

UCLA

UCLA Electronic Theses and Dissertations

Title

Primary Graft Dysfunction and Long-Term Outcomes Following Lung Transplantation

Permalink

<https://escholarship.org/uc/item/3rv9r548>

Author

DerHovanesian, Ariss

Publication Date

2012

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA

Los Angeles

Primary Graft Dysfunction and Long-Term
Outcomes Following Lung Transplantation

A thesis submitted in partial satisfaction
of the requirements for the degree Master of Science
in Clinical Research

by

Ariss DerHovanesian

2012

ABSTRACT OF THE THESIS

Primary Graft Dysfunction and Long-Term
Outcomes Following Lung Transplantation

by

Ariss DerHovanesian

Master of Science in Clinical Research

University of California, Los Angeles, 2012

Professor Robert M. Elashoff, Chair

Background: Primary graft dysfunction (PGD) is an early complication of lung transplantation associated with poor early outcomes, however less is known about its prolonged effects on morbidity and mortality. We hypothesized that PGD is associated with long-term mortality and chronic rejection in the form of bronchiolitis obliterans syndrome.

Methods: A retrospective study of 279 adult lung transplant recipients between 2000 and 2007 was performed. PGD grade was determined both immediately after transplantation

(T0) and at 72 hours post-transplant (T72). Chronic rejection defined as stage 1 bronchiolitis obliterans syndrome (BOS), long-term mortality in 90-day survivors were modeled using competing risk and extended Cox models with time-dependent covariates with internal validation performed via bootstrapping. Cumulative incidence plots for the outcome of BOS were created for each PGD grade and at both time points.

Results: We found that there was a significant stepwise increase in the hazard ratio for both BOS and mortality with increasing PGD grade. This association was most severe among patients with grade 3 PGD at T72, and the association with BOS persisted in adjusted multivariable models with a hazard ratio of 3.75 (95% CI 1.11-21.4, $p < 0.001$). Stratified analyses in recipients with either single or bilateral transplants were also consistent with this finding. The association between PGD and long-term mortality also persisted after adjustment for baseline covariates, but in multivariable models of mortality that also incorporated BOS as a time-dependent variable, PGD was no longer significantly associated with mortality.

Conclusions: These results suggest that severe PGD in the early perioperative period may play a causal role in the subsequent development of BOS after lung transplantation in some patients, and that the development of BOS in patients with PGD may account for increased rates of death even among recipients with severe PGD who survive the early perioperative period.

The thesis of Ariss DerHovanessian is approved.

John A. Belperio

Janet Sinsheimer

Robert M. Elashoff, Committee Chair

The University of California, Los Angeles

2012

TABLE OF CONTENTS

CHAPTER 1 (BACKGROUND)	1
CHAPTER 2 (MANUSCRIPT)	9
TABLE 1	25
TABLE 2	26
TABLE 3	27
TABLE 4	28
TABLE 5	29
FIGURE 1	30
FIGURE 2	31
CHAPTER 3 (APPENDIX)	32
REFERENCES	38

CHAPTER 1: BACKGROUND

Lung Transplantation

Since the first successful human lung transplants performed in the early 1980's, lung transplantation has become an increasingly viable option for patients suffering from a variety of end-stage lung diseases including tobacco-related emphysema, fibrotic lung diseases, cystic fibrosis, and pulmonary vascular disease (1), (2), (3), (4). Over the years the number of lung transplants performed globally has continued to increase with the yearly total more than doubling from 1,559 in 1999 to 3,272 in 2009 (5). Unfortunately, despite improvements in short term morbidity and mortality largely attributable to improved surgical technique and perioperative critical care management, the mortality rate following the first post-transplant year has remained essentially unchanged. As of 2010, the 1-, 3-, and 5-year survival rates following transplantation are 79%, 64%, and 53%, respectively, while the median survival time is 5.5 years (5). Only 30% of patients survive for 10 years or more. In comparison, median survival following both heart and liver transplants are projected to be approximately 11 years (6, 7).

Bronchiolitis Obliterans Syndrome

The leading cause of death after the first year of transplantation continues to be the development of graft failure due to chronic rejection in the form of bronchiolitis obliterans syndrome (BOS) (5, 8-13). BOS is clinically characterized by progressive irreversible airflow obstruction diagnosed based on consensus physiologic criteria defining the first stage as an otherwise unexplained, persistent decline of 20% in the

forced expiratory volume in one second (FEV1) from a patient's post-transplant maximum (14, 15). BOS is rarely seen within the first year of transplantation, however the 5-year cumulative incidence ranges from 43-80% at various centers (5, 16-18). According to the International Society for Heart and Lung Transplantation (ISHLT) registry, 50% of patients will have developed BOS within 5.6 years of transplantation (5). The prognosis is variable in patients with BOS, but the majority of patients have ongoing loss of pulmonary function. As the condition progresses, it ultimately leads to chronic respiratory failure with 5-year survival from the time of diagnosis ranging from only 26 to 43% (19, 20).

In the early phase of BOS, histologic changes may include infiltration of submucosa of small airways by mononuclear cells, leading to an accumulation of foamy macrophages and distal mucostasis, and accompanied by epithelial cell injury and necrosis associated with the recruitment and/or proliferation of fibroblasts and myofibroblasts (14, 21, 22). As this inflammatory response continues, there is ongoing deposition of extracellular matrix proteins culminating in the irreversible fibrosis and obliteration of the bronchioles, referred to as obliterative bronchiolitis (OB), the ultimate pathological correlate of the clinical syndrome of BOS (23).

The immunologic mechanisms involved in pathogenesis of BOS remain complex and incompletely understood. Classically allograft rejection is seen as a host response to allo-antigens driven by either the response of recipient T cells to either intact major

histocompatibility complex (MHC) molecules on the surface of donor antigen-presenting cells (APCs), or processed donor MHC molecules presented as peptides by recipient APCs (12). This leads to the expression of multiple cytokines, chemokines, and growth factors in the local airway microenvironment, which drive feed-forward recruitment of additional mononuclear cells to the site, ongoing inflammation, alloimmune recognition, fibroblast proliferation, and extracellular matrix deposition (24-33). Multiple potentially interacting immunological pathways have been implicated in the process including Type 1, Type 2, and Type 17 responses (12).

A wide variety of approaches to the treatment and prevention of BOS have been reported in the literature including alteration of chronic immunosuppression regimens, augmentation of immunosuppression with high-dose glucocorticoids, immunomodulation with macrolides, monoclonal cytolytic agents (e.g., muromonab, alemtuzumab), polyclonal cytolytic agents (e.g., antithymocyte globulin), interleukin-2 receptor antagonists (e.g., basiliximab, daclizumab), lymphoid irradiation, and extracorporeal photopheresis (34-40). Unfortunately, responses to any of these modalities are at best anecdotal with the literature to date failing to demonstrate consistent benefit from any treatment modality in controlled trials.

Primary Graft Dysfunction

Primary graft dysfunction (PGD) is a form of acute lung injury that arises within the first 72 hours of transplantation, resulting from multiple pathological mechanisms inherent to

the process of transplantation including physiological changes in the donor following brain death, explantation, cold ischemia of the organ during preservation, transplantation, and injury following reperfusion within the recipient (41). Clinically patients develop varying degrees of impaired oxygenation and non-cardiogenic pulmonary edema, and in its more severe form, the incidence ranges from 10% to 25% (42-46). While the leading cause of long-term graft failure is BOS, severe PGD is the leading cause of morbidity and mortality in the first year following transplantation with a 30-day mortality rate as high as 35-60% (45).

Previously also referred to as primary graft failure, ischemia-reperfusion injury, post-transplant acute lung injury, a consensus definition and guidelines for severity grading were first proposed in 2005 by the ISHLT in order to standardize clinical and research efforts (47). The proposed definition paralleled that of non-transplant acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), and graded PGD based on the presence of radiographic findings consistent with pulmonary edema, and the severity of hypoxemia as measured by the ratio of arterial oxygen partial pressure (PaO_2) to the fraction of inspired oxygen (FiO_2) (Table 1).

Central to the pathogenesis of PGD is the process of ischemia-reperfusion injury, which is driven by the tissue ischemia inherent to organ procurement and preservation, and the generation of reactive oxygen species. These oxygen species subsequently cause oxidative stress at the cellular level along with direct epithelial and endothelial injury to

the grafted lung (48). This then precipitates a pro-inflammatory cascade with increased cytokine, chemokine and adhesion molecule expression, which leads to the recruitment of macrophages, recipient lymphocytes, and neutrophils to the sites of injury, further perpetuating the process of lung injury (49-53). Downstream effects include lipid peroxidation driven by neutrophils, activation of the complement cascade, and platelet activation with aggregation and microvascular thrombus formation (54-56). Histologically, this process is indistinguishable from non-transplant ALI and ARDS, and is characterized by the development of diffuse alveolar damage with capillary leak, alveolar and septal edema, alveolar pneumocyte hyperplasia, neutrophil infiltration and the formation of hyaline membranes that line the alveolar spaces (47, 57).

Non-alloimmune Mechanisms for the Development of BOS

The dramatic difference in outcomes between recipients of lung and other solid organ transplants has spurred a great deal of interest in the distinguishing features of BOS and immune response following transplantation. Though indirect and direct alloimmune recognition remain fundamental to the process of rejection following any type of transplant, a growing body of literature now supports the association between the development of BOS and ostensibly non-alloimmune responses to local injury and foreign antigens unrelated to donor-specific MHC within the lung.

Multiple infectious processes have been linked to the development of BOS. The best evidence for this is in patients who develop cytomegalovirus (CMV) pneumonitis (58-

61). CMV infection increases epithelial expression of donor human leukocyte antigen (HLA) in transplant recipients, upregulates proinflammatory cytokine expression, and the virus has been found to share nucleic acid sequence homology with specific HLA antigens (61-63). Two non-randomized trials have found that pharmacological prophylaxis against CMV leads to both a reduced rate of CMV as well as BOS (61, 63-65). Similarly, associations between BOS and community-acquired respiratory viruses, human herpes virus-6 and *Chlamydia pneumonia* infection have been described in retrospective single center series (66-69). Finally, low-level chronic infection or colonization with *Aspergillus* species and *Pseudomonas aeruginosa* have also been linked with BOS (70-72).

Following transplantation, recipients may be at increased risk of developing gastroesophageal reflux due to delayed gastric emptying, lung denervation, impaired cough reflex, and abnormal mucociliary clearance (73). In addition to infections and pathogenic colonization of the allograft, two groups have found that the presence of bile acid in bronchoalveolar lavage from transplant recipients is associated with BOS, while a more recent series has shown that the presence of bile acid in lavage specimens from patients who already have BOS is associated with a more rapid decline in lung function and increased rate of mortality (74-76). The mechanism for this association is unclear, though it has been shown that high levels of bile acids within the allograft of these patients are also associated with lower surfactant collectin proteins and surfactant phospholipids, all components of innate immunity in the lung (77). In transplant

recipients with known reflux, retrospective studies have also found that aggressive therapy with early gastric fundoplication may be associated with greater freedom from BOS and improved lung function (78-80).

Prior to the introduction of the ISHLT consensus grading criteria, studies regarding the association between PGD and BOS were conflicting. Fisher et al. were unable to demonstrate such an association in a cohort of 291 patients, 55 of which had histological evidence of PGD in the form of diffuse alveolar damage (81). In contrast, Fiser et al. demonstrated a statistically significant association between clinically defined PGD and BOS in a cohort of 115 patients (82). More recently, using the formalized ISHLT criteria for PGD, Whitson et al. could only demonstrate a statistical association between grade 3 PGD and BOS (83). Furthermore, this association was not confirmed among single lung recipients.

While prior clinical research has hinted at a potential association between PGD and BOS, the biomolecular underpinnings of a mechanism to explain such an association have yet to be fully described. Preliminary work on this subject has led to the hypothesis that although PGD is generally thought to be an alloimmune-independent form of lung injury, it may trigger a long-term immune response in the transplanted lung. The innate immune response of PGD involves increased expression of interleukin-8 by neutrophils, and increased expression of major histocompatibility class (MHC) II antigens has been demonstrated in animal models of PGD (84-87). Along with increased expression of

MHC II antigens, the recruitment of recipient antigen-presenting cells into allografts, as well as the local release of type V collagen and K- α 1 tubulin, self-antigens not previously exposed to the recipient immune system have also been demonstrated (88-92). In both heart and kidney transplant recipients, similar forms of early graft reperfusion injury have been associated with chronic allograft dysfunction (93-97).

Study Objectives

In summary, one of the distinguishing features of lung transplantation is that multiple forms of non-alloimmune responses to direct injury, infections, and colonizing pathogens in the transplanted lung have been associated with the subsequent development of chronic rejection. While the early literature in lung transplant recipients is somewhat conflicting, data from other solid organ transplants as well as limited findings related to the biomolecular and immunological changes during PGD support the possibility that PGD may also promote BOS. Consequently, the primary aim of this study is to apply the latest standardized ISHLT criteria for grading PGD to our cohort of transplant recipients at the University of California, Los Angeles (UCLA), and assess the association between early as well as prolonged PGD on the subsequent development of BOS. As the association between severe PGD and early perioperative mortality is already well-established, the second aim was to determine the impact of PGD on long-term mortality, and assess the role of BOS in contributing to any such association.

CHAPTER 2: MANUSCRIPT

1. Introduction

Despite ongoing advances in surgical technique, immunosuppressive regimens, and approaches to medical management of recipients, median survival following lung transplantation continues to be less than 6 years, and lags considerably behind other major solid organ transplants (5-7). The primary cause of long-term morbidity and mortality continues to be chronic allograft rejection in the form of bronchiolitis obliterans syndrome (BOS), and as with other solid organ transplants, the most well-established risk factor for chronic rejection is prior acute rejection (AR), both in severity and frequency (5, 11-13). However, intense focus on the distinguishing features of lung transplantation and BOS has lead to a growing body of literature that also supports an association between BOS and a variety of preceding immunological responses to local injury and antigens unrelated to the donor major histocompatibility complex (MHC). Potential risk factors for BOS described in the literature include gastroesophageal reflux as well as infections and/or colonization with a variety of pathogens including cytomegalovirus (CMV), *Pseudomonas aeruginosa*, *Aspergillus* species, community-acquired respiratory viruses (CARVs), human herpes virus-6 and *Chlamydia pneumonia* (58-61, 66-71).

Primary graft dysfunction (PGD) is a form of acute lung injury that arises within the first 72 hours of transplantation, and is the leading cause of mortality within the first year following transplantation (41, 45). The early transplant literature regarding the relationship between PGD and the subsequent development of BOS has been conflicting

(81-83). Using the latest consensus grading criteria for PDG from the International Society for Heart and Lung Transplantation (ISHLT), this study explores the association between PGD and long-term outcomes including BOS and death.

2. Methods

2.1 Study Design and Patient Population

This is a single center retrospective cohort study performed with approval from the University of California, Los Angeles (UCLA) Institutional Review Board. Data were directly extracted from review of the medical record of all patients undergoing bilateral and single lung transplantation at UCLA Medical center between March 2000 and August 2007. Severe PGD has a well-established, profound impact on survival within the first few months of transplantation, prior to the onset of BOS. As the purpose of this study was to assess the long-term effects of PGD on transplant outcomes, and inclusion of early deaths would clearly violate the statistical assumptions of our models, subjects who died within 90 days of transplantation were excluded from the cohort.

2.2 Standard Care and Surveillance of Transplant Recipients

All transplant recipients at UCLA over the period of interest were managed and followed using a standard transplant protocol. Immediately following transplantation, all patients receive induction immunosuppression. Patients over the age of 65, or under 65 with prior malignancy history or elevated risk of infection as judged by their treating physician, receive basiliximab 20 mg intravenously on post-operative days 0 and 4. All other patients received induction therapy of rabbit Anti-Thymocyte-Globulin (ATG) 1.5 mg/kg intravenously on post-operative days 0, 1, and 2. All patients received intra-operative methylprednisolone 1 gram intravenously, followed by 125 mg every 12 hours for 3 doses. They are then initiated on a standard long-term maintenance immunosuppressive

regimen incorporating prednisone tapered to 5 mg daily, tacrolimus titrated to 12-hour trough serum levels between 8-20 ng/mL, and mycophenolate mofetil 1000 mg twice daily.

Patients were seen in clinic every 1-2 weeks for the first 3 months following discharge from the hospital, and every 4-8 weeks thereafter. Lung function was assessed by standard spirometry performed at each visit. In addition, within the first year patients underwent routine surveillance bronchoscopy with bronchoalveolar lavage (BAL) and trans-bronchial biopsies at 1 week, and 1, 3, 6, and 12 months following transplantation, and whenever clinically indicated. When symptoms of productive cough occurred, sputum was also collected at the discretion of the treating physician. During bronchoscopy, BAL was performed with the instillation of 3 standard aliquots of 60 mL of sterile normal saline. Aliquots of the fluid were plated for standard microbiological cultures.

All patients were treated with a regimen of prophylactic agents to prevent infection. Patients received nebulized Amphotericin B 15 mg twice daily (January 2000 to September 2003) or its liposomal formulation 50 mg daily (October 2003 to conclusion), plus caspofungin 50 mg intravenously during their post-transplant hospitalization. Recipients with positive serology for CMV and those with serologic positive donors received routine prophylaxis with intravenous ganciclovir 3 mg/kg/day for 2 weeks, followed by oral valganciclovir 900 mg daily indefinitely. No cases of CMV pneumonitis

were documented. For prophylaxis against *Pneumocystis jiroveci*, all patients received trimethoprim-sulfamethoxazole double-strength tablets twice daily for two days per week.

All patients with acute rejection (AR) of grade 2 or higher, were treated with methylprednisone (500-1000 mg daily for 3 days). Symptomatic grade 1 AR were treated with prednisone burst (0.5 mg/kg daily) followed by taper by 5 mg per week down to the previous dose. Refractory, steroid-resistant AR was treated with ATG (1.5 mg/kg daily for 5 days) unless it was accompanied by high suspicion for infection by the treating physician. In these cases, basiliximab (40 mg daily on days 1 and 4) and sucrose-free intravenous immunoglobulin (500 mg/kg/day for 4 days) were used.

2.3 Definitions of PGD, Viral Infections, Acute Rejection, and BOS

PGD was graded based on the consensus ISHLT criteria from 2005 (Table 1) (47). The results of all arterial blood gases as well as the fraction of inhaled oxygen and oxygen delivery method were obtained for the first 80 hours following transplantation. All chest x-rays over this period were directly reviewed independently by two pulmonary physicians for the presence of findings consistent with edema. There was 85% agreement in these findings, and disagreements were settled by review of the radiologist's report. Patients were graded based on these findings as having PGD 0-3 at arrival to the ICU immediately following surgery (T0), and 72 hours later (T72). All patients had available blood gases, x-rays, and oxygenation data within 6 hours of these time points, and if

multiple blood gases were available, the worst ratio of arterial oxygen partial pressure (PaO₂) to the fraction of inspired oxygen (P/F ratio) was used as per the ISHLT criteria.

Transbronchial biopsy specimens were examined by a pathologist experienced in evaluating transplant tissue, and received AR grades from 0-4 according to standard ISHLT criteria (98). For each patient, an overall AR score was derived as the sum of all AR grades from prior biopsies. This score was felt to reflect the overall burden of AR during the course of each patient, reflects both the severity of and frequency of acute rejection, and has been associated with an increased rate of BOS previously (72).

A CARV pneumonitis (CARV) was defined as the presence of a positive test for respiratory syncytial virus (RSV), parainfluenza, influenza A or B or adenovirus from a respiratory specimen, either BAL or sputum.

BOS was diagnosed based on the development stage 1 of the condition according to the ISHLT guidelines (14). Patients were considered to have BOS after developing a 20% or greater reduction in forced expiratory volume after 1 second on spirometry. This reduction had to persist on repeat testing at least 3 weeks after the initial spirometric result, and had to have occurred in the absence of any confounding cause on review of the medical record such as interval lung resection or bronchial anastomotic stenosis. In the case of the latter, a new maximum baseline was established for assessment of additional future declines in lung function.

2.1 Statistical Analysis

All statistical analyses were performed using SAS software version 9.2, and plots were created using GraphPad Prism version 5.0. At each time point, T0 and T72, PGD grades were incorporated into unadjusted or “crude” Cox models for the outcomes of time-to-BOS and time-to-death. In the case of the former, a competing risk Cox model (also known as a proportional subdistribution hazard model) was employed to avoid the bias that arises from censoring subjects who die prior to developing BOS as this is not an independent outcome for which uninformative censoring can be assumed (99, 100). For the outcome of death a standard Cox model was used. Analyses were further stratified by transplant type: single or bilateral.

Multivariable models adjusting for confounding covariates were then constructed using the automated method of backward elimination based on the Wald test with a removal criteria of a $p > 0.10$. For the variable of CARV, which could occur at any single time point in the post-transplant course, and also BOS as it applies to mortality models, time-dependent covariates were incorporated into the models using a Heaviside function of the hazard at the time of diagnosis. All variables presented in Table 2 (age, gender, pre-transplant diagnosis, transplant type, graft ischemia time, cardiopulmonary bypass, complex surgery, and induction immunosuppression regimen), as well as CARV and AR score were incorporated into the model selection algorithm. The final multivariable models were tested for possible variable interactions by adding interaction terms singly

for each variable pair in a hierarchical fashion. No interaction terms were statistically significant based on a threshold of $\alpha < 0.10$. Cumulative incidence plots for BOS and death at each grade of PGD were constructed using the method described by Rosthøj and colleagues (101). Hypothesis testing was performed with a threshold of $\alpha < 0.05$ for rejection of the null hypothesis. Confidence intervals and p-values for all models were also calculated by bootstrapping 1,000 samples of matching size ($n=279$) with replacement. This yielded results consistent with the maximum likelihood method, and all confidence intervals and p-values reported are based on the results of bootstrapping.

3. RESULTS

3.1 Study Patient Characteristics

Subjects were included in the cohort between March 1, 2000 and August 31, 2007, and follow-up data were collected through August 31, 2009. A total of 279 lung transplants were performed during this period (174 bilateral, and 105 single). Baseline demographics and clinical characteristics for the cohort are summarized in Table 2. At the completion of the study with median follow-up of 2.23 years, 82 patients (33.6%) developed BOS, and 103 patients (36.9%) died or suffered from graft failure (n=5) requiring retransplantation. The average cumulative AR score for the cohort was 1.7, and only 10 subjects were found to have CARV prior to the onset of BOS. At T0, PGD grades 1, 2, and 3 were diagnosed in 63 (23%), 55 (20%), and 72 (26%) patients, respectively. At T72, these grades were diagnosed in 135 (48%), 24 (9%), and 27 (11%) patients, reflecting the fact that many subjects with early PGD at T0 demonstrated improved oxygenation and P/F ratios by T72, but typically continued to have radiographic abnormalities at the later time point, thereby yielding a greater incidence of grade 1 PGD at T72. Moreover, of the 27 patients with PGD grade 3 at T72, 24 (88.8%) had already developed grade 3 dysfunction at T0.

3.2 PGD is a Risk Factor for BOS

Table 3 includes the results of crude and adjusted competing risk Cox models for the outcome of BOS. In the total cohort, there was a stepwise increase in the hazard ratio for BOS with each PGD grade at both time points. In crude analyses, there was a statistically

significant association between BOS and grade 3 PGD at T0, and both grade 2 and 3 PGD at T72. Plots of the cumulative incidence of BOS for all grades of PGD at T0 (Figure 1A) and T72 (Figure 1B) are consistent with this finding. Model selection by backward elimination lead to the inclusion of only AR score as a covariate in the final models. Not surprisingly, this is the best-established risk factor for BOS in the literature, and after adjusting for AR score, the association between PGD and BOS persisted. The most impressive effect, a hazard ratio of 3.75 (95% CI 1.11-21.4, $p < 0.001$), was seen among patients with the most severe form of PGD (grade 3 at T72). Despite prior studies suggesting an association between CARV and BOS, neither bivariate analysis of these variables, nor consideration of CARV as a time-dependent covariate in automated model selection confirmed this association, and CARV was not included in the final models for BOS. A similar analysis was performed at T72 after stratification of the cohort based on transplant type (bilateral or single) (Table 4). Despite the smaller sample sizes in each stratified model, high grade PGD continued to be a statistically significant risk factor for the subsequent development of BOS, both before and after adjusting for AR score. In addition, the effect size for varying grades of PGD was fairly similar in both single and bilateral transplant recipients.

3.3 PGD is a Risk Factor for Death Mediated by BOS

The results of crude and multiple adjusted Cox models for the outcome of death are presented in Table 5 with cumulative incidences plots for mortality by PGD grade presented for T0 and T72 in Figure 2A and 2B, respectively. Prior to adjusting for

relevant covariates, there was a significant association between death and grade 3 PGD at T0 as well as grades 2 and 3 at T72. Backward elimination of covariates presented in Table 2 left only advanced age (above 65 years) in the adjusted models, and the association between PGD and BOS persisted. However after also incorporating BOS as a time-dependent covariate in this model, the absolute estimates for the hazard ratios of all grades of PGD for death were considerably reduced, and no longer met statistical significance. In other words, after adjusting for the effect of BOS, PGD was no longer associated with mortality in subjects surviving at least 90 days.

4. Discussion

As BOS continues to be the leading cause of long-term morbidity and mortality following lung transplantation, efforts to understand its pathogenesis and risk factors are critical. In this retrospective, single center cohort study, we evaluated the association between PGD and the subsequent development of BOS and death. The incidence of PGD in this cohort was comparable to both those reported from the ISHLT transplant registry as well as other single centers (44, 83, 102). We found that there was a step-wise trend of increasing relative hazards for BOS at each PGD grade. At T72, PGD grades 2 and 3 demonstrated a statistically significant association with BOS, while at T0 only grade 3 met statistical significance. As 24 of 27 patients (88.8%) with PGD grade 3 at T72 also had grade 3 dysfunction at T0, much of the association seen even at T0 was driven by subjects with the most severe, prolonged form of PGD. The association between PGD and BOS was also noted in independent stratified analyses of both single and bilateral lung transplant recipients. Finally, in both crude analyses and after adjusting for age, high grade PGD was also a risk factor for late mortality among 90-day transplant survivors. This relationship appears to be mediated to a large extent by the interval development of BOS.

Prior to the publication of the ISHLT consensus criteria for grading PGD, studies of the relationship between PGD and BOS were complicated by a lack of uniformity in defining PGD and its severity. Since these criteria were published, three other centers have reported relevant findings. In the context of the available literature, our results are largely consistent with the findings of Whitson et al. and Huang et al. for bilateral transplant

recipients (83, 102). Interestingly in contrast to our findings in single lung recipients, Whitson and colleagues were unable to find any association between PGD and BOS among single lung transplant recipients, and a smaller study of single lung recipients by Burton and colleagues also failed to find an association (103). In the study by Whitson and colleagues, subjects with PGD grade 3 at any time point within 72 hours of transplantation were compared to a reference group of all other patients, and grading was performed without consideration of x-ray findings. These methodological differences may have biased their results towards the null hypothesis as grade 3 patients without typical radiographic findings may have been misclassified, and relative to a reference group that included patients with possible PGD grade 1-2, one would expect a reduced hazard ratio for grade 3 PGD. In the case of the study by Burton and colleagues, 18% of patients were extubated while on the operating table and excluded from their series. As these subjects likely had little or no PGD, their exclusion might similarly bias this study. In addition, inter-rater reliability in classifying x-rays was poor, and only 1% of patients were classified as having grade 1 PGD in stark contrast to our study and that of Huang and colleagues. Furthermore in both the study by Whitson et al. and Burton et al., the underlying diagnosis was chronic obstructive pulmonary disease (COPD) in the majority of patients, while COPD only accounts for 34.8% of the patients in our study. This is a notable difference, as subjects who receive single lung transplants for COPD may have a considerable degree of native lung hyperinflation. This may lead to further misclassification of PGD because the relative volume loss and lucency of the allograft on x-ray can make assessment of the presence of edema challenging. In addition, ongoing

hyperinflation post-transplant may lead to reduced FEV₁ over time, and potential misclassification of BOS as well.

Independent of the potential for misclassification, application of the ISHLT grading scheme for PGD to single lung transplant recipients may be complicated by the fact that overall oxygenation and the P/F ratio will be affected by the degree of relative ventilation to the native lung, the functional impairment of the native lung, and the extent to which physiological redistribution of perfusion to the allograft occurs. As compared to bilateral recipients, the severity of PGD may potentially be both over- and underestimated in single lung recipients by the P/F ratio. Despite that, if PGD is in fact a risk factor for BOS, the degree to which the association is seen using the set grading criteria may be affected, but the association should ultimately be present in both bilateral and single lung recipients.

The biological mechanisms by which PGD may promote the development of BOS remain poorly elucidated. Though indirect and direct alloimmune recognition are fundamental to the process of chronic rejection, a growing body of literature supports an association between ostensibly *non*-alloimmune forms of lung injury and the subsequent development of BOS (61-80). The local and systemic inflammatory response that occurs during PGD is classically considered a *non*-alloimmune form of lung injury, but has been associated with increased expression of donor MHC and the development of de novo anti-HLA antibodies (84, 88, 104). Interestingly, the association between PGD and BOS

in both our study and that of Huang and colleagues was present after adjusting for the presence of acute rejection, suggesting that if PGD promotes alloimmune recognition, it may do so through mechanisms that are distinct and parallel to those seen in acute rejection. PGD has also been associated with the local release of type V collagen and K- α 1 tubulin, self-antigens not previously exposed to the recipient immune system (89-92). This has led to the emerging alternative hypothesis that autoimmunity to these previously sequestered antigens may potentially lead to chronic lung injury. Finally the development of PGD may itself be an epiphenomenon related to as yet undescribed host or donor factors that similarly predispose recipients to BOS.

We acknowledge that this study has several fundamental limitations, primarily related to its retrospective design. The diagnosis of PGD is potentially confounded by the presence of infection, cardiogenic edema, pulmonary contusion, vascular anastomotic complications, and hyperacute rejection. The medical records from the early transplant period were reviewed, but the potential for misclassification in our cohort persists. While arterial blood gas data was complete in these patients, in practice the FiO₂ setting for patients may be adjusted frequently throughout the day, and is documented at our center in nursing records that may be less reliable. Despite our efforts to adjust for relevant covariates in model selection, as with any regression analysis, the potential for residual confounding still exists. While our study further supports the previously reported association between PGD and BOS particularly among bilateral transplant recipient, this finding requires validation in single lung transplants as the literature remains conflicting.

In conclusion, we find that PGD, particularly at higher grades, is associated with an increased risk of developing BOS. This association was seen in both bilateral and single lung transplant recipients in our cohort, and was independent of acute rejection episodes. In addition, PGD is associated with long-term mortality amongst 90-day transplant survivors, and this affect appears to be mediated to a large extent by the interval development of BOS. These findings suggest that subjects who develop significant PGD warrant close clinical surveillance, and might potentially benefit from altered immunosuppressive or other management strategies. Moreover, as events in the earliest period following transplantation may predict long-term morbidity and mortality, further study of the immunological milieu in patients during this critical period may yield novel insights into the pathogenesis of BOS and PGD, and the mechanisms linking the two.

TABLE 1. ISHLT GRADING OF PRIMARY GRAFT DYSFUNCTION

Grade	PaO ₂ /FiO ₂	Radiographic findings consistent with pulmonary edema
0	>300	Absent
1	>300	Present
2	200-300	Present
3	<200	Present

TABLE 2. CHARACTERISTICS OF THE STUDY COHORT

Characteristic	n = 279
Female Recipient Gender, n (%)	126 (45.2%)
Recipient age, median yr [IQR]	59 [53, 64]
Recipient age $\geq$ 65 yrs, n (%)	63 (22.6%)
Pre-transplant Diagnoses, n (%)	
COPD / Emphysema	97 (34.8%)
Interstitial pulmonary fibrosis (IPF)	101 (36.2%)
Other	81 (29.3%)
Idiopathic pulmonary arterial hypertension	14 (5.0%)
Scleroderma	14 (5.0%)
Cystic Fibrosis	10 (3.6%)
Sarcoidosis	9 (3.2%)
Post-transplant BOS	9 (3.2%)
Bronchiectasis	7 (2.5%)
Alpha1-antitrypsin deficiency	6 (2.2%)
Other lung disease	12 (4.3%)
Transplant Type	
Bilateral or Heart-Lung, n (%)	174 (62.4%)
Single Lung, n (%)	105 (37.6%)
Graft ischemia time, median min [IQR]	321 [267-372]
Graft ischemia time $\geq$ 6 hr, n (%)	73 (31.1%)
Cardiopulmonary bypass (CPB), n (%)	187 (67.0%)
Bypass time, median min [IQR]	191 [171-224]
Complex Surgery	70 (25.1%)
Induction type, n (%)	
Rabbit antithymocyte globulin (ATG)	173 (63.4%)
Basiliximab	100 (36.6%)
Median BOS-free follow-up, yr [IQR]	1.88 [1.03-2.89]
Incidence of BOS, n (%)	82 (33.6%)
Median time to BOS, yr	4.08
Median overall follow-up, yr [IQR]	2.23 [1.23-3.63]
Incidence of death or retransplant, n (%)	103 (36.9%)
Median time to death or retransplant, yr	6.24

TABLE 3. CRUDE AND ADJUSTED COMPETING RISK MODELS OF THE ASSOCIATION BETWEEN PRIMARY GRAFT DYSFUNCTION AND FREEDOM FROM BOS

Predictor	<u>Crude</u>		<u>Adjusted for Acute Rejection</u>	
	Relative Hazard (95% CI)	p-value	Relative Hazard (95% CI)	p-value
<i>T0 Model</i>				
PGD 0	Reference		Reference	
PGD 1	1.49 (0.88-2.63)	0.11	1.37 (0.81-2.40)	0.17
PGD 2	1.72 (0.97-2.95)	0.06	1.62 (0.92-2.82)	0.08
PGD 3	3.45 (2.02-5.84)	< 0.001	3.05 (1.77-5.07)	0.001
<i>T72 Model</i>				
PGD 0	Reference		Reference	
PGD 1	1.68 (0.72-4.01)	0.07	1.55 (1.00-2.53)	0.05
PGD 2	3.49 (1.60-7.60)	0.002	2.71 (1.38-5.25)	0.005
PGD 3	4.46 (2.14-9.28)	< 0.001	3.75 (1.11-21.4)	< 0.001

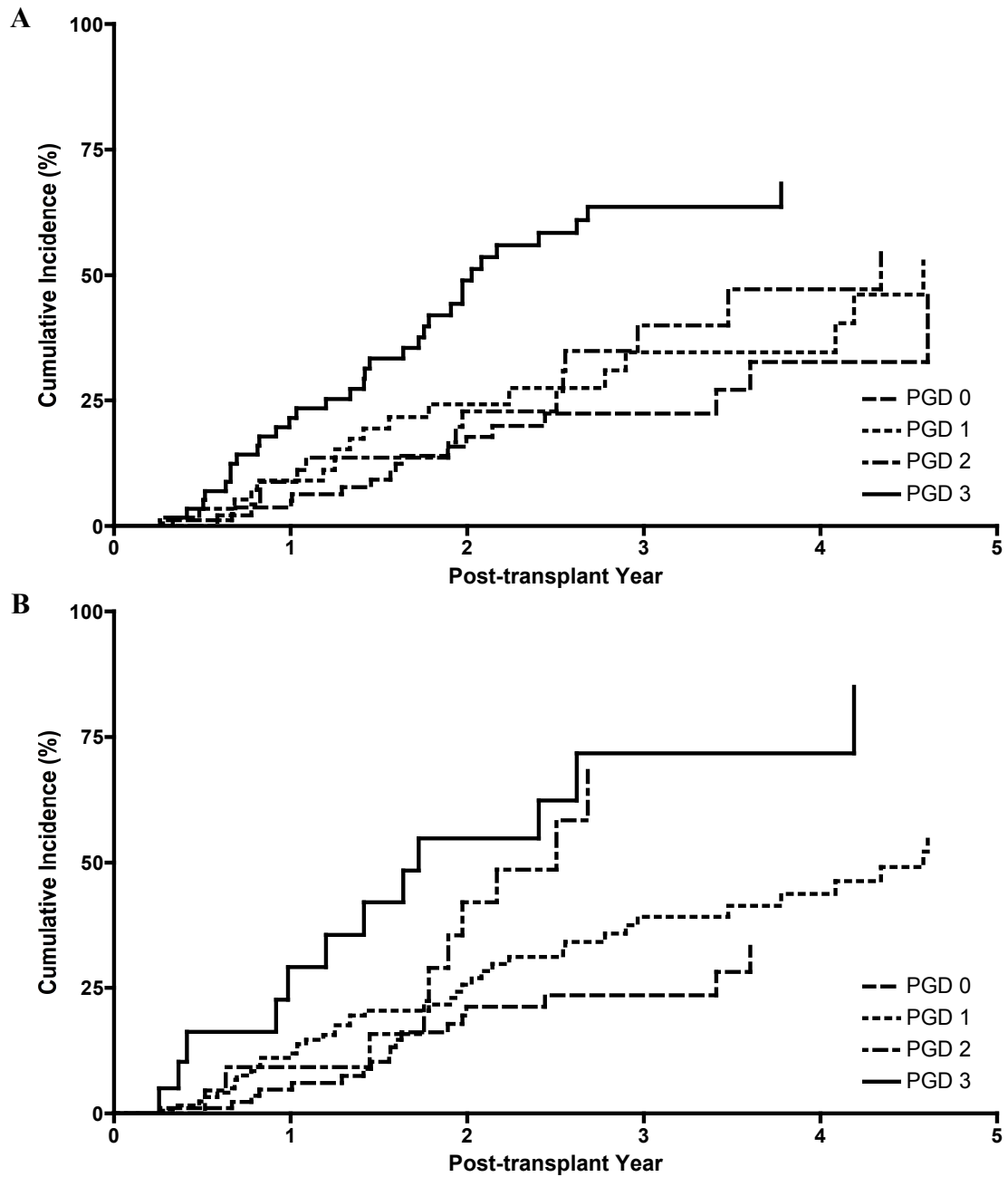
TABLE 4. CRUDE AND ADJUSTED COMPETING RISK MODELS OF THE ASSOCIATION BETWEEN PRIMARY GRAFT DYSFUNCTION AT 72 HOURS AND FREEDOM FROM BOS, STRATIFIED BY TRANSPLANT TYPE

Predictor	<u>Crude</u>		<u>Adjusted for Acute Rejection</u>	
	Relative Hazard (95% CI)	p-value	Relative Hazard (95% CI)	p-value
<i>Single</i>				
PGD 0	Reference		Reference	
PGD 1	1.69 (0.72-4.01)	0.23	1.41 (0.69-3.17)	0.23
PGD 2	3.11 (0.92-10.5)	0.07	1.75 (0.40-5.44)	0.21
PGD 3	4.78 (1.42-16.0)	0.01	3.80 (1.11-21.4)	0.04
<i>Bilateral</i>				
PGD 0	Reference		Reference	
PGD 1	1.65 (0.80-3.43)	0.18	1.58 (0.76-3.29)	0.22
PGD 2	3.69 (1.34-10.2)	0.01	3.24 (1.15-9.16)	0.03
PGD 3	4.05 (1.59-10.3)	0.003	3.64 (1.40-9.50)	0.008

TABLE 5. CRUDE AND ADJUSTED COX MODELS OF THE ASSOCIATION BETWEEN PRIMARY GRAFT DYSFUNCTION AND LONG-TERM MORTALITY

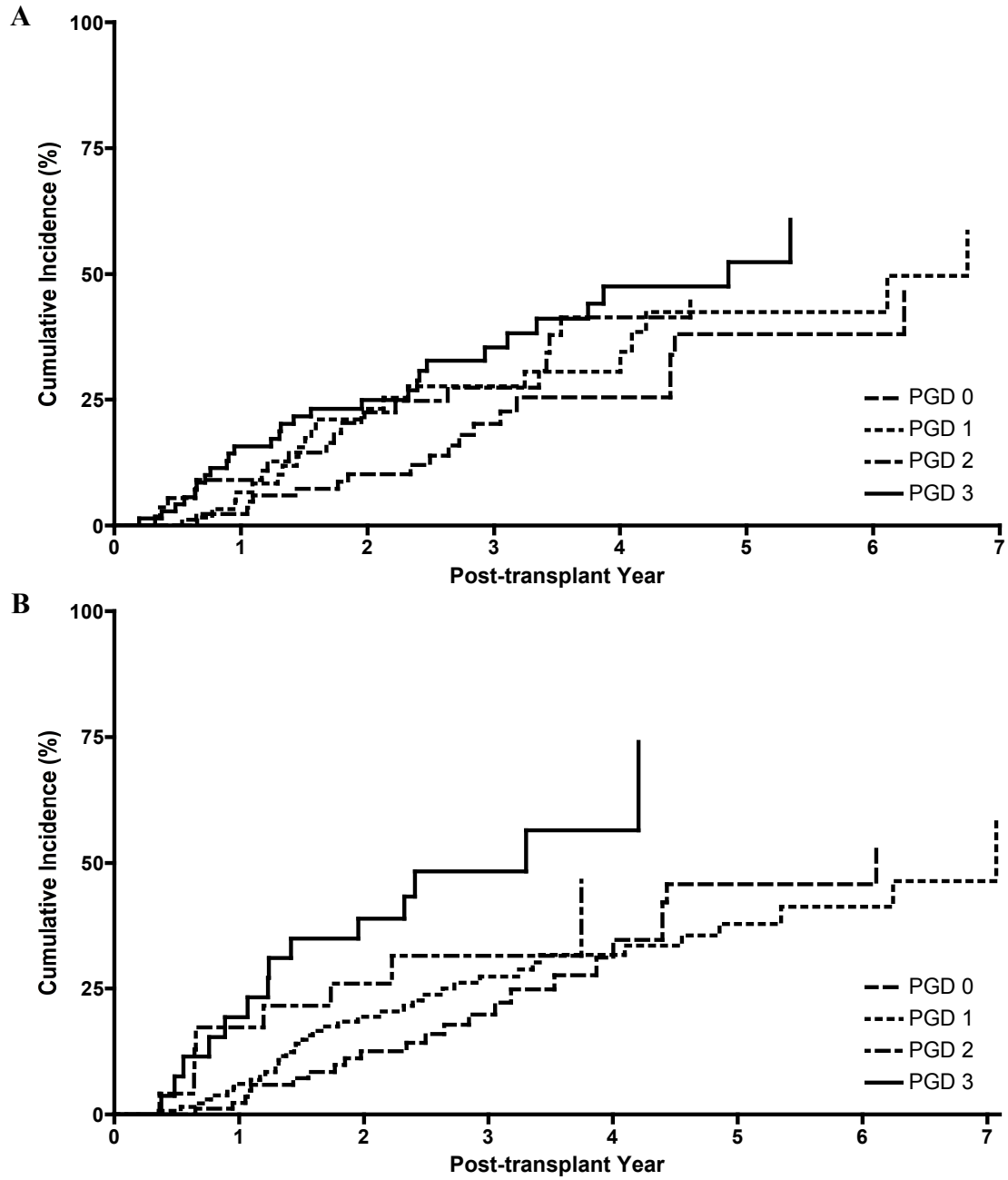
<u>Crude</u>		<u>Adjusted for Age ≥ 65</u>		<u>Adjusted for Age ≥ 65 and BOS (time-dependent)</u>		
Predictor	Relative Hazard (95% CI)	p-value	Relative Hazard (95% CI)	p-value	Relative Hazard (95% CI)	p-value
<i>T0</i>						
PGD 0	Reference		Reference		Reference	
PGD 1	1.52 (0.82-2.83)	0.18	1.62 (0.87-3.02	0.23	1.63 (0.88-3.03)	0.12
PGD 2	1.68 (0.90-3.15)	0.10	1.73 (0.92-3.25)	0.09	1.21 (0.61-2.42)	0.59
PGD 3	2.10 (1.16-3.82)	0.02	2.29 (1.25-4.18)	0.007	1.42 (0.75-2.69)	0.29
<i>T72</i>						
PGD 0	Reference		Reference		Reference	
PGD 1	1.18 (0.71-1.97)	0.53	1.22 (0.73-2.04)	0.45	1.12 (0.66-1.90)	0.67
PGD 2	2.25 (1.09-4.62)	0.03	2.13 (1.00-4.54)	0.05	1.12 (0.45-2.79)	0.81
PGD 3	2.80 (1.36-5.76)	0.005	3.05 (1.48-6.31)	0.003	1.56 (0.68-3.57)	0.29

FIGURE 1.



The cumulative incidence of BOS over time for each grade of PGD at graded at T0 (A) and T72 (B).

FIGURE 2.



The cumulative incidence of death over time for each grade of PGD at graded at T0 (A) and T72 (B).

CHAPTER 3: APPENDIX

1. Study Design and Conduct

This study was performed with approval from the University of California, Los Angeles (UCLA) Institutional Review Board.

1.1. Study Design

This is a retrospective, single center cohort study. The choice of a retrospective design was concerns for feasibility as the conduct of such a study prospectively would be time-prohibitive both from the standpoint of subject accrual and adequate follow-up time. As the population of lung transplant recipients at UCLA is a closely followed group of local patients with common selection and management criteria, and virtually no loss to follow-up, this population lends itself naturally to a cohort design. Moreover, as compared to other study designs such as case-control, a cohort design provides the advantage of permitting direct calculation of incidence or hazard rates, and their accompanying ratios, and allows for multiple relevant outcomes (BOS, death) to be studied in parallel.

Still this approach has multiple disadvantages. First, the study population size was largely limited by circumstance as prior to year 2000, transplant recipients at UCLA were managed by a different group of physicians without clear protocols, and with

limited available medical records for review. Consequently the overall follow-up time was also essentially fixed, and there was no control over the power of the study.

As with any retrospective study, there are inherent limitations related to data availability, reliability, selection bias, and confounding. While the study populations medical care is fortunately documented exceptionally well, we can not fully rule out the possibility of mis-classification of either PGD or BOS in some patients as these are diagnoses of exclusion with alternate diagnoses potentially not documented in the medical record. A prospective design would no doubt yield more reliable data with respect to classification. Furthermore, while a number of key covariates were well documented, some potential risk factors for BOS could not be included in our data set (e.g. gastroesophageal reflux). Finally, while statistical approaches can be applied to correct for confounding, in the absence of proper randomization, residual confounding is virtually guaranteed in this design.

1.2 Data Collection

As previously suggested, a consecutive series of patients transplanted between 2000 and 2007 and surviving at least 90 days were included in the cohort without exception. Intensive effort was applied to obtain as complete a data set as possible. All patients were accounted for at the time of final follow-up. Mortality and censoring data at last follow-up was complete with the exception of 21 patients (7.5%), who were known to have left our care due to moving out of state or to a managed care

system. These patients were censored, and to our knowledge, censoring in these cases was not obviously informative. Data collected from all bronchoscopies, sputum samples, and pulmonary function tests performed at UCLA was complete.

2. Statistical Analyses

2.1. BOS Analyses

As the data was in the form of “time-to-event” with censoring, the most appropriate analyses approaches would be survival analyses. In an effort to adjust for confounding covariates, and minimize model assumptions, we chose Cox proportional hazards models. The basic Cox model is a semi-parametric with the primary assumption that at any follow-up time point, covariates are multiplicatively related to the hazard. This model assumes that all censoring is uninformative, and otherwise leads to biased results. In our case, a conventional approach to analyzing time-to-BOS data would be to censor all patients without BOS at last follow-up *as well as* all patients who died prior to developing BOS. However, this leads to biased estimates of the BOS-specific hazard for relevant covariates as these deaths are informative events that compete with and preclude BOS. Perhaps more importantly, our primary predictor of interest, PGD, is a known risk factor for death independent of BOS. Special techniques for handling this type of competing risk data by directly modeling the subdistribution hazard function of each competing risk were introduced

in the 1990's. This method, described as either a “competing risk Cox” or “proportional subdistribution hazard” model was applied to our BOS data to avoid the bias introduced by the standard Cox model.

2.2 Survival Analysis

In analyzing the outcome of death (both before and after the development of BOS), the competing risk problem no longer applies. All censored subjects were alive at last follow-up. However, accounting for the impact of BOS on mortality introduces a different challenge. In the standard Cox proportional hazards model, covariates are assumed not to have time-varying effects on the outcome of interest. As a covariate, BOS violates this assumption because within an individual subject, BOS may be absent in their early post-transplant course, and occur later, imparting additional hazard for death after its onset. As a result, extended Cox models were used in adjusting for BOS as a time-dependent variable. Specifically, we assumed that a patient's follow-up may be partitioned into accumulated time before and after the onset of BOS, should it occur, and modeled the attributable hazard with a simple Heaviside function. This approach was also applied to another potential time-dependent covariate of interest, CARV, but ultimately CARV was not found to be associated with either relevant outcome in our cohort in unilateral analysis, and were removed from final adjusted models by automated backward selection.

2.3 Model Selection

No universally agree upon method exists for model variable selection when attempting to adjust for confounding. In this study, we present multivariable models were constructed using the automated method of backward elimination based on the Wald test with a removal criteria of a > 0.10 . Alternative methods were also tested (results not shown), including step-wise selection, *a priori* selection of likely confounders (e.g. acute rejection and CARV in models of BOS), and best subsets selection methods incorporating Akaike information criteria for models with up to 5 covariates. None of these alternative model selection methods had a meaning impact on the association found between PGD and BOS or long-term mortality.

2.4 Standard Errors Estimation

Estimation of the presented standard errors and confidence intervals was performed by bootstrapping 1000 samples with replacement from the original data set. This resampling technique yields parallel results to the maximum likelihood method, but uses an empirical derived distribution of the relevant hazards and their ratios. Because bootstrapping does not require distributional assumptions, this approach may potentially better handle outlier effects, particularly in the setting of poorly behaved data or small sample size. Both methods yielded consistent estimates for the standard errors in this study.

2.5 Assumption Testing

Despite the more complex modeling methods required for proper analysis of the data, the fundamental assumption of proportional hazards still applies to all models presented. This assumption was assessed for all covariates in all models via a Kolmogorov-type supremum test based on a sample of 1,000 simulated residual patterns (105). To further test the goodness of fit, we assessed various residuals in the model visually and found that in each case the model behaved appropriately

REFERENCES

1. Orens JB, Garrity ER, Jr. General overview of lung transplantation and review of organ allocation. *Proc Am Thorac Soc* 2009;6:13-19.
2. Kreider M, Kotloff RM. Selection of candidates for lung transplantation. *Proc Am Thorac Soc* 2009;6:20-27.
3. Trulock EP, Egan TM, Kouchoukos NT, Kaiser LR, Pasque MK, Ettinger N, Cooper JD. Single lung transplantation for severe chronic obstructive pulmonary disease. Washington university lung transplant group. *Chest* 1989;96:738-742.
4. Trulock EP. Lung transplantation. *Am J Respir Crit Care Med* 1997;155:789-818.
5. Christie JD, Edwards LB, Kucheryavaya AY, Benden C, Dobbels F, Kirk R, Rahmel AO, Stehlik J, Hertz MI. The registry of the international society for heart and lung transplantation: Twenty-eighth adult lung and heart-lung transplant report--2011. *J Heart Lung Transplant* 2011;30:1104-1122.
6. Taylor DO, Edwards LB, Boucek MM, Trulock EP, Aurora P, Christie J, Dobbels F, Rahmel AO, Keck BM, Hertz MI. Registry of the international society for heart and lung transplantation: Twenty-fourth official adult heart transplant report--2007. *J Heart Lung Transplant* 2007;26:769-781.
7. Lim KB, Schiano TD. Long-term outcome after liver transplantation. *Mt Sinai J Med* 2012;79:169-189.
8. Keller CA, Cagle PT, Brown RW, Noon G, Frost AE. Bronchiolitis obliterans in recipients of single, double, and heart-lung transplantation. *Chest* 1995;107:973-980.
9. Reichenspurner H, Girgis RE, Robbins RC, Yun KL, Nitschke M, Berry GJ, Morris RE, Theodore J, Reitz BA. Stanford experience with obliterative bronchiolitis after lung and heart-lung transplantation. *Ann Thorac Surg* 1996;62:1467-1472; discussion 1472-1463.
10. Scott JP, Higenbottam TW, Sharples L, Clelland CA, Smyth RL, Stewart S, Wallwork J. Risk factors for obliterative bronchiolitis in heart-lung transplant recipients. *Transplantation* 1991;51:813-817.
11. Yousem SA, Dauber JA, Keenan R, Paradis IL, Zeevi A, Griffith BP. Does histologic acute rejection in lung allografts predict the development of bronchiolitis obliterans? *Transplantation* 1991;52:306-309.

12. Belperio JA, Weigt SS, Fishbein MC, Lynch JP, 3rd. Chronic lung allograft rejection: Mechanisms and therapy. *Proc Am Thorac Soc* 2009;6:108-121.
13. Weigt SS, Wallace WD, Derhovanessian A, Saggar R, Lynch JP, Belperio JA. Chronic allograft rejection: Epidemiology, diagnosis, pathogenesis, and treatment. *Semin Respir Crit Care Med* 2010;31:189-207.
14. Estenne M, Maurer JR, Boehler A, Egan JJ, Frost A, Hertz M, Mallory GB, Snell GI, Yousem S. Bronchiolitis obliterans syndrome 2001: An update of the diagnostic criteria. *J Heart Lung Transplant* 2002;21:297-310.
15. Cooper JD, Billingham M, Egan T, Hertz MI, Higenbottam T, Lynch J, Mauer J, Paradis I, Patterson GA, Smith C, et al. A working formulation for the standardization of nomenclature and for clinical staging of chronic dysfunction in lung allografts. International society for heart and lung transplantation. *J Heart Lung Transplant* 1993;12:713-716.
16. Verleden GM, Dupont LJ, Van Raemdonck DE. Is it bronchiolitis obliterans syndrome or is it chronic rejection: A reappraisal? *Eur Respir J* 2005;25:221-224.
17. Burton CM, Carlsen J, Mortensen J, Andersen CB, Milman N, Iversen M. Long-term survival after lung transplantation depends on development and severity of bronchiolitis obliterans syndrome. *J Heart Lung Transplant* 2007;26:681-686.
18. Heng D, Sharples LD, McNeil K, Stewart S, Wreghitt T, Wallwork J. Bronchiolitis obliterans syndrome: Incidence, natural history, prognosis, and risk factors. *J Heart Lung Transplant* 1998;17:1255-1263.
19. Bando K, Paradis IL, Similo S, Konishi H, Komatsu K, Zullo TG, Yousem SA, Close JM, Zeevi A, Duquesnoy RJ, et al. Obliterative bronchiolitis after lung and heart-lung transplantation. An analysis of risk factors and management. *J Thorac Cardiovasc Surg* 1995;110:4-13; discussion 13-14.
20. Knoop C, Estenne M. Acute and chronic rejection after lung transplantation. *Semin Respir Crit Care Med* 2006;27:521-533.
21. Paradis I, Yousem S, Griffith B. Airway obstruction and bronchiolitis obliterans after lung transplantation. *Clin Chest Med* 1993;14:751-763.
22. Stewart S, Fishbein MC, Snell GI, Berry GJ, Boehler A, Burke MM, Glanville A, Gould FK, Magro C, Marboe CC, McNeil KD, Reed EF, Reinsmoen NL, Scott JP, Studer SM, Tazelaar HD, Wallwork JL, Westall G, Zamora MR, Zeevi A, Yousem SA. Revision of the 1996 working formulation for the standardization of nomenclature in the diagnosis of lung rejection. *J Heart Lung Transplant* 2007;26:1229-1242.

23. Tazelaar HD, Yousem SA. The pathology of combined heart-lung transplantation: An autopsy study. *Hum Pathol* 1988;19:1403-1416.
24. Moudgil A, Bagga A, Toyoda M, Nicolaidou E, Jordan SC, Ross D. Expression of gamma-ihf mRNA in bronchoalveolar lavage fluid correlates with early acute allograft rejection in lung transplant recipients. *Clin Transplant* 1999;13:201-207.
25. Iacono A, Dauber J, Keenan R, Spichty K, Cai J, Grgurich W, Burckart G, Smaldone G, Pham S, Otori NP, Yousem S, Williams P, Griffith B, Zeevi A. Interleukin 6 and interferon-gamma gene expression in lung transplant recipients with refractory acute cellular rejection: Implications for monitoring and inhibition by treatment with aerosolized cyclosporine. *Transplantation* 1997;64:263-269.
26. Meloni F, Vitulo P, Cascina A, Oggionni T, Bulgheroni A, Paschetto E, Klersy C, D'Armini AM, Fietta A, Bianco AM, Arbustini E, Vigano M. Bronchoalveolar lavage cytokine profile in a cohort of lung transplant recipients: A predictive role of interleukin-12 with respect to onset of bronchiolitis obliterans syndrome. *J Heart Lung Transplant* 2004;23:1053-1060.
27. Keane MP, Gomperts BN, Weigt S, Xue YY, Burdick MD, Nakamura H, Zisman DA, Ardehali A, Sagggar R, Lynch JP, 3rd, Hogaboam C, Kunkel SL, Lukacs NW, Ross DJ, Grusby MJ, Strieter RM, Belperio JA. IL-13 is pivotal in the fibro-obliterative process of bronchiolitis obliterans syndrome. *J Immunol* 2007;178:511-519.
28. Lama VN, Harada H, Badri LN, Flint A, Hogaboam CM, McKenzie A, Martinez FJ, Toews GB, Moore BB, Pinsky DJ. Obligatory role for interleukin-13 in obstructive lesion development in airway allografts. *Am J Pathol* 2006;169:47-60.
29. Belperio JA, Burdick MD, Keane MP, Xue YY, Lynch JP, 3rd, Daugherty BL, Kunkel SL, Strieter RM. The role of the cc chemokine, rantes, in acute lung allograft rejection. *J Immunol* 2000;165:461-472.
30. Belperio JA, Keane MP, Burdick MD, Lynch JP, 3rd, Xue YY, Berlin A, Ross DJ, Kunkel SL, Charo IF, Strieter RM. Critical role for the chemokine mcp-1/ccr2 in the pathogenesis of bronchiolitis obliterans syndrome. *J Clin Invest* 2001;108:547-556.
31. Belperio JA, Keane MP, Burdick MD, Gomperts B, Xue YY, Hong K, Mestas J, Ardehali A, Mehrad B, Sagggar R, Lynch JP, Ross DJ, Strieter RM. Role of cxcr2/cxcr2 ligands in vascular remodeling during bronchiolitis obliterans syndrome. *J Clin Invest* 2005;115:1150-1162.
32. Hertz MI, Henke CA, Nakhleh RE, Harmon KR, Marinelli WA, Fox JM, Kubo SH, Shumway SJ, Bolman RM, 3rd, Bitterman PB. Obliterative bronchiolitis after lung

transplantation: A fibroproliferative disorder associated with platelet-derived growth factor. *Proc Natl Acad Sci U S A* 1992;89:10385-10389.

33. Charpin JM, Stern M, Grenet D, Israel-Biet D. Insulinlike growth factor-1 in lung transplants with obliterative bronchiolitis. *Am J Respir Crit Care Med* 2000;161:1991-1998.

34. Yates B, Murphy DM, Forrest IA, Ward C, Rutherford RM, Fisher AJ, Lordan JL, Dark JH, Corris PA. Azithromycin reverses airflow obstruction in established bronchiolitis obliterans syndrome. *Am J Respir Crit Care Med* 2005;172:772-775.

35. Rutherford RM, Fisher AJ, Hilton C, Forty J, Hasan A, Gould FK, Dark JH, Corris PA. Functional status and quality of life in patients surviving 10 years after lung transplantation. *Am J Transplant* 2005;5:1099-1104.

36. Ding IB, Baumgartner RA, Schwaiblmair M, Behr J. Administration of anti-interleukin-2alpha monoclonal antibody in bronchiolitis obliterans syndrome after lung transplantation. *Transplantation* 2003;75:1767-1769.

37. Date H, Lynch JP, Sundaresan S, Patterson GA, Trulock EP. The impact of cytolytic therapy on bronchiolitis obliterans syndrome. *J Heart Lung Transplant* 1998;17:869-875.

38. Reams BD, Musselwhite LW, Zaas DW, Steele MP, Garantziotis S, Eu PC, Snyder LD, Curl J, Lin SS, Davis RD, Palmer SM. Alemtuzumab in the treatment of refractory acute rejection and bronchiolitis obliterans syndrome after human lung transplantation. *Am J Transplant* 2007;7:2802-2808.

39. Wiebe K, Harringer W, Wahlers T, Franke U, Struber M, Hamm M, Cremer J, Haverich A. Atg induction therapy and the incidence of bronchiolitis obliterans after lung transplantation: Does it make a difference? *Transplant Proc* 1998;30:1517-1518.

40. Morrell MR, Despotis GJ, Lublin DM, Patterson GA, Trulock EP, Hachem RR. The efficacy of photopheresis for bronchiolitis obliterans syndrome after lung transplantation. *J Heart Lung Transplant* 2010;29:424-431.

41. Lee JC, Christie JD. Primary graft dysfunction. *Clin Chest Med* 2011;32:279-293.

42. Christie JD, Bavaria JE, Palevsky HI, Litzky L, Blumenthal NP, Kaiser LR, Kotloff RM. Primary graft failure following lung transplantation. *Chest* 1998;114:51-60.

43. King RC, Binns OA, Rodriguez F, Kanithanon RC, Daniel TM, Spotnitz WD, Tribble CG, Kron IL. Reperfusion injury significantly impacts clinical outcome after pulmonary transplantation. *Ann Thorac Surg* 2000;69:1681-1685.

44. Christie JD, Kotloff RM, Pochettino A, Arcasoy SM, Rosengard BR, Landis JR, Kimmel SE. Clinical risk factors for primary graft failure following lung transplantation. *Chest* 2003;124:1232-1241.
45. Christie JD, Sager JS, Kimmel SE, Ahya VN, Gaughan C, Blumenthal NP, Kotloff RM. Impact of primary graft failure on outcomes following lung transplantation. *Chest* 2005;127:161-165.
46. Fiser SM, Kron IL, Long SM, Kaza AK, Kern JA, Cassada DC, Jones DR, Robbins MC, Tribble CG. Influence of graft ischemic time on outcomes following lung transplantation. *J Heart Lung Transplant* 2001;20:1291-1296.
47. Christie JD, Carby M, Bag R, Corris P, Hertz M, Weill D, Dysfunction IWGoPLG. 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.
48. de Perrot M, Liu M, Waddell TK, Keshavjee S. Ischemia-reperfusion-induced lung injury. *Am J Respir Crit Care Med* 2003;167:490-511.
49. Moreno I, Vicente R, Ramos F, Vicente JL, Barbera M. Determination of interleukin-6 in lung transplantation: Association with primary graft dysfunction. *Transplant Proc* 2007;39:2425-2426.
50. Serrick C, Adoumie R, Giaid A, Shennib H. The early release of interleukin-2, tumor necrosis factor-alpha and interferon-gamma after ischemia reperfusion injury in the lung allograft. *Transplantation* 1994;58:1158-1162.
51. Karakurum M, Shreeniwas R, Chen J, Pinsky D, Yan SD, Anderson M, Sunouchi K, Major J, Hamilton T, Kuwabara K, et al. Hypoxic induction of interleukin-8 gene expression in human endothelial cells. *J Clin Invest* 1994;93:1564-1570.
52. Ng CS, Wan S, Arifi AA, Yim AP. Inflammatory response to pulmonary ischemia-reperfusion injury. *Surg Today* 2006;36:205-214.
53. Belperio JA, Keane MP, Burdick MD, Gomperts BN, Xue YY, Hong K, Mestas J, Zisman D, Ardehali A, Saggat R, Lynch JP, 3rd, Ross DJ, Strieter RM. Cxcr2/cxcr2 ligand biology during lung transplant ischemia-reperfusion injury. *J Immunol* 2005;175:6931-6939.
54. Gorsuch WB, Chrysanthou E, Schwaible WJ, Stahl GL. The complement system in ischemia-reperfusion injuries. *Immunobiology* 2012;217:1026-1033.

55. Covarrubias M, Ware LB, Kawut SM, De Andrade J, Milstone A, Weinacker A, Orens J, Lama V, Wille K, Bellamy S, Shah C, Demissie E, Christie JD, Lung Transplant Outcomes G. Plasma intercellular adhesion molecule-1 and von willebrand factor in primary graft dysfunction after lung transplantation. *Am J Transplant* 2007;7:2573-2578.
56. Miotla JM, Jeffery PK, Hellewell PG. Platelet-activating factor plays a pivotal role in the induction of experimental lung injury. *Am J Respir Cell Mol Biol* 1998;18:197-204.
57. Matthay MA, Zemans RL. The acute respiratory distress syndrome: Pathogenesis and treatment. *Annu Rev Pathol* 2011;6:147-163.
58. Keenan RJ, Lega ME, Dummer JS, Paradis IL, Dauber JH, Rabinowich H, Yousem SA, Hardesty RL, Griffith BP, Duquesnoy RJ, et al. Cytomegalovirus serologic status and postoperative infection correlated with risk of developing chronic rejection after pulmonary transplantation. *Transplantation* 1991;51:433-438.
59. Cerrina J, Le Roy Ladurie F, Herve PH, Parquin F, Harari S, Chapelier A, Simoneau G, Vouhe P, Darteville PH. Role of cmv pneumonia in the development of obliterative bronchiolitis in heart-lung and double-lung transplant recipients. *Transpl Int* 1992;5 Suppl 1:S242-245.
60. Paraskeva M, Bailey M, Levvey BJ, Griffiths AP, Kotsimbos TC, Williams TP, Snell G, Westall G. Cytomegalovirus replication within the lung allograft is associated with bronchiolitis obliterans syndrome. *Am J Transplant* 2011;11:2190-2196.
61. Luckraz H, Sharples L, McNeil K, Wreghitt T, Wallwork J. Cytomegalovirus antibody status of donor/recipient does not influence the incidence of bronchiolitis obliterans syndrome in lung transplantation. *J Heart Lung Transplant* 2003;22:287-291.
62. Weigt SS, Elashoff RM, Keane MP, Strieter RM, Gomperts BN, Xue YY, Ardehali A, Gregson AL, Kubak B, Fishbein MC, Saggarr R, Ross DJ, Lynch JP, 3rd, Zisman DA, Belperio JA. Altered levels of cc chemokines during pulmonary cmv predict bos and mortality post-lung transplantation. *Am J Transplant* 2008;8:1512-1522.
63. Smith MA, Sundaresan S, Mohanakumar T, Trulock EP, Lynch JP, Phelan DL, Cooper JD, Patterson GA. 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;116:812-820.
64. Ruttman E, Geltner C, Bucher B, Ulmer H, Hofer D, Hangler HB, Semsroth S, Margreiter R, Laufer G, Muller LC. Combined cmv prophylaxis improves outcome and reduces the risk for bronchiolitis obliterans syndrome (bos) after lung transplantation. *Transplantation* 2006;81:1415-1420.

65. Chmiel C, Speich R, Hofer M, Michel D, Mertens T, Weder W, Boehler A. Ganciclovir/valganciclovir prophylaxis decreases cytomegalovirus-related events and bronchiolitis obliterans syndrome after lung transplantation. *Clin Infect Dis* 2008;46:831-839.
66. Khalifah AP, Hachem RR, Chakinala MM, Schechtman KB, Patterson GA, Schuster DP, Mohanakumar T, Trulock EP, Walter MJ. Respiratory viral infections are a distinct risk for bronchiolitis obliterans syndrome and death. *Am J Respir Crit Care Med* 2004;170:181-187.
67. Kumar D, Erdman D, Keshavjee S, Peret T, Tellier R, Hadjiliadis D, Johnson G, Ayers M, Siegal D, Humar A. Clinical impact of community-acquired respiratory viruses on bronchiolitis obliterans after lung transplant. *Am J Transplant* 2005;5:2031-2036.
68. Neurohr C, Huppmann P, Leuchte H, Schwaiblmair M, Bittmann I, Jaeger G, Hatz R, Frey L, Uberfuhr P, Reichart B, Behr J, Munich Lung Transplant G. Human herpesvirus 6 in bronchialveolar lavage fluid after lung transplantation: A risk factor for bronchiolitis obliterans syndrome? *Am J Transplant* 2005;5:2982-2991.
69. Kotsimbos TC, Snell GI, Levvey B, Spelman DW, Fuller AJ, Wesselingh SL, Williams TJ, Ostergaard L. Chlamydia pneumoniae serology in donors and recipients and the risk of bronchiolitis obliterans syndrome after lung transplantation. *Transplantation* 2005;79:269-275.
70. Botha P, Archer L, Anderson RL, Lordan J, Dark JH, Corris PA, Gould K, Fisher AJ. Pseudomonas aeruginosa colonization of the allograft after lung transplantation and the risk of bronchiolitis obliterans syndrome. *Transplantation* 2008;85:771-774.
71. Vos R, Vanaudenaerde BM, Geudens N, Dupont LJ, Van Raemdonck DE, Verleden GM. Pseudomonal airway colonisation: Risk factor for bronchiolitis obliterans syndrome after lung transplantation? *Eur Respir J* 2008;31:1037-1045.
72. Weigt SS, Elashoff RM, Huang C, Ardehali A, Gregson AL, Kubak B, Fishbein MC, Sagar R, Keane MP, Lynch JP, 3rd, Zisman DA, Ross DJ, Belperio JA. Aspergillus colonization of the lung allograft is a risk factor for bronchiolitis obliterans syndrome. *Am J Transplant* 2009;9:1903-1911.
73. Young LR, Hadjiliadis D, Davis RD, Palmer SM. Lung transplantation exacerbates gastroesophageal reflux disease. *Chest* 2003;124:1689-1693.
74. D'Ovidio F, Mura M, Tsang M, Waddell TK, Hutcheon MA, Singer LG, Hadjiliadis D, Chaparro C, Gutierrez C, Pierre A, Darling G, Liu M, Keshavjee S. Bile acid aspiration and the development of bronchiolitis obliterans after lung transplantation. *J Thorac Cardiovasc Surg* 2005;129:1144-1152.

75. Blondeau K, Mertens V, Vanaudenaerde BA, Verleden GM, Van Raemdonck DE, Sifrim D, Dupont LJ. Gastro-oesophageal reflux and gastric aspiration in lung transplant patients with or without chronic rejection. *Eur Respir J* 2008;31:707-713.
76. Mertens V, Blondeau K, Van Oudenhove L, Vanaudenaerde B, Vos R, Farre R, Pauwels A, Verleden G, Van Raemdonck D, Sifrim D, Dupont LJ. Bile acids aspiration reduces survival in lung transplant recipients with bos despite azithromycin. *Am J Transplant* 2011;11:329-335.
77. D'Ovidio F, Mura M, Ridsdale R, Takahashi H, Waddell TK, Hutcheon M, Hadjiliadis D, Singer LG, Pierre A, Chaparro C, Gutierrez C, Miller L, Darling G, Liu M, Post M, Keshavjee S. The effect of reflux and bile acid aspiration on the lung allograft and its surfactant and innate immunity molecules sp-a and sp-d. *Am J Transplant* 2006;6:1930-1938.
78. Cantu E, 3rd, Appel JZ, 3rd, Hartwig MG, Woreta H, Green C, Messier R, Palmer SM, Davis RD, Jr. J. Maxwell chamberlain memorial paper. Early fundoplication prevents chronic allograft dysfunction in patients with gastroesophageal reflux disease. *Ann Thorac Surg* 2004;78:1142-1151; discussion 1142-1151.
79. Hartwig MG, Appel JZ, Davis RD. Antireflux surgery in the setting of lung transplantation: Strategies for treating gastroesophageal reflux disease in a high-risk population. *Thorac Surg Clin* 2005;15:417-427.
80. Davis RD, Jr., Lau CL, Eubanks S, Messier RH, Hadjiliadis D, Steele MP, Palmer SM. Improved lung allograft function after fundoplication in patients with gastroesophageal reflux disease undergoing lung transplantation. *J Thorac Cardiovasc Surg* 2003;125:533-542.
81. Fisher AJ, Wardle J, Dark JH, Corris PA. Non-immune acute graft injury after lung transplantation and the risk of subsequent bronchiolitis obliterans syndrome (bos). *J Heart Lung Transplant* 2002;21:1206-1212.
82. Fiser SM, Tribble CG, Long SM, Kaza AK, Kern JA, Jones DR, Robbins MK, Kron IL. Ischemia-reperfusion injury after lung transplantation increases risk of late bronchiolitis obliterans syndrome. *Ann Thorac Surg* 2002;73:1041-1047; discussion 1047-1048.
83. Whitson BA, Prekker ME, Herrington CS, Whelan TP, Radosevich DM, Hertz MI, Dahlberg PS. Primary graft dysfunction and long-term pulmonary function after lung transplantation. *J Heart Lung Transplant* 2007;26:1004-1011.

84. Waddell TK, Gorczynski RM, DeCampos KN, Patterson GA, Slutsky AS. Major histocompatibility complex expression and lung ischemia-reperfusion in rats. *Ann Thorac Surg* 1996;62:866-872.
85. Fisher AJ, Donnelly SC, Hirani N, Haslett C, Strieter RM, Dark JH, Corris PA. Elevated levels of interleukin-8 in donor lungs is associated with early graft failure after lung transplantation. *Am J Respir Crit Care Med* 2001;163:259-265.
86. De Perrot M, Sekine Y, Fischer S, Waddell TK, McRae K, Liu M, Wigle DA, Keshavjee S. Interleukin-8 release during early reperfusion predicts graft function in human lung transplantation. *Am J Respir Crit Care Med* 2002;165:211-215.
87. DiGiovine B, Lynch JP, 3rd, Martinez FJ, Flint A, Whyte RI, Iannettoni MD, Arenberg DA, Burdick MD, Glass MC, Wilke CA, Morris SB, Kunkel SL, Strieter RM. Bronchoalveolar lavage neutrophilia is associated with obliterative bronchiolitis after lung transplantation: Role of il-8. *J Immunol* 1996;157:4194-4202.
88. Penfield JG, Wang Y, Li S, Kielar MA, Sicher SC, Jeyarajah DR, Lu CY. Transplant surgery injury recruits recipient mhc class ii-positive leukocytes into the kidney. *Kidney Int* 1999;56:1759-1769.
89. Yoshida S, Haque A, Mizobuchi T, Iwata T, Chiyo M, Webb TJ, Baldrige LA, Heidler KM, Cummings OW, Fujisawa T, Blum JS, Brand DD, Wilkes DS. 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.
90. Haque MA, Mizobuchi T, Yasufuku K, Fujisawa T, Brutkiewicz RR, Zheng Y, Woods K, Smith GN, Cummings OW, Heidler KM, Blum JS, Wilkes DS. 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.
91. Burlingham WJ, Love RB, Jankowska-Gan E, Haynes LD, Xu Q, Bobadilla JL, Meyer KC, Hayney MS, Braun RK, Greenspan DS, Gopalakrishnan B, Cai J, Brand DD, Yoshida S, Cummings OW, Wilkes DS. Il-17-dependent cellular immunity to collagen type v predisposes to obliterative bronchiolitis in human lung transplants. *J Clin Invest* 2007;117:3498-3506.
92. Goers TA, Ramachandran S, Aloush A, Trulock E, Patterson GA, Mohanakumar T. De novo production of k-alpha1 tubulin-specific antibodies: Role in chronic lung allograft rejection. *J Immunol* 2008;180:4487-4494.

93. Shoskes DA, Cecka JM. Deleterious effects of delayed graft function in cadaveric renal transplant recipients independent of acute rejection. *Transplantation* 1998;66:1697-1701.
94. Perico N, Cattaneo D, Sayegh MH, Remuzzi G. Delayed graft function in kidney transplantation. *Lancet* 2004;364:1814-1827.
95. Ojo AO, Wolfe RA, Held PJ, Port FK, Schumouder RL. Delayed graft function: Risk factors and implications for renal allograft survival. *Transplantation* 1997;63:968-974.
96. Vassalli G, Gallino A, Weis M, von Scheidt W, Kappenberger L, von Segesser LK, Goy JJ, Working Group Microcirculation of the European Society of C. Alloimmunity and nonimmunologic risk factors in cardiac allograft vasculopathy. *Eur Heart J* 2003;24:1180-1188.
97. El-Hamamsy I, Stevens LM, Vanhoutte PM, Perrault LP. Injury of the coronary endothelium at implantation increases endothelial dysfunction and intimal hyperplasia after heart transplantation. *J Heart Lung Transplant* 2005;24:251-258.
98. Yousem SA, Berry GJ, Cagle PT, Chamberlain D, Husain AN, Hruban RH, Marchevsky A, Ohori NP, Ritter J, Stewart S, Tazelaar HD. 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.
99. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *Journal of the American Statistical Association* 1999;94:496-509.
100. Klein JP, Andersen PK. Regression modeling of competing risks data based on pseudovalues of the cumulative incidence function. *Biometrics* 2005;61:223-229.
101. Rosthøj S, Andersen PK, Abildstrøm SZ. Sas macros for estimation of the cumulative incidence functions based on a cox regression model for competing risks survival data. *Comput Methods Programs Biomed* 2004;74:69-75.
102. Huang HJ, Yusen RD, Meyers BF, Walter MJ, Mohanakumar T, Patterson GA, Trulock EP, Hachem RR. Late primary graft dysfunction after lung transplantation and bronchiolitis obliterans syndrome. *Am J Transplant* 2008;8:2454-2462.
103. Burton CM, Iversen M, Milman N, Zemtsovski M, Carlsen J, Steinbruchel D, Mortensen J, Andersen CB. Outcome of lung transplanted patients with primary graft dysfunction. *Eur J Cardiothorac Surg* 2007;31:75-82.

104. Bharat A, Kuo E, Steward N, Aloush A, Hachem R, Trulock EP, Patterson GA, Meyers BF, Mohanakumar T. Immunological link between primary graft dysfunction and chronic lung allograft rejection. *Ann Thorac Surg* 2008;86:189-195; discussion 196-187.
105. Lin DY, Wei LJ, Ying Z. Checking the cox model with cumulative sums of martingale-based residuals. *Biometrika* 1993;80:557-572.