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Lung Transplant Outcomes are Influenced by Severity of Neutropenia and Granulocyte Colony-Stimulating Factor Treatment

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Abstract

Although neutropenia is a common complication following lung transplantation, its relationship to recipient outcomes remains understudied. We evaluated a retrospective cohort of 228 adult lung transplant recipients between 2008 and 2013 to assess the association of neutropenia and Granulocyte Colony Stimulating Factor (GCSF) treatment with outcomes. Neutropenia was categorized as mild (ANC 1000–1499), moderate (500–999) or severe (<500) and also as a time-varying continuous variable. Association with survival, acute rejection (ACR) and chronic lung allograft dysfunction (CLAD) were assessed by Cox proportional hazards regression. GCSF therapy impact on survival, CLAD and ACR development was analyzed by propensity score matching. 101 of 228 patients (42.1%) developed neutropenia. Recipients with severe neutropenia had higher mortality when compared to recipients with no (adjusted hazard ratio (aHR) 2.97, 95% CI 1.05–8.41, p=0.040), mild (aHR 14.508, 95% CI 1.58–13.34, p=0.018), or moderate (aHR 3.27,

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AUTHORSHIP

LK Tague participated in study design, data collection, data analysis and manuscript preparation. M. Wallendorf and B. Gage participated in data analysis and manuscript preparation. R. Hachem, D. Kreisel, and A. Krupnick participated in study design and manuscript preparation. DE Byers, and D Scozzi participated in manuscript preparation. AE Gelman participated in study design, data analysis and manuscript preparation.

DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

95% CI 0.89–12.01, $p=0.074$) neutropenia. Surprisingly, GCSF treatment was associated with a higher risk for CLAD in mildly neutropenic patients (aHR 3.49, 95% CI 0.93–13.04, $p=0.063$), although it did decrease death risk in severely neutropenic patients (aHR 0.24, 95% CI 0.07–0.88, $p=0.031$). Taken together, our data point to an important relationship between neutropenia severity and GCSF treatment in lung transplant outcomes.

INTRODUCTION

Neutropenia after solid organ transplant is a known risk factor for adverse events.¹ Neutropenia after renal transplant is associated with death and allograft loss.² It is also a risk factor for death after liver transplant.³ Although neutropenia is common after lung transplant, its effects on outcomes in this population has not been as thoroughly investigated and no formal guidelines for management exist. Most neutropenia is predominantly thought to result from treatment with anti-proliferative agents such as azathioprine or mycophenolic acid (MPA) or (cytomegalovirus) CMV prophylaxis or treatment with antiviral drugs such as valganciclovir or ganciclovir.^{1,4} Currently, MPA is the anti-proliferative agent of choice with almost 80% of lung transplant recipients maintained on it through the first year post-transplant according to ISHLT registry data.⁵ Neutropenia is usually managed primarily by a dose reduction and/or discontinuation of immunosuppression therapy, which itself is a risk factor for allograft rejection.⁶ In addition to limiting or discontinuing use of MPA, physicians often manage neutropenia in lung recipients with granulocyte colony stimulating factor (GCSF), but its effects on lung allograft survival and rejection remain unknown.

Studies examining the effectiveness of GCSF in solid organ recipient survival have been primarily limited to its effects on acute cellular rejection (ACR). In kidney and liver transplant recipients, GCSF therapy did not change ACR incidence, but there is conflicting evidence among heart and lung transplant recipients.^{7–9} It also remains unclear whether GCSF treatment improves long-term survival, or if severity of neutropenia plays a role in GCSF-associated outcomes in solid organ transplant recipients. In lung recipients this is especially important to determine given their higher dependence on immunosuppression¹⁰, more frequent acute rejection¹¹, and poorer overall survival⁵, when compared to other solid organ recipients. The goals of this study were to (1) evaluate the association between severity of neutropenia and clinical outcomes, such as allograft rejection and survival, and (2) determine how GCSF administration might attenuate that relationship.

MATERIALS AND METHODS

Study Design and Patient Selection

We conducted a retrospective cohort study of all adult (> 18 years) patients who underwent a lung transplantation at Barnes-Jewish Hospital between 2008 and 2013 who were previously enrolled in a lung transplant recipient database at our institution.¹² Patients were excluded if they were transplanted before the age of 18 or transplanted at another institution. The primary outcome was overall survival. Secondary outcomes included ACR, the development of chronic lung allograft dysfunction (CLAD) and infection.

Diagnosis of Neutropenia.—All blood counts obtained after transplantation were analyzed for each patient. Onset of neutropenia was defined as the first instance of absolute neutrophil count (ANC) less than 1500 and was categorized as mild (ANC < 1500), moderate (ANC < 1000) or severe (ANC < 500) based on the nadir of the ANC during the index episode. Duration was defined as time to next ANC > 1500 and dichotomized with prolonged being more than 1 week.¹³

Diagnosis of ACR and CLAD.—During the study period, all patients at our institution underwent protocolized bronchoscopy with bronchoalveolar lavage and transbronchial biopsy to evaluate for infection and rejection at 1-, 3-, 6- and 12-months as part of standard post-lung transplant care. Additional bronchoscopic evaluations were based on changes in a patient's clinical status at the discretion of the treating transplant pulmonologist. All transbronchial biopsies were evaluated by a trained pathologist and scored for ACR according to guidelines outlined by the International Society for Heart and Lung Transplantation (ISHLT).¹⁴ Severity and timing of each episode of ACR was recorded for all participating patients through the end of the follow up period. CLAD was diagnosed as a persistent drop in forced expiratory volume in one second (FEV1) or forced vital capacity (FVC) of greater than 10% in the absence of infection or other reversible cause as previously described.¹⁵ Although this does not represent the latest guidelines released in early 2019¹⁶, this definition is reflective of clinical practice during the study period¹⁷.

Diagnosis of Infection.—Evaluation for infection was performed at each protocolized and clinically-indicated bronchoscopic exam. Only respiratory infections were considered. Bacterial (gram-positive and gram-negative) and aspergillus infections were diagnosed with positive respiratory cultures from bronchoscopic evaluation, irrespective of clinical symptoms. For CMV, infection was diagnosed if both respiratory and blood CMV PCR were positive and the patient underwent treatment for the positive cultures.

All data were collected through December 31, 2017. Data were maintained in a secure REDCap database with access limited to essential study personnel.

Immunosuppression Regimen.—All patients at our institution are maintained on a standard 3-drug immunosuppression regimen: a calcineurin inhibitor (tacrolimus or cyclosporine), an anti-proliferative agent (MPA) and corticosteroids. Corticosteroids were initiated on postop day 0 at 1 gram of methylprednisolone daily for 3 days followed by 1mg/kg of prednisone (max of 40mg) with a predetermined taper down to 5mg by 3 months. Induction therapy was given per protocol with the preferred agent changing during the study time frame from (anti-thymocyte globulin) ATGAM to basiliximab. Alterations to immunosuppression were made on an individual basis at the discretion of the treating transplant pulmonologist.

This study was approved by the Institutional Review Board at Washington University (IRB#: 201105421).

Statistical Analyses

Baseline demographic and clinical factors were evaluated using the Wilcoxon rank-sum test for continuous variables and the chi-square test for categorical variables.

Construction of Cox Models.—Survival, ACR and CLAD were evaluated as time-to-event outcomes utilizing Cox proportional hazards (PH) regression. Separate Cox models were constructed using neutropenia as a categorical variable of none, mild, moderate, and severe as previously defined (basic Cox model) and using ANC as a continuous time-varying covariate with both a natural log and a quadratic transformation (extended Cox models). For time to ACR, the first episode of ACR after the diagnosis of neutropenia was utilized. For multivariable modelling in both the basic and extended Cox models, covariates of interest were chosen based on prior known or suspected confounding of the outcomes of interest to improve causal inference in this retrospective observational study as previously described.¹⁸ Covariates included in the final Cox model for Survival, ACR and CLAD included age, donor gender, transplant indication, PGD grade at 72 hours and CMV mismatch status. For Survival, CLAD status, CMV infection and gram-negative bacterial infection were included in the final model.

Predictors of Neutropenia.—Predictors of neutropenia were evaluated utilizing Cox regression. For all analyses, univariable analyses were conducted for relevant clinical and biological covariates. Variables with $p > 0.20$ or with prior literature intimating potential confounding in the outcome of interest were offered into the multivariable Cox regression models.

Propensity Score Matching.—Propensity score matching (PSM) for GCSF use was carried out for the entire cohort. Patients were matched on age, gender, neutropenia severity, CMV mismatch status (high risk vs. not high risk where high risk was defined as recipient CMV negative and donor CMV positive) and whether or not they had ACR prior to the first neutropenic episode. Logistic regression was performed to generate propensity scores for GCSF use and patients were matched by score with a match tolerance of 0.1. 51 patients (27 in GCSF and 24 in non-GCSF groups) were included in the PSM analysis. Cox PH regression analyses for survival, time to CLAD and time to ACR were conducted for the matched cohort by severity of neutropenia as outlined above (basic Cox Models).

For all analyses, we considered a two-sided $p < 0.05$ statistically significant. We performed regression diagnostics to ensure proportional hazards assumption was met for Cox regression analyses. We conducted all analyses using SPSS 24 (IBM Corp., Armonk, NY) with the SPSS extension for R work environment (R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>) and SAS v9.4 (copyright ©2013 SAS Institute Inc., Cary, NC, USA).

RESULTS

Patient Population

A total of 228 patients were included in the study. 101 of 228 patients (44.3%) developed neutropenia: 42 Mild, 34 Moderate and 25 Severe. Table 1 depicts the demographics for these patients according to degree of neutropenia. Patients with moderate and severe neutropenia were more likely to have received basiliximab and less likely to receive ATGAM for induction. Additionally, patients with both donor and recipient negative CMV serostatus were more likely to have no neutropenia while patients with donor positive and recipient negative CMV serostatus were more likely to develop neutropenia. 71 of the 101 (70.3%) patients developed neutropenia within the first year after transplant while the other 30 (29.7%) had their first episode after 1 year. Table 2 outlines factors related to neutropenic episodes. The top panel reports differences between neutropenia severity group. The bottom panel reports differences in GCSF therapy within neutropenia severity group. Overall, the median length of neutropenia was 8 days (IQR 5–28). GCSF administration in response to neutropenia was significantly different between the mild, moderate and severe groups with patients more likely to have received GCSF with increasing degree of severity. GCSF was not associated with any difference in the duration of neutropenia for patients with moderate or severe disease. However, patients with mild neutropenia who received GCSF had a significantly shorter duration of neutropenia (9.5 days vs. 5 days, $p=0.023$). Additionally, there was a significant difference among all patients who received GCSF with mild patients having a significantly shorter duration than moderate or severe patients (Table 2, bottom panel, row 1 $p=0.026$). Patients who developed neutropenia late (after the first-year post-transplant) were neutropenic for a longer time than patients who developed it early (within the first-year post-transplant) [median 23 days (5–69) vs. 7.5 (5–20.5) days, $p=0.035$]. Patients with moderate and severe neutropenia were more likely to have at least one dose of MPA held in response to neutropenia than patients with only mild neutropenia. Overall there were no differences in MPA dose between the groups.

Neutropenia and Survival

Overall median survival (IQR) was 86.92 months (79.07–94.77) for patients with no neutropenia, 78.03 months (61.03–95.04) for severe neutropenia, 90.76 months (77.05–104.48) for moderate neutropenia and 93.96 months (80.86–107.07) for mild neutropenia. In the basic Cox model, multivariable modelling revealed severe neutropenia to be an independent risk factor for death when compared to patients with no neutropenia [aHR 2.97 (1.05–8.41), $p=0.040$]. Additionally, severe neutropenia patients were also at a significantly higher risk than patients with mild or moderate neutropenia (Figure 1, Table 3). In the extended Cox model, increasing ANC was significantly associated with improved survival (aHR 3.304, 95% CI 1.982–5.508, $p<0.001$, Supplemental Table 1). Additionally, in an analysis of ANC-squared, an ANC of 3329 (95% CI 1677 to 10,072) appeared to maximize survival (Supplemental Figure 1).

Neutropenia and Rejection

There were no significant differences in time to ACR in either the basic or extended Cox models (data not shown). There was no significant association between severity of

neutropenia and development of CLAD in the basic Cox models (data not shown). However, in extended Cox modelling, increasing ANC was associated with a significantly increased risk for CLAD (aHR 2.64, 95% CI 1.71–4.06, $p < 0.001$, Supplemental Table 2).

Neutropenia and Infection

Table 4 shows the distribution of infection among patients with no, mild, moderate and severe neutropenia. There were no significant differences in rates of CMV infection. For gram positive infections, patients with severe neutropenia had significantly higher rates than patients with no neutropenia ($p = 0.004$) or only mild neutropenia ($p = 0.026$). Moderately and severely neutropenic patients had significantly higher rates of gram-negative infections than mildly neutropenic patients (Moderate vs. Mild, $p = 0.040$; Severe vs. Mild, $p = 0.033$). Mildly neutropenic patients had a significantly lower rate of aspergillus infection than patients without neutropenia ($p = 0.045$). In extended Cox modelling, there was no significant association between ANC and any of the infectious outcomes.

Association between GCSF and Neutropenia Outcomes

Propensity matching resulted in a matched cohort of 51 patients. Supplemental Tables 3 and 4 reveal pre- and post-matching demographics for the cohorts. For patients with mild and moderate neutropenia, GCSF administration had no meaningful association with survival (data not shown). However, for patients with severe neutropenia, GCSF administration was associated with significantly improved overall survival and improved survival after the first episode of neutropenia (overall aHR for death 0.24, 95% CI 0.07–0.88, $p = 0.031$, Figure 2). GCSF had no significant association with the development of ACR in patients with any degree of neutropenia (data not shown). However, for CLAD, GCSF administration was associated with a strong trend towards increased CLAD for patients with mild neutropenia (aHR 3.49 (0.93–13.04), $p = 0.063$) and decreased CLAD for patients with severe neutropenia (aHR 0.25 (0.06–1.02, $p = 0.053$), although these did not reach statistical significance (Figure 3). For infection, stratification according to GCSF use revealed that patients with severe neutropenia had significantly higher rates of aspergillus infection when they did not receive GCSF (83.3% vs. 33.3%, $p = 0.046$) (Supplemental Table 5). There were no other significant differences in infection outcomes among patients within each severity class of neutropenia based on whether or not they received GCSF.

Predictors of Neutropenia

In univariable analysis, high-risk CMV mismatch (seronegative recipient/seropositive donor) was strongly associated with development of post-transplant neutropenia and this relationship remained significant in multivariable analysis (aHR 2.464, 95% CI 1.293–4.696, $p = 0.006$) and male recipient gender was protective (HR 0.654, 95% CI 0.431–0.992, $p = 0.046$, Table 5). No other demographic or clinical factors were found to significantly predict neutropenia.

DISCUSSION

We find here that patients who develop severe neutropenia have decreased survival after transplant when compared to patients with no, mild or moderate neutropenia. GCSF use was

associated with improved outcomes in patients with severe neutropenia, specifically a significantly decreased risk of death. Conversely, in patients with mild neutropenia, GCSF was associated with higher rates of CLAD without any meaningful association with survival or infection outcomes.

This is, to our knowledge, the largest clinical study in lung transplant recipients to evaluate the association between neutropenia and survival and how administration of GCSF may attenuate that association. A similar study among pediatric renal transplant recipients found neutropenia to be similarly frequent (~64%); however, they did not evaluate the effect of neutropenia on survival or other clinical outcomes.¹⁹ Likewise, a large Medicare database study of adult renal transplant recipients found that neutropenia was associated with increased risk of allograft loss and death, and that treatment with GCSF appeared to attenuate that effect², but did not evaluate the differential impact of varying severity of neutropenia.

Neutropenia has been most extensively studied among hematopoietic transplant recipients, where increasing severity and duration of neutropenia is associated with increased infectious outcomes and death.²⁰⁻²² Neutrophils are particularly crucial effector cells in the body's defense against bacterial pathogens, migrating along chemokine gradients to areas of infection and/or inflammation.²³⁻²⁵ In patients receiving MPA for immunosuppression following lung transplantation, the effects of severe neutropenia are likely exacerbated. This is because MPA, in addition to its primary target, also interferes with antigen presentation by dendritic cells, promotes monocyte apoptosis and disrupts leukocyte adhesion.²⁶⁻²⁹ As such, the significantly higher rates of bacterial infections among severely neutropenic patients are to be expected. Additionally, as there were no significant differences in ACR or CLAD for severely neutropenic patients, it is likely that the increase in infectious complications is primarily responsible for the decrement in survival seen in these patients. This supports ISHLT registry data demonstrating that one of the leading causes of death, particularly after the first year post-lung transplant, is infection.⁵

Conversely, patients with mild neutropenia had better survival than all other patients. However, contrary to what was seen in patients with severe neutropenia, GCSF administration was associated with worsened CLAD outcomes among patients with mild neutropenia. GCSF is a cytokine that drives both neutrophil generation from myeloid progenitors within the bone marrow and neutrophil mobilization into the blood.³⁰ However, GCSF promotes immunomodulatory effects on adaptive immune responses that could promote allograft tolerance. There is evidence that GCSF promotes the reduction of alloreactive T lymphocytes and the expansion of regulatory populations of T lymphocytes and antigen presenting cells.³¹⁻³³ Although this might appear to be a paradoxical finding, recent evidence from experimental models of lung transplantation support the notion that GCSF under certain circumstances can promote alloimmunity.³⁴ For example, previous work has shown that GCSF administration to a mouse model of lung transplantation induced neutrophils to activate antigen presenting cells, which in turn, caused the expansion of alloreactive T cells and prevented immunosuppression-mediated tolerance.³⁵ Similarly, GCSF-dependent neutrophilia has been shown to stimulate rejection through neutrophil-mediated T-cell activation during a pseudomonas infection.³⁶ Finally, it has been

demonstrated that blocking GCSF-mediated emergency granulopoiesis prevents acute inflammatory injury.³⁷ Given that patients with mild neutropenia showed no increased risk of infection, and increased rates of CLAD following GCSF administration, it is possible that the emergency granulopoiesis stimulated by GCSF administration in these patients leads to harmful neutrophil infiltration and damage to the allograft. Additionally, this might also explain why patients with mild neutropenia appeared to do better than patients with no neutropenia. This area requires more investigation as prior reports of the impact of GCSF on clinical transplant outcomes have been conflicting, likely related to the heterogeneity both of the patient populations and of the severity of neutropenia.^{7,8,38}

The only significant predictors of neutropenia after transplant in multivariable modelling was recipient female gender and high-risk CMV mismatch recipients, recipients who were seronegative and received lungs from a seropositive donor. The exact relationship between gender and neutropenia is unclear, however the increased risk associated with females might be related to the known increased risk of alloimmunity particularly in women who have had children. This is a phenomenon that requires more investigation. In our institution, the standard protocol for high risk CMV mismatch status is valganciclovir treatment for CMV prophylaxis for the first six months following transplant. Thus, it is interesting to note that pharmacokinetic interactions between valganciclovir and MPA³⁹ have been related to increase rates of neutropenia.^{40,41} It is also possible that there are different etiologies of neutropenia depending on time after transplant. Neutropenia early after transplant, although more common, was shorter and was not associated significantly with overall survival. Given that combination of medications used early after transplant results in higher effective immunosuppression, the transitory nature of early neutropenia could be related to the immunosuppression treatment. In contrast, late neutropenia was significantly longer, was not associated with valganciclovir use, and was associated with significantly decreased survival. Given that prolonged exposure to MPA and other commonly used immunosuppression agents can be cytotoxic, late neutropenia might represent a different phenomenon related to changes in neutrophil biology, production and maturation.⁴²

This study has several limitations. The retrospective design limited the data available to pre-existing patient records. Therefore, incomplete records, or records not in our system (for non-local transplant recipients) may have led to missed episodes of neutropenia or infection. Additionally, there is a selection bias for patients previously enrolled in our lung transplant database. Secondly, the relatively small sample size resulted in fewer numbers of patients available for subgroup analyses. It is possible that type II errors exist in some of the subgroup analyses. In particular, we were unable to assess how changes to clinical management in response to neutropenia altered the association with survival. Additionally, rejection episodes were recorded without controlling for interpatient variability in the number of bronchoscopies performed. Finally, again due to the retrospective nature of the study, it was not possible to control for all potential confounders.

Despite these limitations, we conclude that severe neutropenia is associated with decreased survival and increased rates of infection. Among severely neutropenic patients, GCSF administration may be helpful to attenuate these risks. However, administration to mildly neutropenic patients appears to be detrimental as it was associated with increased rates of

CLAD without any meaningful improvement in allograft survival or reduction in infectious complications. Thus, further investigation into proper patient selection for GCSF therapy is warranted to better optimize neutropenia treatment protocols for lung transplant recipients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

ACR	Acute Cellular Rejection
AHR	Adjusted Hazard Ratio
ANC	Absolute Neutrophil Count
ATG	Anti-thymocyte globulin
CLAD	Chronic Lung Allograft Dysfunction
CMV	Cytomegalovirus
FEV1	Forced Expiratory Volume in One Second
FVC	Forced Vital Capacity
GCSF	Granulocyte Colony Stimulating Factor
IQR	Interquartile Range
ISHLT	International Society for Heart and Lung Transplantation
MPA	Mycophenolic Acid
PSM	Propensity Score Matching

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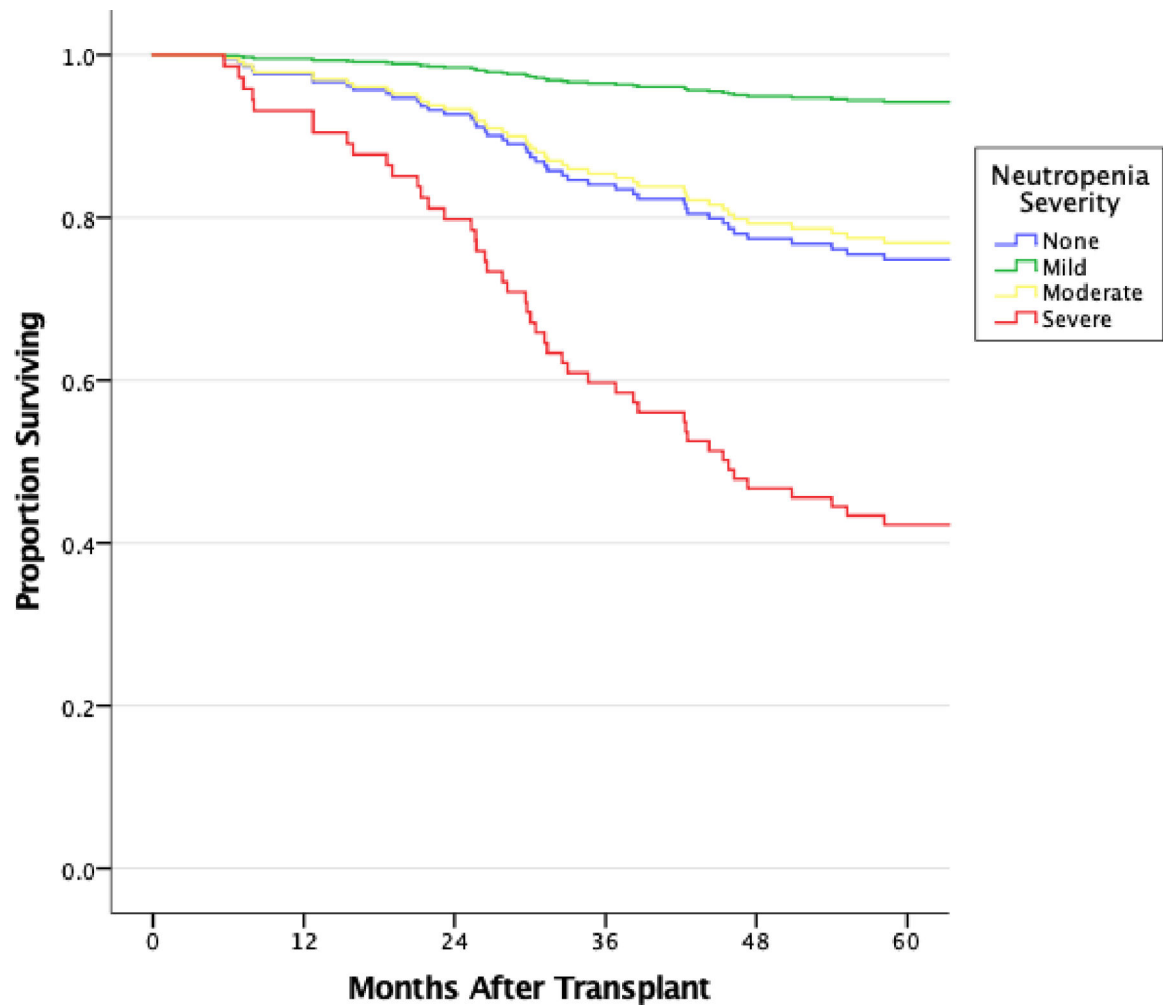


Figure 1: Basic Multivariable Cox Regression Modelling of Survival after Lung Transplantation. All patients who developed neutropenia at any time point after lung transplant were evaluated. Model was adjusted for age, gender, CMV infection, CLAD diagnosis, and gram-negative bacterial infection. Severe neutropenia was associated with poorer survival than no, mild or moderate neutropenia. Severe vs. no neutropenia: aHR=2.97 (1.05–8.41), $p=0.04$; Severe vs. Mild Neutropenia: aHR=14.51 (1.58–33.41), $p=0.018$; Severe vs. Moderate: aHR=3.27 (0.89–12.01), $p=0.07$.

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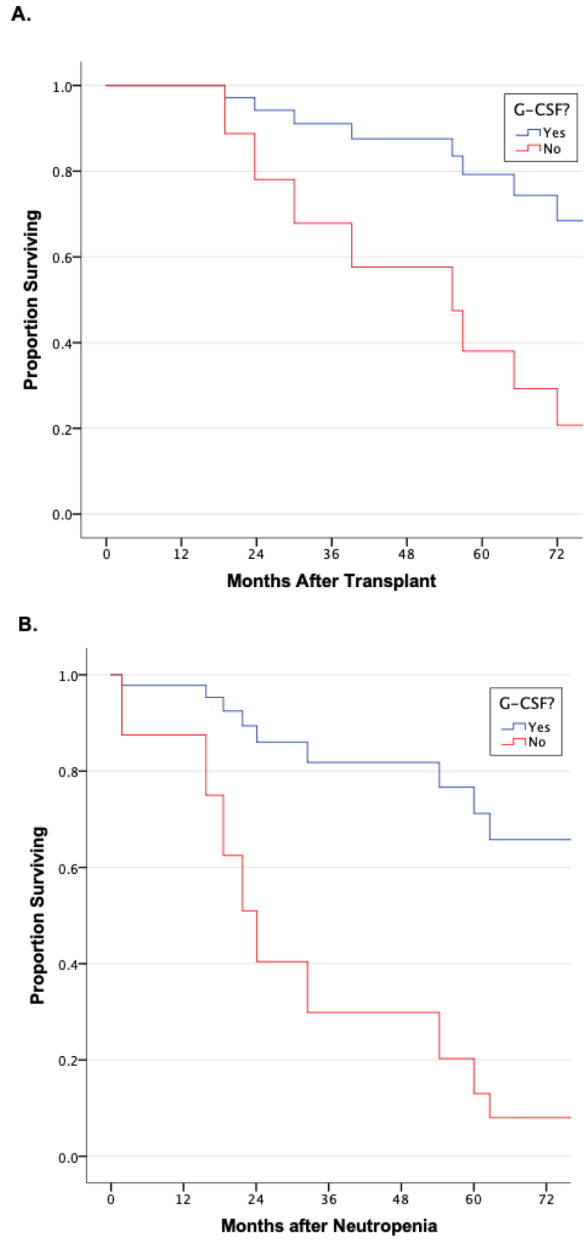


Figure 2: Association Between G-CSF use and Survival Among Propensity-Matched Cohort of Severely Neutropenic Patients

A) Severe neutropenia patients who received G-CSF had significantly improved overall survival than those who did not (aHR for death 0.24, 95% CI 0.07–0.88, p=0.031). B) Severe neutropenia patients who received G-CSF had significantly improved post-neutropenia survival than severe neutropenia patients who did not (aHR for death 0.17, 95% CI 0.04–0.63, p=0.008).

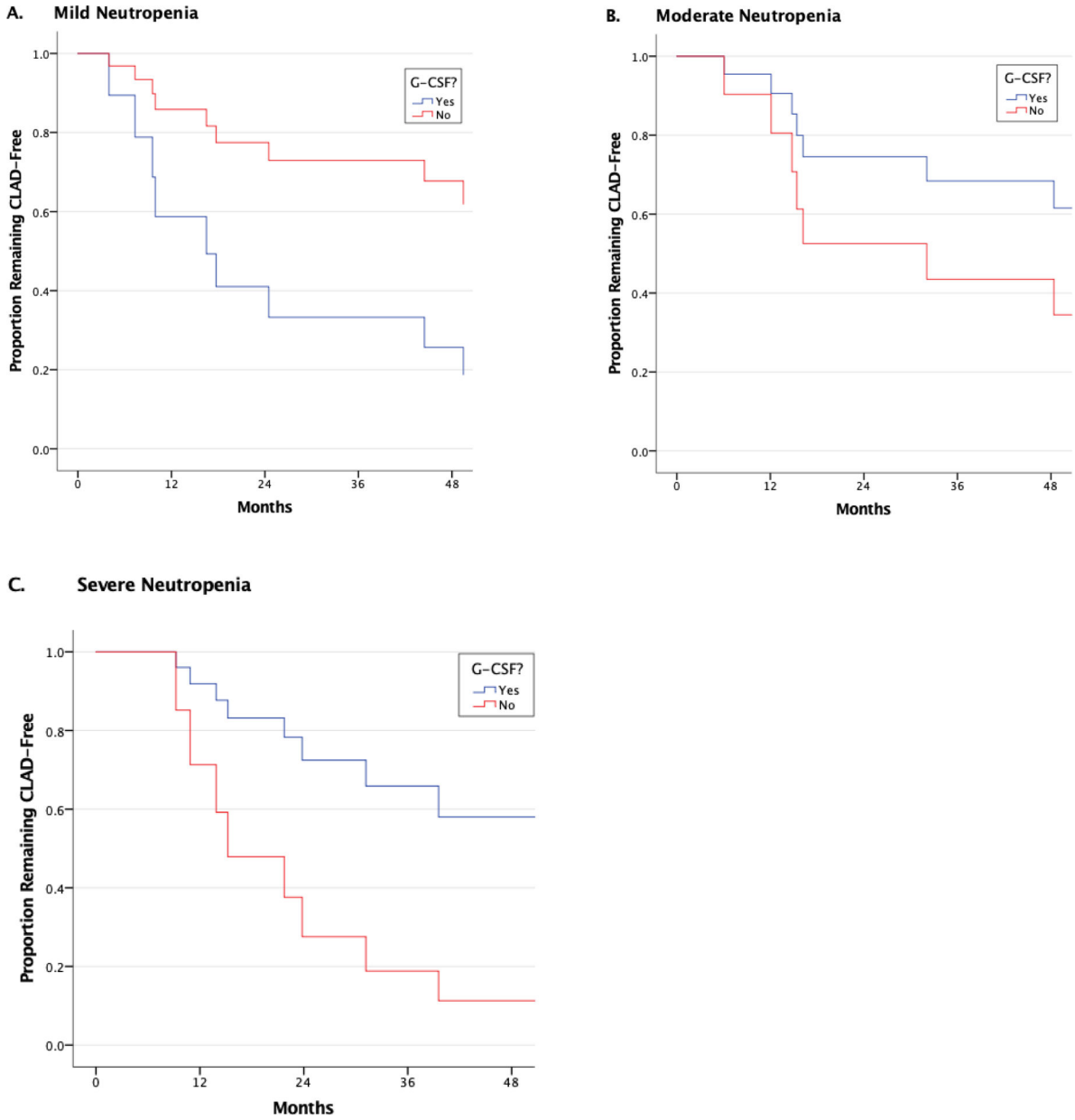


Figure 3: Association Between G-CSF use and CLAD Development Among Propensity-Matched Cohort of Neutropenic Patients

Neutropenia groups represent severity of first episode of neutropenia. Time to CLAD is shown for patients with A) mild, b) moderate and c) severe neutropenia. For patients with mild neutropenia, G-CSF administration was associated with a near significant increased risk for CLAD development (aHR 3.49, 95% CI 0.93–13.04, $p=0.063$). There were no significant differences in CLAD development for patients with moderate neutropenia, but patients with severe neutropenia who received G-CSF had a near significant decreased risk for CLAD development according to whether or not they received G-CSF (aHR 0.25, 95% CI 0.06–1.02, $p=0.053$).

Table 1:

Baseline Characteristics by Degree of Neutropenia

	None (n=125)	Mild (n=42)	Moderate (n=34)	Severe (n=25)	p-value
Recipient Age, years, mean (SD)	54.3 (13.0)	53.7 (12.3)	49.9 (13.4)	49.2 (15.8)	0.287
Recipient Gender					
Female	44 (35.2)	18 (42.9)	13 (38.2)	15 (60)	0.135
Male	81 (64.8)	24 (57.1)	21 (61.8)	10 (40)	
Recipient Race					
White	118 (94.4)	38 (90.5)	32 (94.1)	25 (100)	0.528
African-American	5 (4)	4 (9.5)	2 (5.9)	0 (0)	
Asian	2 (1.6)	0 (0)	0 (0)	0 (0)	
Transplant Diagnosis					
ILD	62 (49.6)	21 (50)	13 (38.2)	11 (44)	
COPD	30 (24)	12 (28.6)	6 (17.6)	4 (16)	
CF	16 (12.8)	7 (16.7)	9 (26.5)	8 (32)	0.570
A1AT	5 (4)	1 (2.4)	2 (5.9)	0 (0)	
PH	2 (1.6)	0 (0)	1 (2.9)	0 (0)	
Other	10 (8)	1 (2.4)	4 (8.8)	2 (8)	
Recipient CMV Positive	75 (60)	23 (54.8)	14 (41.2)	15 (60)	0.258
Donor Age, years, mean (SD)	35.9 (14.9)	34.6 (13.3)	38.9 (14.9)	39.8 (16)	0.771
Donor Male Gender	85 (68)	27 (64.3)	20 (58.8)	14 (56.0)	0.585
Donor Race					
White	101 (80.8)	33 (78.6)	20 (58.8)	20 (80)	0.184
African-American	23 (18.4)	9 (21.4)	14 (41.2)	5 (20)	
Asian	1 (0.8)	0 (0)	0 (0)	0 (0)	
CMV Mismatch Status					
R-/D-	28 (22.4)	8 (19)	3 (8.8)	2 (8)	
R+/D+	51 (40.8)	21 (50)	8 (23.5)	11 (44)	
R+/D-	24 (19.2)	2 (4.8)	6 (17.6)	4 (16)	0.005
R-/D+	22 (17.6)	11 (26.2)	17 (50)	8 (32)	
Transplant Type					
Single	6 (4)	0 (0)	1 (2.9)	1 (4)	0.622
Bilateral	119 (96)	42 (100)	33 (97.1)	24 (96)	
Ischemic Time, minutes, mean (SD)	278.2 (67.6)	264.3 (73.6)	289 (65.8)	288.1 (80.3)	0.322
Induction Therapy					
Basiliximab	81 (65.3)	27 (64.3)	26 (76.5)	18 (72)	
ATGAM	42 (33.9)	9 (21.4)	6 (17.6)	5 (20)	0.007
Thymoglobulin	0 (0)	2 (4.8)	0 (0)	0 (0)	
Other	1 (0.8)	3 (7.1)	1 (2.9)	1 (4)	
None	0 (0)	1 (2.4)	1 (2.9)	1 (4)	
PGD @ 72hours					
0	54 (43.2)	11 (26.2)	10 (29.4)	10 (40)	0.602

	None (n=125)	Mild (n=42)	Moderate (n=34)	Severe (n=25)	p-value
1	60 (48)	26 (61.9)	20 (58.8)	11 (44)	
2	8 (6.4)	4 (9.5)	2 (5.9)	3 (12)	
3	3 (2.4)	1 (2.4)	2 (5.9)	1 (4)	

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Table 2:

Patient Characteristics by Degree of Neutropenia

	Mild (n=42)	Moderate (n=34)	Severe (n=25)	p-value*
Median time to neutropenia, months (IQR)	4.9 (2.4–13.2)	6.2 (3.4–27.2)	3.8 (2.1–6.8)	0.342
Valcyte at time of Neutropenia, n(%)	19 (45.2)	16 (47.1)	14 (56)	0.680
Calcineurin Inhibitor at Neutropenia				
Tacrolimus	41 (97.6)	31 (91.2)	25 (100)	0.181
Cyclosporine	1 (2.4)	3 (.8)	0	
Prednisone Dose at time of Neutropenia, mg, median (IQR)	7.5 (5–60)	10 (5–35)	10 (5–50)	0.296
Mycophenolate Dose at time of Neutropenia, g, Median (IQR)	1 (0.5–2)	1 (0.5–2)	1 (0.5–2)	0.826
Mycophenolate Response to Neutropenia, n (%)				
No Change	12 (28.6)	5 (14.7)	5 (20)	
Dose Decrease	6 (14.3)	3 (8.8)	2 (8)	0.141
Dose Held	24 (57.1)	26 (76.5)	16 (64)	
Medication Change	0	0	2 (8)	
G-CSF Administered for Neutropenia, n(%)	6 (14.3)	9 (26.5)	12 (48)	0.011
Duration of Neutropenia, days, median (IQR)				
GCSF Administered	5 (1–7)	7 (6–37)	8 (4–11)	0.026
GCSF Not Administered	9.5 (6–34.5)	8 (5–28)	8 (3–32)	0.656
p-value for Duration of Neutropenia [^]	0.023	0.274	0.956	

* Reported p-value is for comparison **between** neutropenia severity groups.

[^] reported p-value is for comparison **within** each neutropenia severity group based on whether or not they received GCSF.

Table 3:

Basic Cox Regression Model of Risk of Death after Lung Transplantation

Variable	UNIVARIABLE ANALYSIS		MULTIVARIABLE ANALYSIS	
	Hazard Ratio (95% CI)	p-value	Hazard Ratio (95% CI)	p-value
Degree of Neutropenia (ref: None)		0.020		0.075
Mild	0.16 (0.02–1.18)	0.072	0.20 (0.03–1.49)	0.117
Moderate	0.99 (0.42–2.30)	0.975	0.91 (0.39–2.12)	0.823
Severe	3.26 (1.29–8.21)	0.012	2.97 (1.05–8.41)	0.040
Recipient Age	0.99 (0.98–1.01)	0.524		
Recipient Male Gender	1.24 (0.80–1.90)	0.335		
Recipient Race	1.41 (0.57–3.48)	0.457		
Transplant Diagnosis (ref: COPD)		0.045		
ILD	0.88 (0.52–1.49)	0.623		
CF	1.12 (0.59–2.10)	0.735		
A1AT	2.82 (1.19–6.65)	0.018		
PH	0.88 (0.12–6.58)	0.904		
Other	0.36 (0.11–1.23)	0.103		
Donor Age	0.99 (0.98–1.01)	0.933		
Donor Male Gender	1.51 (0.95–2.40)	0.079	1.94 (1.02–3.67)	0.043
Donor Race	1.37 (0.86–2.18)	0.178		
CMV Mismatch Status (ref: R–/D–)		0.588		
R+/D+	1.31 (0.69–2.46)	0.407		
R+/D–	1.17 (0.54–2.53)	0.683		
R–/D+	1.56 (0.80–3.02)	0.188		
Transplant Ischemic Time, min	1.00 (0.99–1.01)	0.612		
Transplant Type	1.56 (0.38–6.33)	0.537		
ACR	0.75 (0.48–1.17)	0.206		
CLAD	2.98 (1.82–4.86)	<0.001	2.68 (1.42–5.04)	0.002
CMV Infection	1.62 (1.01–2.58)	0.044	1.73 (0.96–3.10)	0.066
Aspergillus Infection	1.72 (1.13–2.63)	0.011		
Gram Positive Bacterial Infection	1.43 (0.93–2.19)	0.102		
Gram Negative Bacterial Infection	1.70 (1.10–2.64)	0.018	1.87 (1.08–3.24)	0.026

Table 4:

Rate of Infection by Overall Degree of Neutropenia

	None	Mild	Moderate	Severe
Aspergillus[*], n(%)	66 (53.6)	15 (35.7)	18 (52.9)	13 (52)
CMV[*], n (%)	80 (64)	26 (61.9)	25 (73.5)	18 (72)
Gram Negative^{**}, n(%)	71 (56.8)	19 (45.2)	23 (67.6)	18 (72)
Gram Positive[^], n(%)	33 (26.4)	12 (28.6)	12 (35.3)	14 (56)

^{*} No Overall significant comparisons for aspergillus or CMV infection

^{**} Severe vs Mild (OR 3.11, 95% CI 1.07–9.02, p=0.033) and Moderate vs Mild (OR 2.78, 95% CI 1.07–7.27, p=0.034) had significantly higher rate of gram-negative infection.

[^] Severe patients had significantly higher rate of gram-positive infection than none (OR 2.89, 95% CI 1.25–6.69, p=0.011) and mild (OR 3.18, 95% CI 1.13–8.96, p=0.026) neutropenia patients.

Table 5:

Cox Regression Analysis for Predictors of Neutropenia

Variable	Univariable Analysis		Multivariable Analysis	
	Hazard Ratio (95% CI)	p-value	Hazard Ratio (95% CI)	p-value
Recipient Age at Transplant	0.989 (0.975–1.003)	0.120		
Recipient Male Gender	0.700 (0.473–1.035)	0.074	0.654 (0.431–0.992)	0.046
Recipient Race (Black vs. White)	1.525 (0.668–3.485)	0.316		
Induction Immunosuppression (ATGAM vs. Simulect)	0.610 (0.370–1.004)	0.052		
Recipient Diagnosis (Reference: COPD)		0.383		
Interstitial Lung Disease	1.066 (0.640–1.776)	0.805		
Cystic Fibrosis	1.696 (0.950–3.028)	0.074		
Alpha-1 Antitrypsin	0.878 (0.263–2.938)	0.833		
Pulmonary Hypertension	0.658 (0.089–4.880)	0.682		
Other	0.870 (0.353–2.146)	0.762		
Donor Age	1.001 (0.989–1.015)	0.822		
Donor Male Gender	0.786 (0.527–1.171)	0.236		
Donor Race (Black v. White)	1.464 (0.947–2.264)	0.087		
CMV Mismatch Status (Reference R–/D–)		0.005		0.007
R+/D+	1.531 (0.819–2.864)	0.182	1.319 (0.694–2.509)	0.398
R+/D–	1.016 (0.463–2.227)	0.969	1.016 (0.463–2.228)	0.969
R–/D+	2.526 (1.337–4.771)	0.004	2.464 (1.293–4.696)	0.006
Transplant Ischemic Time	1.000 (0.998–1.003)	0.732		
Transplant Type (Bilateral vs. Single)	1.526 (0.376–6.191)	0.554		
PGD Grade at 72 hours (Reference: 0)		0.525		
Grade 1	1.355 (0.875–2.099)	0.173		
Grade 2	1.423 (0.677–2.990)	0.352		
Grade 3	1.534 (0.541–4.348)	0.420		
Calcineurin Inhibitor (cyclosporine vs. tacrolimus)	1.817 (0.575–5.746)	0.309		