A Sensitive Cardiac Troponin I Assay to Screen for Acute Rejection in Heart Transplant Patients

Patel et al: Sensitive Cardiac Troponin I in Acute Rejection

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DOI: 10.1161/CIRCHEARTFAILURE.113.000697

Journal Subject Codes: Diagnostic testing:[33] Other diagnostic testing, Cardiovascular (CV) surgery:[37] CV surgery: transplantation, ventricular assistance, cardiomyopathy
Abstract

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

Methods and Results—Stored serum samples were retrospectively matched to EMB in 98 cardiac transplant recipients who survived ≥ 3 months post-transplant. AR was defined as ISHLT grade 2R or higher cellular rejection, acellular rejection, or allograft dysfunction of uncertain etiology leading to treatment for presumed rejection. cTnI was measured with a hs assay (Abbott Diagnostics). Cross-sectional analyses determined the association of cTnI concentrations with rejection and ISHLT grade; and the performance metrics of cTnI for the detection of AR. Among 98 subjects, 37% had at least one 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 vs. 10.2 ng/L, p < 0.0001), and increased in a graded manner with higher biopsy scores (p_trend < 0.0001). The c-statistic to discriminate AR was 0.82 (95% CI 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 hs-cTnI assay appears useful to “rule out” AR in cardiac transplant recipients. If validated in prospective studies, a strategy of serial monitoring with a hs-cTnI assay may offer a low-cost noninvasive strategy for rejection surveillance.

Key Words: biopsy, diagnosis, transplant, troponin, rejection
Approximately 30% of cardiac transplant recipients develop acute allograft rejection (AR) within the first year.\textsuperscript{1-3} Routine surveillance screening for rejection is performed following 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.\textsuperscript{4, 5}

Given that myocytolysis is a key feature of AR,\textsuperscript{6} utilization of serologic 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.\textsuperscript{7-15} New high sensitivity (hs) troponin assays are approximately 10-fold more sensitive than assays evaluated in these previous studies.\textsuperscript{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 at least 3 months post-transplant was created. Charts were retrospectively reviewed for baseline patient characteristics, donor characteristics, surgical variables, and post-transplant variables. Patients were followed through December 31, 2011.
Endomyocardial biopsy protocol

EMB was performed by protocol approximately ten days after transplantation with subsequent frequency dependent upon 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: 1) grade 0: 0R, 2) grades 1A, 1B, and 2: 1R, 3) grade 3A: 2R, and 4) grades 3B and 4: 3R. AR was defined as: a) ISHLT grade 2R or higher acute cellular rejection (ACR); b) hemodynamically significant acellular rejection (antibody-mediated rejection [AMR]) or c) 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 lab at 0, 3, 6, 9 and 12 months post-transplant, then yearly, and during most clinical presentations concerning for rejection. Unused serum from these samples was processed and stored at -70 degrees 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 post-transplant, samples were excluded if obtained ≤ 30 days post-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 “non-rejection” 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 10-30 days of an EMB associated with AR.

The mean (quartiles) days from biopsy to serum acquisition was 0.7 (0, 1, 2) in the rejection group and -0.6 (-1, 0, 0) in the nonrejection group. Baseline troponin was defined as the cTnI concentration from the first serum acquisition at least 30 days after transplantation, provided that this measurement was not matched with rejection. A pre-rejection sample was defined as the sample obtained immediately prior to a sample matched with rejection. A post-rejection 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 utilized in the main analysis. To reduce bias, if there were two 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 due to 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 post-transplant and 175 were obtained after 1 year of transplant.
Measurement of cTnI

For this study, de-identified 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 0-4 times. The stability of cTnI in stored samples has been supported by prior evaluations of cTnI stability during storage and by prior studies utilizing stored specimens to analyze cTnI using a highly sensitive platform.19,20

cTnI levels were measured using a highly sensitive assay on an automated platform (ARCHITECT i2000sr, Abbott Diagnostics, Abbott Park, IL) by personnel blinded to rejection status and clinical data. The between-assay CVs 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 in the transplant clinic frequently in the first year (e.g., initially twice weekly, tapering down to monthly by 9-months post-transplant), quarterly in the second year and twice yearly thereafter. Patients were followed for a mean (quartiles) period of 1575 (773, 1522, 2160) days post-transplant. Our center’s protocols for immunosuppression, rejection treatment, and screening for transplant vasculopathy have been previously described22,23 and are summarized in the online data supplement. The Institutional Review Board of the University of Texas Southwestern Medical Center approved this study. Informed consent of participants was waived due to 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’s chi-square 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 Cochrane-Armitage test for categorical data.

cTnI values stratified by rejection history, biopsy score, and serial assessment (pre-rejection, rejection, and post-rejection) 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, gender, presence of vasculopathy, creatinine, and history of CMV. 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 (ROC) 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 ROC curve, and Youden’s index\textsuperscript{24} 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 online data supplement. Ninety-eight patients were transplanted between 2003 and 2010, had survived over 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 etiology leading to treatment for presumed rejection.

Patients with rejection were more commonly black (42% vs. 18%, \( p = 0.01 \)) with a history of vasculopathy (28% vs. 10%, \( p = 0.02 \)) or CMV infection (53% vs. 21%, \( p = 0.001 \)), and had a higher rate of mortality over the follow-up period (31% vs. 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 (IQR: 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, though there was a strong trend for higher concentrations among older recipients.

Cardiac TnI 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 compared to nonrejection episodes (57.1 vs. 10.2 ng/L, p < 0.0001, Figure 3, panel A). cTnI concentrations increased in a graded fashion across higher ISHLT biopsy scores (Figure 3, panel B, p_trend < 0.0001). There was no significant difference in cTnI concentrations between AMR and ACR episodes (see 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 to immediate pre-rejection samples (p = 0.004) and fell post-rejection (p = 0.002) (Figure 4). The median time between pre-rejection and rejection samples was 89 days and between rejection and post-rejection samples was 94 days.
In multivariable analysis, rejection was significantly associated with higher concentrations of cTnI independent of age, gender, creatinine, presence of vasculopathy, history of CMV, 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% CI 0.76-0.88). Using a ROC-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 two rejection samples with cTnI < 15 ng/L (false negatives), one involved an asymptomatic 56 year old male one year post-transplant with a decline in LVEF to 40% during an annual exam. The EMB was grade 0R and AMR negative. Clinical suspicion and decision to treat for rejection were based primarily on new LV dysfunction. The cardiac index was 2.8 L/min/m². The cTnI was 10.5 ng/L, compared with a baseline of 23.1 ng/L. The second false negative sample involved an asymptomatic 60 year old male one year post-transplant who had grade 2R ACR during an annual exam. LVEF was 56% and cardiac index was 3.0 L/min/m². cTnI was 4.3 ng/L, compared with a baseline of 6.0 ng/L.
Given the concern for the possibility of troponin elevations due to 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% CI 0.66-0.97, NPV 99%).

Subgroup analyses were performed in samples collected a) within the first year of transplant; b) after the first year of transplant; c) without active vasculopathy; d) with serum creatinine < 75th percentile (1.78 mg/dL), e) with serum creatinine ≥ 75th percentile (1.78 mg/dL), f) without acellular rejection, g) without cellular rejection, and h) 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 post transplant compared with 30 days-1 year post-transplant (c-statistic 0.91 vs. 0.75, Sensitivity 100% vs. 91%, NPV 100% vs. 98%, respectively).

**Discussion**

To our knowledge, this is the first study evaluating the association of cTnI levels measured by a highly sensitive 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 rejection, and robust across multiple sensitivity and subgroup analyses. Second, cTnI concentrations were increased during periods of rejection when compared to pre- and post- rejection samples in the same patient. Third, screening characteristics of cTnI demonstrate a potential for clinical utility. 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 appeared 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 “rule out” test for AR.

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 1-3 months post-transplantation due to perioperative ischemia and injury, thereby limiting the efficacy in assessing early rejection. However, utilization of these biomarkers later in transplant appears more promising. One of the largest studies assessing a standard cTnT assay retrospectively evaluated 422 samples from 95 transplant recipients greater than three months post-transplant. The authors demonstrated that cTnT levels increased in parallel with higher histologic 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 troponin concentration and rejection, and many documenting unacceptable false negative rates.

Utilization of a Highly Sensitive Assay to Assess for Rejection

High sensitivity troponin assays are approximately 10 fold more sensitive than current assays and can detect troponin levels well below the 99th percentile cutpoint. To date, only two
small studies have evaluated the performance of the hs-cTnT assay for assessing rejection. In a small retrospective case-crossover study (29 cases, 38 controls), where each patient with rejection served as their own control, cTnT concentrations were significantly higher with versus without rejection (155 ng/L vs. 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.29 cTnT levels were higher in samples with versus without AR (cTnT: 66 ng/L vs. 7 ng/L, p = 0.001) and in exploratory analyses appeared to change within individuals in parallel to rejection status.

Our study is notably larger with more episodes of rejection than prior studies with the hs-cTnT assay. Further, we were able to assess cTnI concentrations in patients with both cellular and acellular rejection. With the hs-cTnI assay, performance metrics were improved compared with prior studies with standard cTnI assays. The c-statistic was 0.82 and at the optimized cutpoint of 15 ng/L sensitivity was 94% and the NPV was 99%, both notably higher than prior 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 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
(~99%), with PPV ranging from 50-79% depending on the threshold used.\textsuperscript{30} Currently, gene expression profiling to rule out ACR in appropriate low-risk patients between 0.5-5 years post-transplant has a Class IIa ISHLT Guideline recommendation.\textsuperscript{32} Despite guideline acceptance, the gene expression strategy has some important limitations including: 1) limited utility while on high dose steroids; 2) high cost; 3) need for concomitant echocardiography and 4) modest PPV.\textsuperscript{33} Furthermore, blood sampling often has to be completed at an off-site lab with a delay in the reporting of results.

In our study, cTnI demonstrates a similarly high NPV in patients beginning one month post-transplant, though with a lower PPV. It is likely that utilization 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 troponin testing average approximately $15 vs. $2821 for gene expression profiling. Further, a multimarker approach using other biomarkers that have been associated with rejection such as BNP\textsuperscript{34,35} and NT-proBNP\textsuperscript{29,36} may improve upon the specificity and PPV of cTnI alone, a hypothesis that will require prospective evaluation in additional studies.

Due to the relatively low incidence of rejection one year post-transplant, some have suggested a strategy in low-risk heart transplant recipients that eliminates routine EMB or gene expression profiling after one year in lieu of echocardiographic assessment.\textsuperscript{37} 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 one year post transplant appeared to be particularly strong.

Utilization of cTnI has the potential to decrease overall biopsy numbers compared to 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, as 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 more than one year post-transplant or other lower 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 due to 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 prior to routine EMB. Given inherent limitations of this retrospective cross-
sectional study, further 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 highly sensitive assay, we demonstrated that higher concentrations of cTnI are robustly and independently associated with acute rejection. The NPV for cTnI using the highly sensitive assay was high, supporting a potential role in screening for rejection and possibly reducing the need for EMB when compared to traditional screening protocols. Further investigation of the clinical utility 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 hs-cTnI assays; however, they were blinded to patient data and did not play any other role in the study.

Disclosures

None.

References


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 trend</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><strong>Baseline Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, yrs</td>
<td>50 ± 11</td>
<td>53 ± 13</td>
<td>56 ± 12</td>
<td>56 ± 12</td>
<td>0.06</td>
</tr>
<tr>
<td>Male, 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>
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<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, mmHg‡</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, mmHg‡</td>
<td>84 ± 13</td>
<td>81 ± 13</td>
<td>77 ± 13</td>
<td>86 ± 12</td>
<td>0.97</td>
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<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><strong>Operative Characteristics‡</strong></td>
<td></td>
<td></td>
<td></td>
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</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>
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<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>
</tbody>
</table>
### Donor Characteristics

<p>| | | | | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Age, yrs</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>
</tr>
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<td>Male, n (%)</td>
<td>13 (76%)</td>
<td>14 (82%)</td>
<td>13 (87%)</td>
<td>7 (78%)</td>
</tr>
<tr>
<td>White, n (%)</td>
<td>15 (88%)</td>
<td>11 (65%)</td>
<td>10 (67%)</td>
<td>6 (67%)</td>
</tr>
</tbody>
</table>

* HTN, hypertension; DM, diabetes; SBP, systolic blood pressure; DBP, diastolic blood pressure; CP, cardiopulmonary

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

† Hypertensive status of 8 subjects, diabetes status of 8 subjects, weight of 25 subjects, systolic blood pressure 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 are unknown.

§ Donor data for 24 subjects without rejection and 8 subjects with rejection are unknown.
Table 2. Multivariable Analysis Evaluating the Association of Acute Rejection with hs-cTnI

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Parameter Estimate</th>
<th>Adjusted p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of Acute Rejection</td>
<td>0.87</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Age</td>
<td>0.01</td>
<td>0.57</td>
</tr>
<tr>
<td>Male</td>
<td>-0.22</td>
<td>0.43</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.49</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Presence of Vasculopathy</td>
<td>0.26</td>
<td>0.007</td>
</tr>
<tr>
<td>History of CMV</td>
<td>0.10</td>
<td>0.67</td>
</tr>
<tr>
<td>Sample Time from Transplant</td>
<td>-0.0002</td>
<td>0.06</td>
</tr>
</tbody>
</table>

CMV, cytomegalovirus.
Table 3. Performance Metrics of the hs-cTnI Assay for the Assessment of Acute Rejection in the Entire Cohort and Selected Subgroups

<table>
<thead>
<tr>
<th></th>
<th>Entire Cohort</th>
<th>&gt;30D and &lt;1 year</th>
<th>&gt;1 year</th>
<th>No CAV</th>
<th>Cr &lt;1.78 mg/dl†</th>
<th>Cr ≥1.78 mg/dl‡</th>
<th>Cellular Rjxn</th>
<th>Acellular Rjxn</th>
<th>No sx or echo findings for rjxn</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>418</td>
<td>243</td>
<td>175</td>
<td>386</td>
<td>323</td>
<td>95</td>
<td>407</td>
<td>394</td>
<td>402</td>
</tr>
<tr>
<td>Rjxn episode, n</td>
<td>35</td>
<td>22</td>
<td>13</td>
<td>29</td>
<td>20</td>
<td>15</td>
<td>24</td>
<td>11</td>
<td>19</td>
</tr>
<tr>
<td>Sens, (%)</td>
<td>94%</td>
<td>91%</td>
<td>100%</td>
<td>93%</td>
<td>100%</td>
<td>87%</td>
<td>96%</td>
<td>91%</td>
<td>89%</td>
</tr>
<tr>
<td>Spec, (%)</td>
<td>60%</td>
<td>57%</td>
<td>65%</td>
<td>63%</td>
<td>61%</td>
<td>66%</td>
<td>60%</td>
<td>60%</td>
<td>60%</td>
</tr>
<tr>
<td>PPV, (%)</td>
<td>18%</td>
<td>17%</td>
<td>19%</td>
<td>17%</td>
<td>15%</td>
<td>33%</td>
<td>13%</td>
<td>6%</td>
<td>10%</td>
</tr>
<tr>
<td>NPV, (%)</td>
<td>99%</td>
<td>98%</td>
<td>100%</td>
<td>99%</td>
<td>100%</td>
<td>96%</td>
<td>100%</td>
<td>100%</td>
<td>99%</td>
</tr>
</tbody>
</table>

CAV, cardiac allograft vasculopathy; Cr, creatinine; Rjxn, rejection; PPV, positive predictive value; NPV, negative predictive value; Sens, sensitivity; Spec, specificity; Sx, symptoms.

*C AV was defined as concurrent or prior angiogram at the time of sample acquisition that demonstrated vasculopathy

† Samples with a creatinine <75th percentile value

‡ Samples with a creatinine ≥75th percentile value
Figure Legends

**Figure 1.** Flow diagram demonstrating the selection of serum samples utilized in the analysis.

**Figure 2.** Distribution of baseline cTnI in transplant patients 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.

**Figure 3.** Troponin I concentrations stratified by the presence or absence of rejection (panel A) and by ISHLT biopsy score (panel B). The line represents the median value, the boxes the 25th and 75th percentile and the error bars the 5th and 95th percentiles.

**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.
Flow Diagram of Derivation Cohort

101 Patients
Transplanted 2003-2010
> 3 months survival

99 Patients
(559 available samples)

19 Samples:
Inadequate Volume

24 Samples:
No Result (Technical Errors)

17 Samples:
>1 Sample Matched to Rejection

81 Samples:
≤ 30 Days Post-Transplant

98 Patients
(418 samples)

243 Samples:
< 1 Year Post-Transplant

175 Samples:
> 1 Year Post-Transplant
Figure 2.

Median: 9.45 ng/L

60th percentile: 15.0 ng/L

Percent of Patients

Troponin I (ng/L)

Circulation
Heart Failure

Journal of the American Heart Association
Figure 3A.
Figure 3B.

P\text{trend} < 0.0001

cTnl, ng/L (log scale)

0R: n = 258
1R: n = 131
2R: n = 15
3R: n = 4
Figure 4.

The graph shows box plots for cTnI levels (ng/L) on a log scale across different stages:

- **Pre-rejection**: n = 21
- **Rejection**: n = 35
- **Post-rejection**: n = 26

Significant differences are indicated by p-values:

- Pre-rejection vs. Rejection: p = 0.004
- Pre-rejection vs. Post-rejection: p = 0.002
A Sensitive Cardiac Troponin I Assay to Screen for Acute Rejection in Heart Transplant Patients

Circ Heart Fail. published online April 14, 2014;
Circulation: Heart Failure is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1941-3289. Online ISSN: 1941-3297

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circheartfailure.ahajournals.org/content/early/2014/04/14/CIRCHEARTFAILURE.113.000697

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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.\textsuperscript{1-3} 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.\textsuperscript{4, 5} A second dose of basiliximab (20mg IV) was administered on postoperative day 4.\textsuperscript{3} 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.\textsuperscript{6}

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 ≥ 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).
Supplemental Tables:

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>
<td></td>
<td></td>
</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>
<td></td>
<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>Time, min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Donor Characteristics†</strong></td>
<td></td>
<td></td>
<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>
</tr>
<tr>
<td></td>
<td>Black</td>
<td>Other</td>
<td>BMI</td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td>---------</td>
<td>--------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td><strong>Vasculopathy, n (%)</strong></td>
<td>16 (16%)</td>
<td>6 (10%)</td>
<td>10 (28%)</td>
<td></td>
</tr>
<tr>
<td><strong>CMV, n (%)</strong></td>
<td>32 (33%)</td>
<td>13 (21%)</td>
<td>19 (53%)</td>
<td></td>
</tr>
<tr>
<td><strong>Death, n (%)</strong></td>
<td>14 (14%)</td>
<td>3 (5%)</td>
<td>11 (31%)</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:


