High-Sensitivity Cardiac Troponin I Assay to Screen for Acute Rejection in Patients With Heart Transplant

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Background—A noninvasive biomarker that could accurately diagnose acute rejection (AR) in heart transplant recipients could obviate the need for surveillance endomyocardial biopsies. We assessed the performance metrics of a novel high-sensitivity cardiac troponin I (cTnI) assay for this purpose.

Methods and Results—Stored serum samples were retrospectively matched to endomyocardial biopsies in 98 cardiac transplant recipients, who survived ≥3 months after transplant. AR was defined as International Society for Heart and Lung Transplantation grade 2R or higher cellular rejection, acellular rejection, or allograft dysfunction of uncertain pathogenesis, leading to treatment for presumed rejection. cTnI was measured with a high-sensitivity assay (Abbott Diagnostics, Abbott Park, IL). Cross-sectional analyses determined the association of cTnI concentrations with rejection and International Society for Heart and Lung Transplantation grade and the performance metrics of cTnI for the detection of AR. Among 98 subjects, 37% had ≥1 rejection episode. cTnI was measured in 418 serum samples, including 35 paired to a rejection episode. cTnI concentrations were significantly higher in rejection versus nonrejection samples (median, 57.1 versus 10.2 ng/L; \( P < 0.0001 \)) and increased in a graded manner with higher biopsy scores (\( P_{\text{trend}} < 0.0001 \)). The \( c \)-statistic to discriminate AR was 0.82 (95% confidence interval, 0.76–0.88). Using a cut point of 15 ng/L, sensitivity was 94%, specificity 60%, positive predictive value 18%, and negative predictive value 99%.

Conclusions—A high-sensitivity cTnI assay seems useful to rule out AR in cardiac transplant recipients. If validated in prospective studies, a strategy of serial monitoring with a high-sensitivity cTnI assay may offer a low-cost noninvasive strategy for rejection surveillance. (Circ Heart Fail. 2014;7:463-469.)

Key Words: biopsy ■ diagnosis ■ graft rejection ■ transplants ■ troponin

Approximately 30% of cardiac transplant recipients develop acute rejection (AR) within the first year.\(^1\)\(^-\)\(^3\) Routine surveillance screening for rejection is performed after transplant to identify subclinical episodes of rejection. Serial endomyocardial biopsy (EMB) has long been the gold standard for surveillance of rejection; however, it is associated with procedural risk, considerable cost, patient discomfort, sampling error, and prolonged processing time.\(^4\)\(^,\)\(^5\)

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Given that myocytolysis is a key feature of AR,\(^6\) the use of serological markers associated with myocardial injury to identify rejection has been proposed. Cardiac troponin T (cTnT) and troponin I (cTnI) have been the biomarkers most extensively studied for the assessment of rejection; however, most studies have documented poor sensitivity and inadequate negative predictive value for clinical use.\(^7\)\(^-\)\(^15\)

New high-sensitivity (hs) troponin assays are ≈10-fold more sensitive than assays evaluated in these previous studies.\(^16\)\(^,\)\(^17\)

The objective of this study was to evaluate the performance of a novel hs-cTnI assay to detect AR in adult cardiac transplant recipients.

Methods

Study Overview and Selection of Subjects

A retrospective cohort of patients who underwent cardiac transplantation at the University of Texas Southwestern Medical Center from January 2003 to December 2010 and survived ≥3 months after transplant was created. Charts were retrospectively reviewed for baseline patient characteristics, donor characteristics, surgical variables, and post-transplant variables. Patients were followed up through December 31, 2011.
EMB Protocol
EMB was performed by protocol ≤10 days after transplantation with subsequent frequency dependent on whether steroids were to be weaned off entirely (20 biopsies in the first year) or down to a maintenance dose of prednisone 0.1 mg/kg (12 biopsies in the first year). Subsequently, biopsies were performed q3 months in the second year, yearly thereafter, and when clinical rejection was suspected. Specimens identified by the 1990 grading system were converted for analysis to the 2004 grading system as described in the International Society for Heart and Lung Transplantation (ISHLT) guidelines.21 (1) grade 0: OR, (2) grades 1A, 1B, and 2: 1R, (3) grade 3A: 2R, and (4) grades 3B and 4: 3R. AR was defined as (1) ISHLT grade 2R or higher acute cellular rejection (ACR);22 (2) hemodynamically significant acellular rejection (antibody-mediated rejection [AMR]);23, or (3) allograft dysfunction without evidence of cellular rejection or transplant vasculopathy leading to treatment for presumed rejection. Mixed rejection episodes, where patients had both ACR and AMR, were categorized as cellular rejection episodes for analysis.

Collection of Serum Samples and Matching to Biopsy Results
As standard clinical practice at our institution, transplanted patients had serum sent to the immunology laboratory at 0, 3, 6, 9, and 12 months after transplant, then yearly, and during most clinical presentations concerning for rejection. Unused serum from these samples was processed and stored at −70°C. Specimens obtained during routine surveillance and during suspected episodes of rejection were processed and handled in the same manner. Stored serum aliquots were retrospectively matched to EMB specimens by an individual without knowledge of rejection status. Given the expected elevation of cTnI immediately after transplant, samples were excluded if obtained ≤30 days after transplant. A rejection sample was defined as any sample obtained within 10 days before or after an EMB that was acquired during AR (as defined above). A nonrejection sample was defined as any sample that was obtained within 30 days of an EMB that was not acquired during AR. There were no samples obtained between 10 and 30 days of an EMB associated with AR.

The median (interquartile range [IQR]) days from the biopsy to serum acquisition was 1 (0–2) in the rejection group and 0 (–1–0) in the nonrejection group. Baseline troponin was defined as the cTnI concentration from the first serum acquisition ≥30 days after transplantation, provided that this measurement was not matched with rejection. A prerejection sample was defined as the sample obtained immediately before a sample matched with rejection. A postrejection sample was defined as the first sample obtained after a sample matched with rejection. Of 101 patients who met inclusion criteria, 99 patients had 559 available samples, with 540 samples having adequate volume for measurement of cTnI (Figure 1). Eighty-one samples were obtained within 30 days of transplant and were not used in the main analysis. To reduce bias, if there were 2 samples obtained within 10 days of rejection for the same subject, the sample closest to the rejection episode was included with the other sample being removed in the analysis (n=17). Twenty-four samples were not included because of technical errors by the analysis platform. This resulted in a final cohort of 98 patients with 418 samples, of which 243 were obtained between 30 days and 1 year after transplant and 175 were obtained after 1 year of transplant.

Measurement of cTnI
For this study, deidentified serum was shipped frozen to Abbott Diagnostics (Abbott Park, IL) for assay performance. Serum samples had been previously thawed for research and clinical purposes between 0x and 4x. The stability of cTnI in stored samples has been supported by previous evaluations of cTnI stability during storage and by previous studies using stored specimens to analyze cTnI using an hs platform.9,20 cTnI levels were measured using the hs assay on an automated platform (ARCHITECT i2000; Abbott Diagnostics) by personnel blinded to rejection status and clinical data. The between-assay coefficients of variation were ≤3.2% and ≤5.8% for control materials. The 99th percentile value for cTnI measured by this assay in a healthy population was 30 ng/L.21

Care After Transplant and Follow-Up
Patients were followed up in the transplant clinic frequently in the first year (eg, initially twice weekly, tapering down to monthly by 9 months after transplant), quarterly in the second year, and twice yearly thereafter. Patients were followed up for a median (IQR) period of 1522 (773-2160) days after transplant. Our center’s protocols for immunosuppression, rejection treatment, and screening for transplant vasculopathy have been previously described22,23 and are summarized in the Data Supplement. The Institutional Review Board of the University of Texas Southwestern Medical Center approved this study. Informed consent of participants was waived because of the retrospective nature of this study.

Statistics
Statistical analyses were performed using SAS 9.2 software (SAS Institute Inc, Cary, NC). Baseline patient, donor, and surgical data were compared between patients with and without subsequent rejection using a t test for normally distributed continuous data, Wilcoxon rank-sum test for non-normally distributed continuous data, and Pearson χ² test for categorical data. Survival was compared between patients with and without subsequent rejection using Kaplan–Meier analysis. Similar analyses were conducted to compare trends across quartiles of baseline cTnI using the Jonckheere–Terpstra test for continuous data and Cochran–Armitage test for categorical data.

cTnI values stratified by rejection history, biopsy score, and serial assessment (prerejection, rejection, and postrejection) were compared via mixed modeling with a random intercept term (patient) and a random slope (sample time from transplant) using a spatial power correlation matrix. These P values were adjusted for multiple comparisons via the Bonferroni procedure.

Multivariable-adjusted associations between cTnI and the presence of rejection were assessed with repeated measures analysis using mixed modeling techniques with log-cTnI as the outcome. The following potential confounders for the association of AR and troponin were included in the model: age, sex, presence of vasculopathy, creatinine, and history of cytomegalovirus. Time-updated variables were used in the analysis. A random intercept (patient) and a random slope (sample time from transplant) were included in the model. Because the time interval between repeat measures was essentially unique to each patient,
a spatial power correlation matrix was imposed, with the time difference between samples used in the exponent of the correlation term.

The performance metrics of cTnI in the diagnosis of rejection were assessed. To determine optimal cut points on the receiver operating characteristics curve and to take into account the repeated nature of the data, generalized linear mixed modeling was first used with a random intercept (patient) term and a logit-link function with a binomial distribution to estimate predicted probabilities for each observation for AR. The predicted probabilities were then used to create the receiver operating characteristics curve, and Youden index was maximized to determine the corresponding cutoff of cTnI concentration.

Results
Baseline patient characteristics of the entire cohort stratified by rejection status are reported in the Data Supplement. Ninety-eight patients were transplanted between 2003 and 2010, had survived ≥3 months, and had serological specimens available for analysis. Thirty-six of the 98 patients (37%) had a history of AR, of whom 29 patients had 35 samples matched to rejection available for analysis. Nineteen samples were matched to ACR, 8 samples to AMR, 5 samples to mixed rejection, and 3 samples to allograft dysfunction of uncertain pathogenesis leading to treatment for presumed rejection.

Patients with rejection were more commonly black (42% versus 18%; \( P=0.01 \)) with a history of vasculopathy (28% versus 10%; \( P=0.02 \)) or cytomegalovirus infection (53% versus 21%; \( P=0.001 \)), and had a higher rate of mortality during the follow-up period (31% versus 5%; \( P=0.02 \)).

Characteristics Associated With Baseline cTnI in Patients Without Early Rejection
The distribution of baseline cTnI in patients without AR during the first sample acquisition is outlined in Figure 2. There was a non-Gaussian distribution with a median concentration of 9.45 ng/L (interquartile range, 6.35–24.4 ng/L). Baseline characteristics of patients without AR stratified by quartiles of baseline cTnI are reported in Table 1. Higher concentrations of baseline cTnI were not associated with donor, recipient, or operative characteristics although there was a strong trend for higher concentrations among older recipients.

![Figure 2. Distribution of baseline cardiac troponin I (cTnI) in patients with transplant without early rejection (n=88). Histogram demonstrates the distribution of cTnI in patients without rejection during first serum acquisition (n=88). Ten outliers were not included in the distribution with cTnI ranging from 60.9 to 268.4 ng/L.](http://circheartfailure.ahajournals.org/Downloaded from)

### cTnI and Rejection
Thirty-five of 418 samples were matched with a rejection episode. Median cTnI concentrations were significantly higher in samples that were matched with rejection when compared with nonrejection episodes (57.1 versus 10.2 ng/L; \( P<0.0001 \); Figure 3A). cTnI concentrations increased in a graded fashion across higher ISHLT biopsy scores (Figure 3B; \( P_{\text{trend}}<0.0001 \)). There was no significant difference in cTnI concentrations between AMR and ACR episodes (in the Data Supplement).

Exploratory analyses were performed in which each episode of rejection was paired with either a measurement before or after the rejection episode (or both). cTnI concentrations increased during periods of rejection when compared with immediate prerejection samples (\( P=0.004 \)) and fell postrejection (\( P=0.002 \); Figure 4). The median time between prerejection and rejection samples was 89 days and between rejection and postrejection samples was 94 days.

In multivariable analysis, rejection was significantly associated with higher concentrations of cTnI independent of age, sex, creatinine, presence of vasculopathy, history of cytomegalovirus, and sample time from transplantation (\( P<0.001 \); Table 2).

### Screening Characteristics of cTnI
The \( c \)-statistic for cTnI to discriminate AR was 0.82 (95% confidence interval, 0.76–0.88). Using a receiver operating characteristics–optimized cut point of 15 ng/L, sensitivity was 94%, specificity 60%, positive predictive value (PPV) 18%, and negative predictive value (NPV) 99%. Using this cut off, 233 samples had a negative cTnI and 185 samples had a positive cTnI. There were 231 true-negatives, 2 false-negatives, 33 true-positives, and 152 false-positives. Of the 33 true-positive tests, 17 (52%) involved asymptomatic patients without echocardiographic evidence of allograft dysfunction and thus would not have been identified without a screening study.

Of the 2 rejection samples with cTnI<15 ng/L (false-negatives), 1 involved an asymptomatic 56-year-old man 1 year after transplant with a decline in left ventricular ejection fraction to 40% during an annual examination. The EMB was grade 0R and AMR negative. Clinical suspicion and decision to treat for rejection were based primarily on new left ventricular dysfunction. The cardiac index was 2.8 L/min per square meter. The cTnI was 10.5 ng/L when compared with a baseline of 23.1 ng/L. The second false-negative sample involved an asymptomatic 60-year-old man 1 year after transplant who had grade 2R ACR during an annual examination. Left ventricular ejection fraction was 56%, and cardiac index was 3.0 L/min per square meter. cTnI was 4.3 ng/L when compared with a baseline of 6.0 ng/L.

Given the concern for the possibility of troponin elevations because of myocyte damage during EMB, sensitivity analysis were performed excluding samples obtained within 2 days after EMB. Performance metrics were not changed when these samples were excluded (\( c \)-statistic, 0.82; 95% confidence interval, 0.66–0.97; NPV, 99%).

Subgroup analyses were performed in samples collected (1) within the first year of transplant; (2) after the first year of transplant; (3) without active vasculopathy; (4) with serum creatinine <75th percentile (1.78 mg/dL); (5) with serum creatinine ≥75th percentile (1.78 mg/dL); (6) without acellular...
Cardiac Troponin as a Potential Biomarker for Rejection

Cardiac troponins have been extensively studied for the assessment of AR using standard assays. cTnI and cTnT levels have been shown to be elevated for 1 to 3 months after transplantation because of perioperative ischemia and injury, thereby limiting the efficacy in assessing early rejection. However, the use of these biomarkers later in transplant seems more promising. One of the largest studies assessing a standard cTnT assay retrospectively evaluated 422 samples from 95 transplant recipients ≥3 months after transplant. The authors demonstrated that cTnT levels increased in parallel with higher histological grades of rejection (ISHLT grade 1, 28 ng/L; grade 2, 33 ng/L; grade 3A, 55 ng/L; and grade 3B/4, 105 ng/L). Using the assay detection limit as a diagnostic cutoff level, the screening characteristics for detecting grade 3A or higher rejection were sensitivity 80.4%, specificity 61.8%, and NPV 96%. Other subsequent studies, also performed with standard assays, demonstrated conflicting results, with most demonstrating a lack of statistical correlation between year than within the first year after transplant. If validated in prospective studies, our findings suggest that hs-cTnI assays may have value as an accurate test to rule out AR.

Discussion

To our knowledge, this is the first study evaluating the association of cTnI levels measured by a high-sensitivity assay with AR. Several findings are of interest. First, cTnI concentrations were significantly higher in serum samples temporally associated with AR, independent of traditional covariates. This association was present for both cellular and acellular rejections and robust across multiple sensitivity and subgroup analyses. Second, cTnI concentrations were increased during periods of rejection when compared with pre- and postrejection samples in the same patient. Third, screening characteristics of cTnI demonstrate a potential for clinical use. In particular, the NPV was extremely high for cTnI concentrations <15 ng/L, a threshold value well below the limit of detection of standard cTnI assays. Fourth, although the sample size was small, performance characteristics seemed to be better after the first year than within the first year after transplant. If validated in prospective studies, our findings suggest that hs-cTnI assays may have value as an accurate test to rule out AR.

Table 1. Baseline Characteristics Stratified by Quartiles of cTnI in Patients Without Rejection During First Serum Acquisition

<table>
<thead>
<tr>
<th>Variable*</th>
<th>Quartile I (n=22)</th>
<th>Quartile II (n=22)</th>
<th>Quartile III (n=23)</th>
<th>Quartile IV (n=21)</th>
<th>P&lt;sub&gt;test&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>cTnI, ng/L</td>
<td>&lt;6.35</td>
<td>6.35–9.44</td>
<td>9.45–24.4</td>
<td>≥24.4</td>
<td>…</td>
</tr>
<tr>
<td>Baseline characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>50±11</td>
<td>52±13</td>
<td>56±12</td>
<td>56±12</td>
<td>0.06</td>
</tr>
<tr>
<td>Men, n (%)</td>
<td>19 (86%)</td>
<td>19 (86%)</td>
<td>17 (74%)</td>
<td>16 (76%)</td>
<td>0.25</td>
</tr>
<tr>
<td>White, n (%)</td>
<td>12 (55%)</td>
<td>18 (82%)</td>
<td>15 (65%)</td>
<td>14 (67%)</td>
<td>0.25</td>
</tr>
<tr>
<td>Ischemic, n (%)</td>
<td>8 (36%)</td>
<td>8 (36%)</td>
<td>10 (43%)</td>
<td>10 (48%)</td>
<td>0.79</td>
</tr>
<tr>
<td>Pretransplant HTN, n (%)</td>
<td>10 (45%)</td>
<td>8 (36%)</td>
<td>10 (43%)</td>
<td>12 (57%)</td>
<td>0.38</td>
</tr>
<tr>
<td>Current HTN, n (%)†</td>
<td>17 (100%)</td>
<td>18 (90%)</td>
<td>19 (86%)</td>
<td>20 (95%)</td>
<td>0.57</td>
</tr>
<tr>
<td>Pretransplant DM, n (%)</td>
<td>5 (23%)</td>
<td>10 (45%)</td>
<td>10 (43%)</td>
<td>10 (48%)</td>
<td>0.12</td>
</tr>
<tr>
<td>Current DM, n (%)†</td>
<td>6 (35%)</td>
<td>13 (65%)</td>
<td>8 (36%)</td>
<td>10 (48%)</td>
<td>0.62</td>
</tr>
<tr>
<td>Weight, kg†</td>
<td>87.3±17.9</td>
<td>97.8±33.9</td>
<td>77.2±19.9</td>
<td>85.3±21.3</td>
<td>0.32</td>
</tr>
<tr>
<td>SBP, mm Hg†</td>
<td>127±16</td>
<td>132±17</td>
<td>126±16</td>
<td>137±13</td>
<td>0.29</td>
</tr>
<tr>
<td>DBP, mm Hg†</td>
<td>84±13</td>
<td>81±13</td>
<td>77±13</td>
<td>86±12</td>
<td>0.97</td>
</tr>
<tr>
<td>Creatinine, mg/dL†</td>
<td>1.47±0.51</td>
<td>1.44±0.32</td>
<td>1.47±0.60</td>
<td>1.46±0.41</td>
<td>0.84</td>
</tr>
<tr>
<td>Operative characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donor ischemic time, min</td>
<td>200±71</td>
<td>196±44</td>
<td>177±50</td>
<td>203±64</td>
<td>0.81</td>
</tr>
<tr>
<td>CP bypass time, min‡</td>
<td>136±47</td>
<td>144±45</td>
<td>131±30</td>
<td>137±40</td>
<td>0.85</td>
</tr>
<tr>
<td>Donor characteristics§</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>27.3±11.7</td>
<td>29.2±10.5</td>
<td>28.5±11.2</td>
<td>34.1±11.6</td>
<td>0.11</td>
</tr>
<tr>
<td>Men, n (%)</td>
<td>13 (76%)</td>
<td>14 (82%)</td>
<td>13 (87%)</td>
<td>7 (78%)</td>
<td>0.74</td>
</tr>
<tr>
<td>White, n (%)</td>
<td>15 (88%)</td>
<td>11 (65%)</td>
<td>10 (67%)</td>
<td>6 (67%)</td>
<td>0.68</td>
</tr>
</tbody>
</table>

*Values are listed as means±SD unless otherwise specified.
†Hypertensive status of 8 subjects, DM status of 8 subjects, weight of 25 subjects, SBP of 26 subjects, diastolic blood pressure of 26 subjects, and creatinine of 18 subjects during sample acquisition are unknown.
‡Cardiopulmonary bypass time of 2 patients is unknown.
§Donor data for 24 subjects without rejection and 8 subjects with rejection are unknown.

CP indicates cardiopulmonary; cTnI, cardiac troponin I; DBP, diastolic blood pressure; DM, diabetes mellitus; HTN, hypertension; and SBP, systolic blood pressure.

rejection; (7) without cellular rejection, and (8) among individuals with no symptoms or echocardiographic findings suggesting AR (Table 3). Although NPV was high in each subgroup, cTnI performed somewhat better >1 year after transplant when compared with 30 days to 1 year after transplant (c-statistic, 0.91 versus 0.75; sensitivity, 100% versus 91%; NPV, 100% versus 98%).
troponin concentration and rejection, and many documenting unacceptable false-negative rates.\(^7\)-\(^{15}\)

**Use of the hs Assay to Assess Rejection**

Hs–troponin assays are \(\approx 10\)-fold more sensitive than current assays and can detect troponin levels well below the 99th percentile cut point.\(^{16,17}\) To date, only 2 small studies have evaluated the performance of the hs-cTnT assay for assessing rejection. In a small retrospective case-crossover study (29 cases and 38 controls), where each patient with rejection served as their own control, cTnT concentrations were significantly higher with versus without rejection (155 versus 47 ng/L; \(P = 0.006\)).\(^{28}\) We have previously evaluated the performance of the hs-cTnT assay to detect AR in pediatric heart transplant recipients.\(^9\) cTnT levels were higher in samples with versus without AR (cTnT, 66 versus 7 ng/L; \(P = 0.001\)) and in exploratory analyses seemed to change within individuals in parallel to rejection status.

Our study is notably larger with more episodes of rejection than previous studies with the hs-cTnT assay. Furthermore, we were able to assess cTnI concentrations in patients with both cellular and acellular rejections. Performance metrics were improved with the hs-cTn assay when compared with previous studies of the standard cTn assay. The \(c\)-statistic was 0.82 and at the optimized cut point of 15 ng/L sensitivity was 94% and the NPV was 99%, both notably higher than previous reports with standard assays (11%–89% and 79%–96%, respectively).\(^7,10,11,13,27\)

**Comparison of cTnI With Other Noninvasive Strategies to Detect Rejection**

Gene expression profiling to assess for rejection has garnered much interest with the recent publications of the Cardiac Allograft Rejection Gene Expression Observation (CARGO)\(^{30}\) and Invasive Monitoring Attenuation through Gene Expression (IMAGE) trials.\(^{31}\) The IMAGE investigators evaluated whether gene expression studies led to the minimization of cardiac biopsies in low-risk patients late after transplantation. They demonstrated noninferiority of the gene expression profiling strategy for a composite outcome of rejection with hemodynamic compromise, allograft dysfunction, death, or retransplantation. The NPV was notably high (\(\approx 99\%\)), with PPV ranging from 50% to 79% depending on the threshold used.\(^{30}\) Currently, gene expression profiling to rule out ACR in appropriate low-risk patients between 0.5 and 5 years after transplant has a class IIa ISHLT guideline recommendation.\(^{32}\) Despite guideline acceptance, the gene expression strategy has some important limitations, including (1) limited use while on high-dose steroids; (2) high cost; (3) need for concomitant echocardiography, and (4) modest PPV.\(^{33}\) Furthermore, blood sampling often has to be completed at an off-site laboratory with a delay in the reporting of results.

In our study, cTnI demonstrates a similarly high NPV in patients beginning 1 month after transplant although with a lower PPV. It is likely that the use of a cTnI-based screening strategy would lead to more false-positive tests than a strategy using gene expression profiling. However, given that cTnI can be obtained faster, with less cost, earlier post-transplant, and on-site at the hospital, this biomarker has the potential to become a suitable low-cost alternative to gene expression profiling. Of note, Medicare reimbursement rates for tropo- nin testing average \(\approx \$15\) versus \$2821 for gene expression profiling. Furthermore, a multimarker approach using other biomarkers that have been associated with rejection, such as B-type natriuretic peptide (BNP)\(^{34,35}\) and N-terminal pro-B–type natriuretic peptide (NT-proBNP),\(^{29,36}\) may improve on the specificity and PPV of cTnI alone, a hypothesis that will require prospective evaluation in additional studies.

![Figure 3. Troponin I concentrations stratified by the presence or absence of rejection (A) and by International Society for Heart and Lung Transplantation biopsy score (B). The line represents the median value, the boxes the 25th and 75th percentile, and the error bars the 5th and 95th percentiles. cTnI indicates cardiac troponin I.](image)

![Figure 4. Serial troponin I concentrations of samples before, during, and after acute rejection. The line represents the median value, the boxes the 25th and 75th percentile, and the error bars the 5th and 95th percentiles. cTnI indicates cardiac troponin I.](image)

![Table 2. Multivariable Analysis Evaluating the Association of Acute Rejection With High-Sensitivity Cardiac Troponin I](image)
Because of the relatively low incidence of rejection 1 year after transplant, some have suggested a strategy in low-risk heart transplant recipients that eliminates routine EMB or gene expression profiling after 1 year in lieu of echocardiographic assessment.57

Given its ease in ascertainment and relatively low cost, the hs-cTnI assay could potentially be used as an adjunct to echocardiographic assessment for rejection. Indeed, although the sample size was small, performance of the hs-cTnI assay as a screening test beyond 1 year after transplant seemed to be particularly strong.

The use of cTnI has the potential to decrease overall biopsy numbers when compared with traditional biopsy strategies. If the present findings are confirmed in prospective studies, we estimate that using the hs-cTnI assay could result in a >50% reduction in biopsies because 56% of samples had cTnI below the 15 ng/L threshold with a NPV of 99%. This reduction could potentially be greater if application of the assay was limited to individuals ≥1 year after transplant or other low-risk subjects.

**Limitations**

This study cohort was assembled retrospectively and the sample size, while larger than any previous study of hs-troponin assays for rejection screening, is still modest. Some serum samples were obtained because of clinical suspicion for rejection or history of elevated post-transplant antibodies, potentially introducing ascertainment bias. However, the results were similar in the subgroup of individuals without symptoms or echocardiographic abnormalities. Blood samples were not collected by protocol on the day of EMB, and samples were matched to rejection episodes if they were obtained within 10 days before or after a rejection episode. However, this limitation would be expected to lead to underestimation of the true association between cTnI and rejection. Future prospective studies should collect blood samples by protocol immediately before routine EMB. Given inherent limitations of this retrospective cross-sectional study, additional evaluation and validation of screening characteristics of cTnI will be necessary through prospective evaluation.

**Conclusions**

In the first study to evaluate the association of cTnI concentrations with acute cardiac rejection using a high-sensitivity assay, we demonstrated that higher concentrations of cTnI are robustly and independently associated with acute rejection. The NPV for cTnI using the hs assay was high, supporting a potential role in screening for rejection and possibly reducing the need for EMB when compared with traditional screening protocols. Further investigation of the clinical use of this biomarker through prospective studies is warranted.

**Sources of Funding**

Abbott Diagnostics provided an investigator-initiated grant to Dr de Lemos and performed the high-sensitivity cardiac troponin I assays; however, they were blinded to patient data and did not play any other role in the study.

**Disclosures**

None.

**References**


High-Sensitivity Cardiac Troponin I in Rejection


**CLINICAL PERSPECTIVE**

Although serial endomyocardial biopsy is currently the gold standard for rejection surveillance in heart transplant recipients, it is associated with procedural risk, considerable cost, patient discomfort, and prolonged processing time. A noninvasive screening marker that decreases the number of biopsies used for rejection screening while decreasing cost and processing time has yet to be developed. In this retrospective study, cardiac troponin I concentrations measured by a high-sensitivity assay were significantly higher in serum samples temporally associated with acute rejection. Cardiac troponin I had a negative predictive value of 99% for acute rejection using a cutoff of <15 ng/L. If validated in prospective studies, our findings suggest that a high-sensitivity cardiac troponin I assay may have value as an accurate rule out test for acute rejection and would be an attractive alternative to either gene expression profiling or endomyocardial biopsy for this purpose.
High-Sensitivity Cardiac Troponin I Assay to Screen for Acute Rejection in Patients With Heart Transplant


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SUPPLEMENTAL MATERIAL

Supplemental Methods:

Immunosuppression Protocol:
An antimetabolite (myfortic 1080 mg po, mycophenolate mofetil 2000 mg IV or azathioprine 1-2 mg/kg IV since 2008 in the case of CMV mismatch [Donor +/ Recipient -]); Basiliximab (20 mg IV) and methylprednisolone (500 mg IV) were given perioperatively. This was followed by methylprednisolone 125 mg q8 hours x 3 on postoperative day 1. Oral prednisone was initiated on postoperative day 2 and was tapered with the goal of discontinuation at 6 months for most patients. A second dose of basiliximab (20mg IV) was administered on postoperative day 4. Antimetabolite doses were initiated on postoperative day 1: mycophenolate mofetil 3000mg/day, myfortic 2160mg/day, or azathioprine 1-2 mg/kg/day in the case of CMV mismatch since 2008. The dose of the antimetabolite was lowered for leukopenia. Cyclosporine or tacrolimus was initiated on days 4 or 5 and was subsequently dosed according to renal function and time post-transplant. Sirolimus was incorporated for transplant vasculopathy or in a renal sparing protocol, as described.

Rejection Treatment Protocol:
Patients with clinical rejection (symptoms or allograft dysfunction) or ISHLT grade 2R or 3R rejection were typically admitted to the hospital and given solumedrol 1 gram IV daily for three days followed by an oral prednisone burst taper starting at 0.5 mg/kg tapered down over 30 days. For advanced hemodynamically significant cellular and acellular rejection, other therapies were added to steroids including anti-thymocyte globulin, photopheresis, plasmapheresis, intravenous immunoglobulin, and/or rituximab. If the donor had not undergone left heart catheterization, a baseline coronary angiogram was completed 6 weeks after transplant.
**Transplant Vasculopathy Screening Protocol:**

Routine coronary angiogram, but not intravascular ultrasound, was completed at 1, 3, 5, 7, 10, and 15 years post-transplant or if there was clinical suspicion for the presence of vasculopathy. Vasculopathy was defined as angiographic evidence of any focal lesion $\geq 50\%$, distal narrowing, or diffuse luminal irregularities.

**Supplemental Results:**

**Cardiac TnI and Rejection**

Eleven samples were matched with acellular rejection episodes (8 samples with AMR and 3 samples with allograft dysfunction of uncertain etiology) and 24 samples were matched with cellular rejection episodes (19 samples with ACR and 5 samples with mixed rejection). Acellular rejection episodes had a significantly lower left ventricular (LV) ejection fraction (EF) (31 ± 14 vs. 56 ± 16, $p = 0.001$) and more frequent hemodynamic compromise (82% vs. 33%, $p = 0.008$) compared to episodes of cellular rejection, but no significant difference was seen in cTnI concentrations between these types of rejection (median 82.0 ng/L [IQR: 46.5-333.1 ng/L] vs. 48.8 [IQR: 20.7-106.1 ng/L], $p = 0.1$, respectively).
### Table 1. Baseline, Transplant, and Post-transplant Characteristics Stratified by Rejection

<table>
<thead>
<tr>
<th>Variable</th>
<th>All Patients (n = 98)</th>
<th>No Rejection (n = 62)</th>
<th>Rejection (n = 36)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline Characteristics</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Age, yrs.</td>
<td>53.2 ± 12.7</td>
<td>54.4 ± 12.5</td>
<td>51.1 ± 12.9</td>
<td>0.19</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>81 (83%)</td>
<td>48 (77%)</td>
<td>33 (92%)</td>
<td>0.07</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>White</td>
<td>66 (67%)</td>
<td>45 (73%)</td>
<td>21 (58%)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>26 (27%)</td>
<td>11 (18%)</td>
<td>15 (42%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>6 (6%)</td>
<td>6 (10%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Ischemic, n (%)</td>
<td>39 (40%)</td>
<td>23 (37%)</td>
<td>16 (44%)</td>
<td>0.63</td>
</tr>
<tr>
<td>History of Hypertension, n (%)</td>
<td>42 (43%)</td>
<td>24 (39%)</td>
<td>18 (50%)</td>
<td>0.28</td>
</tr>
<tr>
<td>History of Diabetes, n (%)</td>
<td>38 (39%)</td>
<td>28 (45%)</td>
<td>10 (28%)</td>
<td>0.09</td>
</tr>
<tr>
<td>BMI, (n = 66)</td>
<td>26.7 ± 5.1</td>
<td>26.1 ± 4.5</td>
<td>27.5 ± 5.7</td>
<td>0.26</td>
</tr>
<tr>
<td><strong>Transplant Characteristics</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Donor Ischemic Time, min</td>
<td>193 ± 57</td>
<td>188 ± 57</td>
<td>202 ± 56</td>
<td>0.19</td>
</tr>
<tr>
<td>Cardiopulmonary Bypass</td>
<td>135 ± 39</td>
<td>132 ± 35</td>
<td>141 ± 45</td>
<td>0.42</td>
</tr>
<tr>
<td><strong>Donor Characteristics†</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Age, yrs</td>
<td>28.9 ± 10.6</td>
<td>28.9 ± 11.2</td>
<td>28.9 ± 10.0</td>
<td>0.68</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>53 (80%)</td>
<td>30 (79%)</td>
<td>23 (82%)</td>
<td>0.74</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.38</td>
</tr>
<tr>
<td>White</td>
<td>46 (70%)</td>
<td>27 (71%)</td>
<td>19 (68%)</td>
<td></td>
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<tr>
<td></td>
<td>10 (15%)</td>
<td>4 (11%)</td>
<td>6 (21%)</td>
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<tr>
<td>Black</td>
<td>10 (15%)</td>
<td>7 (18%)</td>
<td>3 (11%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>28 ± 5.5</td>
<td>28 ± 5.5</td>
<td>28 ± 5.7</td>
<td></td>
</tr>
</tbody>
</table>

**BMI, body mass index; CMV, cytomegalovirus.**

* Values are listed as means ± standard deviation unless otherwise specified.

† Donor data for 24 subjects without rejection and 8 subjects with rejection are unknown.
Supplemental References:


