Troponin T and I are not reliable markers of cardiac transplant rejection

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Abstract

Objective: Heart transplant recipients undergo a number of invasive endomyocardial biopsies to screen for rejection. Serum assays of troponin T and/or I may provide a less invasive alternative. The purpose of this study was to evaluate troponin T and I as markers of cardiac transplant rejection. Methods: We conducted a prospective analysis comparing troponin T and I levels to biopsy results in heart transplant recipients. Plasma was assayed for troponin T and I preoperatively, on the first 3 postoperative days, and with each subsequent biopsy. Results: Twenty-nine patients entered the study. A total of 173 biopsies were performed at a mean follow-up of 129 ± 9 days (range: 12–564 days). There were two rejection episodes (≥ grade 3), one in each of two patients. There were no significant relationships between troponin T or I and biopsy-proven rejection (≥ grade 3; P = 0.59 and 0.54, respectively). There were also no correlations between troponin T or I levels and biopsy grade (P = 0.40 and 0.92, respectively). Troponin T and I levels peaked on postoperative day 1 and fell to baseline over long-term follow-up with no peak in serum markers associated with rejection episodes. Donor ischemic time was significantly correlated to troponin T on postoperative days 1–3 (r = 0.58, P = 0.005; r = 0.61, P = 0.004; and r = 0.61, P = 0.003, respectively). Conclusions: Troponin T and I are not useful indicators of cardiac rejection, but do correlate with donor heart ischemic injury. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Heart transplantation; Graft rejection; Troponin T; Troponin I

1. Introduction

Patients receiving orthotopic heart transplants currently undergo frequent invasive endomyocardial biopsies to assess for postoperative rejection, particularly in the 1st year after transplant. Extensive study has gone into developing a diagnostic tool that is superior in terms of patient comfort, risk, convenience, speed, and expense. One avenue of investigation has been the use of cardiac-specific serum markers to detect the myocardial damage associated with rejection episodes.

Among other cardiac proteins, troponin T (TnT) and troponin I (TnI) are released into the serum during acute myocardial infarction [1–3]. TnT is an extremely sensitive marker of myocyte damage, and it has been hypothesized that it can detect minor damage that remains undetected using less sensitive methods such as creatine kinase MB (CK-MB) [2]. Similarly, serum TnI has been shown to increase following perioperative myocardial infarctions [3].

Previous trials have attempted to use TnT as a marker for rejection with varying success [4–12]. TnI appears to be less tightly bound to the thin filament within cardiac cells and can be extracted from skinned cardiac fibres and myocytes before other muscle proteins [13–15]. This suggests that during cardiac damage, TnI may be the initial protein released into the blood. TnI may therefore be a superior marker to TnT. The purpose of our study was to determine whether TnT and/or TnI are useful markers of rejection following cardiac transplantation.

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2. Materials and methods

All heart transplant candidates were considered for study inclusion. Patients were enrolled subsequent to giving informed consent. Plasma was collected and assayed for CK-MB, TnT, and TnI preoperatively, on the morning of each of the first 3 postoperative days, and at the time of each endomyocardial biopsy. Biopsies were graded according to the standard International Society for Heart and Lung Transplantation criteria [16], omitting the ‘A’ or ‘B’ classifications for statistical analysis. The pathologist was blinded to the study protocol. The detection limits of our TnT and TnI assays were 0.010 and 0.2 μg/l, respectively.

Statistical analysis was performed using SPSS statistical software (SPSS Inc., 444 N Michigan Avenue, Chicago, IL). TnT and TnI were compared to biopsy results by one-way analysis of variance (ANOVA). Additionally, biopsies were grouped as < grade 3 and ≥ grade 3 and the analysis was repeated using the Mann–Whitney U non-parametric test. Correlations between donor ischemic time and plasma markers were analyzed by Pearson bivariate correlation.

Data are expressed as the mean ± standard error of the mean. A P-value ≤0.05 was considered statistically significant.

This study was approved by the University of Alberta Faculty of Medicine Research Ethics Board and was conducted within the guidelines of the board.

3. Results

Twenty-nine patients were included in the study. A total of 173 biopsies were performed at a mean follow-up of 129 ± 9 days (range: 12–564 days). Demographics and clinical data are presented in Table 1.

TnT and TnI levels peaked on postoperative day 1 and returned to baseline on long-term follow-up, as shown in Fig. 1. No variation was seen in the two patients experiencing grade 3 rejection, as seen in Figs. 2 and 3. Table 2 presents TnT and TnI levels compared to biopsy results and Table 3 presents the comparison with biopsy results grouped as <3 and ≥3. No significant relationships were noted.

The correlations between donor ischemic time and CK-MB, TnT and TnI for the first 3 postoperative days are shown in Fig. 4. Statistically significant relationships to
donor ischemic time were seen in TnT on each postoperative day. A scatter plot of the postoperative day 1 result is presented in Fig. 5, which is representative of the correlations on the subsequent days. Although postoperative CK-MB was not significantly correlated to donor ischemic time, it was highly correlated with both TnT ($r = 0.52$, $P < 0.001$) and TnI ($r = 0.71$, $P < 0.001$).

4. Discussion

The results of our study do not support the use of cardiac TnT or TnI assay to screen for rejection following cardiac transplantation. We found no significant relationship between plasma TnT or TnI levels and rejection grade seen on biopsy. These findings are consistent with those of a number of recent studies [4–9,11].

Histologically, myocyte breakdown is not seen with grade 1 rejection and becomes apparent only at higher levels [16]. Since TnT and TnI are only released with myocyte damage, elevations in these proteins would likely only occur in severe rejection episodes. Re-analysis of the data using two groups based on biopsy results (<grade 3 and ≥grade 3) yielded a similar non-correlation. This lends evidence against the release of TnT and TnI during rejection. Similar analyzes performed in previous trials have yielded comparable results [5,7,9].

There are a number of possible reasons for the disappointing performance of these markers. The degree of myocyte necrosis seen with rejection may be insufficient to significantly elevate TnT or TnI [5,7]. Since the myocardial dysfunction associated with severe rejection is reversible, irreversible myocyte necrosis may not occur [5]. Timing of serum sampling may also be an issue. It is possible that myocyte damage is occurring before it becomes apparent on biopsy. Due to its short half-life (2 h) [8], troponin may no longer be present in serum samples drawn at the time of biopsy. This hypothesis is supported by the findings of Hossein-Nia et al. [11]. The investigators found no rejection-associated elevations of TnT when samples were drawn at the time of biopsy. They did, however, find TnT to be elevated at a median of 13 days prior to showing rejection on biopsy. These results have not been confirmed in subsequent trials. Although endomyocardial biopsy may not be a true gold standard [5,7], it remains the benchmark for other assays.

A study by Dengler et al. [10] compared biopsy results to TnT in 422 blood samples from 95 heart transplant recipients. Mean TnT concentrations increased in parallel with biopsy grade and were significantly greater in cases of grade 3 or 4 rejection compared to lower levels of rejection. Neither our study nor others have corroborated these findings [5,7,9].

A temporal analysis of our data revealed that TnT and TnI

<table>
<thead>
<tr>
<th>Biopsy grade</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>108</td>
<td>62</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Troponin T (μg/l)</td>
<td>0.08 ± 0.02</td>
<td>0.07 ± 0.02</td>
<td>0.36</td>
<td>0.02 ± 0.02</td>
<td>–</td>
<td>0.4</td>
</tr>
<tr>
<td>Troponin I (μg/l)</td>
<td>0.10 ± 0.05</td>
<td>0.07 ± 0.02</td>
<td>0.00</td>
<td>0.00 ± 0.00</td>
<td>–</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Fig. 3. Plasma troponin levels following cardiac transplantation in a patient experiencing grade 3 rejection.
become elevated following transplantation, peaking on postoperative day 1 and dropping to baseline on long-term follow-up. This finding is consistent with the literature [4,5,8]. It is probable that this related to donor heart ischemia or perioperative damage [5]. A study by Halwachs et al. [4] showed TnT release to be elevated by higher pre-existing pulmonary artery pressure. This suggests that the mechanical stress being placed on the donor heart while it adapts to recipient hemodynamics may result in right-ventricular overload and subsequent myocardial damage. No obvious difference was seen between the two patients in our trial experiencing significant rejection and the study population as a whole. This confirms previous results [5] and suggests that TnT and TnI levels are not predictive of rejection.

To further delineate the mechanism of postoperative TnT and TnI elevation, we correlated their levels on the first 3 postoperative days to donor ischemic time. We found TnT to be significantly correlated to donor ischemic time on all 3 postoperative days. This is the first finding of this type, although Carrier et al. [17] did note that the initial slope of release of TnT in the first 48 h post-transplant is significantly related to donor ischemic time. They did not find a correlation between donor ischemic time and maximum TnT in the first 48 h, however. Other studies have not made this finding [4,7]. It is likely that this is due to increased myocardial damage associated with longer donor ischemic time. TnT and TnI may be useful markers of ischemic myocardial injury following heart transplantation, but further investigation is warranted.

We also correlated CK-MB levels on the first 3 postoperative days to donor ischemic time and to both TnT and TnI. CK-MB was not significantly related to donor ischemic time, but was highly correlated to TnT and TnI. This is likely because CK-MB is a less sensitive marker of myocardial damage than troponin [2].

This study is limited by a small sample size. There is a possibility that the negative result is actually a Type II error. Furthermore, the large fluctuation in troponin levels in the early postoperative period could mask any early rejection episodes, rendering these assays less useful.

We conclude that TnT and TnI are not useful markers for predicting cardiac transplant rejection. Troponin T and I correlate with donor ischemic time and CK-MB release and as such, are useful markers of the degree of ischemic injury immediately after cardiac transplantation. The search for sensitive and specific non-invasive assessments of cardiac rejection will continue.

Table 3
Biopsy-proven rejection compared to troponin T and I following cardiac transplantation

<table>
<thead>
<tr>
<th>Biopsy grade</th>
<th>&lt; 3</th>
<th>≥ 3</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>171</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Troponin T (µg/l)</td>
<td>0.08 ± 0.01</td>
<td>0.02 ± 0.02</td>
<td>0.6</td>
</tr>
<tr>
<td>Troponin I (µg/l)</td>
<td>0.09 ± 0.03</td>
<td>0.00 ± 0.00</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Fig. 4. Correlations between donor ischemic time and serum markers for the first 3 postoperative days following cardiac transplantation.

Fig. 5. Troponin T versus donor ischemic time on postoperative day 1 following cardiac transplantation.
Acknowledgements

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References