The case for cardiac troponin T: marker for effective risk stratification of patients with acute cardiac ischemia

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Availability of markers such as cardiac troponin T (cTnT) has brought new insights into ischemic heart disease (IHD). cTnT is a distinct protein that differs from other markers in biological function, molecular mass, and cytosolic pool. cTnT has been utilized for diagnosis of acute myocardial infarction (AMI) and risk stratification of patients with IHD. For AMI diagnosis, cTnT showed high sensitivity (94–100%) but generally lower specificity (46–99%), possibly because of increases in non-AMI patients with minor myocardial damage. Outcome studies have demonstrated that IHD patients with increased cTnT are at significantly greater risk for cardiac events; revascularization in patients with increased cTnT may improve outcome. Estimated costs for batched ES 300 cTnT results and for a cTnT rapid assay run “on demand” were $17.48 and $21.65, respectively. cTnT currently has no specific common procedure test code; expected reimbursement is $18.32 for the ES 300 and is not established for the rapid assay.

INDEXING TERMS: myocardial infarction • heart disease markers • cost analysis • creatine kinase MB isoenzyme • electrocardiogram

According to the National Heart Attack Alert Program (NHAAP), an estimated 1.25 million Americans have an acute myocardial infarction (AMI) each year.1 Of these, 500 000 are classified as “sudden deaths” and 700 000 AMI patients are hospitalized. Further, ~3 million additional patients are admitted to medical centers for whom AMI eventually is ruled out. This conservative admission practice is necessary because of the morbidity, mortality, and diagnostic difficulty involved with AMI. Despite this approach, AMI goes undiagnosed in ~30 000 to 50 000 individuals each year, accounting for the largest source of malpractice dollars in US emergency departments (EDs) [1]. Overall, the NHAAP estimates that coronary artery disease affects 6 million individuals in this country.

The 1990s have been a period of active evolution for the treatment, monitoring, and assessment of the acute coronary syndromes, including AMI. In 1993 data from the “Global Utilization of Streptokinase and Tissue Plasminogen Activator” (GUSTO) megatrial were released [2]; this important study elucidated the relative efficacy of various thrombolytic regimens and confirmed the open artery hypothesis. The 1990s have also been a time for the advancement of technology used for electrocardiographic (ECG) monitoring [3]. In fact, specific ECG observations are the criteria for thrombolytic therapy in the US and improvements in ECG technology have undoubtedly streamlined care for many MI patients. Although the ECG is a vital component of care for the AMI patient, it is important to note that only 24–60% of AMI patients show diagnostic ECG on admission and are thus candidates for thrombolytics [4]. Biochemical markers of myocardial injury are considered the “gold standard” for the diagnosis of AMI [5] and are particularly important in the nondiagnostic ECG patient. Care for the nondiagnostic ECG AMI patient has been facilitated by implementation of specific areas (mainly in the ED termed chest pain evaluation centers (CPEC) [6]. These specific areas are intended for the systematic and cost-effective care of the “rule out MI patient” [6]. Biochemical markers are particularly important in the CPEC environment and will continue to play an essential role in the context of cardiac ischemia.

Various biochemical markers used in the context of cardiac ischemia are listed in Table 1. The MB isoenzyme of creatine kinase (CK-MB) represents the benchmark for comparison with other biochemical markers because the characteristic rise and fall of CK-MB in serial measurements is nearly pathognomonic for AMI [7]. Utilizing “mass” CK-MB assays and a strategy that included sampling at presentation, 3 h, 6 h, and 9 h, Gibler et al.
Table 1. Characteristics of selected biochemical markers.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Diagnostic window</th>
<th>Heart/skeletal</th>
<th>Molecular mass</th>
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<tbody>
<tr>
<td>Myoglobin</td>
<td>2–18 h</td>
<td>++++/+/-/++</td>
<td>17.8 kDa</td>
</tr>
<tr>
<td>CK-MB</td>
<td>4 h to 3 days</td>
<td>++++/+/-/++</td>
<td>85 kDa</td>
</tr>
<tr>
<td>MB isoforms</td>
<td>3 h to 1.5 days</td>
<td>++++/+/-/++</td>
<td>85 kDa</td>
</tr>
<tr>
<td>cTnT</td>
<td>3 h to 14–21 days</td>
<td>++++/0*</td>
<td>37 kDa</td>
</tr>
<tr>
<td>cTnI</td>
<td>3 h to 7 days</td>
<td>++++/0</td>
<td>24 kDa</td>
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*Reportedly increased in some renal failure patients and extreme skeletal muscle injury/disease.

documented a sensitivity of 100% and specificity of 98.3% for diagnosis of AMI [8]. Despite these findings, CK-MB is not a perfect marker because characteristic release requires 8–12 h after onset of symptoms for efficient AMI diagnosis with high sensitivity and specificity [7]. Also, CK-MB is not cardiac specific [9]; this nonspecific issue is particularly problematic for interpretation in patients with concomitant myocardial and skeletal muscle damage [7, 9]. Myoglobin measurement has also been used in the context of cardiac ischemia because of its early release after AMI [10]. Brogan et al. reported that myoglobin was a more sensitive marker of myocardial necrosis than CK-MB during the first 3 h of ED presentation [11]. In a similar study, Tucker et al. showed that the sensitivity within 4 h of the onset of symptoms was 78% for myoglobin vs 63% for CK-MB [12]. Although myoglobin is rapidly released after myocardial injury, it must be considered a nonspecific marker because of its abundance in both skeletal and cardiac muscle [10]. However, the early release and the excellent negative predictive value demonstrated suggests that myoglobin may be a powerful tool for triage of chest pain patients [10–14]. The MB isoforms have also demonstrated promise for diagnostic use early in the course of myocardial damage [15–17]. However, the MB isoforms have not commanded widespread clinical use to date because the MB isoforms have the same tissue specificity issues as CK-MB, and rapid and simple techniques for routine analysis are not fully developed. Inadequacies of biochemical markers currently in use have generated considerable interest towards development of more cardiac-specific markers such as troponin T (cTnT) and troponin I (cTnI).

The troponin complex plays an essential role in the contraction of striated muscle. This complex consists of three subunits: troponin C, which binds to calcium and regulates contraction, M, 18; troponin I, which inhibits actomyosin ATPase, M, 24; and troponin T, which binds the troponin complex to the tropomyosin strand, M, 37 [18]. Both troponin T and troponin I have amino acid sequences that differ between adult skeletal and cardiac muscle at numerous locations [18]. Antibodies have been developed that recognize these cardiac-specific sequences; these antibodies form the basis for cTnT and cTnI immunoassays [18]. Troponin C has no potential as a cardiac-specific marker because its amino acid sequence is identical in both skeletal and cardiac muscle [19].

cTnT and cTnI must be considered two distinct proteins. A most important contrast between the proteins is the larger cytosolic pool for cTnT, quoted at 6% [20] and 8% [21], vs the smaller 2.8% cytosolic pool for cTnI [22]. The combination of a relatively large cytosolic pool combined with cTnT’s extended lifetime in blood may explain, in part, cTnT increases in patients with the minor myocardial damage that can occur during acute cardiac ischemia. Also, there may be an analytic component to this issue. In data available in package inserts from the respective manufacturers, the analytical sensitivity of Boehringer Mannheim’s cTnT assay is listed as 0.03 µg/L, whereas the sensitivity of Dade International’s cTnI assay is listed as 0.4 µg/L. Although standardization of results may be an issue, the higher apparent analytical sensitivity of the cTnT assay may account for detection of cTnT release with minor myocardial injury.

Concerns classified as analytical, biological, and issues with unknown significance have been discussed regarding the clinical utilization of cTnT. The analytical aspect refers to the fact that the specificity of the cTnT assay has been challenged by reports of nonspecific binding in cases of extensive skeletal muscle injury [23]. This issue has been attributed to a 12% cross-reactivity of the detection antibody (1B10) with skeletal muscle troponin T. A second-generation cTnT assay that incorporates more cardiac-specific antibodies for both capture and detection will apparently address this analytical worry [24, 25]. Biological issues involve reports documenting cTnT increases in the absence of cardiac injury when skeletal muscle is regenerating, such as with polymyositis and dermatomyositis [26]. These false positives are thought to occur because cTnT is expressed in skeletal muscle during fetal development [27]; proteins expressed during development may be reexpressed during tissue regeneration. Although one study in rats showed that cTnT was reexpressed in response to skeletal muscle injury [28], recently published data on a test population subjected to arduous physical training provide indirect evidence that cTnT was not reexpressed during regeneration [29]. Alternatively, it is possible that increased cTnT in polymyositis may reflect a cardiac component seen as a complication with disease progression. Apparent “false-positive” cTnT increases have been documented with end-stage renal disease patients [30–32]. The true clinical significance of cTnT in these patients must be considered unknown at present because sorting out if the increases are analytical interferences, from reexpression due to uremic polyneuropathy, or are cardiac in origin can only be reliably determined in an appropriately designed outcome study. Of interest, cTnI was shown to be increased in critically ill patients without recognized cardiac involvement [33].

Utilization of cTnT for the assessment of suspected AMI patients is possibly a major application. Wu et al. [34] showed that cTnT was a sensitive marker of myocardial injury during the first 6 h after chest pain and was comparable with a “gold standard” CK-MB mass assay. Thereafter, the clinical sensitivity of cardiac TnT exceeded CK-MB [34] because of the prolonged release of the structurally bound cTnT pool. However, this study also found that cTnT’s diagnostic specificity was only 46%, possibly because a substantial number of patients in this study had minor myocardial damage due to unstable angina [34]. Table 2 shows data from studies indicating that peak cTnT
Table 2. Diagnostic sensitivity and specificity of peak cTnT.

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<tr>
<th>Reference</th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tr>
<td>20</td>
<td>100%</td>
<td>78%</td>
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<tr>
<td>35</td>
<td>97%</td>
<td>99%</td>
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<tr>
<td>36</td>
<td>100%</td>
<td>69%</td>
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<tr>
<td>37</td>
<td>100%</td>
<td>93%</td>
</tr>
<tr>
<td>34</td>
<td>94%</td>
<td>46%</td>
</tr>
<tr>
<td>38</td>
<td>100%</td>
<td>88%</td>
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Note: The different patient populations and comparative methods included in these studies may explain, in part, the different diagnostic performances.

concentration shows sensitivities between 94% and 100% and specificities between 46% and 99% for diagnosis of AMI [20, 34-38]. It is worthwhile to consider that different patient populations were studied and the CK-MB “benchmark” used to arrive at the data in Table 2 was determined by a variety of analytical methods, which may partly explain differences in diagnostic performance.

Numerous studies have examined cTnT’s role in reperfusion assessment [39], infarct sizing [40], perioperative myocardial infarction [41, 42], and detection of minor myocardial damage in acute cardiac ischemia [20, 35]. This final role is very significant because several studies have indicated that patients with acute cardiac ischemia in whom myocardial infarction was ruled out, but in whom the cTnT value exceeded a defined cutoff, were at increased risk for frank AMI or cardiac death [36, 37, 43-46] (see Table 3). Although different cutoffs were used in these studies, the association of cTnT with increased risk of adverse events was clearly demonstrated in a metaanalysis study by Wu and Lane [47]. This metaanalysis demonstrated that patients with increased cTnT have fourfold increased odds for an adverse event compared with those patients for whom cTnT was negative [47].

cTnT utilization for risk stratification is of particular importance if outcome can be improved through intervention in patients for whom cTnT is increased. One study [48] showed such importance in a group of 62 patients, all of whom had cTnT values ≥0.2 μg/L. Of the 22 patients in this group who underwent revascularization, only one died; this compared with 11 deaths in the remaining 40 patients who did not undergo revascularization [48]. Although the number of patients was not large, these data suggest a role for cTnT in revascularization decisions.

Several studies suggested that markers such as CK-MB and the ECG may also have roles for risk stratification in patients for whom AMI is ruled out, leading to the question: Does cTnT reveal any new information? An outcome study was undertaken to investigate the relative usefulness of cTnT, the ECG, and CK-MB for risk assessment as part of the Global Use of Strategies to Open Occluded Coronary Arteries IIa (GUSTO IIa) trial [46]. The GUSTO IIa substudy included 854 patients, all of whom had symptoms of cardiac ischemia within 12 h of enrollment and an abnormal ECG showing transient or persistent ST elevation or depression, or a persistent T-wave inversion. CK-MB was measured by a state-of-the-art mass assay on the Stratus II analyzer (Dade International, Miami, FL) in serum samples taken at patient presentation. Concurrent cTnT measurements were made with the enzyme immunoassay on the ES 300 (Boehringer Mannheim Corp., Indianapolis, IN). The reference ranges used were 0–7 μg/L for CK-MB and <0.1 μg/L for cTnT. Although earlier studies used a 0.2 μg/L cutoff for cTnT (see Table 3), a lower cutoff was used in the GUSTO IIa study to better discriminate between patients with no injury vs those with minor injury [34]. Table 4 shows results of a model developed with logistic regression for the GUSTO IIa substudy.

<table>
<thead>
<tr>
<th>Table 3. Literature review of publications with outcome events in patients for whom AMI was ruled out.</th>
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<th>Table 4. Logistic regression model for risk stratification with cTnT.</th>
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<td>Model used</td>
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<tr>
<td>Overall model</td>
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<tr>
<td>Univariate model</td>
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<td>Univariate model</td>
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<tr>
<td>Univariate model</td>
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<tr>
<td><strong>Clinical utilization strategy</strong></td>
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<tr>
<td>Perform ECG first</td>
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<tr>
<td>Then measure cTnT</td>
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<tr>
<td>Measure CK-MB instead of cTnT</td>
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<tr>
<td>Perform ECG, cTnT &amp; CK-MB</td>
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* C Index = concordance index; this index represents the area of the receiver operator characteristic curve.

°Note that $\chi^2$ and C Index are the same as strategy with ECG and cTnT without CK-MB. Thus CK-MB provides no added value to model with ECG and cTnT.
data [46]. Univariate analysis in GUSTO IIa showed that cTnT was the most powerful indicator of adverse outcome in the 30 days subsequent to their clinical presentation, compared with either CK-MB mass or the ECG. Further, combined analysis of ECG, cTnT, and CK-MB confirmed that cTnT added the most information; CK-MB provided no added value to the model including ECG and cTnT.

GUSTO IIa’s finding that cTnT was the strongest indicator of adverse events [46] may be viewed as being in contrast to the conclusion of a study by Ravkilde et al. [44], who reported that neither CK-MB nor cTnT added information to that provided by the ECG. The conflicting results may be due to the larger number of patients included in GUSTO IIa [46], the different test populations included, and the higher cTnT decision limit (0.2 μg/L) used by Ravkilde et al. [44], as well as the different CK-MB assays used in these two studies.

Because available data indicate that measurement of cTnT is an important adjunct for risk stratification and triage of patients with acute cardiac ischemia, it is prudent to assess the logistic feasibility, cost, and reimbursement expectations for providing cTnT results to caregivers. cTnT measurements are available in two formats: the ES 300, a batch analyzer utilizing a single-step enzyme immunoassay and streptavidin-coated tubes [49], and as a rapid assay that uses whole blood and no additional equipment [50, 51]. The ES 300 is a quantitative method that requires a serum sample and has an on-analyzer time to first result of 85 min. Alternatively, a cTnT rapid assay has been cleared recently by the Food and Drug Administration. The rapid assay is a self-contained device that yields qualitative results, with a cutoff of 0.2 μg/L, within 20 min of sample application. The rapid assay shows substantial promise for either in-laboratory and (or) point-of-care testing.

Costs and reimbursement for all laboratory testing, including cTnT, are an increasingly important issue. Costs will differ depending on whether the test will be utilized for batch testing or “on demand.” Because of the characteristics of the ES 300 cTnT format, the cost for performance will be calculated on a batch testing basis. Costs for the rapid assay will be estimated for utilization singly, on demand. Relatively simplistic approaches will be used to estimate costs here because methodology for calculating actual expense can vary greatly and these issues must be evaluated at each individual institution.

As an example analysis for the ES 300, we will assume that the cost per reagent tube is $8.00, which includes rental of the ES 300 equipment. We will assume that analytical runs will be performed once each shift, 7 days per week. Each batch will include five patients’ samples, run singly, on the ES 300; one-point calibration and two levels of control are required laboratory practice for each run. The reagent component per reportable result for the ES 300 is:

Reagents = $8.00 × 8 tubes/5 results = $12.80 per reportable result.

To cover for any specimen repeats, a 10% repeat/dilution rate will be assumed:

Specimen repeats = $12.80 × 0.10 = $1.28 for repeats/dilutions.

At the manufacturer’s suggested calibration schedule (6 calibrators in duplicate) performed every 2 weeks, the cost will be spread over 14 days at 3 shifts/day:

Calibration = {[$8.00 × 12]/(14 days/calibration × 3 runs/day)}/5 results = $0.46 per result for calibration.

Thus,

Estimated supply cost = $12.80 (reagents) + $1.28 (repeats/dilutions) + $0.46 (calibration) = $14.54 for supplies per reportable result.

For estimating the technical component of ES 300 testing, we will assume that 45 min of hands-on time is required for setting up, running, and reporting this same batch of 5 patients’ specimens. At a cost of $20.00 per hour ($0.333 per min) for technical time, the cost is:

Technical = (45 min/5 results) × $0.33 per min = $3.00 per reportable result.

Thus the total estimated cost per reportable cTnT result with the ES 300 is:

Total estimate = supply + technical = $14.54 + $3.00 = $17.48 total per reportable result.

The cTnT rapid assay cost analysis will assume that testing is performed in the central laboratory and that the per-device cost is $20.00. Regulatory indications are that controls with the rapid assay need only be performed with each lot of reagent; no calibration will be required for the rapid assay. Thus, disregarding the cost of running the controls once per lot:

Reagents = cost for a rapid assay device = $20.00 per reportable result.

Although repeat testing could substantially affect these cost estimates, retesting has represented <5% of the total reagent cost in our experience. To run a single sample on the rapid assay will require ~5 min of technologist time at a cost of $0.33/minute:

Technical = (5 min/sample) × $0.33 per min = $1.65 per reportable result.

Thus the estimated total cost for the rapid assay would be:

Total estimate = supply + technical = $20.00 + $1.65 = $21.65 per reportable result.

Reimbursement for cTnT testing is complicated to estimate. Of course, for inpatients that are covered under “diagnosis-related groups” (DRGs), specific reimbursement for cTnT testing cannot be expected. For outpatients, there is currently no specific common procedure test (CPT) code for the ES 300 quantitative cTnT. It therefore seems that reimbursement under CPT code 83520 (immunoassay quantitative, not otherwise specified) is most appropriate at a rate of $18.32 in most areas. Rapid assay reimbursement is even more complicated; the established CPT code is 83516, described as: multiple step; immunoassay qualitative analyte, other than antibody or infectious disease. Reimbursement for this CPT code is highly variable, apparently ranging from $2.00 to $50.00 across the US. Currently it appears that for cTnT measurement with the ES 300 the cost per reportable result would be $17.48 with reimbursement at $18.32. cTnT rapid assay measurement would be $21.65 per reportable result with reimbursement still to be established.
**Summary**

cTnT is a structural protein that is a specific component of the contractile apparatus in cardiac muscle. cTnT should not be confused with or "lumped" with cTnI because it is distinctly different with regards to biological function, molecular mass, and, perhaps most importantly, the cytosolic pool available for rapid release after injury. Although the diagnostic sensitivity of cTnT is very similar to CK-MB and the protein remains increased for many days after AMI, the diagnostic specificity of cTnT is lower in most studies. In a simple example, the estimated cost for providing cTnT is \$17.48 for the quantitative ES 300 method and \$21.65 for a 20-min rapid assay. Reimbursement is expected to be \$18.32 for the ES 300 and is as yet undefined for the cTnT rapid assay.

Numerous outcome-based studies have shown that cTnT measurements provide important and independent information for the identification of high-risk patients with acute cardiac ischemia. Although large intervention studies in patients who are cTnT positive have not been completed to date, early work suggests that clinical decisions and the course of therapeutic intervention may be significantly affected by availability of cTnT measurements, particularly in patients with minimal myocardial damage undetected by other biochemical markers.

**References**


