Procalcitonin, A Donor-Specific Predictor of Early Graft Failure–Related Mortality After Heart Transplantation

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Background—To date, donor-specific markers to predict outcome after heart transplantation (HTx) are unknown. Increased procalcitonin (PCT) levels have been found in infectious inflammation with systemic reactions and/or poor organ perfusion but have not been studied in heart donors. We evaluated PCT as a predictor of early graft failure–related mortality after HTx.

Methods and Results—PCT and C-reactive protein (CRP) serum concentrations were measured in samples collected immediately before pericardium opening from 81 consecutive brain-dead multiple-organ donors. Donors for high-urgency-status recipients (n=2) were excluded from analysis. The remaining donors were retrospectively divided into 2 groups: donors for recipients who died within 30 days after HTx, after an early graft failure (group II, n=8), and all other donors (group I, n=73). No differences in donor and recipient demographic characteristics were found between groups. Areas under the receiver operating characteristic curves for graft failure–related mortality were 0.71 for PCT and 0.64 for CRP. A PCT value >2 ng/mL as a predictor of graft failure–related mortality had a specificity of 95.8% and sensitivity of 50.0%. The odds ratio for graft failure–related mortality for recipients of hearts from donors with PCT levels >2 ng/mL was 22.7 (unadjusted, 95% CI 3.7 to 137.8, P=0.0007) and 43.8 (after adjustment for prespecified potential confounders, 95% CI 1.4 to 1361.0, P=0.031).

Conclusions—A PCT level >2 ng/mL in a cardiac donor at the time of explantation appears to predict early graft failure–related mortality. (Circulation. 2001;104[suppl I]:I-192-I-196.)

Key Words: transplantation ▪ mortality ▪ heart failure ▪ infection ▪ procalcitonin

The donor pool for heart transplantation (HTx) worldwide has plateaued in recent years. Expansion of the donor pool by inclusion of donors who previously would not have been accepted for HTx is the only way to increase the number of heart transplantsations.1 However, data on donor-specific parameters to predict success or failure of HTx and thus prevent a higher risk to the recipient are limited.2 Moreover, donor-specific biochemical markers to predict outcome after HTx are currently unknown, with the exception of a recent report on troponins as indicators of myocardial damage.3 Myocardial dysfunction occurs to various degrees in all brain-dead donors, and in up to 20% of cases, it precludes the use of these hearts for HTx.4 Experimental and clinical studies have suggested that brain death may induce irreversible myocardial damage.5 In clinical practice, the potential donors are mechanically ventilated and may develop systemic infections before brain death and subsequent donation. However, brain death–associated physiological changes and antibiotic therapy may conceal the symptoms. After the first reports that calcitonin precursor molecules were found to be elevated in patients with bacterial infection and sepsis,6 procalcitonin (PCT) was proposed as a marker of severe infection and systemic inflammatory response.7 Recent studies indicated that an acute infection or inflammation may impair myocardial function,8 which affects outcome after HTx. Therefore, knowledge of a specific and sensitive marker of systemic inflammation in heart donors would help to avoid early graft failure, which causes up to one third of early postoperative mortality.9 Moreover, such knowledge could prevent transmission of an unrecognized infection to the recipient.10

The objective of the present study was to evaluate PCT for selection of heart donors in relation to clinical outcome and as a predictor of early graft failure–related mortality after HTx. C-reactive protein (CRP) was used as a reference parameter because it is the most widely used marker for assessing the severity of the inflammatory response to infection or tissue injury.11

Methods

From November 1998 to May 2000, 81 consecutive potential multiorgan donors above the age of 10 years who were accepted for
HTx were included in the study. The study was approved by the Institutional Review Committee.

At the donor hospital, an arterial blood sample for subsequent analysis of PCT and CRP was taken immediately before the donor’s pericardium was opened. The blood was stored in dry tubes and in an icebox. After the return of the harvesting team to our institution, the blood was centrifuged, and the serum was stored at −70°C until analysis. The time between collection of the blood samples and storage at −70°C was <12 hours. Additionally, demographic data of the donors, time frame between diagnosis of brain death and explantation, laboratory values including white blood cell (WBC) count, catecholamine support, and other parameters before explantation were analyzed.

Routine donor criteria of our institution were used for donor selection, as reported previously.11 Preservation of the hearts was performed with 3 L of ice-cold HTK-Bretschneider solution. Immunosuppression was based on a triple-drug regimen in both groups (cyclosporine A, azathioprine, and prednisone) in accordance with our institutional protocol.11

Donors scheduled for acute re-HTx (within 30 days, n = 2) were excluded from the analysis because the recipients were on high inotropic support, had multiorgan failure, and were moribund before transplantation. The remaining 79 donors formed the study population; the recipients were hemodynamically stable, elective patients. They were retrospectively divided into 2 groups: donors for recipients who died within 30 days after HTx, after an early graft failure (group II, n = 8), and all other donors (group I, n = 71). Demographic data and preoperative recipient characteristics were considered. The postoperative clinical course of recipients and myocardial function documented by transesophageal echocardiography were analyzed.

Early graft failure after HTx was defined if at least 1 of the following 3 criteria was fulfilled: (1) patients died intraproactively of cardiac failure; (2) an intra-aortic balloon pump had to be inserted for weaning from cardiopulmonary bypass or for hemodynamic support within 12 hours after surgery; or (3) left ventricular ejection fraction was <30%, as revealed by postoperative transesophageal echocardiography (within 12 hours after surgery). Early graft failure–associated mortality was defined as mortality in recipients presenting with early graft failure after HTx, who died within 30 days in the hospital (group II). None of the recipients died of an acute rejection or failure of surgical technique according to biopsy or autopsy protocols.

**Analytical Methods**

Measurements of PCT and CRP in the sera of all 79 donors were performed centrally at the end of the sampling period once the collection of samples was complete, 19 months after commencement of the study. The respective CRP measurements were performed at the department of the PCT analysis; PCT determinations were performed with a commercially available assay kit (LUMTest, Brahms Diagnostics) with a “sandwich”-type luminescence immunnoassay and a coated-tube technique. Two monoclonal antibodies are directed against the C-terminal and mid-regional katacalcin sequences. The anti-catacalcin antibody is immobilized on the surface of the coated tube, and the anti-kalcitonin antibody is labeled with a luminescent acridine derivative. The assay remains unaffected by calcitonin and katacalcin levels even at high concentrations. The analytical assay sensitivity was 0.1 ng/mL. Interassay coefficients of variation (CV) at low and high concentrations were 7% and 8%, respectively. The upper reference limit (URL) was 0.5 ng/mL.

CRP was measured by direct immunoandria (Tina-Quam, Roche Diagnostics). The analytical assay sensitivity was 3 mg/L. Interassay CVs at low and high concentrations were 6% and 1.3%, respectively. The URL was 5 mg/L.

**Statistical Analysis**

Statistical analyses of the data were performed with SPSS 10.0.5 for Windows (SPSS Inc). Continuous data are reported as medians and ranges and binary data in absolute and relative frequencies. Nonparametric Mann-Whitney U tests and likelihood-ratio $x^2$-tests, respectively, were used for univariate comparisons between mortality groups. Values of $P<0.05$ were considered statistically significant.

The associations between PCT and CRP (as reference inflammation parameter), taken as continuous variables, and graft failure–related mortality were more closely inspected with receiver operating characteristic (ROC) curve analyses. ROC curves were drawn, and areas under ROC curves were calculated with 95% confidence limits. To assess sensitivities and specificities for PCT, a cutoff of $>2.0$ mg/L for systemic inflammation was chosen according to the manufacturer’s recommendation. For CRP, a cutoff of $>10$ mg/L was chosen according to the literature. To assess whether PCT $>2.0$ mg/L has to be viewed as a surrogate parameter for other potentially prognostic parameters rather than as an independent parameter on its own, unadjusted odds ratios and odds ratios adjusted for all other variables listed in Table 1 were calculated for PCT and CRP with respect to the above-defined cutoff, with standard logistic regression models. Finally, Kaplan-Meier survival curves were used to illustrate the prognostic value of PCT for graft failure–related mortality. In this analysis, deaths without graft failure were censored.

**Results**

There were no significant differences between groups with respect to donor age, donor sex, time ensuing between brain death and explantation, central venous pressure, norepinephrine used, arterial oxygen tension/fractional inspired oxygen ratio (PaO$_2$/FiO$_2$), body temperature, antibiotics used, episodes of hypoxemia and/or hypotension and/or cardiac arrest in the donor, and laboratory parameters (platelets, creatinine kinase, blood glucose, glutamate oxaloacetate transaminase, urea, creatinine, and bilirubin). CRP levels were locally available in only 28 of 79 donors. These were too few measurements for a meaningful analysis. Instead, retrospective central CRP determinations were performed at the time of the PCT analyses. Thus, PCT and CRP values used in the analysis were complete for all 79 donors. Only PCT values, ischemic time, and frequency of dobutamine use were significantly different. With respect to recipients, no between-group differences were found in age, sex, renal function, origin of heart failure, or preoperative hemodynamic status.

<table>
<thead>
<tr>
<th>Table 1. Medians and Ranges of Donor and Recipient Parameters in Groups Divided According to 30-Day Graft Failure–Related Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Graft Failure–Related Mortality</strong></td>
</tr>
<tr>
<td>No (n=71)</td>
</tr>
<tr>
<td><strong>Donor parameters</strong></td>
</tr>
<tr>
<td>Age, y</td>
</tr>
<tr>
<td>Female, %</td>
</tr>
<tr>
<td>PCT, ng/mL</td>
</tr>
<tr>
<td>CRP, mg/L</td>
</tr>
<tr>
<td>WBC count, Gpt/L</td>
</tr>
<tr>
<td>Ischemic time, min</td>
</tr>
<tr>
<td>Dobutamine, %</td>
</tr>
<tr>
<td>Norepinephrine, %</td>
</tr>
<tr>
<td><strong>Recipient parameters</strong></td>
</tr>
<tr>
<td>Age, y</td>
</tr>
<tr>
<td>Female, %</td>
</tr>
<tr>
<td>Preoperative use of VAD, %</td>
</tr>
</tbody>
</table>

VAD indicates ventricular assist device. Values in parentheses are ranges.
failure–related mortality. Group I consists of donors for recipients without early graft fail-
ure–related mortality. Horizontal line represents cutoff of >2 ng/mL.

Figure 2 shows PCT and CRP ROC curves for the outcome of graft failure–related mortality. The corresponding areas under the ROC curves were 0.71 (95% CI 0.48 to 0.95, \( P = 0.048 \)) for CRP and 0.64 (95% CI 0.43 to 0.85, \( P = 0.205 \)) for PCT, respectively.

Table 2 shows sensitivities and specificities of binary versions of PCT and CRP for both outcome parameters under consideration. Unadjusted and adjusted ORs for graft failure–related death, 3 died of infectious causes after a prolonged period of a low cardiac output syndrome with hemodynamic instability that necessitated high doses of catecholamines and support with an intra-aortic balloon pump. The remaining 5 recipients died of primary cardiac failure without evidence of infection at the time of death.

The sample characteristics of the variables that were initially judged to be potential PCT confounders and thus were prespecified for adjustment of PCT and CRP effects in the multivariate models are shown in Table 1. The distribution of PCT values in the groups is given in Figure 1. Figure 2 shows PCT and CRP ROC curves for the outcome of graft failure–related mortality. The corresponding areas under the ROC curves were 0.71 (95% CI 0.48 to 0.95, \( P = 0.048 \)) for PCT and 0.64 (95% CI 0.43 to 0.85, \( P = 0.205 \)) for CRP, respectively.

Table 2 shows sensitivities and specificities of binary versions of PCT and CRP for both outcome parameters under consideration. Unadjusted and adjusted ORs for graft failure–related death after HTx for donors with PCT >2 ng/mL versus PCT ≤2 ng/mL and for donors with CRP >10 mg/L versus CRP ≤10 mg/L are given in Table 3. Kaplan-Meier curves with respect to 30-day graft failure–related mortality for PCT >2 ng/mL versus PCT ≤2 ng/mL are given in Figure 3.

**Discussion**

Precise assessment of the heart donor is essential because this is an important prognostic factor for outcome after HTx.\(^2\) The decision to accept a donor heart is based on many variables, such as medical history, hemodynamic parameters, inotropic support, laboratory parameters, ECG, echocardiography, and in selected cases, angiography. The visual inspection is a subjective parameter to rule out major myocardial dysfunc-
tion and palpable coronary artery sclerosis. To assess the diagnosis of infection, WBC count and CRP are the most widely used laboratory parameters. Brain death, the associ-
ated adrenergic storm,\(^13\) and subsequent pathophysiological changes make the diagnosis of systemic infection and sepsis/severe sepsis difficult. Many multiple-organ donors require fluid resuscitation with plasma expanders and vasopressor therapy. WBC count and body temperature are easily obtain-
able parameters available in any hospital but are relatively unspecific.\(^14\)

CRP, an acute-phase protein synthesized in the liver, increases in response to trauma or infection. Although CRP values rise markedly in response to infection, a definite correlation between infection and the magnitude of CRP response has not been found.\(^15\) In addition, CRP is thought to reflect and amplify overall cytokine activation in the body.\(^16\)

Recently, PCT, a 116-amino acid precursor of calcitonin, has been identified as a marker for inflammatory host responses that is particularly induced in severe bacterial infections and sepsis.\(^6\) In healthy individuals, PCT plasma concentrations are very low, but they increase markedly after voluntary injection of endotoxin.\(^17\) The site of PCT production and its biological function during severe systemic infection are still unknown. In patients with viral infections or locally confined bacterial infections, PCT values do not increase or increase only slightly.\(^6\) Not infection per se but infection associated with a severe systemic response or poor organ perfusion is thought to induce PCT release.\(^7\) It has been proposed that PCT might be a better marker of elevated cytokines than the routinely used criteria of inflammation.

**TABLE 2. Sensitivities and Specificities of PCT and CRP With Respect to 30-Day Graft Failure–Related Mortality**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCT &gt;2 ng/mL</td>
<td>50.0</td>
<td>95.8</td>
</tr>
<tr>
<td>CRP &gt;10 mg/L</td>
<td>87.5</td>
<td>32.9</td>
</tr>
</tbody>
</table>

**TABLE 3. Unadjusted and Adjusted ORs With Respect to 30-Day Graft Failure–Related Mortality**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unadjusted OR (95% CI), ( P )</th>
<th>OR Adjusted for all Other Variables in Table 1 (95% CI), ( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCT &gt;2 ng/mL</td>
<td>22.7 (3.7–137.8), 0.0007</td>
<td>43.8 (1.4–1361.0), 0.0310</td>
</tr>
<tr>
<td>CRP &gt;10 mg/L</td>
<td>3.4 (0.4–29.5), 0.26</td>
<td>8.7 (0.3–271.2), 0.22</td>
</tr>
</tbody>
</table>
PCT curves (Figure 2). The differences were largest in regions of high specificity. However, only PCT was significantly associated with early graft failure mortality in the present study.

A closer inspection of the PCT differences by application of ROC analysis, with CRP as a reference parameter, revealed that CRP curves were almost uniformly inferior to PCT curves (Figure 2). The differences were largest in regions of high specificity. However, only PCT was significantly associated with early graft failure mortality in the present study.

ROC analysis further demonstrated the near optimality of the prespecified cutoffs for PCT and CRP. The corresponding binary variable PCT $> 2$ ng/mL was highly specific but only moderately sensitive, whereas CRP $> 10$ mg/L was highly sensitive but not specific for early graft failure–related mortality (Table 2).

The predictive value of PCT $> 2$ ng/mL was even higher than the predictive value of PCT taken as a continuous variable, as the OR of 22.7 ($P=0.0007$) demonstrates (Table 3). After adjustment for potential confounders, the OR remained significant, which indicates that PCT is not a surrogate parameter for other indicators of infection but rather an independent predictor of graft failure mortality. Again, no significant association could be assessed between CRP, this time in its binary version, and graft failure–related mortality (Table 3). The time-to-event curves (Figure 3) demonstrate the differences in graft failure–free survival times between recipients of hearts from donors with PCT $> 2$ ng/mL versus donors with PCT $\leq 2$ ng/mL in the study population.

The exact reason for the increased PCT values in donors of hearts that subsequently developed early graft failure remains unclear. Elevated PCT levels at the time of explantation might indicate an ongoing systemic infection with subsequent impairment of myocardial function, even if medical history, clinical investigation, laboratory values, and catecholamine dose showed no evidence of this phenomenon. We can neither confirm nor exclude that PCT elevations were caused by systemic infections in the donors, because we have no access to the details of the clinical course. We compared antibiotic therapy and norepinephrine administration in donors and did not find intergroup differences.

Yet, PCT values may well have been increased for reasons other than a manifest infection in these patients. In multiple-trauma patients, high PCT values correlated with more severe injury, a more frequent onset of shock, and multiple-organ dysfunction. Moreover, a PCT value $> 2$ ng/mL at 12 hours after the initial trauma was a strong predictor of a fatal outcome without evidence of infection and sepsis.19

Although the level of PCT has been shown to be related to the systemic reaction to severe infection,14 there is evidence to support the hypothesis that PCT may also be induced by endotoxins and proinflammatory cytokines in the absence of bacterial and fungal infections. In experimental endotoxemia, tumor necrosis factor-α (TNF-α) and interleukin-6 peaked before the appearance of PCT.17

There is growing evidence that myocardial insufficiency itself may induce immune activation, one explanation being intestinal malperfusion.20 Endotoxemia, elevated soluble CD14 receptors, and soluble TNF receptors have been found in patients with congestive heart failure out of hospital.21 In contrast to these patients, in whom only slightly increased PCT values were reported, exceptionally high levels of PCT were found in patients in cardiogenic shock associated with fever and a hemodynamic profile similar to that of a systemic inflammatory response syndrome in the absence of bacterial infection.23 It has been speculated that PCT may be induced by proinflammatory cytokines and bacterial translocation in these situations.24

Although originally proposed as a marker to indicate severity in the presence of systemic bacterial and fungal infection, systemic inflammatory syndromes of noninfectious causes may also trigger PCT release. Thus, PCT is currently hypothesized as a parameter of the severity of systemic inflammatory responses, irrespective of an infectious cause. This fuels the idea of PCT as a proinflammatory parameter in the potential heart donor, indicating organ dysfunction that may eventually lead to recipient death related to early graft failure in the present study.

The fact that transplanted organs from brain-dead donors have a consistently inferior success rate compared with that of living sources led to the discovery of cytokine activation after brain death in the animal model, suggesting a progressive detrimental effect of brain death on donor organ quality.25-26 Inflammatory activation was found in dysfunctional donor hearts, which lent support to the hypothesis that brain death causes inflammatory activation and may lead to apo-
ptosis, with a possible important effect on myocardial function.27

The cytokines TNF-α and interleukin-6 were elevated in the myocardium and sera of donor hearts with poor myocardial function compared with donor hearts with good ventricular function.28 Expression of TNF-α in donor hearts has been shown to predict the development of right ventricular failure early after HTx.29 These findings and the results of the present study suggest a significant correlation between the inflammatory status of the donor and the clinical course of the recipient.

A limitation of the present study is its retrospective nature. Although increased PCT levels in donor hearts were predictive of early graft failure–related mortality, a causal relationship to infection or systemic inflammatory changes in the donor has not been established.

Conclusions

Among different markers of acute infection investigated in the present study, an elevated PCT level was the single best predictor of early graft failure–related mortality after HTx. Considering the difficulties in diagnosis of systemic inflammation in multiple-organ donors, an additional biochemical marker to predict outcome related to graft function after HTx would assist clinical decision making in accepting donor organs and help to more safely expand the donor pool. The inclusion of PCT measurements in our routine for donor selection by use of a semiquantitative bedside test (Brahms PCT-Q, Brahms Diagnostica) is currently under consideration.

Elevated levels of PCT were found in donors with ongoing impairment of cardiac function, even when direct inspection and hemodynamic and echocardiographic measurements showed no evidence of compromised heart function. PCT values >2 ng/mL had a high specificity and predicted early graft failure–related mortality after HTx, but values ≤2 ng/mL were less sensitive and did not predict good graft function.

PCT was combined with other potential prognostic parameters and proved to be an independent predictor of early graft failure–related mortality. A PCT level >2 ng/mL in a cardiac donor at the time of explantation is a candidate prognostic indicator of early graft failure–related mortality. This merits validation in a controlled prospective study.

Acknowledgment

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References


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