Comparable Detection of Acute Myocardial Infarction by Creatine Kinase MB Isoenzyme and Cardiac Troponin I

Jesse E. Adams, III, Kenneth B. Schechtman, Yvonne Landt, Jack H. Ladenson, and Allan S. Jaffe

Although measurement of cardiac troponin I (cTnI) is, in some situations, more specific for detection of cardiac injury than is measurement of the MB isoenzyme of creatine kinase (MBCK), its sensitivity and specificity relative to MBCK for detection of myocardial infarction has not been established. Accordingly, we studied prospectively 199 consecutive patients admitted to the coronary care unit. Values of MBCK and cTnI mass were determined in all samples. Of the 186 patients admitted with a suspicion of acute myocardial ischemia, 89 were diagnosed as having an acute myocardial infarction on the basis of the patterns of MBCK values. Eighty-six of these patients also had increased cTnI (concordance, 96.6%); three did not. Of the patients diagnosed as without infarction, five with unstable angina and symptoms in the day(s) prior to admission had increased cTnI, for a cTnI specificity of 94.9%. Receiver operating characteristic curve analysis indicated that cTnI and MBCK had statistically indistinguishable diagnostic accuracies for the detection of acute myocardial infarction.

Indexing Terms: heart injury/diagnostic efficiency

The MB isoenzyme of creatine kinase (EC 2.7.3.2) (MBCK) has been the marker protein of choice for the diagnosis of acute myocardial infarction for many years. However, findings that increased values of MBCK can occur in patients with acute or chronic muscle disease in the absence of detectable cardiac injury (1–3) have led to efforts to develop more specific markers. Recently, we documented (3) that high values of cardiac troponin I (cTnI) are not present in patients with acute or chronic muscle disease despite frequent and marked increases of MBCK unless concomitant acute cardiac injury is present. These findings are consistent with the developmental molecular biology of cTnI (4, 5) and MBCK (6, 7) and suggest that increases of cTnI should be more specific than increases of MBCK for the diagnosis of myocardial injury. A highly specific myocardial marker such as cTnI would improve the accuracy of diagnosis in patients with acute or chronic skeletal muscle injury and possible concomitant myocardial injury. However, the apparent superior cardiac specificity of cTnI could simply be the result of lower sensitivity for detecting myocardial injury. Accordingly, we studied the diagnostic accuracies of cTnI and MBCK for detecting myocardial infarction in patients admitted to the coronary care unit with possible acute myocardial infarction. We also related the patients' results to the relative amounts of cTnI and MBCK found in myocardial tissue.

Materials and Methods

Patient Population

Consecutive patients (n = 201) admitted to the coronary care unit were prospectively enrolled. For each patient, either the history, electrocardiogram, or clinical situation suggested the possibility of acute myocardial ischemia. Samples for cTnI were not obtained from two patients, who were then excluded from further analysis. All patients had serial clinical evaluations by cardiologists and at least daily electrocardiograms. Blood samples were obtained at least every 12 h for the first 2 days of hospitalization for measurement of MBCK, total CK, and cTnI. The diagnosis of acute myocardial infarction was made by clinicians responsible for the patient, based on the history, electrocardiogram, and a rising and falling pattern of MBCK, according to the guidelines of the World Health Organization (8). The cTnI values were not available to the clinicians caring for the patients. Increases of total CK, as in other studies, were not required for the diagnosis of myocardial infarction (9). Of the study group of 199 patients, 11 were admitted primarily for medical illnesses with hemodynamic and (or) respiratory compromise rather than consideration of acute myocardial infarction. These 11 were analyzed separately.

Measurement of Molecular Markers

Every 12 h after the patients' admission to the coronary care unit, samples were drawn into tubes with no preservatives, centrifuged at 2000g for 15 min, and stored at −70°C; stored samples were thawed once and assayed in batches. Total CK, MBCK, and cTnI are stable when handled in this manner (10–12). Results of the CK, MBCK, and cTnI assays were classified as normal or abnormal by individuals unaware of the clinical status of the patients.

Total CK activity (upper reference limit ≤220 U/L, lower limit of detection 25 U/L) was measured on a Flexigem centrifugal analyzer (Electro-Nucleonics, Fairfield, NJ) with a kinetic enzymatic method as previously described (11).

MBCK (upper reference limit ≤6.7 μg/L, lower limit of detection 2.2 μg/L) was measured with a commercially available immunoabsorbant assay (Stratus CK-
MB; Baxter Dade, Miami, FL), in which a monoclonal antibody recognizes MBCK but neither BBCK nor MMCK isoenzymes (10).

cTnI was assayed by an immunoassay in a preliminary research application on the Baxter Stratus analyzer. The method utilizes two cTnI-specific monoclonal antibodies, each of which recognizes different epitopes (12). Values of cTnI in healthy volunteers are undetectable by this method. Studies performed with this assay on samples of hospitalized patients without established myocardial injury defined the upper limit of the reference range to be ≤3.1 μL/L (95% cutoff value by nonparametric analysis) (12). The cTnI immunoassay has no detectable cross-reactivity with human skeletal muscle TnI (3, 12).

Measurements of MBCK and cTnI in Myocardium

Human heart tissue was obtained at autopsy or from explanted hearts at the time of transplantation. Bovine heart was obtained from a local slaughter house, and dog heart was obtained from Keystone Biological, Cleveland, OH. Cytosolic and myofibrillar fractions were prepared according to previously described methods (13). Small pieces of heart (~0.25 g) were homogenized in 10 volumes of cytosolic buffer: per liter, 1 mmol of potassium phosphate, pH 6.8, 1 mmol of EGTA, 20 mmol of KCl, and various protease inhibitors (0.5 mg of soybean trypsin inhibitor, 1 mmol of phenylmethylsulfonyl fluoride, 20 mg of leupeptin, and 1 mg of pepstatin). After the homogenate was centrifuged at 20,000g for 15 min, the pellet was reoversuspended in 10 volumes of cytosol buffer and the centrifugation repeated. The supernates of each centrifugation were assayed separately, and the data were combined to calculate the cytosolic fraction.

The final pellet was reoversuspended in 10 volumes of extraction buffer: per liter, 40 mmol of sodium pyrophosphate, pH 9.0, 1 mmol of EGTA, 1 mmol of MgCl₂, and protease inhibitors (as above). After 30 min, the extract was centrifuged at 20,000g for 15 min. The pellet was reoversuspended twice. Each supernate was assayed separately, and the data were combined to give the myofibrillar fraction.

Total protein concentrations in the cytosol and myofibrillar fractions were determined by protein assay of Lowry et al. (14). MBCK and cTnI concentrations were determined by the immunoassays described above except that, before assaying, we diluted the fractions in pooled normal human serum with undetectable values of MBCK and cTnI. The cytosolic fraction was divided by the total fraction (cytosolic plus myofibrillar) to obtain the percentage of cTnI and MBCK in the cytosolic preparation. Values are expressed as milligrams per gram of tissue (mean ± SD).

Statistical Analysis

Sensitivity and specificity were calculated for cTnI and MBCK relative to the clinical diagnosis. Receiver operating characteristic (ROC) curves were developed for MBCK and cTnI values and compared (15, 16).

Results

The characteristics of the patients are described in Table 1. Of the 188 patients admitted for the evaluation and treatment of known or suspected ischemic heart disease, the clinical diagnosis of acute infarction was made in 89. All patients diagnosed with acute infarction had at least one value of MBCK above the upper bound of the reference range (≥6.7 μL/L), either during hospitalization or at an outlying hospital before transfer. Of the patients diagnosed as having had myocardial infarction, 86 patients (96.6%) also had increases in cTnI; 3 did not (Table 2). In each of the three patients without increased cTnI, values for total CK (peak total CK = 308, 98, and 207 U/L, respectively) and MBCK (peak MBCK = 6.8, 8.5, and 11.1 μL/L, respectively) were modest. None of the three patients developed Q waves on their electrocardiograms. Two of these patients had a very slow decline in total and MBCK values; in the third patient, only one sample was obtained before the patient was taken for emergency coronary artery bypass grafting.

Of the 99 patients diagnosed as not having myocardial infarction, none had a value of MBCK above the upper bound of the reference range (all were <6.7 μL/L). Five of these patients had increased cTnI, however, yielding a specificity of 94.4% for cTnI in the patients

### Table 1. Characteristics of patients.

<table>
<thead>
<tr>
<th></th>
<th>Acute myocardial infarction</th>
<th>No myocardial infarction</th>
<th>Complex medical problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>89</td>
<td>99</td>
<td>11</td>
</tr>
<tr>
<td>Age, years*</td>
<td>62.4 ± 12.9</td>
<td>61.1 ± 15.0</td>
<td>53.4 ± 11.2</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>50</td>
<td>63</td>
<td>9</td>
</tr>
<tr>
<td>Female</td>
<td>39</td>
<td>36</td>
<td>2</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>70</td>
<td>76</td>
<td>7</td>
</tr>
<tr>
<td>Black</td>
<td>19</td>
<td>23</td>
<td>4</td>
</tr>
<tr>
<td>Type of infarction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q wave</td>
<td>28</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td>Non-Q wave/LBBB*</td>
<td>61</td>
<td>NA</td>
<td>3*</td>
</tr>
<tr>
<td>Location of infarction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterolateral</td>
<td>48</td>
<td>NA</td>
<td>2</td>
</tr>
<tr>
<td>Inferior</td>
<td>28</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Undetermined*</td>
<td>13</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± SD.

*Undetermined" indicates those patients whose electrocardiograms [including those patients with left bundle branch block (LBBB)] precluded determination of the site of the infarction.

* 8 had no infarction.

NA, not applicable.

### Table 2. Relative diagnostic sensitivity of cTnI assay.

<table>
<thead>
<tr>
<th></th>
<th>No. of patients</th>
<th>No. with increased cTnI</th>
<th>% concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with AMI and increased MBCK*</td>
<td>89</td>
<td>86</td>
<td>96.6</td>
</tr>
<tr>
<td>Patients without AMI, no increase in MBCK</td>
<td>99</td>
<td>5</td>
<td>94.9</td>
</tr>
</tbody>
</table>

* Only the MBCK values were available to the clinicians for diagnosis.
studied (Table 2). In four, the increases of cTnI were present at admission; in the fifth, the value of cTnI was at the upper bound of the reference limit at admission and continued to rise. All had a history of recurrent episodes of angina in the 1–7 days prior to hospitalization. None of these patients developed Q waves in their subsequent electrocardiograms.

Forty-three patients presented sufficiently early that values of MBCK and cTnI were either normal (n = 22) or were beginning to increase (n = 21), permitting more complete data acquisition on the time course of changes in MBCK and cTnI. These patients manifested a broad range of MBCK and cTnI values. Mean ± SD peak MBCK was 60 ± 80 µg/L (range 6.7–393 µg/L), and peak cTnI was 126 ± 309 µg/L (6–1455 µg/L). Values also were similar when we considered only samples assayed simultaneously for both MBCK and cTnI (to correct for the more frequent measurements of MBCK): 62 ± 86 µg/L (2.7–393 µg/L) for MBCK and 128 ± 246 µg/L (1.5–1485 µg/L) for cTnI. Of these 43 patients, 23 had only modest increases in peak MBCK (all <25 µg/L); in 12, the peak MBCK was <12 µg/L. All of these 23 patients had increased cTnI (peak cTnI = 18.4 ± 20.1 µg/L).

The ROC curve for cTnI (Fig. 1) confirmed the high sensitivity and specificity of this assay. The area under the cTnI (0.987) was not significantly different from the area under the MBCK curve (area = 1) (15, 16).

Of the 11 patients with above-normal values of MBCK admitted for management of severe multiple medical problems, 3 were diagnosed as having a concomitant acute myocardial infarction; each had both cTnI and MBCK increased, and ancillary invasive and noninvasive tests were supportive of the diagnosis. In the remaining 8 patients, MBCK was increased but not cTnI. Five of these patients had well-documented acute or chronic muscle disease, and the clinicians caring for them eventually believed that the increases of MBCK were due to skeletal muscle release. Two patients had a slowly decreasing pattern of MBCK after peak values of 10.0 and 9.0 µg/L, while a third had a pattern of rising and slowly falling MBCK after a peak value of 6.8 µg/L, all in the absence of increases of cTnI. None of these patients had any other evidence of cardiac injury, and none developed Q waves on their electrocardiograms. Because of the severity of their illnesses, all had prolonged hospital courses and were treated as if myocardial injury had occurred.

Table 3 lists the amounts of cTnI and MBCK found in the cytosolic and myofibrillar fractions of human heart. Similar percentages of cTnI were measured in the fractions of bovine and dog myocardium (2.0% and 2.1%, respectively).

Discussion

These data document that cTnI and MBCK have similar sensitivity and specificity for the detection of myocardial injury during the first 48 h of acute myocardial infarction. There was concordance between increases in MBCK and cTnI in 96.6% of patients admitted for evaluation of symptoms of acute myocardial ischemia. Measurements of cTnI and MBCK were similarly sensitive for the diagnosis of small as well as larger myocardial infarctions. These results support previous contentions that the lack of increase of cTnI in patients with acute and chronic skeletal muscle disease despite increases of MBCK represents a greater specificity of cTnI for myocardium rather than a less robust sensitivity (3, 12, 17–19). Thus, measurement of cTnI should be useful to clarify which increases in MBCK are due to myocardial injury and which ones reflect acute or chronic skeletal muscle abnormalities.

Although concordance between values for MBCK and cTnI was high, eight patients admitted for evaluation of ischemic heart disease displayed discordant values. In five, cTnI was increased but not MBCK. Each patient had had episodes of angina before the symptoms that led to their hospitalization. These patients are probably like those described by others (20, 21), who had unstable angina and increased values of cardiac troponin T, another long-lived marker of myocardial injury. The increases of cTnI in these patients may have been due to myocardial injury occurring long enough before hospitalization that concentrations of MBCK had returned to the normal range. It is also possible that increases in MBCK were missed due to infrequent sampling, as previously reported (22); this is not a problem with cTnI, which appears to persist in plasma for at least 5–7 days (12, 18, 19). It is less likely but cannot be excluded that these five patients with normal values of MBCK and increased values of cTnI released more cTnI per gram of myocardium injured than did other patients. We also

<table>
<thead>
<tr>
<th>Conc, mg/g tissue</th>
<th>Cytosol fraction</th>
<th>Myofibrillar fraction</th>
<th>Total</th>
<th>% from cytosol</th>
</tr>
</thead>
<tbody>
<tr>
<td>cTnI</td>
<td>0.12 ± 0.04</td>
<td>3.9 ± 0.8</td>
<td>4.0 ± 0.9</td>
<td>2.8 ± 0.5</td>
</tr>
<tr>
<td>MBCK</td>
<td>0.30 ± 0.04</td>
<td>0</td>
<td>0.3 ± 0.04</td>
<td>100</td>
</tr>
</tbody>
</table>

Fig. 1. ROC curve for values of cardiac troponin I (cTnI). Numbers on the curve indicate selected cutoff values in µg/L.
cannot exclude, but believe it unlikely given previous data (3), that the increases in cTnI are due to a lack of specificity either of cTnI or of the assay used for this analysis.

MBCK, but not cTnI, was increased in three patients admitted with possible myocardial injury. In one patient, this was the finding in the one sample available before emergency coronary artery bypass surgery; possibly, parallel increases would have been apparent had additional blood samples been obtained. In the two other patients, the increases of MBCK were minor (maximum 8.5 µg/L) and decreased slowly over several days. A slowly clearing pattern in patients with higher concentrations of MBCK is suggestive of skeletal muscle release; at lower concentrations, where biological and analytical variance are greater, it is more difficult to judge. Thus, we suspect but cannot confirm that the discordance observed in these two patients resulted from skeletal muscle release of MBCK and reflects the increased specificity of cTnI for myocardium rather than a difference in sensitivity between MBCK and cTnI.

Despite minor differences, the area under the cTnI ROC curve was almost the same as the unit area under the MBCK curve. Analysis of the ROC curve must be tempered by the diagnostic bias present in an institution where heavy reliance is placed on MBCK for the diagnosis of acute infarction: that is, for the patients we studied, there was no discordance between a value of MBCK above the upper bound of the reference range and the clinical diagnosis. In this situation, evaluation of other cutoff values for MBCK has little value. This fact similarly puts measurements of cTnI at a disadvantage from the perspective of sensitivity and specificity because any differences from MBCK are thus presumed to be due to inaccuracy of cTnI. This is particularly problematic when assessing specificity because increases of cTnI can persist in plasma for 5–7 days (12), whereas MBCK increases generally resolve much earlier. Despite these problems, the sensitivity of cTnI was 96.6% and the specificity 94.9% at a cutoff value of 3.1 µg/L. Conceivably, future studies may find the upper bound of our reference range for cTnI for detection of infarction to be too high. This relatively high value may reflect the presence of occult myocardial injury in some of the hospitalized patients used to determine the reference range.

Our data documenting the comparable relative clinical sensitivity of cTnI and MBCK for detection of acute infarction are useful in the interpretation of the results of studies quantifying the amounts of cTnI and MBCK in myocardium. There is much more cTnI in myocardium than MBCK, 13.3:1 by weight vs only 0.4:1 in the cytosolic fractions. In the serum samples from patients with myocardial infarction, mean peak concentrations of cTnI of 128.4 µg/L and of MBCK and 61.7 µg/L yields a ratio of roughly 2:1. This high ratio implies that there is at least some release from the myofibrillar compartment and not just the cytosolic compartment during the first 24 to 48 h of infarction. Our data are similar to those reported by LaRue et al. (23), who found similar amounts of cTnI in cardiac muscle (4.35 mg/g), and Katus et al. (24), who reported that unbound cardiac troponin T is 6% of the total cardiac troponin T found in human myocardium. Our results are also similar to those reported previously for MBCK (25). Given that we cannot exclude the possibility that the release ratios for these proteins also are different, this issue will require further study.

Increased MBCK, but not cTnI, also occurred in eight patients with complex medical problems. In five, concomitant acute or chronic skeletal muscle disease was clearly present. This is consistent with previous reports, where MBCK values were increased in the absence of confirming evidence of infarction, including increases of cTnI (1, 2, 26). Of the other three patients, none had evidence of cardiac injury on invasive or noninvasive tests, and none developed Q waves on subsequent electrocardiograms. cTnI was detectable in each patient, but values remained below the upper bound of the reference range. We cannot state with confidence whether the increases of MBCK observed in these few complex patients represent a greater sensitivity of this marker for myocardial injury or result from its release from skeletal muscle. Although these few exceptions exist, it is clear that, for patients in whom myocardial injury is a primary consideration, the sensitivities of MBCK and cTnI are essentially equivalent.

We conclude that these findings support previous data demonstrating the superior specificity of cTnI for cardiac injury, consistent with its developmental molecular biology. cTnI is present only in myocardium throughout ontogeny, and it has a unique amino acid sequence (27). The assay we used employs two monoclonal antibodies directed against different epitopes on cTnI; no cross-reactivity with the skeletal muscle forms of troponin has been observed (18). Furthermore, and in contrast to other troponins (C and T), studies in rats have found that cTnI is not expressed in skeletal muscle after skeletal muscle injury (28). The superior specificity of cTnI shown in other studies coupled with the comparable sensitivity and specificity of cTnI to MBCK in detection of patients with myocardial injury has important clinical implications. Patients with the potential for concomitant myocardial and skeletal muscle injury are common, and there is often considerable controversy concerning whether increases in MBCK are due to skeletal muscle or cardiac injury, a distinction that the percentage criteria (% of total CK) are unable to make (2, 3). Measurement of cTnI should clarify the diagnosis in these situations that occur postoperatively (29), after traumatic injury, in patients with seizures, in patients with renal failure, and in those with skeletal muscle myopathies (3). Definitively excluding or confirming the presence of myocardial injury in these patients should simplify their care.

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References