Published in final edited form as:

Int J Cardiol. 2018 January 15; 251: 58–64. doi:10.1016/j.ijcard.2017.10.060.

Change in lymphocyte to neutrophil ratio predicts acute rejection after heart transplantation

Dong-Hyun Choi, M.D.^{1,2}, Yuhei Kobayashi, M.D.¹, Takeshi Nishi, M.D.¹, Helen Luikart, R.N.¹, Sadia Dimbil, B.S.³, Jon Kobashigawa, M.D.³, Kiran Khush, M.D.¹, and William F. Fearon, M.D.¹

¹Division of Cardiovascular Medicine, Stanford University Medical Center, Stanford, California, USA

²Department of Internal Medicine, Chosun University School of Medicine, Gwangju, Republic of Korea

³Heart Institute, Cedars-Sinai Medical Center, Los Angeles, California, USA

Abstract

Aims: Most immunosuppressive drugs provide targeted immunosuppression by selective inhibition of lymphocyte activation and proliferation. This study evaluated whether a change in the lymphocyte to neutrophil ratio (LNR) is related to acute rejection.

Methods: In 74 cardiac transplant recipients peripheral blood lymphocyte and neutrophil counts were measured soon after (baseline) and three, six, and 12 months after heart transplantation. The primary endpoint was the incidence of acute rejection.

Results: Significant acute rejection after heart transplantation occurred in 20 patients (27%) during a median follow-up of 49.4 [IQR 37.4–61.1] months. LNR significantly increased over time (0.1149 ± 0.1354 at baseline, 0.2330 ± 0.2266 at 3 months, 0.2961 ± 0.2849 at 6 months, and 0.3521 ± 0.2383 at 12 months; $P < 0.001$), especially during the first 3 months in the group without acute rejection. The area under the curve of the change in LNR during the first three months (LNR) for acute rejection was 0.565 (95% CI 0.420 to 0.710, $P = 0.380$) on ROC curve analysis. The best cutoff value of LNR to differentiate those with and without acute rejection was 0.046 by ROC curve analysis. Kaplan-Meier analysis revealed that the low LNR group (< 0.046) had a significantly higher rate of acute rejection than the high LNR group (> 0.046) (37.5% vs. 19.0%, log-rank: $P = 0.0358$). The low LNR for the first 3 months was an independent predictor of clinically significant acute rejection after adjusting for cytomegalovirus donor seropositive and recipient seronegative.

Address for correspondence: William F. Fearon, M.D.: Interventional Cardiology, H2103, 300 Pasteur Drive, Stanford University Medical Center, Stanford, CA 94305, United States. Tel.: +1 650 725 2621, Fax: +1 650 725 6766, wfearon@stanford.edu.

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Conflict of interest

There are no financial conflicts of interests of any of the authors.

Conclusions: The results of this study suggest that LNR over the first 3 months after heart transplantation is a strong and independent predictor of acute rejection after heart transplantation.

LNR can be used as an early biomarker for predicting of acute rejection after heart transplantation.

Keywords

lymphocyte neutrophil ratio; heart transplantation; acute rejection

1. Introduction

Heart transplantation is a life-saving therapy for patients with end stage heart failure.(1) In spite of extensive developments in immunosuppressive therapies, allograft rejection remains a major problem leading to allograft loss and death.(1) Most immunosuppressive drugs provide targeted immunosuppression by selective inhibition of lymphocyte activation and proliferation.(2–7) Recently, Lida et al. demonstrated that transient lymphopenia initiated rejection of stable cardiac allografts.(8) Thus, it is necessary to find an optimal balance of lymphocyte inhibition which avoids accumulation of pathogenic donor-reactive cell varieties that could cause graft rejection and which avoids lymphopenia and an increased chance for rejection. (8)

Several investigations have revealed the possible harmful role of neutrophils during myocardial reperfusion just after heart transplantation in mice, particularly through neutrophil activated β_2 -integrin such as $\alpha_M\beta_2$ -integrin complexes (CD11b/CD18).(9, 10) Neutrophils are an important subtype of immune cells that mediate the progression of acute rejection of cardiac allografts. Inhibition of neutrophil infiltration into the allograft results in reduced rejection and improved graft survival.(11–13)

So far, lymphocyte to neutrophil ratio (LNR) has not been interrogated in heart transplant patients. On the basis of the need to develop a biomarker to detect over immunosuppression and persistent neutrophilia, here, we addressed the association of LNR, especially early change in LNR, with the occurrence of acute rejection after heart transplantation.

2. Material and Methods

2.1. Study Design

This study is a retrospective analysis of prospectively collected data from a randomized, controlled, multicenter study exploring the role of the angiotensin converting enzyme (ACE) inhibitor, ramipril, in preventing the development of cardiac allograft vasculopathy early after heart transplantation conducted at Stanford University, the Palo Alto VA Health Care System, and the Cedars-Sinai Medical Center between 2009 and 2014 (NCT01078363). Patients underwent coronary angiography with measurement of fractional flow reserve (FFR), coronary flow reserve (CFR), and the index of microcirculatory resistance (IMR) and performance of intravascular ultrasound (IVUS) in the left anterior descending (LAD) coronary artery within 8 weeks of heart transplantation. Patients were then randomized in a double blind fashion to either ramipril or placebo. After 1 year, the aforementioned

examinations were repeated. The absolute lymphocyte count (LC) and neutrophil count (NC) were measured at baseline (soon after heart transplantation), and at 3, 6 and 12 months after heart transplantation. Subjects were enrolled if they were 18 years of age or older, had a serum creatinine less than 2.0 mg/dL, received their first heart transplant, and were willing and able to provide informed written consent. Subjects were excluded if they had more than one solid organ transplant at the time of heart transplant, had serum creatinine greater than 2.0 mg/dL, or were pregnant. Patients were further excluded from this substudy if the baseline and 3 month LNR data were not available, or event of acute rejection occurred within the first 3 months to evaluate the prognostic significance of early change of LNR during the first 3 months (LNR). The study was approved by an institutional review committee from each participating site, and informed consent was obtained from all patients.

2.2. Imaging and assays

Transthoracic echocardiography was executed by standard methods, and left ventricular ejection fraction (LVEF) was measured by means of the biplane method of discs formula.

White blood cell (WBC) count and its subtypes, including absolute LC and NC, were measured by an automated blood cell counter. LNR was calculated as the ratio of lymphocyte and neutrophil counts obtained at baseline (soon after heart transplantation), and at 3, 6 and 12 months post-transplant.

2.3. Clinical follow-up

The main outcome measure was the occurrence of clinically significant acute rejection. Clinically significant acute rejection was defined as an event that led to an acute augmentation of immunosuppression in conjunction with an International Society for Heart and Lung Transplantation grade 2R right ventricular endomyocardial biopsy result or non-cellular rejection (biopsy-negative rejection) with hemodynamic compromise (decrease in LVEF by >25%). The main outcome was prospectively recorded and obtained from retrospective review. When patients had multiple acute rejection events, the time to first event was counted as the censored outcome.

2.4. Statistical analysis

The values are expressed as mean $\pm$ SD or as numbers (percentages). Normality of the continuous variables was confirmed using the Shapiro–Wilk test. Continuous variables were compared using Student's t-test or the Mann–Whitney U-test, as appropriate. Categorical variables were summed up as percentages and compared using χ^2 statistics. Comparisons between the different time points (at baseline, 3, 6 and 12 months) were performed using one way analysis of variance (ANOVA) on ranks test. If the overall difference was significant, differences between the different time points were assessed by Games-Howell test. Receiver operating characteristic (ROC) analysis was carried out to establish the best cutoff value of LNR to differentiate those with and without acute rejection. The area under the curve (AUC) was calculated with 95% confidence intervals. The freedom from event of acute rejection according to the cut-off value of LNR was estimated using the Kaplan-Meier method, and results were compared using the log-rank test. Independent predictors of acute rejection were calculated by Cox proportional hazards regression. Baseline clinical

factors with a P value <0.15 were then entered into a forward stepwise multivariate Cox proportional hazards model. Multivariate logistic regression was performed with covariates included if P value <0.15 in univariate analysis to select variables associated with low LNR. In this model, mean glucose level and mean prednisone dosage during 3 months were computed by averaging values of baseline, 2 months, and 3 months. The correlation between

LNR and time to acute rejection were evaluated using Spearman's coefficient of rank correlation analysis. A P value of <0.05 was regarded as statistically significant. Statistical analyses were conducted using SPSS, version 24 for Windows (SPSS Inc., Chicago, Illinois, USA) and MedCalc Statistical Software version 16.8.4 (MedCalc Software bvba, Ostend, Belgium).

3. Results

3.1. Baseline Clinical Characteristics

Out of a total of 104 heart transplant recipients enrolled, we included 74 patients who were 18 years of age or older, without acute rejection within the first 3 months, and who had absolute lymphocyte and neutrophil counts measured soon after heart transplantation and at 3 months. Patient characteristics, including donor-recipient sex mismatch, comorbidities, cytomegalovirus status, donor ischemic time, and incidence of rejection, were similar between included and excluded patients.

The mean age was 56 ± 13 years, and 67.6% of recipients were men. Thirty four patients (45.9%) were transplanted due to dilated cardiomyopathy, and 14 patients (18.9%) were transplanted due to ischemic cardiomyopathy. Other etiologies of heart failure were amyloidosis in 12.2 and valvular heart disease in 1.4%. All patients received standard immunosuppressive therapy. Among the donors, the mean age was 33 ± 13 years, and 62.2% were men (Table 1).

3.2. LC, NC, and LNR level of all cohort

The mean LC and NC were 620 ± 480 , 750 ± 510 , 780 ± 410 , $900 \pm 410/\text{mm}^3$ and 6680 ± 2540 , 4590 ± 2690 , 3840 ± 2420 , $3220 \pm 1570/\text{mm}^3$ at baseline, three, six and twelve months, respectively. The mean LC significantly increased over time and the NC significantly decreased over time. The mean LC was in the lymphopenia range and the mean NC was in the normal range at all-time points. The mean LNR was 0.1149 ± 0.0780 , 0.2330 ± 0.2266 , 0.2961 ± 0.2849 , and 0.3521 ± 0.2383 at baseline, three, six and twelve months, respectively. The mean LNR significantly increased over time ($P < 0.001$), especially between baseline and 3 months ($P < 0.001$).

3.3. Acute rejection and LC, NC, and LNR level

Significant acute rejection after heart transplantation occurred in 20 patients (27.0 %) during a median follow-up of 49.4 [IQR 37.4–61.1] months. Most episodes occurred within 37 months (median 10.1 [IQR 4.2–26.5] months). Among them, 13 patients (65.0%) had International Society for Heart and Lung Transplantation grade 2R right ventricular endomyocardial biopsy result and 7 patients (35.0%) had noncellular rejection. The mean LC significantly increased in patients without acute rejection, but increased less in patients

with acute rejection (650 ± 520 , 780 ± 530 , 790 ± 430 , $930 \pm 420/\text{mm}^3$ vs. 540 ± 360 , 670 ± 470 , 750 ± 360 , $830 \pm 370/\text{mm}^3$; $P=0.001$ vs. $P=0.052$, respectively). The mean NC significantly decreased in both patients with and without acute rejection (6520 ± 2530 , 4450 ± 2700 , 3520 ± 2330 , $3080 \pm 1470/\text{mm}^3$ vs. 7120 ± 2570 , 4970 ± 2700 , 4710 ± 2510 , $3560 \pm 1800/\text{mm}^3$; $P<0.001$ vs. $P=0.001$, respectively). The mean LNR significantly increased in both patients with and without acute rejection (0.1249 ± 0.1533 , 0.2469 ± 0.2288 , 0.3217 ± 0.3127 , 0.3634 ± 0.2179 vs. 0.0879 ± 0.0622 , 0.1954 ± 0.2218 , 0.2287 ± 0.1839 , 0.3235 ± 0.2182 ; ANOVA $P<0.001$ vs. $P<0.001$, respectively, figure 1-A and B). However, the mean LNR significantly increased between baseline and 3 months only in patients without acute rejection events (0.1249 ± 0.1533 and 0.2469 ± 0.2288 ; $P=0.008$), but did not change significantly during the first 3 months in patients with acute rejection (0.0879 ± 0.0622 and 0.1954 ± 0.2218 ; $P=0.188$, figure 1-A and B).

3.4. Acute rejection and change in LC and NC for the first 3 months (LC and NC)

The mean change in LC and NC for the first 3 months (LC and NC) were $134 \pm 273/\text{mm}^3$ and $-2085 \pm 2725/\text{mm}^3$. In the ROC curve analysis of LC and NC to differentiate those with and without acute rejection, AUCs were 0.508 (95% CI: 0.365–0.651, $P=0.909$) and 0.504 (95% CI: 0.357–0.650, $P=0.961$), respectively.

3.5. Acute rejection and change in LNR for the first 3 months (LNR)

The mean LNR between baseline and 3 months was 0.1181 ± 0.2116 . In the ROC curve analysis of LNR to differentiate those with and without acute rejection, the AUC was 0.565 (95% CI: 0.420–0.710, $P=0.380$). Using ROC curve analysis to identify the best cutoff value of LNR to differentiate those with and without acute rejection, those patients with a LNR 0.046 had a higher rate of acute rejection compared to those patients with a LNR > 0.046 (37.5% vs. 19.0%, $P=0.077$). In Kaplan-Meier analysis, patients with the lower LNR (0.046) were characterized by an increased rate of acute rejection (Log-rank $P=0.0358$; figure 1-C).

3.6. Independent predictors of acute rejection

Univariate analysis indicated that LNR 0.04 and cytomegalovirus donor seropositive and recipient seronegative were associated with acute rejection (P value < 0.15). The Cox proportional hazards model included these univariate variables. From this model, the variables that were independent risk factors for acute rejection were LNR 0.046 and cytomegalovirus donor seropositive and recipient seronegative (Table 2).

3.7. Independent risk factors of LNR 0.046

Univariate analysis showed that the mean daily dosage of prednisone during 3 months after heart transplantation (16 mg/day), mean glucose level during 3 months (92 mg/dL) and diabetes mellitus before heart transplantation were associated with LNR 0.046 (P value < 0.15). The logistic regression model included these univariate variables. From this model, the mean prednisone dosage during 3 months and diabetes mellitus were independent risk factors for LNR 0.046 (Table 3).

3.8. Correlation between LNR and time to acute rejection

We performed Spearman's coefficient of rank correlation analysis to demonstrate the relationship between the degree of LNR and the time to acute rejection. There was a positive trend of correlation between LNR and time to acute rejection among patients with acute rejection (Spearman $\rho=0.436$, $P=0.055$; figure 1-D). The mean time to acute rejection was significantly shorter in those recipients with low LNR (233 ± 130 vs. 740 ± 313 days; $P=0.001$).

3.9. Etiology of acute rejection (cellular rejection vs. non-cellular rejection)

We separated acute rejection into cellular rejection and non-cellular rejection to demonstrate the impact of etiology of acute rejection on predictive value of LNR. Cellular rejection occurred more frequently in low LNR group (31.3% vs. 7.1%, $P=0.007$) but rate of non-ACR showed no significant difference between low LNR and high LNR group (6.3% vs. 11.9%, $P=0.410$). In Kaplan-Meier curves, LNR predict cellular rejection rather than non-cellular rejection (Figure 1-E).

3.10. Subgroup analysis

We performed subgroup analysis according to presence of de novo donor specific antibodies (DSA) or not and thymoglobulin induction therapy. In Kaplan-Meier curves, LNR predict acute rejection in group without de novo DSA rather than with de novo DSA (Figure 1-F). Moreover, LNR predict acute rejection in group with induction therapy with thymoglobulin rather than without induction therapy with thymoglobulin (Figure 1-G).

4. Discussion

This study investigated the relationship between LNR and clinically acute rejection after heart transplantation. The primary result is that low LNR early after the transplant is a significant predictor of acute rejection. Thus, LNR may help to predict patients with a high immunologic hazard of acute rejection events. In addition, the mean daily prednisone dosage during 3 months and diabetes mellitus before heart transplantation were risk factors for LNR 0.046. To the best of our knowledge, the current study is the first longitudinal clinical outcome study in heart transplantation patients demonstrating the predictive significance of LNR.

Most immunosuppressive medications offer selective inhibition of lymphocyte activation and proliferation.(2–7) Previous studies have predominantly focused on early T-cell lymphopenia, which has been broadly ascribed to lymphodepletive therapies.(14, 15) However, Iida et al. reported that transient lymphopenia was a problem that initiated rejection of stable cardiac allografts.(8) Therefore, the goal is to achieve a degree of lymphocyte depletion that avoids the subsequent buildup of pathogenic cell types that may cause graft rejection.(8) However, up to the present time, no biomarker has been recognized to identify over immunosuppression.(16) Accordingly, here, we propose a new biomarker, LNR, for acute rejection. On the basis of the requirement to develop a biomarker to identify over immunosuppression, we addressed the associations of LNR, especially early change in

LNR during the first 3 months (LNR), with the occurrence of acute rejection after heart transplantation.

In this study, LC and NC by themselves were not associated with acute rejection but LNR was associated with acute rejection. It may mean that the combination of lymphopenia and neutrophilia contribute to acute rejection. LNR reveals the balance between lymphocyte and neutrophil counts. Several investigations have revealed the possible harmful role of neutrophils during myocardial reperfusion just after heart transplantation in mice, particularly through neutrophil activated β_2 -integrin such as $\alpha_M\beta_2$ -integrin complexes (CD11b/CD18).(9, 10) Furthermore, β_2 -integrin-mediated neutrophil penetration and gathering in the cardiac allograft tissue was associated with acute rejection in rodents.(9) Neutrophils are one of the immune cells that aggravate the progression of acute rejection of cardiac allografts, while inhibition of neutrophil infiltration into the allograft results in reduced rejection and improved survival of the allograft.(11–13) This may explain why LNR was a strong and powerful independent predictor for acute rejection.

Even though novel immunosuppressive drugs have been shown to prevent acute rejection of allogeneic grafts, rejection remains a serious issue. Currently common maintenance immunosuppressive regimens include calcineurin inhibitor (cyclosporin or tacrolimus), antiproliferative agents (mycophenolate mofetil), and steroids (prednisone).(4) In terms of dosage of prednisone, mean prednisone dosage for the first 3 months was a significant risk factor for low LNR in this study. Thus, rapid tapering of prednisone during the first 3 months might improve outcomes. Lymphocyte depletion is the central antilymphocyte action of steroids. They reversibly block T cell and antigen-producing cell derived cytokine and cytokine-receptor expression that otherwise would happen as a consequence of T-cell activation.(17–19) On the other hand, one study reported that neutrophil counts were significantly higher in patients with rheumatoid arthritis receiving oral prednisone.(20) In case of cyclosporine, tacrolimus, and mycophenolate, these immunosuppressive drugs have narrow therapeutic windows and the balance between noxious adverse events and rejection remains challenging. However, it is unclear why serum concentrations and cumulative dosages of these immunosuppressive drugs were not associated with a low LNR in the current study. A possible explanation is that the mean values of the serum concentration during the first 3 months of these immunosuppressive drugs were all within recommended serum levels.(4, 5) Whether more rapid prednisone tapering changes the LNR or the incidence of acute rejection needs further study. Moreover, whether the correlation between

LNR and time to acute rejection allows one to predict the timing of subsequent episodes of rejection requires further testing (Figure 1-D).

We encompassed all of the cellular rejection and the non-cellular rejection as a primary outcome. However, in this study, LNR had a predictive value only for the cellular rejection but not for the non-cellular rejection. It may mean that the potential biological explanation of the role of LNR only supports the cellular rejection pathophysiology, but not the antibody mediated one. Furthermore, current study showed that LNR was predictive for acute rejection only in the subgroup without de novo DSA. DSA may be associated to the non-cellular rejection and it is unlikely that they may be associated with cellular rejection. This is also supportive finding that LNR is related with cellular rejection rather than non-

cellular rejection. In addition, we found that LNR was more predictive in the subgroup treated with thymoglobulin induction. Thymoglobulin do induce lymphopenia. Therefore, LNR is a more valid biomarker in patients receiving thymoglobulin.

Our findings must be understood in view of the following limitations. First, this study had a relatively small sample size although it was the first study exploring the prognostic value of LNR for subsequent rejection after heart transplantation. Second, because only patients in whom absolute lymphocyte and neutrophil count were measured soon after heart transplantation were included, there may be a selection bias. Third, the current study was not pre-specified, but is a post hoc analysis from a prospective, randomized controlled trial that was not intended to assess impact of LNR on acute rejection after heart transplantation. Thus, further prospective study is required.

In conclusion, low LNR after cardiac transplantation is a strong and independent predictor for acute rejection. Therefore, LNR may represent a novel biomarker after heart transplantation for earlier prediction of subsequent rejection especially for cellular rejection, in patients without de novo DSA, or in patients received thymoglobulin induction therapy. A larger, prospective study is warranted to confirm these initial exploratory results.

Acknowledgment

This study was supported by research fund from Chosun University, 2016 and by the NIH: 5 R01 HL093475-02 (WFF).

Abbreviations and Acronyms

ACE	angiotensin converting enzyme
ANOVA	analysis of variance
AUC	area under the curve
CFR	coronary flow reserve
LNR	change of LNR during the first 3 months
FFR	fractional flow reserve
IMR	the index of microcirculatory resistance
IVUS	intravascular ultrasound
LAD	left anterior descending
LC	lymphocyte count
LNR	lymphocyte to neutrophil ratio
LVEF	left ventricular ejection fraction
NC	neutrophil count

ROC	receiver operating characteristic
WBC	white blood cell

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Figure 1.

A. Non acute rejection group. LNRs significantly increased from baseline to 3 months.

Means and corresponding standard errors are given.

B. Acute rejection group. There was no significant difference between baseline and 3

months LNRs. Means and corresponding standard errors are given.

C. Kaplan-Meier Curves for acute rejection based on cut-off value of LNR.

D. Correlation between LNR and time to acute rejection among patients with acute rejection.

E. Kaplan-Meier Curves for acute cellular rejection or non-cellular rejection based on cut-off value of LNR.

F. Kaplan-Meier Curves for acute rejection according to presence of de novo donor specific antibodies (DSA) or not based on cut-off value of LNR.

G. Kaplan-Meier Curves for acute rejection according to thymoglobulin induction or not based on cut-off value of LNR.

Table 1.**Baseline Characteristics (n=74)**

Recipient age, y	56±13
Recipient sex, male/female (%)	50/24 (67.6/32.4)
History of diabetes mellitus, n (%)	24 (32.4)
History of hypertension, n (%)	47 (63.5)
History of hyperlipidemia, n (%)	34 (45.9)
CMV (donor+/recipient-), n (%)	13 (17.6)
Donor age, y	33±13
Donor sex, M/F (%)	56/28 (62.2/37.8)
Donor ischemic time, min	216±48
Presence of de novo donor specific antibodies, n (%)	20 (27.0)
Reason for transplant	
Dilated cardiomyopathy, n (%)	34 (45.9)
Ischemic cardiomyopathy, n (%)	14 (18.9)
Amyloidosis (wild-type transthyretin), n (%)	9 (12.2)
Restrictive cardiomyopathy, n (%)	7 (9.5)
Congenital heart disease, n (%)	6(8.1)
Hypertrophic cardiomyopathy	2 (2.7)
Valvular heart disease, n (%)	1 (1.4)
Arrhythmia, n (%)	1 (1.4)
Immunosuppressive regimen	
Induction with anti-thymocyte globulin, n (%)	48 (64.9)
Cumulative dosage of anti-thymocyte globulin (mg)	218±47
Prednisone, n (%)	74 (100)
Tacrolimus, n (%)	69 (93.2)
Cyclosporine, n (%)	5 (6.8)
Mycophenolate, n (%)	74 (100)
Cumulative dosage of prednisone during 3months (mg)	2082±306
Cumulative dosage of tacrolimus during 3months (mg)	176±85
Cumulative dosage of cyclosporine during 3months (mg)	9438±2563
Cumulative dosage of mycophenolate during 3months (mg)	169147±17951
Mean daily dosage of prednisone during 3months (mg)	18.3±6.9
Mean serum concentration of tacrolimus during 3months (ng/mL)	11.9±2.7
Mean serum concentration of cyclosporine during 3months (ng/mL)	272.5±27.7
Mean serum concentration of mycophenolate during 3months µg/mL)	2.71±1.91
Other medical therapy	
ACE inhibitor, n (%)	37 (50.0)
Calcium channel blocker, n (%)	44 (59.5)
Beta blocker, n (%)	2 (2.7)
Diuretics, n (%)	44 (59.5)
Statin therapy, n (%)	71 (95.9)

CMV prophylaxis with valgancyclovir, n (%)	61 (82.4)
Race/Ethnicity	
Whites, n (%)	59 (79.7)
Blacks, n (%)	6(8.1)
Asians, n (%)	9 (12.2)

Values are mean±SD when appropriate. CMV indicates cytomegalovirus; ACE, angiotensin converting enzyme.

Table 2.

Cox proportional hazard analyses for determining the significant and independent predictors of acute rejection

Factors	Univariate Hazard ratio (95% CI), P value	Multivariate Hazard ratio (95% CI), P value
LNR 0.046	2.53 (1.03–6.20), 0.043	2.77 (1.12–6.87), 0.028
CMV (donor+/recipient–)	2.31 (0.89–6.04), 0.086	2.65 (1.003–7.02), 0.049
Graft ischemic time	1.01 (0.998–1.02), 0.152	
Cumulative dosage of prednisone during 3months	1.001 (0.999–1.003), 0.178	
Cumulative dosage of tacrolimus during 3months	1.001 (0.996–1.01), 0.646	
Cumulative dosage of cyclosporine during 3months	1.001 (0.997–1.01), 0.610	
Cumulative dosage of mycophenolate during 3months	1.00 (1.00–1.00), 0.169	
Mean prednisone dosage during 3months	1.02 (0.97–1.07), 0.487	
Serum concentration of tacrolimus during 3months	1.03 (0.88–1.22), 0.699	
Serum concentration of cyclosporine during 3months	0.997 (0.94–1.05), 0.908	
Serum concentration of mycophenolate during 3months	0.70 (0.41–1.21), 0.201	
Induction with anti-thymocyte globulin	0.77 (0.31–1.88), 0.566	
Cumulative dosage of anti-thymocyte globulin	1.003 (0.99–1.02), 0.690	
CMV prophylaxis with valgancyclovir	0.87 (0.29–2.60), 0.799	
Presence of de novo donor specific antibodies	1.55 (0.62–3.90), 0.350	
African American	1.77 (0.52–6.05), 0.362	
Recipient Age	0.99 (0.96–1.02), 0.344	
LVEF	0.97 (0.90–1.04), 0.380	
Recipient Female Sex	1.13 (0.43–2.94), 0.803	
Donor-recipient sex mismatch	0.85 (0.29–2.56), 0.778	
Hypertension	0.73 (0.30–1.75), 0.476	
Hyperlipidemia	0.96 (0.40–2.32), 0.929	
Diabetes mellitus	0.94 (0.36–2.46), 0.904	
Mean glucose level for 3months	1.002 (0.99–1.01), 0.677	
Glucose level at 3months	0.99 (0.98–1.01), 0.309	

LNR, change in lymphocyte neutrophil ratio overtime; CMV, cytomegalovirus; LVEF, left ventricular ejection fraction

Table 3.

Logistic regression model for determining the significant and independent predictors of LNR 0.046

Factors	Univariate Odds ratio (95% CI), P value	Multivariate Odds ratio (95% CI), P value
Mean prednisone dosage during 3months 16 mg/day	4.46 (1.44–13.8), 0.010	5.14(1.23–21.4), 0.025
Diabetes mellitus	3.24 (1.18–8.91), 0.023	5.80 (1.65–20.3), 0.006
Mean glucose level during 3months 92 mg/dL	3.25 (1.16–9.13), 0.026	2.23 (0.63–7.85), 0.211
LVEF	0.96 (0.88–1.05), 0.355	
Recipient Age	1.002 (0.97–1.04), 0.903	
Recipient Female Sex	0.67 (0.25–1.78), 0.417	
Donor-recipient sex mismatch	0.74 (0.24–2.30), 0.601	
CMV (donor+/recipient–)	0.79 (0.23–2.68), 0.702	
Graft ischemic time	0.99 (0.98–1.01), 0.286	
Induction with anti-thymocyte globulin	1.35 (0.51–3.58), 0.542	
Cumulative dosage of anti-thymocyte globulin	0.998 (0.98–1.01), 0.734	
CMV prophylaxis with valgancyclovir	0.87 (0.26–2.89), 0.816	
Presence of de novo donor specific antibodies	1.92 (0.68–5.42), 0.217	
Cumulative dosage of prednisone during 3months	0.999 (0.997–1.001), 0.493	
Cumulative dosage of tacrolimus during 3months	0.997 (0.989–1.01), 0.412	
Cumulative dosage of mycophenolate during 3months	1.00 (1.00–1.00), 0.465	
Serum concentration of tacrolimus during 3months	1.05 (0.88–1.25), 0.587	
Serum concentration of mycophenolate during 3months	0.96 (0.73–1.26), 0.768	
Hypertension	0.73 (0.28–1.90), 0.519	
Hyperlipidemia	1.67 (0.66–4.22), 0.281	

LNR, change in lymphocyte neutrophil ratio over time; LVEF, left ventricular ejection fraction; CMV, cytomegalovirus