BOS development (stage 1) and progression (stages 2 and 3). Our data demonstrate that PGD at all time points is a significant risk factor for both BOS development and progression independent of other recognized risk factors including acute rejection, lymphocytic bronchitis, and CARV infections. Furthermore, there is a consistent direct relationship between the severity of PGD and the risk of BOS development. Indeed, PGD grade 3 was associated with the highest risk of BOS development and progression at all time points. In addition, our results suggest that worsening PGD grade in the first 72 hours after transplantation increases the risk of BOS, while an improvement in PGD grade may be associated with a lower risk although this was not statistically significant. Lastly, while PGD grade 2 and grade 3 were significant risk factors for late mortality in univariable analyses, these associations appear to be dependent, at least in part, on BOS development.

In the context of previous studies examining the association between early graft dysfunction and BOS, our results expand on our previous findings (8). Indeed, an important limitation of our previous study is that we only analyzed PGD as a risk factor for BOS at T0. Thus, a recipient who has PGD grade 0 at T0 but develops grade 1 or grade 2 over the next 72 hours would have the reference relative risk (RR = 1) for developing BOS. Clearly, this scenario could confound the analysis. Therefore, our current study extends and complements our previous findings by analyzing the impact of PGD graded at later time points (T24, T48, and T72) on the risk of BOS development (stage 1) and progression to stages 2 and 3. Taken together, our data implicate PGD as an important risk factor for BOS development and progression and underscore the significance of PGD on outcomes after lung transplantation. In addition, our results corroborate those of Whitson and colleagues who demonstrated that recipients who developed PGD grade 3 had a significantly lower BOS-free survival than those who did not (6). Our methods, study design, and patient population differ from those used by Whitson and colleagues. They divided their cohort into those who developed PGD grade 3 and those who did not (PGD grades 0, 1, and 2) and compared BOS-free survival between the two groups. However, it is important to distinguish the presence of disease from its absence in a staging system. Specifically, PGD grade 0 is not equivalent to grade 1 or grade 2. Indeed, we have demonstrated in this study and in our previous report (8) that recipients who developed PGD grade 1 have a significantly higher risk of BOS development and progression than those who did not (grade 0). In fact, those who developed PGD grade 1 had a 2 fold higher risk of BOS stage 1 and a 3 fold higher risk of BOS stage 3 than those who had PGD grade 0. Furthermore, the direct relationship between the severity of PGD and the risk of BOS validates the operational definition of PGD proposed by the ISHLT. In

addition, we used multivariable analyses to examine the risk of BOS attributable to PGD in the context of other recognized risk factors while Whitson and colleagues used univariable analyses. Lastly, Whitson and colleagues did not find an association between PGD and BOS among single lung recipients. Our cohort consisted primarily of bilateral recipients and only 26 of the 334 had a single lung transplant. Thus, our data are not sufficient to evaluate the impact of PGD on BOS among single lung recipients. Finally, differences in study design and patient population notwithstanding, both studies reach the similar conclusion that PGD increases the risk of BOS months or years later.

However, our findings and those of Whitson and colleagues contrast with those of Burton and colleagues who evaluated the impact of PGD on BOS and found no correlation between PGD and BOS (15). It is likely that the different definitions of PGD contributed to these disparate conclusions. Burton and colleagues identified PGD when radiographic infiltrates were present diffusely in the allograft and excluded cases where only peri-hilar infiltrates were present. This definition is more stringent than the ISHLT definition based on the presence of “infiltrates consistent with pulmonary edema” (4). This suggests that some patients who were classified as not having PGD in Burton and colleagues’ study would have been classified as having PGD in our study and would have a higher risk of BOS. This would then diminish the apparent difference in the risk of BOS between those who had PGD and those who did not. A primary goal of the ISHLT working group on PGD was to standardize the definition so that independent groups can study this complication and interpret their findings appropriately.

The mechanisms by which PGD predisposes to BOS development and progression have yet to be elucidated. Recipients who develop PGD and those who eventually develop BOS have a marked systemic inflammatory response early after transplantation characterized by significant elevations in serum levels of IP-10, MCP-1, and Th1-cytokines (16,17). These inflammatory mediators can upregulate human leukocyte antigens (HLA), costimulatory molecules, and cell adhesion molecules on donor airway epithelial cells promoting alloimmunity (16-19). Furthermore, recipients who have PGD are more likely to develop anti-HLA antibodies *de novo* after transplantation (17), and these have clearly been linked to the subsequent development of BOS (20-22). However, despite these immunologic associations between PGD and BOS, there does not appear to be a relationship between PGD and acute rejection or lymphocytic bronchitis (8,23,24). Nonetheless, these findings do not necessarily conflict with an alloimmune explanation for the relationship between PGD and BOS because the immune mechanisms that mediate acute rejection may be distinct from those that mediate BOS. Indeed, if anti-HLA antibodies play a role in the association between PGD and BOS, there may be a humoral component of BOS that is independent of acute (cellular) rejection and lymphocytic bronchitis (25,26).

Additionally, recent evidence suggests that BOS may be driven by autoimmunity. Type V collagen is an immunogenic minor sequestered antigen intercalated in the major collagen fibrils in the lung (27,28). Animal and human studies demonstrate that allograft injury such as acute rejection and ischemia-reperfusion injury can expose and release fragments of type V collagen (29,30). In fact, stable lung transplant recipients have circulating collagen V-specific T cells that suppress the proliferation of collagen V-specific Th-1 cells through an IL-10 dependent pathway (31,32). However, the onset of BOS is characterized by a decline in the IL-10 producing collagen V-specific T cells and an expansion of collagen V-specific Th-1 cells (31,32). These findings suggest that recipients become immunized to collagen V, but this autoimmunity is limited by regulatory T cells until the onset of BOS when the suppression of autoimmunity is lost. Similarly, a recent study demonstrates that some recipients develop antibodies against K alpha 1 tubulin, a highly conserved and normally concealed cellular protein, after transplantation and this is strongly associated with the

development of BOS (33). Clearly, autoimmunity is an emerging paradigm for BOS and studies are ongoing to further our understanding of the potential links between PGD, autoimmunity, and BOS development.

Alternatively, the association between PGD and BOS may be independent of immune-mediated mechanisms, and BOS may be the stereotypic lung allograft response to injury as recovery results in an exuberant and disordered repair process. Indeed, a significant increase in the ratio of matrix metalloproteinase-9 to tissue inhibitors of metalloproteinase-1 has been reported in the bronchoalveolar lavage fluid of recipients with BOS (34). Similarly, pro-fibrotic mediators including platelet-derived growth factor, insulin-like growth factor, and transforming growth factor- β have been linked to BOS in human and animal studies (35).

This study has inherent limitations because of the retrospective design. PGD grade assignment is perhaps the most serious potential bias. For this reason, a single investigator assigned PGD grades to avoid inter-observer variability. Furthermore, we used the chest x-ray report that had been prospectively interpreted by a thoracic radiologist and noted the presence or absence of radiographic evidence of pulmonary edema. We considered only the following terms as synonymous with pulmonary edema: re-implantation edema, re-implantation response, reperfusion edema, reperfusion injury, and ischemia-reperfusion lung injury. In addition, because exclusion of other potential causes of early graft dysfunction, such as hyperacute rejection, vascular anastomotic complications, cardiogenic pulmonary edema, and pneumonia, is implicit in the PGD definition, we excluded the post-operative courses of three recipients who developed hyperacute rejection. Furthermore, we excluded vascular anastomotic complications with a nuclear perfusion lung scan and cardiogenic pulmonary edema with the pulmonary capillary wedge pressure in all recipients. However, it is difficult to exclude the possibility of superimposed pneumonia, especially for PGD grades at later time points (T48 and T72). Therefore, PGD grades immediately after transplantation (T0) may be most accurate, especially in a retrospective study. Nonetheless, the results of our analyses at T48 and T72 are in agreement with those at T0 and T24 (8). An additional limitation is the small sample sizes of the PGD grade 2 and grade 3 groups, especially at T48 and T72; this may have inflated the risk of BOS. However, we made only one variable time-dependent in the multivariable analysis to avoid risk inflation and mitigate this bias. Lastly, we have not validated our models using an independent cohort because of the limited sample size. Nevertheless, a bias toward a specific cohort would be necessary for any of these limitations to significantly alter our results, and none is apparent.

In conclusion, our results demonstrate that PGD at all time points is an independent risk factor for BOS development and progression. In addition, there is a direct relationship between the severity of PGD and the risk of BOS. These findings suggest that the long-term outcome after transplantation may be determined in the immediate post-operative period as the die is cast. This underscores the importance of future studies to evaluate therapeutic interventions that might reduce the risk or severity of PGD or that might uncouple its association with BOS.

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References

1. de Perrot M, Liu M, Waddell TK, Keshavjee S. Ischemia-reperfusion-induced lung injury. *Am J Respir Crit Care Med*. 2003; 167:490–511. [PubMed: 12588712]

2. Christie JD, Van Raemdonck D, de Perrot M, Barr M, Keshavjee S, Arcasoy S, et al. Report of the ISHLT working group on primary lung graft dysfunction part I: Introduction and methods. *J Heart Lung Transplant*. 2005; 24:1451–1453. [PubMed: 16210115]
3. Christie JD, Kotloff RM, Ahya VN, Tino G, Pochettino A, Gaughan C, et al. The effect of primary graft dysfunction on survival after lung transplantation. *Am J Respir Crit Care Med*. 2005; 171:1312–1316. [PubMed: 15764726]
4. Christie JD, Carby M, Bag R, Corris P, Hertz M, Weill D. Report of the ISHLT working group on primary lung graft dysfunction part II: definition. A consensus statement of the International Society for Heart and Lung Transplantation. *J Heart Lung Transplant*. 2005; 24:1454–1459. [PubMed: 16210116]
5. Prekker ME, Nath DS, Walker AR, Johnson AC, Hertz MI, Herrington CS, et al. Validation of the proposed International Society for Heart and Lung Transplantation grading system for primary graft dysfunction after lung transplantation. *J Heart Lung Transplant*. 2006; 25:371–378. [PubMed: 16563963]
6. Whitson BA, Prekker ME, Herrington CS, Whelan TPM, Radosevich DM, Dahlberg PS. Primary graft dysfunction and long-term pulmonary function after lung transplantation. *J Heart Lung Transplant*. 2007; 26:1004–1011. [PubMed: 17919620]
7. Whitson BA, Nath DS, Johnson AC, Walker AR, Prekker ME, Radosevich DM, et al. Risk factors for primary graft dysfunction after lung transplantation. *J Thorac Cardiovasc Surg*. 2006; 131:73–80. [PubMed: 16399297]
8. Daud SA, Yusen RD, Meyers BF, Chakinala MM, Walter MJ, Aloush AA, et al. Impact of immediate primary lung allograft dysfunction on bronchiolitis obliterans syndrome. *Am J Respir Crit Care Med*. 2007; 175:507–513. [PubMed: 17158279]
9. Hachem RR, Khalifah AP, Chakinala MM, Yusen RD, Aloush AA, Mohanakumar T, et al. The significance of a single episode of minimal acute rejection after lung transplantation. *Transplantation*. 2005; 80:1406–1413. [PubMed: 16340783]
10. Yousem SA, Berry GJ, Cagle PT, Chamberlain D, Husain AN, Hruban RH, et al. Revision of the 1990 working formulation for the classification of pulmonary allograft rejection: Lung Rejection Study Group. *J Heart Lung Transplant*. 1996; 15:1–15. [PubMed: 8820078]
11. Yousem SA. Lymphocytic bronchitis/bronchiolitis in lung allograft recipients. *Am J Surg Pathol*. 1993; 17:491–496. [PubMed: 8470763]
12. Khalifah AP, Hachem RR, Chakinala MM, Schechtman KB, Patterson GA, Schuster DP, et al. Respiratory viral infections are a distinct risk for bronchiolitis obliterans syndrome and death. *Am J Respir Crit Care Med*. 2004; 170:181–187. [PubMed: 15130908]
13. Cooper JD, Billingham M, Egan T, Hertz MI, Higenbottam T, Lynch J, et al. A working formulation for the standardization of nomenclature and for clinical staging of chronic dysfunction in lung allografts. *J Heart Lung Transplant*. 1993; 12:713–716. [PubMed: 8241207]
14. Estenne M, Maurer JR, Boehler A, Egan JJ, Frost A, Hertz MI, et al. Bronchiolitis obliterans syndrome 2001: an update of the diagnostic criteria. *J Heart Lung Transplant*. 2002; 21:297–310. [PubMed: 11897517]
15. Burton CM, Iversen M, Milman N, Zemtsovski M, Carlsen J, Steinbruchel D, et al. Outcome of lung transplanted patients with primary graft dysfunction. *Eur J Cardiothorac Surg*. 2007; 31:75–82. [PubMed: 17134909]
16. Bharat A, Narayanan K, Street T, Fields RC, Steward N, Aloush A, et al. Early posttransplant inflammation promotes the development of alloimmunity and chronic human lung allograft rejection. *Transplantation*. 2007; 83:150–158. [PubMed: 17264811]
17. Bharat, A.; Kuo, E.; Hachem, R.; Trulock, EP.; Patterson, GA.; Meyers, BF., et al. Immunological link between primary graft dysfunction (PGD) and bronchiolitis obliterans syndrome (BOS) [abstract]; Abstract presented at the Annual Meeting of the Society of Thoracic Surgeons; Fort Lauderdale FL. January 26-29, 2008;
18. Mauck KA, Hosenpud JD. The bronchial epithelium: a potential allogeneic target for chronic rejection after lung transplantation. *J Heart Lung Transplant*. 1996; 15:709–714. [PubMed: 8820787]

19. Waddell TK, Gorczynski RM, DeCampos KN, Patterson GA, Slutsky AS. Major histocompatibility complex expression and lung ischemia-reperfusion in rats. *Ann Thorac Surg.* 1996; 662:866–72. [PubMed: 8784021]
20. Smith MA, Sundaresan S, Mohanakumar T, Trulock EP, Lynch JP, Phelan DL, et al. Effect of development of antibodies to HLA and cytomegalovirus mismatch on lung transplantation survival and development of bronchiolitis obliterans syndrome. *J Thorac Cardiovasc Surg.* 1998; 116:812–20. [PubMed: 9806388]
21. Palmer SM, Davis RD, Hadjiliadis D, Hertz M, Howell DN, Ward FE, et al. Development of an antibody specific to major histocompatibility antigens detectable by flow cytometry after lung transplant is associated with bronchiolitis obliterans syndrome. *Transplantation.* 2002; 74:799–804. [PubMed: 12364858]
22. Girmata AL, Duquesnoy R, Yousem SA, Iacono AT, Corcoran TE, Buzoianu M, et al. HLA-specific antibodies are risk factors for lymphocytic bronchitis and chronic lung allograft dysfunction. *Am J Transplant.* 2005; 5:131–138. [PubMed: 15636621]
23. Hachem RR, Yusen RD, Patterson GA, Trulock EP. Impact of primary graft dysfunction on bronchiolitis obliterans syndrome after lung transplantation [abstract]. *J Heart Lung Transplant.* 2007; 26(S2):109.
24. Elidemir O, Doan ML, McKenzie ED, Heinle JS, Morales DL, Mallory GB. Primary graft dysfunction and acute rejection in pediatric lung transplant recipients [abstract]. *Proc Am Thorac Soc.* 2006; 3:A366.
25. Magro CM, Ross P, Kelsey M, Waldman WJ, Pope-Harman A. Association of humoral immunity and bronchiolitis obliterans syndrome. *Am J Transplant.* 2003; 3:115–1166.
26. Jaramillo A, Smith CR, Maruyama T, Zhang L, Patterson GA, Mohanakumar T. Anti-HLA class I antibody binding to airway epithelial cells induces production of fibrogenic growth factors and apoptotic cell death: a possible mechanism for bronchiolitis obliterans syndrome. *Human Immunology.* 2003; 64:521–529. [PubMed: 12691702]
27. Madri JA, Furthmayr H. Collagen polymorphism in the lung. An immunochemical study of pulmonary fibrosis. *Human Pathology.* 1980; 11:353–366. [PubMed: 6997183]
28. van Kuppevelt TH, Veerkamp JH, Timmermans JA. Immunoquantification of type I, III, and V collagen in small samples of human lung parenchyma. *Int J Biochem Cell Biol.* 1995; 27:775–782. [PubMed: 7584611]
29. Haque MA, Mizobuchi T, Yasufuku K, Fujisawa T, Brutkiewicz RR, Zheng Y, et al. Evidence for immune responses to a self-antigen in lung transplantation: role of type V collagen-specific T cells in the pathogenesis of lung allograft rejection. *J Immunol.* 2002; 169:1542–1549. [PubMed: 12133982]
30. Yoshida S, Haque A, Mizobuchi T, Iwata T, Chiyo M, Webb TJ, et al. Anti-type V collagen lymphocytes that express IL-17 and IL-23 induce rejection pathology in fresh and well-healed lung transplants. *Am J Transplant.* 2006; 6:724–735. [PubMed: 16539629]
31. Bharat A, Fields RC, Steward N, Trulock EP, Patterson GA, Mohanakumar T. CD4+25+ regulatory T cells limit Th1-autoimmunity by inducing IL-10 producing T cells following human lung transplantation. *Am J Transplant.* 2006; 6:1799–1808. [PubMed: 16889540]
32. Bharat A, Fields RC, Trulock EP, Patterson GA, Mohanakumar T. Induction of IL-10 suppressors in lung transplant patients by CD4+25+ regulatory T cells through CTLA-4 signaling. *J Immunol.* 2006; 177:5631–5638. [PubMed: 17015751]
33. Goers TA, Ramachandran S, Aloush A, Trulock EP, Patterson GA, Mohanakumar T. De novo production of K- α 1 tubulin-specific antibodies: role in chronic lung allograft rejection. *J Immunol.* 2008; 180:4487–4494. [PubMed: 18354170]
34. Hubner RH, Meffert S, Mundt U, Bottcher H, Freitag S, El Mokhtari NE, et al. Matrix metalloproteinase-9 in bronchiolitis obliterans syndrome after lung transplantation. *Eur Respir J.* 2005; 25:494–501. [PubMed: 15738294]
35. Nicod LP. Mechanisms of airway obliteration after lung transplantation. *Proc Am Thorac Soc.* 2006; 3:444–449. [PubMed: 16799090]