International Journal of Cardiology 251 (2018) 58–64

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

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

Dong-Hyun Choi [a,b], Yuhei Kobayashi [a], Takeshi Nishi [a], Helen Luikart [a], Sadia Dimbil [c], Jon Kobashigawa [c], Kiran Khush [a], William F. Fearon [a,*]

[a] Division of Cardiovascular Medicine, Stanford University, Stanford, CA, USA
[b] Department of Internal Medicine, Chosun University School of Medicine, Gwangju, Republic of Korea
[c] Heart Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA

ARTICLE INFO

Article history:
Received 12 May 2017
Received in revised form 7 September 2017
Accepted 17 October 2017
Available online 22 October 2017

Keywords:
Lymphocyte neutrophil ratio
Heart transplantation
Acute rejection

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 (ΔLNR) 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 Δ LNR to differentiate those with and without acute rejection was ≤0.046 by ROC curve analysis. Kaplan-Meier analysis revealed that the low ΔLNR group (≤0.046) had a significantly higher rate of acute rejection than the high ΔLNR group (>0.046) (37.5% vs. 19.0%, log-rank: $P = 0.0358$). The low ΔLNR for the first 3 months was an independent predictor of clinically significant acute rejection after adjusting for cytomegalovirus donor seropositive and recipient seronegative.

Conclusions: The results of this study suggest that ΔLNR over the first 3 months after heart transplantation is a strong and independent predictor of acute rejection after heart transplantation. ΔLNR can be used as an early biomarker for predicting of acute rejection after heart transplantation.

Published by Elsevier Ireland Ltd.

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, Iida 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 $\alpha_{M\beta2}$-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.

Abbreviations: ACE, angiotensin converting enzyme; ANOVA, analysis of variance; AUC, area under the curve; CFR, coronary flow reserve; Δ 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.

* Corresponding author at: Interventional Cardiology, H2103, 300 Pasteur Drive, Stanford University Medical Center, Stanford, CA 94305, USA.
E-mail address: wfearon@stanford.edu (W.F. Fearon).

https://doi.org/10.1016/j.ijcard.2017.10.060
0167-5273/Published by Elsevier Ireland Ltd.

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 <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 >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 (Δ 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 ± 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 ΔLNR 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 ΔLNR 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 Δ 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 Δ 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 ± 410/mm^3 and 6680 ± 2540, 4590 ± 2690, 3840 ± 2420, 3220 ± 1570/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 ± 420/mm^3 vs. 540 ± 360, 670 ±