Cardiac Troponin-T Immunoassay for Diagnosis of Acute Myocardial Infarction

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We evaluated the analytical and clinical performance of an immunoassay for cardiac troponin T (cTnT). Within-run and total imprecision ranged from 1.6 to 11.3%. The sensitivity and linear range was 0.015 and 13 μg/L, respectively. Frozen samples were stable for at least 8 weeks. No interferences were seen with lipids or bilirubin (total and conjugated). Hemoglobin caused a negative bias at concentrations > 4 g/L. Heparinized plasma showed a 6% negative bias compared with serum. The clinical utility of cTnT was compared with that of creatine kinase (CK)-MB (mass assay). The sensitivity of cTnT measurements from 63 patients with acute myocardial infarction (AMI) (cTnT cutoff 0.1 μg/L) was 90% at 0–3 h, 95% at 3–6 h, 94% at 6–9 h, 90% at 9–12 h, 99% at 12–24 h, 92% at 24–48 h, 83% at 48–72 h, and 100% at 72–96 h. Corresponding results for CK-MB (cutoff 5.0 μg/L and 2.5% relative index) were 45%, 84%, 82%, 97%, 87%, 81%, 54%, and 59%. The specificity of the markers from 49 non-AMI patients was 46% and 79% for cTnT and CK-MB, respectively. We show that CK-MB is more specific for diagnosis of AMI, and propose that cTnT is more sensitive to myocardial injury.

Indexing Terms: sandwich immunoassay/creatine kinase isoenzymes/serum cardiac marker/skeletal muscle injury/receiver-operating characteristic curves

Creatine kinase (EC 2.7.3.2) isoenzyme MB (CK-MB) in serum is the most widely used marker for the diagnosis of acute myocardial infarction (AMI) (1, 2). The development of automated immunoassays for CK-MB has led to improvements in analytical sensitivity and turnaround time (2–4). Clinical studies have shown that assay of cardiac troponin may challenge the role of CK-MB in diagnosis of AMI (5–10). Cardiac troponin T (cTnT) is a 37-kDa polypeptide subunit of the myofibrillar regulatory troponin complex (11, 12). It is one of the three major types (troponin-C, -I, and -T) found in striated muscle. Since most of the troponin complex is bound to contractile elements, a prolonged release of troponin following AMI is expected because of the destruction and clearance of structural elements from muscle cells. However, a small amount of troponin is present free in the cytoplasm, and this may be released within the first few hours after cell injury (13).

Assays for measuring serum concentrations of cTnT (8–10, 13–16) and cardiac troponin-I (cTnI) (6, 8, 17) have been reported; research assays for cTnT have led to the development of an automated 90-min immunoassay (16). Clinical trials conducted in Europe have reported the use of cTnT for the detection of AMI (7, 9, 10, 16). These studies either used earlier versions of the cTnT assay (9) or analyzer (ES22) (5, 10), or compared results with CK-MB by immunoinhibition (5, 9, 10).

The purpose of this multicenter study was to evaluate the analytical and clinical performance of the cTnT assay on the ES 300 for imprecision, transferability, detection limit, linearity, sample stability, interferences, and specimen collection requirements. We determined the reference range and verified a cutoff concentration for cTnT in serum for detecting myocardial injury. The clinical sensitivity and specificity of the cTnT assay for diagnosis of AMI was compared with the Stratus II mass assay of CK-MB. Assays involving cardiac-specific monoclonal antibodies to cTnT show promise because such antibodies exhibit little or no cross-reactivity with skeletal muscle troponin (18). Thus patients with skeletal muscle disease or injury, in whom serum CK-MB concentrations are increased, should not have high and potentially interfering concentrations of cardiac troponin (19).

Materials and Methods

Analytical Study

Testing sites. Three laboratory sites were included in this study. The primary and tertiary care hospitals were Hennepin County Medical Center (HCMC), Minneapolis, MN; University of Louisville Hospital (ULH), Louisville, KY; University of Texas Medical School (UTMHS), Houston, TX. The study was coordinated by Boehringer Mannheim, Indianapolis, IN. The same protocol was followed at all sites. Transferability studies were conducted at ULH, UTMSH, and Boehringer Mannheim. A study of blood collection tubes was performed only at HCMC. This protocol was reviewed and approved by Institutional Review Boards at each study site.

Analytical methods. All measurements of cTnT were made with an enzyme immunoassey on the ES300 (CardiATroponin T, Boehringer Mannheim) with streptavidin-coated tubes (16). This assay involves two
monoclonal antibodies. The capture antibody (M7) is specific for cTnT; the enzyme-labeled (horseradish peroxidase) antibody (1B10) has a 12% cross-reactivity with skeletal muscle troponin T (smTnT). Although these antibodies were raised against human cTnT, they also cross-react with bovine and rabbit cardiac tissue because of the high degree of interspecies homology (20). The calibration curve was constructed from 6 calibrators ranging from 0 to 13 μgL. Calibrators were prepared from purified bovine troponin T and put into a human serum protein matrix. The assay requires two on-instrument incubation steps of 60 and 25 min at 25°C.

For the clinical studies, CK-MB was measured by a mass assay on the Stratus II analyzer (Baxter Healthcare, Miami, FL). Total CK was measured with the Ektachem 700RX (Eastman Kodak, Rochester, NY) at HCMC, and with the Hitachi 717 analyzers (Boehringer Mannheim) at ULH and UTMSH. The percent relative index (%RI) was calculated as: [CK-MB (μgL)/total CK (UL)] × 100. Each of these assays was in routine use at these respective institutions. Quality control was performed according to current procedures at these hospitals. The reference ranges for total CK (UL), CK-MB (μgL), and RI (%) were 40–200, 0–7, and 0–2.0, respectively, at HCMC; 19–205 (males), 17–111 (females), 0–5, and 0–3 at ULH; and 83–200, 0–7, and 0–2.5 at UTMSH. We considered these differences in the reference range to be negligible, considering the range of values observed after AMI, and used a single limit for CK-MB of 0–5.0 μgL and 0–2.5% RI.

Imprecision and transferability. A modified “midi” EP3-T protocol was performed (21). For the external sites, three pools (low, medium, and high) from patients and one commercial control (Precitrol-N, Boehringer Mannheim) were assayed six times (24 samples/run) each day for 10 days (240 total samples). To assess transferability, we assayed 19 samples of fresh human serum at ULH and Boehringer Mannheim, and 10 samples at ULH, UTMSH, and Boehringer Mannheim, using the same lot of reagents. We also assayed identical-lot (lyophilized) kit controls at each clinical site.

Analytical sensitivity, linearity, and recovery. To determine the lowest detectable concentration, we measured the within-run (n = 20) precision of the absorbance measurement of the zero calibrator supplied with the reagent kit. Detection limits were defined as the mean absorbance values plus 3 SD relative to their respective concentrations on the calibration curve. To assess linearity, we diluted a serum sample containing a high cTnT concentration with the zero calibrator supplied by the kit. Measured cTnT concentrations were plotted against expected cTnT values, and a linearity fit plot was computed to indicate the goodness-of-fit to a straight line in terms of residual percent recovery from expected linearity. For recovery, to a serum sample containing no cTnT, we added human serum from a pooled sample containing a high concentration of cTnT to final concentrations ranging from ~3 to 7 μgL. Percent recovery was computed as the measured value/expected value × 100.

Stability of cTnT. Both short-term and long-term studies of sample stability were performed. We aliquoted and tested 12 serum samples from different patients in which cTnT concentrations ranged from 0.46 to 6.9 μgL. The tests were conducted at various time intervals up to 194 h after storage of the samples at 25°C, 2–8°C, and −20°C. cTnT results were plotted vs time of storage. A limit of ± 10% was used to assess stability. To assess long-term stability we stored samples from 40 patients with initial cTnT concentrations of 0.04–6.45 μgL for 3–8 weeks at −20°C. Results after storage were compared with measurements obtained before storage. Using SDs from our precision studies, we used acceptance criteria of <2 SD for samples with cTnT concentrations <1.0 μgL, and <3 SD for samples ≥1.0 μgL.

Interference studies. Various interferences were added to serum samples containing a measured concentration of cTnT. The interferences were as follows: Intralipid (Cutter Labs., Berkeley, CA) was added to produce lipid concentrations up to 12.5 g/L; a hemolysate prepared from heparinized blood was added to produce hemoglobin concentrations up to 10 g/L; unconjugated bilirubin (Fluka, Buchs, Switzerland) dissolved in 0.1 mol/L NaOH was added to produce bilirubin concentrations up to 645 mg/L; and an icteric serum sample containing 158 mg/L conjugated bilirubin (total bilirubin 208 mg/L) and no cTnT was added to produce bilirubin concentrations up to 13 μgL. A limit of ± 10% of the unsupplemented serum sample was used.

Plasma collection tubes. At one site (HCMC), nine patients presenting to the coronary care unit had blood drawn into four different collection tubes: red (no anticoagulant), green (lithium heparin, 14.4 USP mL); purple (K₂EDTA, 1.5 g/L), and blue (sodium citrate, 25.8 mmol/L), all from Beckton Dickinson (Rutherford, NJ). These tubes were used to assess the effects of anticoagulant interference in the assay; a 95% confidence (P <0.05) interval was used for comparing differences.

Reference range. We randomly selected and equally distributed 112 healthy ambulatory subjects from each hospital site for the reference range study. These subjects were deemed free of clinical complications or medications on the basis of interviews. CK-MB in these samples was measured by immunoassay. The reference range for cTnT was determined from the central 95% of values from this population. Many of these individuals were employees of the participating hospitals.

Clinical Study

Subjects. We obtained serial blood samples from 63 patients diagnosed by attending physicians at the time of discharge as having had AMI. The diagnosis, based on criteria established by the World Health Organization, included typical or atypical chest pain, unequivocal changes in the electrocardiogram (EKG), and increased activity of CK and concentrations of CK-MB on serial blood collections (22). In the absence of EKG evidence and a consistent clinical history, diagnosis of AMI may have been based solely on increases in total CK and CK-MB. The concentration of cTnT was not made
known to attending physicians and was not used in making the diagnosis. The onset of chest pain was noted in all patients included in this study. Patients were excluded if the time of onset was not known or not documented in the record. We also collected 116 serial blood samples from 49 patients with chest pain in whom a diagnosis of AMI was ruled out.

Sample collection. All samples collected for this study were ordered by attending or resident physicians as part of the management of patients; no additional samples were drawn solely for the purposes of this study. Informed consent was therefore deemed unnecessary by the Institutional Review Boards at the study sites. Samples were grouped into time intervals after the onset of chest pain: 2–6, 6–12, 12–24, 24–48, and 48–96 h. All were collected into evacuated red-top tubes containing no preservatives. The blood was allowed to clot and was centrifuged before analysis. Total CK and CK-MB were assayed within 8 h after collection and stored at 4°C. For cTnT, samples were stored at 4°C for up to 72 h or frozen at −20°C for up to 3 weeks before batch analysis. For AMI patients, samples were collected at regular intervals for up to 7 days from presentation in the emergency department. For non-AMI patients, samples were collected on the first and second day after admission. We prepared receiver-operating characteristic (ROC) curves for cTnT vs CK-MB at various time intervals after the onset of chest pain.

Results
Analytical Study

Assay calibration. In Fig. 1 we show several calibration curves obtained from all three sites. These data demonstrate the consistency in range of absorbance values and response slopes between laboratory sites and for several individual assay runs from each site. The curves selected (of 12 from each site) represent the beginning, middle, and end of the 90-day evaluation period. The curves for the low concentrations are expanded in Fig. 1A to contrast the reproducibility of the standards between sites.

Imprecision and transferability. Table 1 shows representative data for within-run and total imprecision at one of the testing sites. The values are within limits established by the manufacturer. Results of split samples demonstrated good transferability of results between sites (BMC vs ULH, y = 1.037x + 0.008 μg/L, r = 0.999, n = 21; and UTMSH vs ULH, y = 1.050x − 0.055 μg/L, r = 0.991, n = 10). The relative accuracy between laboratory sites was also tested with controls provided with each reagent kit and assayed once during each run of the project. Mean results from the three laboratories for two levels of controls were 0.14, 0.17, and 0.17 μg/L for the low control, and 4.81, 4.60, and 4.71 μg/L for the high control.

Analytical sensitivity, linearity, and recovery. To determine the lowest detection limits, we calculated the average absorbance values (SD) for the zero calibrator using 20 samples: 0.0153 (0.0011), 0.0194 (0.0017), and 0.0066 (0.0010) μg/L for the HCMC, ULH, and UTMSH sites, respectively. Corresponding detection limits computed from these data were 0.0187, 0.0246, and 0.0007 μg/L, respectively, with an average of 0.015 μg/L. For linearity, the plots of expected concentrations of cTnT (y) vs measured values (x) produced a regression curve of y = 1.00x − 0.0004 μg/L, r = 0.997, n = 30, for the three sites combined. These results show that the cTnT assay is linear up to at least 13 μg/L. With a sensitivity of 0.015, the dynamic range of the assay covers more than three orders of magnitude. The recovery values for a blank serum sample supplemented with high cTnT were 93%, 100%, and 101% for expected cTnT concentrations of 3.84, 6.33, and 6.90 μg/L, respectively.

Stability of cTnT. In Fig. 2 we show the percent recovery from the starting concentration for all samples combined for the three temperatures tested. cTnT is stable for at least 194 h at −20°C and 146 h at 2–6°C. Stability at room temperature is lost after 24 h. For long-term stability, 38 of 40 samples (95%) met our criteria for stability when stored at −20°C for 8 weeks.
These results were measured within the first 6 hours of blood collection, and the recovery for each group was calculated.

**Interference studies.** In Fig. 3 we show the results of the interference studies with Intralipid, hemoglobin, and total (unconjugated) direct bilirubin. Only hemoglobin at concentrations >4 g/L demonstrated interference in the cTnT assay. For direct bilirubin at concentrations of 53, 79, 105, and 132 mg/L, the recovery values for cTnT were 100%, 98%, 100%, and 102%, respectively (data not plotted), indicating no significant interference.

**Plasma collection tubes.** To determine the acceptability of drawing blood in different blood collection tubes, we collected blood from nine patients at HCMC into each of the four different tubes. Mean (SD) results for blood collected in heparin, EDTA, citrate, and no preservatives (serum) were 4.52 (6.55), 5.67 (7.26), 4.20 (5.58), and 4.82 (6.15) µg/L, respectively. A >5% average deviation of recovery compared with serum was observed with all the anticoagulants used. A t-test comparison of means relative to serum produced results of t = 0.94, 1.86, and 2.52 for heparin, EDTA, and citrate, respectively (t < 2.306 was required for P = 0.05). Thus, heparin had the lowest deviation compared with serum.

**Reference range.** All normal subjects had CK-MB values that were within the reference range established at each institution. The results for cTnT are shown in Fig. 4 and indicate a skewed distribution. Using the central 95%, we calculated the reference range to be 0–0.08 µg/L. This is within the cutoff limit of 0.10 µg/L recommended by the manufacturer for diagnosis of myocardial injury.

**Clinical Study**

**Diagnostic efficiency for AMI.** Table 2 shows the data for cTnT and CK-MB for all the patients studied. For AMI patients, the data are further grouped according to the reported time of onset of chest pain. In Fig. 5, ROC curves are presented that indicate the clinical sensitivity and specificity at different cutoff limits. Fig. 6 shows results with single cutoff limits of 0.1 µg/L for cTnT and a combined cutoff of 5.0 µg/L and 2.5% RI for CK-MB. These data show that during the critical first 6 h after onset of chest pain, the clinical sensitivity for cTnT and CK-MB are roughly equivalent. The sensitivity for CK-MB is highest between 9 and 48 h, after which time values begin to decrease. In contrast, the sensitivity for cTnT remains high 96 h after onset. Fig. 7 shows mean results for all patients in the study.

The clinical specificities of cTnT and CK-MB in 49 patients in whom the diagnosis of AMI was ruled out were 46% and 79%, respectively. Increasing the cutoff concentration for cTnT to 0.2 µg/L improved the specificity to 54%, as shown in Fig. 5. Retrospective reviews of the medical records indicated that many of the patients that were positive for cTnT and negative for CK-MB had multiple illnesses besides cardiovascular disease, such as pneumonia, liver disease, stroke, cancer, and cardiac failure.
Fig. 4. Distribution of cTnT values measured in serum from 112 healthy adults on the ES-300.
Numbers on bars are the number of subjects with values at the cTnT concentrations indicated.

Discussion

This study describes the first evaluation of a cTnT assay performed on the ES300 analyzer in the US. The imprecision studies for cTnT from three laboratories demonstrated CVs that compare well with other reports (5, 10, 13, 16). Between sites, the day-to-day reproducibility of controls gave consistent values with no drifts or shifts, demonstrating assay transferability. The linear range for the cTnT assay was broad. Of serum samples obtained from AMI or non-AMI patients, <10% required dilution. The absorbance response of the calibration curve within the concentration range of the calibrators was reproducible between sites (Fig. 1). Dilution of serum specimens gave linear recoveries of expected cTnT values throughout a wide concentration range, sufficient to meet clinical needs reported by others (16, 17). With regard to plasma samples, only tubes containing heparin produced results comparable to those obtained with serum. We did not attempt to explain why citrate and EDTA tubes were unacceptable.

Interference studies showed that hemoglobin concentrations (>4 g/L) reduced the recovery of cTnT in serum by >10%. Although the mechanism was not studied, the interference may be related to the initial absorbance at 405 nm, with the reaction rate being influenced by non-specific adherence and absorbance of hemoglobin or red cell constituents remaining after the washing procedure. In any event, slight to moderate hemolysis (hemoglobin <4 g/L) did not cause interference. Bilirubin and lipids did not interfere with this assay.

Serum samples from 112 healthy individuals had cTnT values ≤0.1 μg/L; most (96%) had <0.05 μg/L. Although such concentrations produce an upper reference limit of 0.08 μg/L, we used a decision limit of 0.1 μg/L for the clinical studies. A higher cutoff concentration of 0.2 μg/L was used in studies performed in Europe (5, 16). Our cutoff limit was selected to optimize clinical sensitivity to minor myocardial injury.

An objective of the clinical study was to determine if cTnT was effective for diagnosis of AMI during the first 6 h after onset of chest pain. Release of cTnT from the cytosolic pool may enable earlier detection of AMI than other cardiac markers if a low cutoff concentration is used. However, our results, showed a clinical sensitivity of only 63% from 0–6 h for cTnT, which is insufficient for effective early diagnosis. These findings are in agreement with those of Mair et al. (10), who reported a 57% sensitivity for emergency department patients (mean time of presentation 4 h); Katus et al. (9), who reported a 50% sensitivity for patients presenting within 3 h; and Bodor et al. (17), who reported sensitivities of 17% between 0 and 4 h and 77% between 4 and 8 h after chest pain for cTnI.

Our results also confirm other reports that cTnT remains abnormally increased in serum for a much longer time after onset of AMI than does CK-MB. This corresponds to the gradual release of proteins due to degradation of structural elements (10, 11, 17, 23). The cTnT assay will eventually obviate the need for assays of lactate dehydrogenase isoenzymes.

The clinical specificity of 46% reported in this study is much lower than in previous reports. Bodor et al. found cTnI sensitivities of 78% and 83% in patients with and without chronic ischemic cardiac disease, respectively (17). Gerhardt et al. reported a 98% specificity, although some non-AMI cases with minor myocardial damage

### Table 2. cTnT and CK-MB in AMI and non-AMI patients.

<table>
<thead>
<tr>
<th>Hours after onset of chest pain</th>
<th>No. of subjects</th>
<th>cTnT, μg/L</th>
<th>Total CK, U/L</th>
<th>CK-MB, μg/L</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Range</td>
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<tr>
<td>0–3</td>
<td>20</td>
<td>0.48</td>
<td>1.03</td>
<td>0–4.8</td>
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<tr>
<td>3–6</td>
<td>22</td>
<td>0.54</td>
<td>0.83</td>
<td>0–3.5</td>
</tr>
<tr>
<td>6–9</td>
<td>17</td>
<td>6.1</td>
<td>7.3</td>
<td>0–25.4</td>
</tr>
<tr>
<td>9–12</td>
<td>29</td>
<td>4.4</td>
<td>8.5</td>
<td>0.04–42.1</td>
</tr>
<tr>
<td>12–18</td>
<td>31</td>
<td>9.2</td>
<td>11.3</td>
<td>0.17–45.5</td>
</tr>
<tr>
<td>18–24</td>
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<td>6.5</td>
<td>9.0</td>
<td>0.05–33.7</td>
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<tr>
<td>24–36</td>
<td>53</td>
<td>5.9</td>
<td>14.1</td>
<td>0–98.2</td>
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<tr>
<td>36–48</td>
<td>11</td>
<td>14.7</td>
<td>41.2</td>
<td>0–146</td>
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<tr>
<td>48–72</td>
<td>24</td>
<td>12.4</td>
<td>24.3</td>
<td>0.04–95.8</td>
</tr>
<tr>
<td>72–96</td>
<td>17</td>
<td>4.2</td>
<td>7.7</td>
<td>0.12–34.2</td>
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<tr>
<td>&gt;96</td>
<td>18</td>
<td>3.0</td>
<td>1.5</td>
<td>0.87–5.78</td>
</tr>
<tr>
<td>Non-AMI</td>
<td>116</td>
<td>1.2</td>
<td>2.7</td>
<td>0–16.0</td>
</tr>
</tbody>
</table>
were added to the AMI group on the basis of increased CK-MB (5). (When this minor injury group was included in the non-AMI group, the specificity decreased to 87%.) Mair et al. reported a sensitivity of 96% from 96 patients in the emergency department (10). Patients in this population included those with unstable angina, other cardiovascular diseases, and miscellaneous disorders. We believe that the discordance in our data was due to differences in the inclusion criteria for patients selected in our study as compared with others. We purposely included serum from critically ill patients and those with multisystem disorders. We suggest that increased cTnT in some of these non-AMI patients was due to the existence of minor myocardial injuries not detected by CK-MB.

Other explanations for high cTnT in these patients are possible. Patients with rhabdomyolysis are likely to have high smTnT concentrations. Any degree of cross-reactivity or nonspecific binding to smTnT in the cTnT assay will produce serum concentrations that exceed the cutoff. Previous studies have shown a 3.8% nonspecific binding to streptavidin-coated tubes with the Boehringer Mannheim assay of purified smTnT (16). However, this appears to be an artifact of the preparation of smTnT, because there was no cross-reactivity when equivalent smTnT concentrations from serum samples...
were used (16). Also, cTnT may be upregulated after skeletal muscle injury. Although cTnT is absent in normal human skeletal muscles, cardiac and skeletal muscle forms are coexpressed during fetal development (24). Fetal cTnT isoforms that are normally absent in adult heart tissue are transiently expressed in adult patients with failing hearts (25). Moreover, fetal expression of cTnT has been demonstrated in regenerating skeletal muscle fibers of rats subjected to cold injury and denervation (26). However, there are no reports to date to suggest that cardiac expression of troponin T occurs after repair of skeletal muscle injury in humans.

The ROC curves (Fig. 5) show that cTnT has a lower clinical specificity and diagnostic efficiency than CK-MB in the diagnosis of AMI. If the objective of the cTnT assay is diagnosis of AMI, our data suggest that CK-MB may be superior to cTnT during the first 48 h. However, if detection of myocardial injury (to include AMI) is the objective, then cTnT maybe more sensitive and specific than CK-MB. As shown in Fig. 8, the cutoff concentration of CK-MB is set a few units above the upper reference limit so that patients with minor myocardial injury may have higher-than-normal CK-MB concentrations that are still below the cutoff. Some investigators have lowered CK-MB limits to detect minor myocardial injury (27, 28). However, the utility of CK-MB will ultimately be limited by the inability of the marker to distinguish myocardial injury from skeletal muscle damage. For cTnT, selecting a cutoff at the upper limit of normal (Fig. 8) could be useful for detecting minor myocardial injury because there is no cTnT contribution from skeletal muscles. A higher cTnT cutoff limit could be used to improve clinical specificity for AMI diagnosis; however, the detection of minor myocardial injury will be lost.

The use of cTnT for detecting evidence of minor ischemia may have new applications in the management of patients with unstable angina. Hamm et al. showed that patients with unstable angina and abnormal cTnT concentrations were more likely to develop AMI or to die during hospitalization (29). Collinson et al. reported four patients with unstable angina and abnormal cTnT who developed cardiac events during the subsequent 6 months (30). Unstable angina patients identified as being at high risk may prompt cardiologists to be more aggressive in treating their patients.

Our results for cTnT are consistent with the concept that irreversible myocardial injury follows a progressive and continuous pattern of injury. This pattern is characterized by (a) minimal release of cytoplasmic proteins after reversible injury; (b) development of a subendocardial MI with a higher degree of protein release (small, irreversible injury); and (c) progression to a transmural infarct with the highest degree of enzyme release (substantial irreversible injury). Under this assumption, many patients with unstable angina will have injury detectable by increased concentrations of a sensitive and specific marker such as cTnT.

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