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PII: S1053-2498(18)31511-0
DOI: 10.1016/j.healun.2018.06.009
Reference: HEALUN 6769

To appear in: *Journal of Heart and Lung Transplantation*

Received date: 14 December 2017
Revised date: 5 April 2018
Accepted date: 20 June 2018

Please cite this article as: Ann B. Nguyen MD , Laura Lourenço PharmD, BCPS , Ben Bow Chung MD , Teruhiko Imamura MD , Daniel Rodgers BA , Stephanie A. Besser MS , Catherine Murks PhD, APN , Tiana Riley MSN, RN, ACNP-BC , JoDel Powers MSN, FNPBC-BC , Jayant Raikhelkar MD , Sara Kalantari MD , Nitasha Sarswat MD , Valluvan Jeevanandam MD , Gene Kim MD , Gabriel Sayer MD , Nir Uriel MD, MSc , Increase in Short-term Risk of Rejection in Heart Transplant Patients Receiving Granulocyte Colony-Stimulating Factor, *Journal of Heart and Lung Transplantation* (2018), doi: 10.1016/j.healun.2018.06.009

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Total Pages: 15

Figures and Tables: 9

Word Count: 3,344

Type of Contribution: Original Research Communications

Key Words: neutropenia, G-CSF, acute rejection, heart transplantation

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ABSTRACT

Background: Neutropenia is a significant adverse event following heart transplantation (HT) and increases infection risk. Granulocyte colony-stimulating factor (G-CSF) is commonly used in patients with neutropenia.

Objectives: Assess the adverse effects of G-CSF treatment.

Setting: University Hospital

Methods: Data on HT patients from January 2008 – July 2016 were reviewed. Patients who received G-CSF were identified and compared to patients without history of therapy. Baseline characteristics, rejection episodes, and outcomes were collected. Data was analyzed by incidence rates, time to rejection and survival were analyzed using Kaplan-Meier curves, and odds ratios were generated using logistic regression analysis.

Results: 222 HT patients were studied and 40 (18%) received G-CSF for 85 total neutropenic events (0.79 events/patient year). There were no differences in baseline characteristics between the groups. In the 3 months after G-CSF, the incidence rate of rejection was 0.067 events/month. In all other time periods considered free of G-CSF effect, the incidence rate was 0.011 events/month. This rate was similar to the overall incidence rate in the non-GCSF group, which was 0.010 events/month. There was a significant difference between the incidence rates of the G-CSF group 0-3 months after G-CSF administration and the non-GCSF group ($p = 0.04$), but no difference between the incidence rates of the G-CSF group at all other time periods and the non-GCSF group ($p = 0.5$). Freedom from rejection in the 3 months after G-CSF administration was 87.5%, compared to 97.5% in the non-GCSF group ($p = 0.006$).

Conclusions: G-CSF administration was associated with significant short-term risk of rejection. This suggests the need for increased surveillance during this time period.

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INTRODUCTION

Neutropenia is known to frequently occur in heart transplantation (HT) patients, however, published data are scarce regarding the true incidence of neutropenia and consequences of its treatment following HT. The cause for neutropenia in this patient population is multifactorial, with contributory effects of bone marrow toxicity from immunosuppressive (anti-thymocyte globulin, mycophenolate mofetil) and anti-infective medications (ganciclovir, valganciclovir, sulfamethoxazole-trimethoprim), and systemic infections, particularly cytomegalovirus. The goal of treatment of neutropenia in these patients is to prevent infection, but this is countered by a concern for an increase in rejection risk. Additionally, in cases of severe infection or refractory neutropenia, there is sometimes a need to reduce immunosuppressive therapy, further increasing the risk of rejection (1). Therefore, a high threshold is usually maintained for treatment of neutropenia with granulocyte-colony stimulating factors (G-CSF).

G-CSF is a cytokine that is made by many different tissues (fibroblasts, endothelial cells, stromal cells, macrophages, and lymphocytes) and stimulates the bone marrow to produce granulocytes and release them into the bloodstream (2). The pharmaceutical analogs of these naturally occurring cytokines stimulate production of neutrophil colonies, and shorten the time required for neutrophil precursors to mature and appear in the circulation. G-CSF administration for neutropenia has been studied most widely in bone marrow transplantation and oncology, and has been shown to reduce duration of neutropenia and increase nadir level (3-6). However, G-CSF may also lead to slight increases in circulating monocytes (4). The important role monocytes play in transplant rejection raise the question as to whether G-CSF may be harmful to graft function.

Few reports on the use of G-CSF in the solid organ transplantation population have been published, and it is not clear what the efficacy and safety of G-CSF is in this group of patients (7-17). Most of these studies were done in kidney, liver, and pancreas transplant, and while the general consensus is that G-CSF is safe within this population, most do report some instances of acute rejection following G-CSF administration. Additionally, there is more ambiguity about the effect of G-CSF in the HT

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population as this is the least studied population. In this study, we aim to evaluate the safety of G-CSF administration in HT patients and to specifically evaluate its effect on the risk of rejection.

METHODS

Patient Population

Electronic medical records (EMR) of all adult HT recipients at the University of Chicago Medicine from January 2008 to July 2016 were reviewed. Patients who received G-CSF for treatment of neutropenia were included in the G-CSF group, and those who did not receive G-CSF therapy were included in the non-GCSF group. Decisions to treat or not treat neutropenia with G-CSF were made by clinicians caring for the patients at the point of care. All patients included in this study received Neupogen® (filgrastim). Other formulations of G-CSF were given for neutropenia, however, these patients were excluded to avoid incongruent data. All patients received immunosuppressive and anti-infective agents according to standard protocol at the University of Chicago Medicine with adjustments made based on the clinical status of the patient. Data collection was not started until approval was obtained from our Institutional Review Board.

Acute Rejection

Episodes of acute rejection in both groups were identified by EMR review. Acute rejection was defined as biopsy-proven cellular rejection that was International Society for Heart and Lung Transplantation (ISHLT) grade 1B or greater, or noncellular rejection. The rejection had to be treated with intravenous or oral immunosuppressive therapy in order to be considered a true episode of acute rejection.

Follow-Up and End-Points

Patients were followed from time of transplant to death or time of data analysis (October 10, 2016). In the G-CSF group, all episodes of treated neutropenia were identified. We defined the time

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effect of G-CSF to be 3 months after administration of the medication. All other time periods in which the patients were followed were considered to be free of G-CSF effects. This included time periods before G-CSF administration and the time periods after months 0-3 after G-CSF administration. We collected data on rejection episodes during these pre-specified time periods. The primary endpoint consisted of the incidence rate per month of acute rejection during months 0-3 after administration of G-CSF, and the incidence rate per month of acute rejection during all other times of the patient's follow-up. This was compared to the overall incidence rate of acute rejection in the non-GCSF group starting four months after transplantation. This time period was chosen because the median time to first neutropenic episode was 119.5 days in the G-CSF group, giving us the most matched comparator. An additional primary endpoint compared freedom from rejection in the G-CSF group during months 0-3 after administration of G-CSF to freedom from rejection in the non-GCSF group. Secondary endpoints consisted of left ventricular ejection fraction (LVEF) at end follow-up and cumulative survival at 3 years. Finally, univariate and multivariate analyses were performed to identify significant predictors of rejection.

Statistical Analysis

Continuous variables were expressed as median (interquartile range IQR), or mean $\pm$ standard deviation. Independent t-test and Mann-Whitney U Test were used to analyze differences in continuous variables. Categorical variables were expressed as n (%) and compared using either the chi-square test or Fisher's exact test as appropriate. Multivariate analysis was conducted to assess whether there were significant associations between risk factors and rejection. Logistic regression analysis was performed to generate odds ratios (OR) and 95% confidence intervals (95% CI). Kaplan-Meier curves were generated to describe time to rejection and survival, and tested using log rank tests. Incidence rates were calculated utilizing STATA MP Version 14 (College Station, TX) and its tables for epidemiologists, specifically incidence-rate ratios. All other statistical analyses were performed using IBM SPSS Statistics Version 24 (Armonk, NY: IBM Corp). A p-value of < 0.05 was considered significant.

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RESULTS

Patient Characteristics

Patient characteristics are summarized in Table 1. Of 222 HT patients studied, 40 (18.0%) received G-CSF for a total of 85 neutropenic events (0.79 events per patient year). There were no significant differences in baseline characteristics between the two groups (Table 1). There was, however, a trend towards significance of older patients (60.5 years vs. 56 years, p = 0.07) and high-risk CMV patients (35% vs. 20.3%, p = 0.08) in the G-CSF group. Of the 40 patients treated with G-CSF, 22 patients had one episode of treated neutropenia, and 18 patients had 2 or more episodes of treated neutropenia (Figure 1). G-CSF doses separated by 7 days or more were considered different courses of therapy. The presumed etiologies of neutropenia were medication-induced in 59 (71%) (Table 2). Median time from transplant to first neutropenic event was 119.5 days (57.3-236.5). Median duration of neutropenia was 3 days (1-7). Absolute neutrophil count (ANC) prior to initiation of G-CSF was 775.0 K/μL ± 288.3 K/μL and the average cumulative dose of G-CSF given per neutropenic episode was 1017.2μcg ± 823.3μcg. The average peak ANC was 4217.3 K/μL ± 4241.3 K/μL, the average nadir ANC was 703.3 ± 312.9 K/μL, and the average increase in ANC per neutropenic episode was 3521.3 ± 4371.6 K/μL (Table 3). Initial medication dose changes in response to neutropenia varied, however in 43.4% of neutropenic episodes, no changes were made (Table 4).

Incidence of Rejection and Freedom from Rejection

In the G-CSF group, the incidence rate per month of rejection was 0.067 during months 0 to 3 after administration of G-CSF for a total of 11 rejections out of 85 neutropenic episodes. During all other times considered free of G-CSF effect, the incidence rate per month was 0.011. In the non-GCSF group, the overall incidence rate of rejection was 0.010 events per month (Table 5). There was a significant difference between the incidence rates of the G-CSF group during months 0-3 after G-CSF administration compared to the non-GCSF group (p = 0.04), however there was no difference between the incidence rates of the G-CSF group during times considered free of G-CSF and the non-GCSF group (p = 0.5). The

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3-month freedom from rejection in the G-CSF group from the time of G-CSF administration was 87.5%. In the non-GCSF group, the 3-month freedom from rejection from 4 months post-transplant was 97.5%. There was a statistical significance between these groups with a p value of 0.006 (Figure 2).

Factors Associated with Rejection

On the basis of univariate analysis, female gender, African American race, ischemic etiology of heart failure, high-risk CMV status, use of both basiliximab and anti-thymocyte globulin for induction, diabetes mellitus, and G-CSF use were chosen based on near statistical significance for the multivariate risk analysis for development of rejection. In the multivariate analysis, African American race (OR 6.1; 95% CI, 2.1 – 18.3; p=0.001), use of both basiliximab and anti-thymocyte globulin for induction (OR 7.8; 95% CI, 1.2 – 49.6; p=0.03), diabetes mellitus (OR 3.48; 95% CI, 1.2 – 10.2; p=0.02), and G-CSF use (OR 33.9; 95% CI, 11.1 – 103.7; p<0.001) were independent risk factors for developing rejection (Table 6).

Ejection Fraction (EF) and Survival

At time of death or end of follow-up, there was no significant difference in LVEF between the non-GCSF and G-CSF groups (59.8% vs. 64.7%, p = 0.12). At 3 years, there was a trend toward reduced survival in patients who received G-CSF, however it did not reach statistical significance (83% vs. 72.5%, p = 0.17).

DISCUSSION

In this study, we examined the safety of G-CSF for the treatment of neutropenia in HT patients and its association with rejection and survival. First, 1 out of 5 HT patients experience neutropenia requiring G-CSF. Second, in the first 3 months following G-CSF therapy, there was a 6-fold increase in the rate of cellular rejection. Thirdly, G-CSF therapy was found to be a significant and independent predictor of rejection. And lastly, there was a trend toward increased mortality among patients with

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neutropenia treated with G-CSF. To our knowledge, this is the first clinical report to suggest an increased risk of rejection with the use of G-CSF for neutropenia in HT patients. A previous retrospective study suggested a decreased incidence of rejection in HT patients who received G-CSF for neutropenia (18). This study evaluated patients between 2000-2007, while our study evaluated patients between 2008-2016. Immunosuppressive regimens have changed over that time period, possibly explaining the different findings in our study. Additionally, the patient population studied by Vrotec et al differed greatly from ours, and could account for the differences in outcome. Our patient population had a greater percentage of African-Americans, non-ischemic etiology for heart failure, and more high-risk CMV patients. Other studies in solid organ transplantation have also suggested a decreased risk of rejection in patients treated with G-CSF. Proposed mechanisms to explain this include significant reductions in serum TNF levels, inhibition of T-cell proliferation, and mobilization of regulatory T-cells associated with G-CSF treatment (11,19). These conflicting results from our study require further understanding of the proposed mechanisms by which G-CSF affects the risk of rejection.

Neutropenia following HT is mostly due to immunosuppressive medications used to prevent rejection, prophylactic medications used to prevent opportunistic infection, and CMV disease. The induction agents used currently include anti-thymocyte immune globulin (Thymoglobulin) and basiliximab (Simulect). Anti-thymocyte immune globulin is a formulation of polyclonal anti-lymphocyte antibodies produced in rabbits that leads to substantial lymphocyte depletion with complement-dependent opsonization and cell lysis. Granulocytes and platelets are also bound by these antibodies, causing neutropenia and thrombocytopenia in the peripheral circulation (20). The antiproliferative agents azathioprine (AZA) and mycophenolate mofetil (MMF) are also important contributors to the development of neutropenia. Both prevent the synthesis of DNA and thus proliferation of both T and B lymphocytes. In general, the side effect of myelosuppression is dose-dependent for both medications (21). The calcineurin inhibitors cyclosporine (CSA) and tacrolimus work by inhibiting transcription of IL-2 and other cytokines to suppress the immune system. This class of immunosuppression mainly causes nephrotoxicity and in general, is not usually implicated in the development of myelosuppression

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after heart transplantation (21). Notably, case reports have been published that show neutropenia improves after discontinuation of tacrolimus and improvement with switching to cyclosporine (22,23). Furthermore, a retrospective study examining post-HT patients grouped according to postoperative peripheral cytopenias showed a significant correlation between tacrolimus serum concentration and risk for leukopenia (24). The mechanism by which tacrolimus is thought to cause neutropenia is unknown. Finally, medications used to prevent opportunistic infection are important etiologies of neutropenia following HT. Ganciclovir and valganciclovir are widely used in the prevention of CMV disease after solid organ transplantation, and commonly cause neutropenia. The mechanism of neutropenia appears to be dose-related inhibition of DNA polymerase in hematopoietic progenitor cells (25). Cessation of these medications can lead to CMV disease, which is also associated with neutropenia. A delicate balance therefore is necessary for the management of these patients. Finally, trimetoprim/sulfamethoxazole (Bactrim) is used in post-HT patients for prophylaxis against Toxoplasma gondii and Pneumocystis jiroveci. Though the effects of trimethoprim/sulfamethoxazole prophylaxis have not been described in post-HT patients, they have been described in the HIV population who also require infection prophylaxis. In such a study, leukopenia from trimethoprim/sulfamethoxazole was seen in up to 26% of patients (26).

The risk of neutropenia as an adverse event following HT has not been formally described in large registries. However, small studies have evaluated the risk of neutropenia following HT. The abovementioned study looked at 22 HT patients divided into 2 groups: cytopenic group and non-cytopenic group. In comparing the 2 groups, basiliximab induction, higher average tacrolimus concentration, and longer duration of care in the intensive care unit were found to be risk factors for leukopenia following transplantation (24). Another small study sought to understand the role of CMV disease in leukopenia following HT. HT patients with no CMV disease were compared to those with CMV disease. The major findings of this study were that leukopenia mostly developed prior to the diagnosis of CMV disease, neutrophils and monocytes are most affected by CMV disease, and lastly, the reduction of leukocytes during CMV disease was independent of immunosuppression (27). Finally, a recent study evaluated the risk for development of leukopenia in relation to immunosuppressive

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medications. In this study, 2 groups were identified. Group 1 consisted of patients who were on an immunosuppressive regimen of tacrolimus or cyclosporine, prednisone, MMF, with the addition of valganciclovir and trimethoprim/sulfamethoxazole for prophylaxis. Group 2 consisted of tacrolimus or cyclosporine, prednisone, and mycophenolate mofetil only (the older regimen). In comparing the two groups, there was a significant increase in leukopenia in group 1, implicating valganciclovir and trimethoprim/sulfamethoxazole as the key to development of leukopenia. In this study, decreasing the dose of MMF led to normalization of blood counts without an increase in risk for rejection (28). In our study, we identified a very high rate of neutropenia (18%) concurrent with the immunosuppressive regimen described above of tacrolimus or cyclosporine, prednisone, and MMF, with the addition of valganciclovir and trimethoprim/sulfamethoxazole for prophylaxis. However, we describe that those patients experience multiple episodes of rejection (0.79 events per patient year) requiring adjustment of both immunosuppressive regimen and prophylaxis therapy.

The use of G-CSF for treatment of neutropenia following solid organ transplant has been evaluated in several small reports, predominantly in kidney and liver transplantation (7-17). Most reports did not find an increased risk of rejection, although the data are mixed. In general, cardiac allografts are viewed as more immunogenic than kidney and liver allografts, which may explain why our findings differ from the previous literature in other organs. In fact, several investigators have implied a protective role of the donor liver in inducing a degree of protection toward other tissues (29-31). The mechanism of this protective effect of the liver on other tissues and on preformed antibody states is not clear, though the relative resistance of the liver allograft to antibody damage is well demonstrated.

Our main finding is that the risk of rejection is increased in the early period (first 3 months) after G-CSF administration. This timing is congruent with the above previous reports in kidney and liver transplantation (7-17). It is important to highlight that in our study, the risk of rejection in all other time periods was not significantly different from the baseline risk we identified in the rest of the patient population. Neutropenia may itself be protective against rejection; of 85 neutropenic episodes, there were 2 episodes of rejection in the month preceding G-CSF administration compared to 11 afterwards. We

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also found that G-CSF use was an independent and significant predictor of rejection in multivariate analysis. Our data thus suggest administration of G-CSF is associated with a significant short-term increase in rejection. The mechanism by which this occurs is possibly due to an increase in immune activity from mobilization of neutrophil precursors, though an increased risk of rejection due solely to an increased WBC has not been described in the literature. Our data does show that the average increase in neutrophil count was 3521.3 ± 4371.6 K/μL, which is a significant change and can explain the increased rejection rate in the G-CSF group.

In the first-year post-transplant, rejection is a major cause of morbidity and mortality. A review of the Cardiac Transplant Research Database (CTRD) from 1990 to 2008 showed post-transplant death occurred in 2 phases: an early phase <1 year after transplant, and a late phase of low, gradually increasing risk. In the first early phase of acute risk, acute rejection was a cause of death in 12.5% (32). Acute rejection is not a significant contributor to death beyond this acute phase. The implications of our results are thus important, and suggest that patients who receive G-CSF should undergo increased surveillance for rejection during the 3 months following administration of G-CSF, especially in the first-year post-transplantation.

Study Limitations

This is a retrospective analysis and we recognize the inherent limitations in such a review. Additionally, our results are not widely applicable as they are based on chart review at a single institution. Furthermore, the management of neutropenic patients after heart transplant is not standardized, and doses of G-CSF given can vary widely by institution. This study calls for a multicenter prospective registry to assess the safety and efficacy of G-CSF therapy in HT patients.

Conclusions

In conclusion, G-CSF therapy appears to be associated with an increased short-term risk of rejection. This risk of rejection returns to the baseline risk of patients without history of G-CSF therapy

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within 3 months after receiving G-CSF. G-CSF therapy has no long-term effects on survival or graft function. As such, enhanced surveillance of rejection in the 3 months following G-CSF use should be considered.

RELEVANT DISCLOSURES: All authors have nothing to disclose.

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Table 1. Baseline Patient Characteristics

	G-CSF Group (N=40)	Non-GCSF Group (N=182)	P value
Age at Transplant, years	60.50 (54.25 – 64.00)	56.00 (46.00 – 63.00)	P = 0.08
Sex			P = 0.41
Male	29 (72.5%)	143 (78.6%)	
Female	11 (27.5%)	39 (21.4%)	
Race			
Caucasian	21 (52.5%)	102 (56.4%)	P = 0.68
AA	16 (40.0%)	69 (37.9%)	P = 0.81
Other	3 (7.5%)	11 (6.1%)	P = 0.72
HF Etiology			P = 0.98
NICM	26 (65.0%)	118 (64.8%)	
ICM	14 (35.50%)	64 (35.2%)	
CMV Risk			
Low	3 (7.5%)	29 (15.9%)	P = 0.22
Intermediate	23 (57.5%)	116 (63.7%)	P = 0.46
High	14 (35%)	37 (20.3%)	P = 0.07
Induction Agent			
Basiliximab	21 (52.5%)	104 (57.1%)	P = 0.59
Anti-thymocyte Globulin	15 (37.5%)	69 (37.9%)	P = 0.96
Basiliximab & Anti-thymocyte Globulin	2 (5.0%)	4 (2.2%)	P = 0.30
None	2 (5.0%)	5 (2.7%)	P = 0.61
Diabetes	11 (27.5%)	39 (21.4%)	P = 0.41
Lab Values Prior to Transplant			
Creatinine (mg/dL)	1.20 (1.00 – 1.95)	1.4 (1.00 – 1.60)	P = 1.00
Total Bilirubin (mg/dL)	0.55 (0.40 – 1.00)	0.70 (0.40 – 1.08)	P = 0.21
White Blood Cells (10^3/μL)	7.35 (5.43 – 8.35)	7.20 (5.70 – 8.45)	P = 0.76
Hemoglobin (g/dL)	11.43 ± 2.32	11.40 ± 1.91	P = 0.94
Platelets (10^3/μL)	166 (145.00 – 229.75)	202 (157.00 – 249.00)	P = 0.17

Table 2. Etiology of Neutropenia

	Percentage (n)
Medication-Induced	71.1% (59)
Infection-Induced	12% (10)
CMV-Induced	16.9% (14)

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Table 3. Characteristics of Neutropenia

	Median or Mean
Time from Transplant to 1st Neutropenic Episode	119.5 days (57.3-236.5)
Duration of Neutropenia	3 days (1-7)
ANC prior to G-CSF	779.2 K/μL ± 288.3 K/μL
Average dose of G-CSF given per episode	1,017.2μcg ± 832.3μcg
Average Nadir of ANC per episode	703.3 ± 312.9 K/μL
Average Peak of ANC per episode	4217.3 ± 4241.3 K/μL
Average Increase in ANC per episode	3521.3 ± 4371.6 K/μL

ANC: absolute neutrophil count

Table 4. Medication Changes in Response to Neutropenia

	Percentage (n)
MMF only	20.5% (17)
TMP-SMX only	1.2% (1)
Valganciclovir only	7.2% (6)
Everolimus only	1.2% (1)
MMF & TMP-SMX	8.4% (7)
MMF & Valganciclovir	8.4% (7)
MMF , TMP-SMX, Valganciclovir	9.6% (8)
None	43.4% (36)

MMF: mycophenolate mofetil; TMP-SMX: trimethoprim sulfamethoxazole

Table 5. Incidence Rates of Rejection

	Incidence Rate (Events per month)	**P value**
Non-GCSF Group	0.010	
G-CSF Group		
Months 0-3 after G-CSF	0.067	0.04
All other time periods	0.011	0.50

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Table 6. Multivariable Risk Factor Analysis

	Univariate Analyses			Multivariate Analyses		
	OR	**95% CI**	**P value**	**OR**	**95% CI**	**P value**
Female Gender	0.65	0.24 – 1.81	0.41			
African American Race	3.30	1.48 – 7.34	0.03*	6.10	2.04 – 18.3	0.001*
Ischemic Etiology	0.42	0.16 – 1.07	0.068			
High-Risk CMV Status	1.26	0.52 – 3.03	0.61			
Basiliximab and Anti-thymocyte Globulin Induction	7.00	1.34 – 36.5	0.021*	7.80	1.22 – 49.6	0.030*
Diabetes Mellitus	2.70	1.20 – 6.09	0.016*	3.48	1.19 – 10.2	0.023*
G-CSF use	21.2	8.52 – 53.0	<0.01*	33.9	11.09 – 103.7	<0.001*

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REFERENCES

1. Rubin RH, Young LS. Clinical approach to infection in the compromised host. 3rd ed. New York: Plenum Medical Book Co., 1994.
2. Metcalf D. The molecular control of cell division, differentiation commitment and maturation in haemopoietic cells. Nature 1989;339:27-30.
3. Lieschke GJ, Burgess AW. Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor (2). N Engl J Med 1992;327:99-106.
4. Lieschke GJ, Burgess AW. Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor (1). N Engl J Med 1992;327:28-35.
5. Lindemann A, Herrmann F, Oster W et al. Hematologic effects of recombinant human granulocyte colony-stimulating factor in patients with malignancy. Blood 1989;74:2644-51.
6. Hartmann LC, Tschetter LK, Habermann TM et al. Granulocyte colony-stimulating factor in severe chemotherapy-induced afebrile neutropenia. N Engl J Med 1997;336:1776-80.
7. Tajima A, Aso Y, Kawabe K et al. Colony-stimulating factor for treatment of leukopenia after kidney allografting. Transplant Proc 1991;23:1369-70.
8. Colquhoun SD, Shaked A, Jurim O, Colonna JO, Rosove MH, Busuttil RW. Reversal of neutropenia with granulocyte colony-stimulating factor without precipitating liver allograft rejection. Transplantation 1993;56:1593-5.
9. Page B, Morin MP, Mamzer MF, Thervet E, Legendre C. Use of granulocyte-macrophage colony-stimulating factor in leukopenic renal transplant recipients. Transplant Proc 1994;26:283.
10. Squiers EC, Elkhammas EA, Henry ML. Use of granulocyte-macrophage colony-stimulating factor for reversal of neutropenia following combined kidney-pancreas transplantation. Transplant Proc 1995;27:3092-3.
11. Foster PF, Mital D, Sankary HN et al. The use of granulocyte colony-stimulating factor after liver transplantation. Transplantation 1995;59:1557-63.
12. Peddi VR, Hariharan S, Schroeder TJ, First MR. Role of granulocyte colony stimulating factor (G-CSF) in reversing neutropenia in renal allograft recipients. Clin Transplant 1996;10:20-3.
13. Minguez C, Mazuecos A, Ceballos M, Tejuca F, Rivero M. Worsening of renal function in a renal transplant patient treated with granulocyte colony-stimulating factor. Nephrol Dial Transplant 1995;10:2166-7.
14. Kutsogiannis DJ, Crowther MA, Lazarovits AI. Granulocyte macrophage colony-stimulating factor for the therapy of cytomegalovirus and ganciclovir-induced leukopenia in a renal transplant recipient. Transplantation 1992;53:930-2.
15. Patel HD, Anderson JR, Duncombe AS, Carrington D, Murday A. Granulocyte colony-stimulating factor. A new application for cytomegalovirus-induced neutropenia in cardiac allograft recipients. Transplantation 1994;58:863-7.
16. Gordon MS, O'Donnell JA, Mohler ER, Cooper MA. The use of granulocyte colony-stimulating factor in the treatment of fever and neutropenia in a heart transplant patient. J Heart Lung Transplant 1993;12:706-7.
17. Turgeon N, Hovingh GK, Fishman JA et al. Safety and efficacy of granulocyte colony-stimulating factor in kidney and liver transplant recipients. Transpl Infect Dis 2000;2:15-21.
18. Vrtovec B, Haddad F, Pham M et al. Granulocyte colony-stimulating factor therapy is associated with a reduced incidence of acute rejection episodes or allograft vasculopathy in heart transplant recipients. Transplant Proc 2013;45:2406-9.
19. Foster PF, Kociss K, Shen J et al. Granulocyte colony-stimulating factor immunomodulation in the rat cardiac transplantation model. Transplantation 1996;61:1122-5.
20. Lindenfeld J, Miller GG, Shakar SF et al. Drug therapy in the heart transplant recipient: part I: cardiac rejection and immunosuppressive drugs. Circulation 2004;110:3734-40.

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21. Lindenfeld J, Miller GG, Shakar SF et al. Drug therapy in the heart transplant recipient: part II: immunosuppressive drugs. Circulation 2004;110:3858-65.
22. Amorim J, Costa E, Teixeira A, Rebocho MJ, Loureiro M, Barbot J. Tacrolimus-induced neutropenia in a cardiac transplant patient. Pediatr Transplant 2014;18:120-1.
23. De Rycke A, Dierickx D, Kuypers DR. Tacrolimus-induced neutropenia in renal transplant recipients. Clin J Am Soc Nephrol 2011;6:690-4.
24. Urbanowicz T, Straburzyńska-Migaj E, Klotzka A et al. Induction therapy, tacrolimus plasma concentration, and duration if intensive care unit stay are risk factors for peripheral leucopenia following heart transplantation. Ann Transplant 2014;19:494-8.
25. Fishman JA, Emery V, Freeman R et al. Cytomegalovirus in transplantation - challenging the status quo. Clin Transplant 2007;21:149-58.
26. Bozzette SA, Finkelstein DM, Spector SA et al. A randomized trial of three antipneumocystis agents in patients with advanced human immunodeficiency virus infection. NIAID AIDS Clinical Trials Group. N Engl J Med 1995;332:693-9.
27. Cooke ME, Potena L, Luikart H, Valantine HA. Peripheral blood leukocyte counts in cytomegalovirus infected heart transplant patients: impact of acute disease versus subclinical infection. Transplantation 2006;82:1419-24.
28. Pazdernik M, Malek I, Koudelkova E, Sochman J, Kautzner J. Bone marrow suppression and associated consequences in patients after heart transplantation: A 6-year retrospective review. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2015;159:372-7.
29. Kamada N, Davies HS, Roser B. Reversal of transplantation immunity by liver grafting. Nature 1981;292:840-2.
30. Iwatsuki S, Iwaki Y, Kano T et al. Successful liver transplantation from crossmatch-positive donors. Transplant Proc 1981;13:286-8.
31. Houssin D, Gigou M, Franco D et al. Specific transplantation tolerance induced by spontaneously tolerated liver allograft in inbred strains of rats. Transplantation 1980;29:418-9.
32. Tallaj JA, Pamboukian SV, George JF et al. Have risk factors for mortality after heart transplantation changed over time? Insights from 19 years of Cardiac Transplant Research Database study. J Heart Lung Transplant 2014;33:1304-11.

Figure 1. Frequency of Neutropenia

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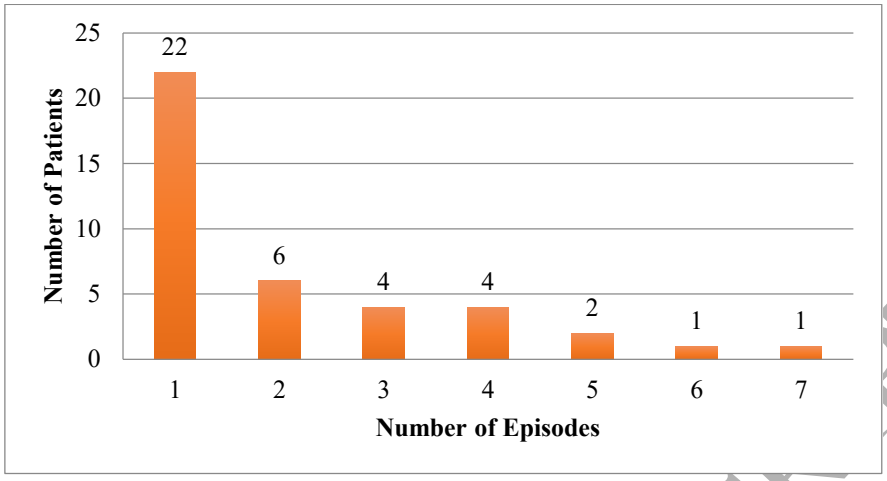

Figure 2. Freedom from Rejection

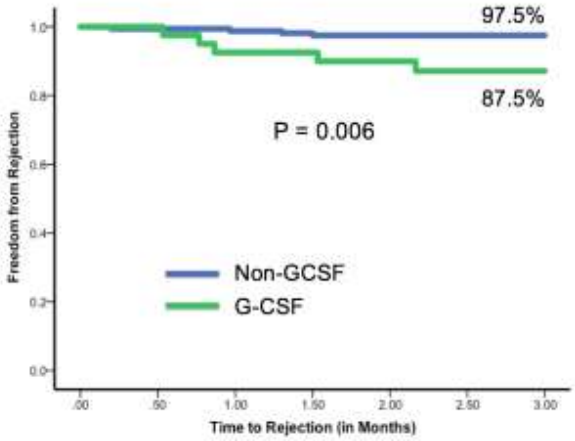

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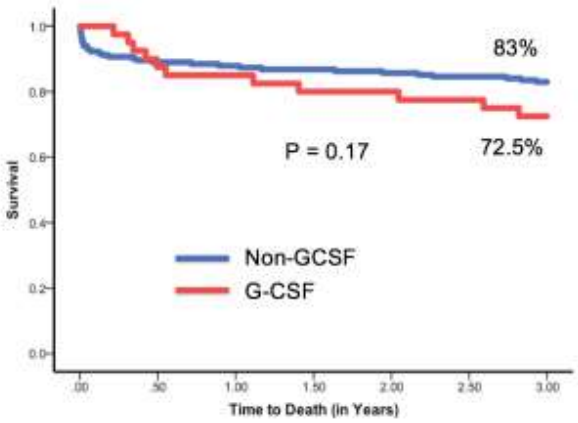
Figure 3. Survival at 3 years



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