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The Cardiac Troponins

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POSTER SESSIONS INDICATE THAT cTnI AND cTnT ARE MORE SPECIFIC, SENSITIVE, AND MORE CLINICALLY USEFUL THAN CKMB

The poster presentations at the 1997 American Association for Clinical Chemistry (AACC) Annual Meeting held in Atlanta, GA suggested that immunoassays for the cardiac isoforms of troponin I (cTnI) and troponin T (cTnT) may soon replace those for the conventional biochemical markers of myocardial infarction (MI), such as total creatine kinase (total CK) and creatine kinase - MB isoenzyme (CKMB). The results from several large-scale clinical studies were presented as cTnI and cTnT were extensively evaluated. Several reports compared the diagnostic value of the cardiac troponins directly against that of CKMB, while other studies investigated the use of a combination of two or more markers (i.e., the cardiac troponins with CKMB and/or Mb) to rule-in or rule-out MI. The results obtained were convincing enough to prompt some investigators to recommend an increase in the use of cTnI assays and a corresponding decrease in the use of CKMB assays in the assessment of patients with suspected MI.

Cardiac troponins vs. CKMB

In one of the larger studies, cTnI was compared to total CK and CKMB in 593 consecutive patients admitting to a hospital emergency department (ED) with chest pain.[1] Total CK and CKMB values were used to calculate the CK/CKMB index (MB index). Based on WHO-defined criteria for MI, 53 patients were positive for both cTnI and the MB index, 21 were cTnI-positive and MB index-negative (9 with myocardial damage), 25 were cTnI-negative and MB index-positive (23 with no myocardial damage), and 492 were negative for both markers. The following summarized results, indicated that cTnI was significantly more sensitive and specific than the MB index for the diagnosis of MI.

	cTnI	MB index
Sensitivity	97.4%	76.5%
Specificity	99.6%	95.7%
Positive Predictive Value	97.4%	76.3%

(cut-off values: cTnI, 1.5 ng/ml; MB index, 2.5)[1]

Several other studies also demonstrated a clear superiority of cTnI over CKMB.[2,3,4,5] One study of note involved 130 suspected MI patients in a comparison of cTnI with CKMB and the relative index value (RIV).[5] The results of this study were so significant as to prompt the investigators to recommend that their institution increase the use of cTnI assays and decrease the use of CKMB assays in the biochemical confirmation of MI. Results are summarized below.

	cTnI	CKMB or RIV	CKMB and RIV
Sensitivity	96%	93%	81%
Specificity	99%	89%	100%
Negative Predictive Value	99%	98%	95%
Positive Predictive Value	96%	69%	100%

(cut-off values: cTnI, 1.5 ng/ml; RIV, 3.0; CKMB, 6.0 ng/ml)[5]

Similar results were reported for cTnT in comparison with CKMB. In an evaluation of 293 patients with chest pain, cTnT displayed substantially better overall sensitivity and specificity than CKMB in MI diagnosis.[6] In addition, cTnT was elevated in both Q-wave and non-Q-wave MI and correlated with typical MI-associated chest pain. Closer investigation revealed that cTnT's specificity markedly increased approximately 3-4 hours after the onset of chest pain. Results follow:

	cTnT	CKMB
Overall Sensitivity	86.7%	46.7%
Overall Specificity	94%	84.5%

	cTnT 2-4 hr	cTnT 4-6 hr
Sensitivity	98%	100%
Specificity	66.7%	89.7%[6]

In another investigation, 107 suspected MI patients were measured for cTnT, total CK, CKMB, and myoglobin (Mb).[7] As in the above study, cTnT showed a distinct advantage over CKMB after approximately 3 hours post-admission. The results also indicated that no test was more sensitive than Mb 0-3 hours post-admission, although the specificity of Mb was low throughout the test period (approximately 80%).

The specificity of cTnT for MI diagnosis exceeded 99% in another study of 294 patients admitting to a hospital ED with chest pain.[8] Highlighting the benefit of serial measurements of biochemical cardiac markers, a 20% increase in diagnostic sensitivity was noted in this study when patient samples were available both before and after reperfusion therapy, rather than before therapy only.

Combining markers

The above studies evaluated cTnI, cTnT, CKMB, and Mb in an effort to determine the best single marker for MI confirmation. The results suggested that both cTnI and cTnT are superior to CKMB and Mb as single biochemical markers of MI. Other studies, however, investigated whether or not diagnostic efficacy could be improved by evaluating the cardiac troponins, not alone, but in combination with CKMB and/or Mb.

In a study involving 22 WHO-defined MI patients, single, parallel,

Tests	Sensitivity at Admission	3 hours	6 hours	Specificity
single Mb	82%	100%	95%	55%
single cTnI	59%	100%	100%	100%
single CK	36%	86%	100%	66%
single CKMB	54%	100%	100%	77%
parallel Mb or cTnI	86%	100%	100%	55%
parallel Mb or CKMB	86%	100%	100%	55%
parallel CK or CKMB	54%	100%	100%	66%

	Sensitivity admission/3 hours	Specificity
serial Mb and cTnI	82%	100%
serial Mb and CKMB	82%	77%
serial CK and CKMB	36%	77%

Table 1. The diagnostic sensitivities and specificities of single, parallel, and series measurements of cTnI, Mb, total CK, and CKMB. The mean time of admission was 3.3 hours after the onset of chest pain; range: 0.5 - 6 hours.[9]

cTnI in Clinical Algorithms

The feature article in this issue of Scripps News presents data supporting the superior clinical efficacy of the cardiac troponins vs. CKMB. The studies discussed here, however, differ in that they investigate the fiscal and practical impact of cTnI in a clinical setting.

cTnI, CKMB, total CK, and Mb were measured in 77 suspected MI patients.[32] Careful evaluation of the assay data resulted in the following clinical algorithm, which successfully ruled-in all 19 confirmed MI cases and ruled-out the other 58:

- Sample collection is scheduled at 0, 2, 4, 6, and 12 hours post-admission;
- At 0 hour, CKMB is measured if total CK ≥ 85 U/L;
- After 0 hour, CKMB is measured if total CK is ≥ 85 U/L and is on the increase;
- cTnI is measured if CKMB is elevated;
- Elevated CKMB and cTnI confirm MI;
- If both markers are not elevated by 12 hours, MI is ruled-out;
- Once an elevated test result is obtained, neither marker is repeated.

In this study, CKMB and cTnI were positive by 6 hours in all 19 MI cases. This algorithm required CKMB assays in 23% of the cases studied, and cTnI in 11%. The authors estimated that this algorithm would reduce the average length of stay in the hospital by 0.35 days per patient without MI, resulting in an approximate savings of $310,000 per year.

Another study investigated the clinical and fiscal impact of adding cTnI to their current MI test protocol of total CK, CKMB, and Mb.[33] The parameters evaluated were length of stay, time to cardiac catheterization, laboratory costs laboratory charges, hospital costs, and hospital charges. The authors concluded that cTnI assays did not significantly increase laboratory or hospital costs and that certain patients subgroups (i.e., those with UA or non-Q-wave MI) may benefit from cTnI measurements.

In a similar study, cTnI was added to the test protocol of total CK, CKMB, and Mb in the evaluation of 100 consecutive chest pain patients.[34] 42% of the MI patients had elevated serum markers within 2 hours of hospital admission, and 54% of the non-MI patients were eligible for early discharge. Adding cTnI to the current MI test protocol resulted in a difference in treatment of 12.5% of patients, increasing the accuracy of MI diagnosis and decreasing hospital costs and patient length of stay. As a result of this study, the researchers created a Cardiovascular Observation Unit for patients with chest pain and non-diagnostic ECGs.

and serial measurements of cTnI, Mb, total CK, and CKMB were taken upon admission, and at 3 and 6 hours post-admission.[9] The data are reported in Table 1 to the left. All parallel mode tests involving Mb showed good sensitivity, but unacceptable specificity. The method with the best diagnostic efficacy was the serial combination testing of the most sensitive marker, Mb, and the most specific marker, cTnI.

In another multiple marker study involving 298 patients, not only did cTnI demonstrate greater diagnostic efficiency (98%) than both CKMB (94%) and Mb (85%), but combining cTnI with CKMB and Mb assay results allowed 11 of the 53 confirmed-MI patients to be identified earlier than with the CKMB assay alone.[10] Combining cTnI with CKMB identified 4 confirmed-MI patients earlier, while combining Mb with CKMB identified 9 confirmed-MI patients earlier than with CKMB alone. It should be noted that the result of combining cTnI and Mb assay results was not reported.

Undoubtedly, the cardiac troponins are more specific than currently used biochemical markers of MI, but further study is needed to determine whether or not a combination of markers will prove more efficacious in diagnosing MI than any single marker.

Risk stratification

The cardiac troponins are also proving useful in evaluating patients with MI-like symptoms but without clinically-defined MI. Previous reports have indicated that approximately one-third of unstable angina (UA) patients with elevated serum levels of cTnT have a prognosis equally poor to MI patients.[11] These reports indicated that cTnT elevations were due to myocardial damage that was otherwise clinically undetectable (i.e., patients with a non-diagnostic ECG). It is desirable to identify those patients without clinically-defined MI but who are at a high risk for MI in the near future. These patients would be monitored closely and administered thrombolytic therapy upon confirmation of the imminent MI.

Two studies indicated that cTnI levels are elevated longer and more frequently than total CK, CKMB, and Mb in patients with UA,[12] and accurately reflect minor degrees of myocardial damage in patients with unstable coronary artery disease (CAD; i.e., UA and/or non-Q-wave MI).[13]

Three studies compared cTnI to cTnT in the risk stratification of suspected MI patients and presented discrepant results. In a study of 755 patients with acute coronary syndromes, samples drawn upon admission and at 8 and 16 hours post-admission indicated that cTnT was more predictive of future cardiac events than cTnI, based on 30-day

mortality data.[14] In two other studies, however, cTnI and cTnT categorized suspected MI patients and predicted 30-day mortality outcomes approximately equally.[11,15]

The presented evidence indicates that cTnT and perhaps cTnI are useful in the risk assessment of suspected MI patients. The discrepant results mentioned above, however, suggest that further study is required before cTnT, cTnI, or both markers may be recommended for widespread use.

Troponins in special cases

Several posters discussed the clinical-utility of the cardiac troponins, particularly cTnI, in special clinical situations. It is known that renal failure patients may have associated cardiac conditions that are difficult to diagnose biochemically, as both CKMB and cTnT are elevated in renal disease.[16,17] In a study of renal dialysis patients, cTnI was significantly more specific than cTnT in detecting myocardial damage.[16] Of the patients measured for cTnT, 21 (54%) had elevated cTnT levels. In contrast, of those patients measured for cTnI, only 1 (3%) had elevated cTnI, and this patient had suffered MI two days prior to the dialysis.

Several other studies reported similar results as cTnT was elevated in 37% of chronic renal failure patients,[18] 11.5% of end-stage renal disease patients undergoing transplant surgery,[17] and displayed a 16% false-positive rate in suspected MI patients due to renal dysfunction.[19] As in the study cited above, cTnI elevations in these cases were either not present or were attributable to a concurrent cardiac injury.

The diagnosis of peri-operative MI (POMI) during various surgical procedures is also complicated by an increase in the conventional biochemical markers. Total CK, CKMB, and Mb are often elevated as a result of the skeletal muscle damage caused by the surgical procedure.[20] As shown below, in an evaluation of POMI in 11 patients undergoing abdominal aortic surgery, cTnI and cTnT were considerably more specific for POMI than total CK, CKMB, and Mb.[20]

Marker	Specificity for POMI
cTnI	100%
cTnT	100%
Total CK	78%
CKMB	92%
Mb	43%

cTnI and/or cTnT also accurately reflected myocardial ischemia in coronary artery bypass grafting (CABG) patients,[21] cardiac allograft recipients,[22,23] and in children undergoing surgery for congenital heart disease.[24] In addition, cTnT identified thosesurgery patients with no in-hospital indication of POMI who were at risk for future cardiac events,[25] while cTnI helped diagnose cardiac injury in carbon monoxide poisoning[26] and in myocardial contusion injuries.[27]

Troponin release kinetics and conformation in circulation

The overwhelming evidence presented above clearly indicates that the cardiac troponins are useful in detecting myocardial injury both in suspected MI patients and in several clinical conditions in which conventional biochemical markers typically provide ambiguous data. Less information is available, however, regarding the physiology of the cardiac troponins during myocardial necrosis. In fact, until recently, little was known about the release of the troponin subunits from ischemic myocardium. Several poster presentations addressed this topic, however, as free cTnI, cTnI bound to cTnC (IC complex), and cTnI bound to both cTnC and cTnT (ICT complex) were studied.

Using an immunofluorescence detection method, the release of cTnI was studied in sera samples from 30 MI patients.[28] Highly selective antibodies were used to identify and quantitate both free cTnI and IC complex. Total cTnI, defined as the free cTnI concentration plus that of the IC complex, levels were found to peak 10-20 hours after the onset of chest pain and remained elevated for 60-80 hours. At the beginning of the observation period, total cTnI and free cTnI were present at approximately the same concentrations. At peak levels, total cTnI was approximately 6-12 times more abundant than free cTnI. Toward the end of the observation period, the concentrations returned to approximately equal values. The authors concluded that during MI, the greatest fraction of cTnI in serum is present as the IC complex.

Other studies, involving Western blot analysis[29] and gel filtration chromatography,[30] also indicated that the predominant form of immunoreactive cTnI in patient plasma is present as IC or ICT complex. The predominant form of cTnT, on the other hand, appears to be free cTnT.[30]

The chemistry of the troponin subunits may elucidate why cTnI is present in serum as a complex, while cTnT appears to be unbound. cTnI exists as both an oxidized and a reduced form. In general, the oxidized form is more stable than the reduced form, as reduced cTnI has a tendency to oxidize, then form disulfide bonds.[31] In a study using highly selective antibodies, plasma from MI patients was analyzed for the various forms of cTnI: oxidized and reduced cTnI, IC complex, and ICT complex.[31] The authors found that most cTnI in plasma was present as the IC complex (range: 20-60% IC complex), although in one patient 50% was present as the ICT complex. In addition, up to 30% of the free cTnI was present in the reduced form. The authors suggested that the IC and ICT complexes can be either released from the heart or formed in the blood of MI patients after the oxidation of reduced free cTnI.

Closing remarks

The 1997 AACC Poster Sessions presented convincing evidence that the cardiac troponins are superior to the conventional biochemical markers of MI: total CK, CKMB, and Mb. In addition, cTnI and cTnT may provided distinct diagnostic data, suggesting that both assays may provide useful information in a cardiac panel of biochemical markers. At this point, this panel of markers should include an assay for Mb, despite its lack of specificity, as no marker is consistently elevated as early as Mb. Although it remains to be confirmed, preliminary data indicates that combining results from the earliest and most sensitive marker, Mb, with the most specific marker(s), cTnI and/or cTnT, will provide diagnostic data that is superior to that provided by any single marker.

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