Elevated Levels of Plasma C-Reactive Protein Are Associated With Decreased Graft Survival in Cardiac Transplant Recipients

Marc S. Eisenberg, MD; Hong Jun Chen, MD; Mark K. Warshofsky, MD; Robert R. Sciacca, EngScD; Hal S. Wasserman, MD; Allan Schwartz, MD; LeRoy E. Rabbani, MD

Background—Inflammation may be involved in the origin of transplant coronary artery disease. We hypothesized that plasma levels of C-reactive protein (CRP) and interleukin-6 (IL-6), markers for systemic inflammation, would correlate with cardiac transplant graft survival.

Methods and Results—We studied 99 consecutive cardiac transplant recipients who were referred for routine endomyocardial biopsy and/or surveillance coronary angiography. Plasma levels of CRP and IL-6 were measured by their respective ELISAs. Patients were divided into 2 groups: those who died or required retransplantation and those who survived without the need for retransplantation. During the follow-up period of 5.0 ± 2.7 years (range, 0.2 to 15.1 years) after transplant, 20 patients died and 9 required retransplantation. There was no significant difference in age, race, sex, cause of native myopathy, presence of diabetes, or use of aspirin, statins, or calcium channel blockers between the 2 groups. Although IL-6 did not relate to graft failure, CRP level was predictive of allograft failure (P = 0.003). The risk of allograft failure increased 36% for every 2-fold increase in CRP level. Moreover, CRP levels also correlated significantly with the frequency of grade 3 rejection (P = 0.02). In multivariate analysis, when combined with other significant predictors such as donor age and sex mismatching of the graft, CRP still significantly predicted graft failure (P = 0.025) with a 32% increase in the risk of graft failure for every 2-fold increase in CRP level.

Conclusions—These findings suggest that elevated plasma levels of CRP are associated with subsequent allograft failure in cardiac transplant recipients. (Circulation. 2000;102:2100-2104.)

Key Words: transplantation n inflammation n proteins n survival

Recent prospective studies suggest that chronic low-grade inflammation plays an important role in the pathogenesis of cardiovascular disease.1–4 C-reactive protein (CRP), an established marker for underlying systemic inflammation, is a pentameric protein produced by hepatocytes under the influence of inflammatory cytokines, primarily interleukin-6 (IL-6).5 Elevated plasma levels of CRP have been shown to be predictive of subsequent cardiovascular events among apparently healthy men6–9 and women,10 as well as among patients with stable and unstable angina11–13 and those with a prior history of myocardial infarction.16 Moreover, the efficacy of established therapeutic interventions, such as the use of aspirin6 and HMG-CoA reductase inhibitors,16 relates directly to baseline levels of CRP. Thus, plasma levels of CRP appear to be useful not only in stratifying the risk of future cardiovascular events but also in selecting which patients may benefit from established forms of therapy.

Transplant coronary artery disease (TCAD) remains the leading cause of death or retransplantation in cardiac transplant recipients surviving >6 months.17 It is characterized by a diffuse, proliferative vasculopathy limited to the allograft coronary arteries and is associated with the development of myocardial infarction, ventricular failure, malignant arrhythmias, and sudden death.18,19 The pathogenesis of graft atherosclerosis is not fully elucidated and is likely multifactorial. However, one hypothesis is that TCAD arises from chronic immune stimulation involving the donor-derived endothelium with vascular and inflammatory cell activation.20–23 Because of the high incidence of graft failure, cardiac transplant recipients are subject to routine endomyocardial biopsy and surveillance coronary angiography that are invasive, time consuming, and expensive and are associated with risk. An inflammatory marker that is sensitive for assessing the risk of graft failure in a stable cardiac transplant population may allow the elimination of a large number of invasive procedures and might target a group of patients who are at increased risk for subsequent cardiovascular events.

We hypothesized that plasma levels of circulating inflammatory markers relate to the risk of allograft failure among stable cardiac transplant recipients. In particular, we sought to...
demonstrate that elevated levels of CRP and IL-6 would be associated with decreased allograft survival.

Methods

Study Population
Cardiac transplant patients who presented to the cardiac catheterization laboratory for either annual surveillance coronary angiography and endomyocardial biopsy or only endomyocardial biopsy were eligible for enrollment. Patients were recruited between December 1994 and September 1995. Ninety-nine consecutive patients were enrolled. The follow-up period ended on December 1, 1997. The median interval between transplantation and study enrollment was 2 years (range, 5 days to 14 years). Immunosuppressive regimens included cyclosporine, prednisone, and azathioprine, adjusted at the physician’s discretion. Cellular-rejection episodes of biopsy grade 3A or greater were treated with either steroid pulses (oral or intravenous) or cytolytic therapy (OKT3 or antithymocyte globulin). Treatment of cellular-rejection episodes of biopsy grades below 3A depended on individual physicians, patient symptoms, and abnormal hemodynamic values.26 The study was approved by the Columbia-Presbyterian Medical Center Institutional Review Board. All patients gave informed consent before entry into the study.

Plasma Samples and Laboratory Analysis
Blood samples for plasma were collected from a central venous line on the day of recruitment before the patient’s annual surveillance coronary angiography and/or endomyocardial biopsy. Blood was stored on ice in a tube containing sodium citrate until plasma was separated by centrifugation at 2000g for 15 minutes. Plasma samples were frozen at −80°C until their use for inflammatory factor level determination and lipid analysis. For each patient, plasma was thawed and assayed for CRP by an ELISA based on purified protein and polyclonal anti-CRP antibodies (Calbiochem).27 Levels of IL-6 were measured by an ELISA according to the commercial IL-6 ELISA kit from Immunotech. Lipid analysis was performed by conventional enzymatic methods.

Statistical Analysis
Patients were divided into 2 groups for comparison. One group consisted of patients who survived without the need for retransplantation, and the other group consisted of patients who died or required retransplantation by the end of the follow-up period. Data are presented as mean±SD for continuous variables and as frequency for categorical variables. Continuous variables were compared between groups with the use of Student’s t test, except for CRP and IL-6 levels, which were not normally distributed. For these variables, the nonparametric Mann-Whitney test was used to test for the significance of differences between the groups. Categorical variables were compared through χ² analyses. Kaplan-Meier curves were constructed for the frequency of graft failure as a function of time after transplantation. The significance of potential predictors of graft failure was assessed with the log-rank test. Hazard ratios were calculated, and a multivariate proportional-hazards model was used to determine which set of variables best predicted graft survival.

Results

Baseline Clinical Characteristics
Ninety-nine patients were enrolled in the study. Of the 99 patients, 29 died or required retransplantation (20 patients died, and 9 underwent retransplantation). Table 1 reveals the characteristics of the patients in both the graft failure and graft survival groups. There was no significant difference in mean age, sex, or use of diabetic medication between the 2 groups. No patient admitted to smoking at the time of the study. The white blood cell count, hematocrit, platelet count, blood urea nitrogen, creatinine, lipid levels, and cytomegalovirus (CMV) status were similar in both groups. The origin of the cardiomyopathy leading to transplantation was similar in each group. There was no difference in the use of aspirin, calcium channel blockers, or statins at the time of enrollment between the 2 groups, as well as no difference in immunosuppressive regimens used. There was no significant difference in the time from transplant to the 2 groups. The mean time to follow-up in the graft survival group (n=70) was 5.2±2.4 years, whereas in the graft failure group (n=29), it was 4.5±3.4 years. Of the baseline clinical variables, only donor age, female donor, and sex mismatch were significantly different between the 2 groups.

CRP levels were not normally distributed; therefore, the log transform was performed. As demonstrated in Figure 1, the levels were significantly higher in the group with graft failure. There was no significant difference in plasma IL-6 levels between the 2 groups.

Predictors of Allograft Failure
Table 2 shows the results of univariate analysis to identify potential predictors of graft failure. The presence of female donor, sex mismatch, increased donor age, and elevated levels of CRP were all univariate predictors of allograft failure. IL-6 levels did not relate to graft failure. Table 3 shows the results of the multivariate analysis. In this analysis,

| Table 1. Patient Characteristics |
|-------------------------------|-----------------|-----------------|
| Variable                      | Graft Failure   | Graft Survival  |
|                               | (n=29)          | (n=70)          |
| Age, y                        | 48±13           | 52±13           |
| Male sex, n (%)               | 18/29 (62)      | 55/70 (79)      |
| Ischemic origin, n (%)        | 11/29 (38)      | 30/70 (43)      |
| Diabetes, n (%)               | 2/26 (7)        | 10/70 (14)      |
| ASA use, n (%)                | 17/27 (63)      | 49/65 (75)      |
| Calcium channel blocker use, n (%) | 10/27 (37)   | 35/60 (58)      |
| Hematocrit, %                 | 35±6            | 34±5            |
| Platelets, 10⁹/L              | 238±74          | 231±81          |
| WBC, 10⁹/L                    | 7.1±2.5         | 7.5±4.0         |
| BUN, mg/dL                    | 33±16           | 38±15           |
| Creatinine, mg/dL             | 1.7±1.8         | 1.9±1.2         |
| Cholesterol, mg/dL            | 205±51          | 216±46          |
| LDL, mg/dL                    | 124±39          | 129±38          |
| HDL, mg/dL                    | 46±16           | 51±20           |
| TG, mg/dL                     | 217±256         | 200±207         |
| Statin use, n (%)             | 1/27 (4)        | 12/67 (18)      |
| CMV risk,* n (%)              | 3/28 (12)       | 7/53 (13)       |
| Donor age, y                  | 33±16†          | 28±10           |
| Male donor, n (%)             | 15/28 (54)†     | 51/65 (78)      |
| Sex mismatch, n (%)           | 12/28 (43)‡     | 10/65 (15)      |
| IL-6, pg/mL                   | 5.7±0.3         | 14.0±45.3       |
| CRP, mg/L                     | 3.23±4.20†      | 1.98±2.66       |

ASA indicates acetylsalicylic acid; WBC, white blood cell count; BUN, blood urea nitrogen; and TG, triglycerides. Values are mean±SD for continuous variables and as frequency for categorical variables.

*CMV-positive donor/CMV-negative recipient. †p<0.05, ‡p<0.01.
donor age (OR, 1.61 per 10-year increase; 95% CI, 1.16 to 2.28; P = 0.005), sex mismatch (OR, 3.06; 95% CI, 1.39 to 6.76; P = 0.006), and CRP level (OR, 1.32 for every 2-fold increase; 95% CI, 1.04 to 1.69; P = 0.025) were independent predictors of allograft failure. Thus, independent of the other univariate predictors of graft failure, CRP level significantly predicted graft failure with a 32% increase in the risk of graft failure for every 2-fold increase in CRP level. Figure 2 shows the Kaplan-Meier curve depicting the effect of enrollment CRP levels on graft survival with patients dichotomized into those with levels >1.06 mg/L and those with levels ≤1.06 mg/L. This cutoff corresponded to the median level of CRP in the study population. Patients with CRP levels >1.06 mg/L had significantly worse graft survival.

Although CRP levels did not relate to the frequency of either grade 1 or 2 rejections per year, CRP levels did correlate significantly with the frequency of grade 3 rejections per year (correlation coefficient, 0.25; P = 0.02). In addition, CRP levels correlated significantly with the donor age (correlation coefficient, 0.30; P = 0.004) and recipient age (correlation coefficient, 0.20; P = 0.05); however, CRP levels did not correlate with either the donor sex or the cause of the native myopathy.

**Figure 1.** Semilog graph of distribution of CRP levels in patients with and without graft failure. Levels were significantly higher in group with graft failure (P<0.05).

**TABLE 2. Univariate Predictors of Graft Survival**

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor age</td>
<td>1.76*</td>
<td>1.28–2.39</td>
<td>0.0004</td>
</tr>
<tr>
<td>Female donor</td>
<td>2.33</td>
<td>1.10–5.00</td>
<td>0.027</td>
</tr>
<tr>
<td>Sex mismatch</td>
<td>3.13</td>
<td>1.46–6.71</td>
<td>0.003</td>
</tr>
<tr>
<td>CRP</td>
<td>1.36†</td>
<td>1.11–1.68</td>
<td>0.003</td>
</tr>
</tbody>
</table>

*Per 10-year increase in age. †Per doubling of value.

correlated with allograft failure among stable cardiac transplant patients.

Inflammation may play an important role in the initiation and progression of native atherosclerosis and likely plays an important role in the pathogenesis of graft atherosclerosis, which remains the leading cause of death or retransplantation in cardiac transplant recipients surviving >6 months. Our finding that elevated plasma levels of CRP are associated with subsequent cardiovascular events among stable cardiac transplant patients extends previous observations about inflammation and cardiovascular risk. It is unclear whether CRP is just a marker for underlying systemic inflammation; thus, elevated levels may reflect enhanced immune or inflammatory activity in these patients. Several prior studies have reported the association between CMV infection and cardiac allograft vasculopathy. In one study, CMV infection was associated with an overall decreased survival, with only 32% of CMV-positive patients surviving 5 years after transplantation compared with 68% of CMV-negative patients. However, our study failed to show a significant relationship between CMV status and cardiac graft failure. Other immunological and nonimmunological factors that have been associated with increased risk for TCAD include human leukocyte antigen mismatch, humoral rejection, donor and recipient age, obesity, and hyperlipidemia. In this study, CRP levels did correlate significantly with the donor age and recipient age. Thus, CRP may in fact reflect other immunological or nonimmunological factors correlated with allograft failure among stable cardiac transplant patients.

**Discussion**

In this study, we demonstrate that elevated plasma levels of CRP are associated with subsequent allograft failure in stable cardiac transplant recipients. Moreover, we found that CRP levels correlated significantly with the frequency of grade 3 rejections per year. This is the first report to demonstrate that plasma levels of a circulating inflammatory marker are correlated with allograft failure among stable cardiac transplant patients.

**Figure 2.** Kaplan-Meier curve depicting effect of enrollment CRP levels on graft survival with patients dichotomized into those with levels >1.06 mg/L and those with levels ≤1.06 mg/L. This cutoff corresponded to median level of CRP in study population. Patients with CRP levels >1.06 mg/L had significantly worse graft survival (P<0.005).

**TABLE 3. Multivariate Predictors of Graft Survival**

<table>
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<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor age</td>
<td>1.61*</td>
<td>1.16–2.28</td>
<td>0.005</td>
</tr>
<tr>
<td>Sex mismatch</td>
<td>3.06</td>
<td>1.39–6.76</td>
<td>0.006</td>
</tr>
<tr>
<td>CRP</td>
<td>1.32†</td>
<td>1.04–1.69</td>
<td>0.025</td>
</tr>
</tbody>
</table>

*Per 10-year increase in age. †Per doubling of value.

<table>
<thead>
<tr>
<th>Effect of CRP Levels on Graft Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Graft Survival</td>
</tr>
<tr>
<td>Years Following Transplant</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>8</td>
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<tr>
<td>10</td>
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<td>12</td>
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</tbody>
</table>

CRP ≤ 1.06 CRP > 1.06

P < .005
inflammatory mechanisms that predispose to increased risk of allograft failure.

Instead of being just a marker for underlying immune or inflammatory activity, CRP may have a pathogenic role. CRP has been shown to activate complement and to stimulate the production of tissue factor by mononuclear cells. Impaired hemostatic function has been shown to be predictive of future cardiovascular events in healthy men and patients with known CAD. Moreover, impaired fibrinolysis has been demonstrated in heart transplant recipients and has been correlated with both the presence and severity of TCAD in these patients. In fact, the distribution of certain hemostatic factors in endomyocardial biopsy specimens has been shown to be an important predictor of the clinical outcome of cardiac allografts. Patients whose transplanted hearts developed depletion of tissue plasminogen activator in arteriolar smooth muscle had a significantly higher rate of subsequent graft failure.

Whether CRP serves as a marker for underlying systemic inflammation or in fact has a prothrombotic role, it appears to be useful in assessing the risk of graft failure in a stable cardiac transplant population and thus may be effective in targeting which patients might benefit from therapeutic interventions. Moreover, Kobashigawa et al found that after cardiac transplantation, the early use of pravastatin decreases the incidence of cardiac rejection accompanied by hemodynamic compromise, improves 1-year survival, and reduces the development of coronary vasculopathy. This may be due in part to a cholesterol-independent effect of pravastatin on immune or inflammatory function. It has been shown that the efficacy of pravastatin in survivors of myocardial infarction is greater among those with elevated levels of both CRP and serum amyloid A. Furthermore, the use of pravastatin among survivors of myocardial infarction resulted in significant reductions in CRP levels over 5 years of follow-up that were not related to the magnitude of lipid alterations. In view of the latter studies, our study suggests not only that plasma levels of CRP may be useful in selecting which cardiac transplant patients may benefit from established forms of therapy but that CRP may actually be a modifiable marker of risk.

In this study, there was no significant difference in the use of aspirin, statins, or calcium channel blockers at the time of enrollment between the graft failure and graft survival groups. Only 14% of our study population were being treated with statins at the time of study enrollment. The reason is that our recruitment period ended before the publication of the study by Kobashigawa et al. The beneficial effects of pravastatin on outcomes after cardiac transplantation. Also, we studied design only located at the use of these therapies at the time of recruitment and did not take into account the duration of treatment or dosage. Thus, we are unable to address whether graft failure is influenced by these therapies or whether these therapies influence plasma CRP levels. Moreover, the analyses are based on 1 determination of CRP level, and the effect of changes in CRP levels could not be assessed.

Plasma levels of IL-6 did not relate to graft failure in our stable cardiac transplant patients. IL-6 is known to induce the production of CRP by hepatocytes, and levels of IL-6 have been shown to be elevated in and predictive of outcome in acute coronary syndromes. Reports of an association between episodes of cardiac transplant rejection and elevated plasma levels of IL-6 have been variable. This variability may be accounted for in part by differences in assay sensitivity and detection limits among the various studies and may explain why our study did not show an association between graft failure and plasma IL-6 levels.

One limitation of our study was that the median interval between transplantation and study enrollment was 2 years (range, 5 days to 14 years). However, there was no significant correlation between the time after surgery and CRP level in patients without graft failure. In addition, 3 of the 99 subjects—2 from the graft failure group and 1 from the graft survival group—had grade 3A rejection at the time of blood sampling. Reanalyzing the data without these 3 subjects made no significant difference in the results.

In summary, we have demonstrated that elevated plasma levels of CRP are associated with subsequent allograft failure in stable cardiac transplant recipients. In addition, we found that CRP levels correlated significantly with the frequency of grade 3 rejections per year. These findings confirm previous observations about inflammation and cardiovascular risk and extend them to the cardiac transplant population.

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References


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