Cardiac Troponin Assays
A View From the Clinical Chemistry Laboratory
Stacy E.F. Melanson, MD, PhD; Milenko J. Tanasijevic, MD, MBA; Petr Jarolim, MD, PhD

Many clinical chemistry laboratories perform troponin testing to aid in the diagnosis of acute coronary syndrome (ACS). Laboratories must interact with clinicians to establish diagnostic cut points, achieve acceptable assay performance, and troubleshoot questionable findings. Here we present 3 hypothetical scenarios related to troponin testing in the clinical chemistry laboratory.

Troponin: Its Utility in Acute Coronary Syndrome
Case Presentation 1: J.P. is an obese 55-year-old male with a history of gastroesophageal reflux disease who presents to the emergency department with substernal chest pain that he attributes to a “large meal.” J.P. is pale and diaphoretic. An ECG shows tachycardia with normal sinus rhythm. J.P.’s troponin levels (decision limit \(0.04 \mu g/L\)) at presentation and at 6 and 12 hours after presentation are 0.09, 4.41, and 19.62 \(\mu g/L\), respectively.

Performance and Analytical Accuracy of Troponin Assays
Cardiac troponin (cTn) has established itself firmly as the “gold standard” in the diagnosis of ACS. cTn should be measured in all patients presenting with symptoms suggestive of ACS, in conjunction with physical examination and ECG. Because of the specificity of cTn for myocardial damage, a single cTn above the decision limit, along with clinical evidence, is indicative of myocardial injury. However, serial testing can be useful, depending on clinical presentation and onset of symptoms.

cTn is a protein complex, located along the thin filaments of myofibrils, that regulates the contraction of cardiac muscle. It is composed of 3 distinct gene products. cTnC binds calcium, cTnT attaches to tropomyosin on thin filaments, and cTnI inhibits actomyosin ATPase. Expression of each subunit is cardiac restricted. The cardiac isoforms of cTnI and cTnT have additional unique N-terminal amino acid sequences, which allows for specific antibody and assay development and detection of each component in the bloodstream. The diagnostic utilities of cTnI and cTnT are comparable. Most cTn assays are sandwich immunoassays that use either monoclonal or polyclonal antibodies. It is essential that at least 1 of these antibodies be directed against the stable epitope of cTnI. Because cTn circulates in various forms and complexes, including free cTn, I-C binary complex, T-I-C ternary complex, and oxidized, reduced, and phosphorylated cTn, antibodies should detect all circulating forms, and the specificity for each should be described by the manufacturer.

The American College of Cardiology and the European Society of Cardiology in conjunction with the National Academy of Clinical Biochemistry recommend the use of a decision limit for myocardial injury at the 99th percentile of the reference population for cTnT and cTnI. Although it is acceptable to use the manufacturer’s decision limits, each laboratory should, if possible, define its own cutoff value based on its reference population. Furthermore, assays should ideally not exceed a total imprecision of 10% at the diagnostic cutoff. Table 1 lists troponin assays of leading in vitro diagnostic manufacturers and their claims for precision at the 99th percentile limit. These claims were reviewed by other investigators who reported long-term variations in the analytical performance. For this reason, each laboratory should validate the accuracy and precision of its troponin assay both on initial implementation and also every few months to verify assay performance.
Table 1. Manufacturer Claims for Decision Limits and Precision*

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Platform</th>
<th>Assay</th>
<th>99th Percentile Cutoff, µg/L</th>
<th>CV (%) at 99th Percentile</th>
<th>Achieving 10% CV Cutoff (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott</td>
<td>Architect</td>
<td>cTnI</td>
<td>0.022</td>
<td>NA</td>
<td>0.032</td>
</tr>
<tr>
<td>Beckman</td>
<td>Access 2</td>
<td>cTnI</td>
<td>0.04</td>
<td>14</td>
<td>0.06</td>
</tr>
<tr>
<td>Dade</td>
<td>Dimension Vista</td>
<td>cTnI</td>
<td>0.045</td>
<td>10</td>
<td>0.04</td>
</tr>
<tr>
<td>Ortho</td>
<td>Vitros Eci</td>
<td>cTnI</td>
<td>0.08</td>
<td>12</td>
<td>0.12</td>
</tr>
<tr>
<td>Roche</td>
<td>Cobas e601</td>
<td>cTnT</td>
<td>0.01</td>
<td>20</td>
<td>0.03</td>
</tr>
<tr>
<td>Siemens†</td>
<td>ADVIA</td>
<td>TnI</td>
<td>0.04</td>
<td>10</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Centaur</td>
<td>Ultra</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NA indicates not available.
*Data provided by manufacturer.
†Results verified by our laboratory.

Assay Interpretation
Despite continuous advances in troponin assays, there is still significant room for improvement, particularly in the area of assay standardization and elimination of interferences and various preanalytical factors. The absolute concentrations of cTnI are currently not comparable between manufacturers because of the different antibodies used in the cTnI assays. Although many laboratories calibrate using National Institute of Standards and Technology standards to minimize inter-laboratory variations, complete standardization would only be achieved if all manufacturers utilized the same antibodies, which is a difficult goal because of intellectual property and economic issues. In the absence of cTnI standardization, clinicians should not base clinical judgment on results obtained from different analyzers or laboratories.

Case 1: Discussion
J.P.’s presentation and troponin results are consistent with the diagnosis of ACS (Figure 1A). An additional cTnI measurement, 48 hours after admission, revealed a downward trend in his troponin level. Because of the improved sensitivity and precision at low cTnI concentrations, the initial cTnI result indicated myocardial injury and the subsequent diagnosis of ACS.

Increasing Sensitivity of Troponin Assays: A Diagnostic Dilemma
Case Presentation 2: R.S. is an 80-year-old female with a history of breast cancer who presents with “chest tightness.” Her ECG shows normal sinus rhythm. Troponin levels (decision limit >0.04 µg/L) at presentation and 9 and 20 hours after presentation are 0.07, 0.06, and 0.09 µg/L, respectively.

Interpretation of Assays With Higher Analytical Sensitivity
The threshold for myocardial injury has been lowered as cTnI assay performance has improved and as clinical data supporting the importance of cTnI in defining ACS have become available. Laboratories are implementing more sensitive cTnI assays. Although any troponin concentration above the 99th percentile of the reference range is indicative of myocardial injury, it may be difficult to determine the cause and clinical significance of minor troponin elevations (Figure 1B). The appearance of troponin 4 to 6 hours after the onset of chest pain, a peak in troponin at 12 to 16 hours, and a subsequent fall in troponin level would be consistent with the diagnosis of ACS; however, other conditions that result in myocardial cell damage, such as myocarditis, cardiac surgery, and sepsis, can increase cTnI levels.

The Future of Troponin Assays
Clinicians and scientists have advocated for better assays with improved clinical sensitivity and precision, and many manufacturers are developing new assays to meet these criteria. Current assays marketed by major in vitro diagnostic manufacturers’ measure concentrations of troponin in nanograms per milliliter, whereas assays in development by several smaller companies can detect concentrations in picograms per milliliter. The clinical impact of such changes for diagnostic applications requires careful assessment. Although assays with improved sensitivity will improve the diagnosis of ACS, they will also lead to an increase in the number of patients with slightly elevated troponin concentrations due to “minor” myocardial damage.

Increasing the sensitivity of cTnI assays will require changes in both reporting and interpretation of the results. Although clinical laboratories strive to provide cTnI assays with improved technical performance as they become available, it is understandable that clinicians may face difficulties interpreting analytically significant yet clinically minor cTn elevations. Close collaboration between pathologists and cardiologists is needed before and during implementation of more sensitive assays. On the basis of our experience, it may be possible to detect cTnI elevations in 1 to 3 hours from the onset of symptoms rather than the current 4 to 6 hours, thus allowing diagnostic intervals to be shortened.5 It may also be necessary to define age-, gender-, or race-specific cutoffs, because, for example, more sensitive assays may reveal increases in the 99th percentile limit with increasing age. Additional studies will be needed to define the clinical significance of minor releases in cTnI and its relation to cardiac tissue viability.

Case 2: Discussion
R.S. presents a diagnostic dilemma. Although her troponin concentrations are elevated above the decision limit, they do not change significantly for 24 hours after presentation and do not follow a classic rise and fall typical for ACS (Figure 1C). The differential diagnosis in this patient may include...
other causes, such as myocarditis or sepsis.

**Troponin: Is It Real?**

*Case Presentation 3:* L.M. is a 53-year-old male who presents with fever and “burning” in his chest after recent treatment for non-Hodgkin’s lymphoma. Cardiac biomarkers are ordered as part of his workup, and the results are listed in Table 2. ECG and echocardiogram are performed and reveal no significant findings. The disproportionately high troponin concentration in relation to the normal results of other tests and studies prompts the clinician to call the laboratory and question the troponin result.

**Human Anti-Mouse Antibodies**

Human anti-mouse antibodies (HAMAs) represent 1 of many types of human anti-animal antibodies. HAMAs are typically IgG and recognize epitopes on the Fc portion of the foreign immunoglobulin. The rapidly increasing incidence of therapy with mouse monoclonal antibodies is the main reason for the increasing prevalence of HAMAs in the population. Titers of HAMAs can remain elevated for years. Importantly, HAMAs can lead to analytical errors in immunoassays that use mouse monoclonal antibodies. False increases in analyte concentration can be seen when the HAMAs cross-link the capture and labeled antibodies in the absence of a specific analyte (Figure 2). Falsely low results can be obtained if the HAMAs react with 1 of the assay reagents and prevent reaction with the analyte.

Most immunoassays contain a non-specific “blocker” immunoglobulin designed to prevent interference from HAMAs, but the problem still exists. Several simple measures can be used to demonstrate the presence of or prevent the interference by HAMAs. Typically, if there is suspected interference by HAMAs, the laboratory may use an assay from a different vendor if available. Such an assay may use different antibodies and reagents that may not be prone to the interference in a particular specimen. Subsequently, serial dilutions of the specimen may be performed. If HAMAs are present, test results in diluted specimens are usually out of proportion to the dilutions. Finally, the sample can be pretreated with a commercially available heterophilic antibody-blocking reagent, which removes HAMAs before analysis.

**Table 2. Laboratory Results for Cardiac Enzymes for Patient L.M. (Case 3)**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Concentration</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatine phosphokinase</td>
<td>21 U/L</td>
<td>Male 41–266 U/L, female 27–218 U/L</td>
</tr>
<tr>
<td>Creatine kinase–MB</td>
<td>1.0 µg/L</td>
<td>0.0–5.0 µg/L</td>
</tr>
<tr>
<td>Troponin I</td>
<td>225.8 µg/L</td>
<td>&gt;0.04 µg/L, indicative of myocardial injury</td>
</tr>
</tbody>
</table>
Case 3: Discussion

Interference by HAMAs causing a falsely elevated troponin reading was suspected. The laboratory performed serial dilutions and incubated the specimen in the heterophilic blocking tube. Troponin readings were not proportional to the dilutions, and treatment with the blocking reagent led to a dramatic decrease in measured cTnI, from 225.8 to 0.09 μg/L. These results showed that L.M. had HAMAs that interfered with the troponin assay and yielded a falsely elevated result. The development was most likely triggered by rituximab used to treat L.M.’s non-Hodgkin’s lymphoma. The increasing incidence of therapy with incompletely humanized mouse monoclonal antibodies is considered the cause of the higher frequency of incorrect results caused by HAMA interference.

Disclosures

Dr. Jarolim serves as a consultant for Siemens Medical Solutions Diagnostics. The remaining authors report no conflicts.

References

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