Detection of Transient Myocardial Ischemia Using a Highly Sensitive, Single Molecule, Troponin I Assay

Petr Jarolim,1 Marc S. Sabatine,1 David A. Morrow,1 Stacy E.F. Melanson,1 Quynh Anh Lu,2 James A de Lemos,3 John A Todd,2 Eugene Braunwald,1

1Brigham and Women’s Hospital, Harvard Medical School, Boston, MA, 2 Singulex Inc., Hayward, CA, 3 University of Texas Southwestern Medical Center, Dallas, TX

Background
Small amounts of cardiac troponin may be released from cardiac myocytes in the setting of a reversible, ischemia-related, myocardial injury. We hypothesized that a highly sensitive troponin assay could permit the quantification of transient myocardial ischemia. The objective of the study was to ascertain whether or not an ultrasensitive assay can permit quantification of changes in circulating cardiac troponin (Tn) in the setting of stress test-induced myocardial ischemia.

Methods
A total of 120 patients without recent ischemia undergoing stress testing with myocardial perfusion imaging enrolled in the PROHeart Markers of Ischemia using Point-of-Testing (PROMPT) – TIMI 35 prospective cohort study. The baseline patient characteristics are summarized in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number</th>
<th>Mean ± SD</th>
</tr>
</thead>
</table>
| Age (years)               | 63 ± 11
| Male                      | 62 (52%) |
| Body mass index (kg/m²)   | 28 ± 5.9
| Diabetes                  | 86 (72%) |
| Hypertension              | 93 (78%) |
| Smoking                   | 79 (66%) |
| Hyperlipidemia            | 53 (44%) |
| Prior MI                  | 41 (34%) |
| Prior PCI                 | 62 (52%) |
| Prior CABG                | 28 (23%) |

Blood samples were obtained immediately before, immediately after, 2 hours and 4 hours after stress testing. Cardiac Troponin I (cTnI) was measured using the ultrasensitive Singulex Erenna System based upon capillary flow single molecule counting with nanoparticle immunoassay technology. The assay was standardized to National Institute of Standards and Technology material and validated with a lower limit of detection of 0.0002 ng/ml or 0.20 pg/ml. The coefficient of variation (CV) is 10% at 0.81 pg/ml and the 99th percentile in a healthy control population is 4 pg/ml. Cardiac troponin I was measured in 165 subjects using two other assays: the ACS-180 (cTnI assay; Life Sciences, Deerfield, IL), which has a limit of detection of 0.03 ng/ml and a CV of 10% at 0.48 ng/ml, and the cardiac troponin I assay on the Diaspace 1010-10 (Roche Diagnostics, Indianapolis, IN), which has a limit of detection of 0.01 ng/ml and a CV of 10% at 0.03 ng/ml. The ultrasensitive troponin assay offered the best accuracy (sensitivity 60%, specificity 69%, accuracy 64%) for prediction of ischemia. As shown in Figure 3, an increase in troponin I of >1.3 pg/ml after stress testing was strongly associated with ischemia and compared favorably with traditional metrics such as ST depression and limiting angina.

When patients were categorized on the basis of how many of the five risk variables were present (male sex, limiting angina, ST depression >0.1 mV, post-stress test BNP ≥1 mm ST depression 1.28 (0.35-4.71) 0.71 Limiting angina 2.61 (0.46-14.81) 0.28 1 mm ST depression 1.28 (0.35-4.71) 0.71 Limiting angina 2.61 (0.46-14.81) 0.28

Results
The median duration of angina during testing was 0, 0, and 3 min in patients with none, mild, and moderate/severe ischemia. Figure 1 shows cTnI levels measured using the Singulex assay in patients with none (blue circles), mild (green triangles), and moderate-to-severe (red squares) ischemia at baseline, immediately after stress testing, 2 hours after stress testing, and 4 hours after stress testing. P values are for trend across ischemic categories at each timepoint.

Patients were categorized for the primary analysis on the basis of the severity of ischemia as determined by myocardial perfusion imaging. Troponin concentrations were compared across groups using a non-parametric test for trend across ordered groups (Jonckheere-Terpstra test). The differences between baseline and post-stress test troponin levels within groups were compared using Signed Rank tests.

Using older troponin assays comparable to those used in prior studies investigating exercise-induced troponin release, the change in troponin I (troponin I at 4 hours minus troponin I at 0 hours) was a mediator of 0.00 mg/ml (in patients in each of the three myocardial ischemia categories (P=0.52) and 0.10 mg/ml (comparison across ischemic categories for troponin I and T, respectively).

We also measured troponin I using our current commercial assay, Tri-Labs. Only 0.2% of cTnI levels were above the 10% CV threshold (0.05 ng/ml at baseline and 0.15% at 4 hours. Using Tri-Labs, the change in troponin I by 4 hours was not significantly different across the three myocardial ischemia categories (P=0.36).

A cutpoint of >1.3 ng/ml for the rise in cardiac troponins 1 by 4 hours using the ultrasensitive assay offered the best accuracy (sensitivity 60%, specificity 69%, accuracy 64%) for prediction of ischemia. As shown in Figure 4, an increase in troponin I of >1.3 ng/ml after stress testing was strongly associated with ischemia and compared favorably with traditional metrics such as ST depression and limiting angina.

Conclusions
Transient stress-test-induced myocardial ischemia is associated with a quantifiable increase in circulating troponin levels, which is detectable with a novel, ultrasensitive Tri assay. This finding has potentially important clinical implications for the use of troponin for diagnosis and risk stratification for multiple cardiac conditions.

References


