Uttam C. Garg*  
Gloria Austin  
Charles Barnes  
Marilyn Hamilton  
Pathology and Laboratory Medicine  
Children’s Mercy Hospital  
2401 Gillham Rd.  
Kansas City, MO 64108

*Author for correspondence. E-mail ugarg@cmh.edu.

Increased Concentrations of Cardiac Troponin I Are Equivalent to Increased Cardiac Troponin T in Identifying Chest Pain Patients at Short-Term Risk of Myocardial Infarction

To the Editor:

A recent paper by Christenson et al. (1) presented a comparative analysis of cardiac troponin I (cTnI) and T (cTnT). Their objective was to stratify patients for short-term risk of an acute coronary event on the basis of initial marker concentration as determined within 3.5 h of ischemic symptoms. The authors suggest that cTnT is a better predictor than cTnI on the basis of comparisons of the area under the curve (AUC) of ROC curves for cTnT (AUC = 0.68) and cTnI (AUC = 0.64). However, although weakly significant (P = 0.0375), the 95% confidence interval (CI) for the two areas overlap almost completely (95% CI for cTnI, 0.56–0.72; 95% CI for cTnT, 0.6–0.75). The fact that the samples were not tested for the two analytes simultaneously, but >1 year apart, could easily account for this small difference.

Using cutoffs of >0.1 ng/mL (0.1 µg/L) for cTnI and >1.5 ng/mL (1.5 µg/L) for cTnI, the authors found 90.4% concordance between the two assays. After correction for samples falling within the 95% CI around the cutoffs, the overall concordance rose to 93%. Of the 66 cTnT-positive/cTnI-negative patients, 7.5% died and 70% suffered from an acute myocardial infarction (AMI). The authors use this as evidence that cTnT is more sensitive than cTnI for risk stratification but neglect to present data recalculating the cTnI sensitivity/specificity profile using a cutoff of 0.4 ng/mL (0.4 µg/L), which has been demonstrated to be the more appropriate concentration for cTnI in risk stratification of chest pain patients (2). The authors cite that the cutoffs used are in accordance with the manufacturer’s specifications; however, the Dade Stratus® Cardiac Troponin-I package insert (3) describes in detail the multicenter, outcomes-based study of Antman et al. (2), where 0.4 ng/mL (0.4 µg/L) was used as a cutoff. The insert also states that in two separate studies, the 97.5 percentile distribution of ostensibly healthy individuals (n = 156) and patients with chest pain but confirmed not to have AMI (n = 149) was 0.4–0.6 ng/mL (0.4–0.6 µg/L).

Both the Christenson et al. study (1) and a previous report describing the use of cTnT in risk assessment of patients with myocardial ischemia (4) used a high-risk population in whom >72% of the patients were diagnosed as having an AMI. The patient population upon which both studies were based came from the GUSTO IIa thrombolytic trial (5), which studied the efficacy of thrombolytic therapy (streptokinase or tissue plasminogen activator–alteplase) for those who had ST-segment elevation in randomization with administration of either the anticoagulant heparin or hirudin (4, 5). An analysis of the electrocardiographic (ECG) classifications reveal that 435 of 755 (58%) of that population had ST-segment elevations sufficient to qualify them for thrombolytic therapy. Based on the WHO criteria, these patients were diagnosed with AMIs based on chest pain and ECG ST-segment elevation and did not require any biochemical marker for initial AMI diagnosis. Regardless of cTnI or cTnT values at presentation, 83% of the patients had a cardiac event, defined as death, AMI, or revascularization therapy (1). In their study, the single best predictor of 30-day mortality was not cTnT, but the ECG categorization (P = 0.045 for cTnI and 0.019 for ECG).

In clinical studies using the recently developed Chiron Diagnostics ACS:180 cTn assay, which is calibrated to the Dade Stratus cTn assay, the cTn concentrations in 158 ostensibly healthy patients were <0.1 ng/mL (0.1 µg/L), and the median values in 73 unstable angina patients were 0.8 µg/L and 7.6 µg/L in 130 confirmed AMI patients (data on file, Chiron Diagnostics Corp.). These findings corroborate the use of lower cutoffs for cTnI in risk stratification. Why the authors did not simply recalculating the comparative data using this lower cutoff for cTnI is questionable, because they clearly were aware of the Thrombolitics in Myocardial Infarction (TIMI IIIB) trial and the subsequent cTnI substudy (2, 6).

The use of cTnI as a predictor of short-term acute coronary events in chest pain patients based on marker values on presentation has now been demonstrated in a number of studies (1, 2, 7, 8). The TIMI IIIB substudy specifically analyzed cTnI concentrations on the most difficult to diagnose chest pain population, patients with no or transient ST-segment elevation or depression who are not candidates for acute thrombolytic therapy (2). By using the lower 0.4 µg/L cutoff, this study on 1404 patients showed that patients presenting between 0 and 24 h after chest pain onset with a cTnI concentration above the cutoff were 3.8-fold more likely to die within 42 days of presentation compared with patients with concentrations <0.4 µg/L. When the initial measurement was taken after at least 6 h after onset of chest pain, consistent with the temporal profile of increased cTnI in the majority of AMI patients, the risk ratio of mortality at 42 days was 9.5-fold higher in patients with cTnI concentrations >0.4 µg/L. In terms of overall risk of a short-term acute cardiac event, this population was at much lower risk compared with the population described in the GUSTO IIa studies (1, 4, 5). Comparisons show that the death rate was 2.7-fold
lower (2.4% vs 6.4% of the study population) and that the rate of AMI was ~13-fold lower (5.4% vs 72%) compared with the population studied by Christenson et al. (1). This was expected, because 58% had severe ST-segment elevation in the GUSTO IIa study, compared with <10% with only transient elevations in the TIMI IIB study. Given the lower overall risk of the TIMI IIB population, the ability to identify candidates at high risk of an acute coronary event is important because, typically, patients with unstable angina are not monitored as rigorously as patients who have a confirmed AMI. The ability to stratify and closely monitor such a subpopulation during this interval of heightened risk will likely have impact on the morbidity and mortality of unstable angina patients who progress to AMI.

Finally, a recent paired study used both point-of-care qualitative and laboratory-based quantitative tests for cTnT and cTnI to assess the efficacy of initial diagnosis and short-term risk stratification based on marker concentrations at presentation (7). Of 773 patients presenting to an emergency department, 171 were positive for cTnI and 123 were positive for cTnT. Among 47 patients with AMI that evolved while at the hospital, 94% were positive for cTnT and 100% were positive for cTnI. Among 315 patients with unstable angina, 70 patients were positive for cTnT (22%) and 114 were positive for cTnI (36%). During 30 days of follow-up, 34 cardiac events were reported, 20 fatal and 14 nonfatal AMIs. Of these, 20% of the deaths (4 of 20) had a negative cTnT result and only 5% (1 of 20) had a negative cTnI result. Of the nonfatal AMI patients, 21% (3 of 14) had negative cTnT values and 7% (1 of 14) had negative cTnI values. As predictors of a cardiac event at 30 days, both assays were highly significant predictors regardless of the ECG value; however, the odds ratio for cTnI was >2.4-fold that of cTnT. The authors’ conclusions were exactly the opposite of Christenson et al. (1), i.e., cTnI is slightly more sensitive than cTnT.

At this stage in the evolution of understanding of how to best utilize cTnT and cTnI results, on balance, careful analysis of the literature suggests that both proteins are about comparable in terms of initial diagnosis of AMI and prediction of short-term cardiac events. Selection of one assay vs the other may therefore be made more on the basis of convenience, system availability, and analytical rather than clinical performance. Additional well-controlled studies with a primary objective of direct cardiac marker comparisons similar to that of Hamm et al. (7) are required to determine if cTnI or cTnT is truly the better marker. Any assessment of the utility of troponins in assigning predictive value must clarify the status of the unstable angina/non-Q wave myocardial infarction population because they are the most problematic to diagnose and manage. In that regard, cTnI data from the TIMI IIB study (2, 6) provide convincing evidence of the utility of cTnI in risk stratification. Similar comprehensive studies on unstable angina/non-Q wave myocardial infarction are required of cTnT to assure comparability.

References


Barry Bluestein*  
George Parsons  
Kimberly Foster  
Chiron Diagnostics Corporation  
333 Coney Street  
East Walpole, MA 02032

*Author for correspondence.

Another reader comments on the same article:

To the Editor:

I commend Christenson et al. (1) for performing analysis of both cardiac troponin T (cTnT) and I (cTnI) in a large population of patients with acute coronary syndromes to compare the abilities of these cardiac markers to stratify risk. I’d like to comment on three points regarding findings from this paper. First, one issue that should be addressed more rigorously by the authors involves the 1.5 μg/L cTnI (Stratus) cutoff concentration used for predicting clinical outcomes. Although it is true that the Dade Stratus® package insert states an upper reference limit for acute myocardial infarction as 1.5 μg/L, the 97.5% percentile of the distribution of 150 individuals presenting with chest pain but subsequently diagnosed as non-acute myocardial infarction was 0.6 μg/L. Thus, 0.6 μg/L should be the cutoff value used for comparison with the cTnI cutoff value of 0.1 μg/L, which is the upper reference limit established in clinical trial studies for cTnT (2); Christenson et al. reference 24). If the authors were to establish for themselves appropriate cutoff concentrations using ROC curve analyses using the same population of patients, they would find that the Stratus cTnI cutoff of 1.5 μg/L corresponds to a cTnT cutoff of 0.2 μg/L and that a cTnI cutoff of 0.6 μg/L corresponds to a cTnT value of 0.1 μg/L (unpublished results from our laboratory). Thus, it would be of value to report the cTnI concentrations of the 66 patients who were
cTnT-positive and cTnI-negative in their study (1).

Second, all samples were frozen after collection for 12–18 months prior to cTnI analysis. However, cTnI analysis was performed within 60 days after collection. Unfortunately, no data are given in the paper by Antman et al. (3); Christenson et al. reference 5) regarding stability of cTnI. It would have been more appropriate to measure cTnT at the same time samples were analyzed for cTnI.

Finally, the authors note several times in their discussion that differences in the release patterns of cTnT and cTnI, with cTnT being released earlier, are a rationale for why cTnT shows favorable results. However, none of the references cited in their paper actually compare these two markers. In a study of acute myocardial infarction patients in which hourly cTnT and cTnI measurements were followed during the first 6 h after admission, no statistical differences in release patterns were observed (4). Furthermore, this study (4) also demonstrates equivalent early release of troponin and creatine kinase (CK) MB mass, and does not support an early increase of cTnT compared to CK-MB, as noted by Christenson et al. (1).

In conclusion, because these investigators will continue to make valuable contributions to this clinically important area, it is extremely important that they establish in their own laboratories, using identical patient populations, statistically derived decision cutoffs, so that fewer questions are raised when cTnT and cTnI concentrations are compared in risk stratification studies in patients with acute coronary syndromes.

References

Fred S. Apple
Department of Laboratory Medicine and Pathology
Hennepin County Medical Center
701 Park Ave.
Minneapolis, MN 55415
Fax 612-904-4229
E-mail fred.apple@co.hennepin.mn.us

Two of the authors of the article cited in the two preceding letters respond:

To the Editor:

We appreciated the comments of Drs. Bluestein and Apple regarding our recent paper comparing cardiac troponin T (cTnT) and cardiac troponin I (cTnI) for risk stratification (1). Because their comments addressed similar issues, the following represents our responses to both communications.

One issue raised involved the significance (P = 0.0375) of differences between the ROC curve areas. The comparison of ROC curve areas has been commonly performed in laboratory medicine to evaluate the relative usefulness of tests. Such comparison provides practical information for partly meeting this objective, so long as method limitations such as the shapes of the curves and the tradeoff of sensitivity and specificity are recognized. It must also be remembered that the ROC statistical method is akin to the Wilcoxon test, so the reader must interpret differences that achieve statistical significance with regard to their practical meaning. The ROC analysis for cTnT and cTnI was included in our paper (1) for the sake of completeness, because they are a commonly utilized tool. Although the ROC data are supportive of the overall findings of the study, it is critical that these data be considered in light of the logistic regression analysis.

The issue of which cutoff is appropriate for use with the Stratus cTnI assay was raised. In our study, a cTnI cutoff of 1.5 µg/L and a cTnT cutoff of 0.1 µg/L were used, because this was the information provided for the diagnosis of myocardial infarction (MI) in the respective manufacturers’ inserts. Unfortunately, data supporting the 1.5 µg/L cutoff in the Stratus cTnI package insert have never been published in the peer-reviewed literature. Institution-specific data were quoted, from which it was summarized that a 0.6 µg/L Stratus cTnI value correlates with a 0.1 µg/L cTnT result and that 1.5 µg/L from Stratus would correlate with a 0.2 µg/L for cTnT. Other anecdotal cutoffs for MI that I have been told for the Stratus cTnT system are any detectable amount (>0.35), 0.8, 1.2, and as high as 3.2 µg/L! In total, this information underscores the difficulty (folly) of selecting comparative cutoffs without carefully defined protocols designed to compensate for the assays, patient populations, different institutions located in diverse geographic locations, and different philosophies of (and reimbursement for) medical practice. Readers must be keenly aware of the issues of cutoffs when reading this or any other paper. Most importantly, readers must focus on the body of our paper (1), which examines cTnT and cTnI as continuous variables with logistic regression analysis, a strategy that altogether avoids the caveats (and possible bias) of cutoffs.

Obviously the concept and caveats of cutoffs are impossible to avoid when using positive/negative qualitative tests, as was done comparing rapid assays for cTnT and cTnI in the study of Hamm et al. (2). To compare our study (1) with that of Hamm et al. (2) is inappropriate because the tests run in the latter were both qualitative and therefore could not be treated as continuous variables. Suffice it to say that readers need to keep in mind that that our paper (1) compared two markers, cTnT and cTnI, whereas the paper of Hamm et al. compared two tests, a qualitative rapid assay for cTnI (Spectral Diagnostics) and the cTnT Rapid Assay (Boehringer Mannheim Corp.). When reading the study of Hamm et al. (2), one should be cognizant of the relative concentrations of cTnI or
cTnT needed to produce a positive response (i.e., the cutoffs). These differences probably explain why there is some disparity in diagnostic performance between the tests (2). It is noteworthy that the cTnT assay used in the Hamm et al. study is no longer available; evidently the cTnT Rapid Assay has been reformulated to have a cutoff of <0.1 μg/L, which would be expected to yield different results.

A similar issue was raised about using a different (presumably lower) cutoff for risk stratification. This is, of course, a good idea, but has its own set of issues. The paper of Galvani et al. (3) had insufficient data to allow further analysis. However, consider the Stratus cTnI data and risk of short-term mortality among the 1404 patients included in the study of Antman et al. (4), in which 0.4 μg/L was used as the cutoff. Fig. 3 of this paper (4) shows a clear increase in 42-day mortality risk with increasing concentrations in cTnI when the data are divided into six strata. Analysis of these data shows that there is no significant difference in mortality rate between the group having cTnI values <0.4 μg/L (the limit of detection of the assay) and patients having cTnI values between 0.4 μg/L and <1.0 μg/L (χ² = 0.77; P = 0.38). In fact, statistical analysis presented in the paper showed that an increment of 1.0 μg/L was associated with a significant increase in 42-day mortality (4). Although a cutoff of 0.4 μg/L was able to discriminate risk of mortality with significance (1.0% vs 3.7% mortality; χ² = 12.2; P <0.0005), a cutoff of 1.0 μg/L showed better discrimination for risk stratification (1.1% vs 4.5% death rate; χ² = 16.8, P <0.0001). Based on this evidence, should a cutoff of 1.0 μg/L be used instead of <0.4 μg/L? If so, this would obviously be higher than the 0.6 μg/L MI cutoff recommended in Dr. Apple’s letter. Using lower cutoffs for MI may mean that low-risk patients will be labeled as having had MI, which has many insurance, psychological, employment, and lifestyle implications for these patients.

Rather than a cutoff for risk stratification, we agree with the concept implied from the work of Antman et al. (4), that any detectable cTnI should be considered an indicator of increased risk and that the higher the cTnI concentration the greater the risk of short-term mortality. This situation underscores why quantitative cTnT and cTnI should be considered continuous variables with the logistic regression analysis for risk stratification, a strategy that altogether avoids the caveats of using cutoffs.

The issue of cutoffs and the outcome or endpoint studied is also worthy of comment. Generic problems associated with establishing diseased populations include differences in diagnostic criteria, low disease prevalence, and generally a low number of diseased patients in the area of the putative cutoff values. Cutoffs are particularly confounded when they are developed using an outcome of “MI diagnosis”, either by chart review or World Health Organization guidance, which includes two of the following three criteria: symptoms, specific electrocardiographic (ECG) changes, and/or an increase in biochemical markers. Not only is there variability in this endpoint because of different markers, different cutoffs, different practice philosophies, and other factors, there is great risk of circular reasoning when examining the diagnostic performance of biochemical markers, when markers are a critical part of the diagnostic criteria. To avoid this issue, GUSTO IIa was designed to use an outcome of death by 30 days after the index event. (In contrast with the confounders of WHO criteria for MI, few argue whether the patient is alive or dead.) Thus, the relative value of cTnT and cTnI as continuous variables for predicting an outcome of 30-day mortality in the population included in GUSTO IIa does not suffer the limitation of which cutoff was used for which marker, nor was the endpoint used open to question.

It was suggested that the cTnI concentrations for the 66 patients who were cTnI-positive and cTnI-negative be reported. These specific data have been presented (5), and a full-length manuscript containing them along with other data has been prepared.

Another issue deals with the fact that the specimens were frozen for ~12 months before Stratus cTnI measurement. We agree that, ideally, measurement of cTnT would have occurred at the same time that samples were analyzed for cTnI. However, GUSTO IIa was designed to have additional specimens collected and appropriately stored until analysis (1). The study of Antman et al. (4) did address the point of cTnI measurement in frozen specimens stored for a longer period of time and measured with the identical assay. It is noteworthy that our study was in agreement with that of Antman et al. (4), in that cTnI showed a strong relationship with short-term mortality (P = 0.002) (1). Although stability upon freezing is apparently an issue with some cTnI assays, we are aware of no data suggesting that (even small) differences are observed with the Stratus cTnI assay after appropriate frozen storage for 1 year.

The possibility of release differences between cTnT and cTnI (or immunoreactivity of the released forms) was indeed suggested as the reason that cTnT was more predictive of 30-day mortality than was cTnI in the GUSTO IIa population (1). Certainly there are other possibilities. It must be remembered that lack of a statistically significant difference is often because of an “n” deficiency. There have been few studies that have directly compared cTnT and cTnI, and none have had sufficient statistical power to show that release of cTnT and cTnI are equivalent. To gleam some idea of the timing, users of cardiac marker assays should be encouraged to examine the package inserts and specific publications for their cTnT, cTnI, CK-MB, or other assays to compare when high, e.g., >90%, diagnostic sensitivity is achieved.

Reiterating the need to validate cutoffs in one’s own laboratory is music to the choir of all that are in compliance with regulatory requirements. However, readers must be cautioned that the development of institution-specific cutoffs for risk...
stratification is difficult and resource-intensive, and takes a long time even at high-volume institutions. This is partly because important outcomes, e.g., 30-day mortality, have low incidence in low-risk patients. Worse, results of inappropriately designed, incomplete, and/or poorly conducted studies can be misleading. Thus, just as our clinical colleagues rely on appropriately designed and powered multicenter pharmaceutical trials to guide the use of medications and interventions based on outcome, we must rely (at least in part) on appropriately designed studies including carefully characterized patients to help provide the evidence base for guiding use of biochemical markers in risk stratification.

Confidence Intervals and Free Prostate-specific Antigen

To the Editor:

Estimation of the concentration of prostate-specific antigen (PSA) in serum has been widely used in recent years in the early detection and monitoring of patients with prostate cancer. PSA concentrations may also be moderately increased in patients with benign prostatic hypertrophy (BPH), and differentiating between patients with these two conditions may lead to considerable extra investigation with techniques such as trans-rectal ultrasound and biopsy.

PSA exists in several forms in blood. The majority is bound to the protease inhibitor antichymotrypsin, whereas a variable portion exists in an unbound (“free”) form. It is now well established that a higher proportion of the total PSA exists in the free form in BPH than in prostate cancer, and it is proposed that determination of the percentage of free PSA improves discrimination between BPH and prostate malignancy (1).

There is an important consideration with regard to free PSA that is being largely ignored. Every measurement is associated with an analytical error, and when these errors are added together as occurs, for example, in the calculation of the anion gap (2), or used in calculation of a ratio, e.g., the percentage of free PSA, this error may grow to be large. Below we calculate, from published data, the errors in the calculation of the percentage of free PSA at concentrations close to the important 4.0 µg/L total PSA concentration, with ~20% free PSA:

\[
CV^2_{\text{total}} = CV^2_{\text{analytical}} + CV^2_{\text{biological}}
\]

The biological CV for free PSA is 17.0% and for total PSA is 15.0% (3). The analytical CV for free PSA is 5.2% and for total PSA is 4.5% (4). Therefore:

\[
\text{total } CV^2_{\text{free}} = 17.0^2 + 5.2^2 = 316.0
\]

i.e., \( CV_{\text{free}} = 17.8\% \), whereas:

\[
\text{total } CV^2_{\text{total}} = 15.0^2 + 4.5^2 = 245.3
\]

i.e., \( CV_{\text{total}} = 15.7\% \)

The CV² of a proportion is approximately the sum of the CV’s of the individual components (5). Therefore:

\[
CV^2_{\text{free/total PSA}} = CV^2_{\text{free}} + CV^2_{\text{total}}
\]

i.e., \( CV_{\text{total}} = 17.8^2 + 15.7^2 \) and

\[
CV_{\text{free/total PSA}} = 23.7\%
\]

Thus, at typical values where the percentage of free PSA will be particularly useful in decision making, for example, 20% free PSA at a total PSA concentration of 4 µg/L, the 95% confidence intervals for the percentage of free PSA will be 10.7–29.3%.

The majority of results will of course be close to the central value, and certainly population studies using the percentage of free PSA are of great value in understanding the disease process. However, great caution must be exercised in using results in individual cases to decide therapeutic options. This important consideration is largely lacking from discussions of the value of free PSA.

References


Tony Badrick¹
Peter E. Hickman²

¹Sullivan, Nicolaides and Partners
Taringa
Queensland, Australia 4068

²Department of Pathology
Princess Alexandra Hospital
Woollongabba
Queensland, Australia 4102

*Author for correspondence. Fax 61 7 3240 7070; e-mail PHickman@medicine.pa.uq.edu.au.