Diagnostic Marker Cooperative Study for the Diagnosis of Myocardial Infarction

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Background—Millions of patients present annually with chest pain, but only 10% to 15% have myocardial infarction. Lack of diagnostic sensitivity and specificity of clinical and conventional markers prevents or delays treatment and leads to unnecessary costly admissions. Comparative data are lacking on the new markers, yet using all of them is inappropriate and expensive.

Methods and Results—The Diagnostic Marker Cooperative Study was a prospective, multicenter, double-blind study with consecutive enrollment of patients with chest pain presenting to the emergency department. Diagnostic sensitivity and specificity and frequency of increase in patients with unstable angina were determined for creatine kinase-MB (CK-MB) subforms, myoglobin, total CK-MB (activity and mass), and troponin T and I on the basis of frequent serial sampling for 24 hours. Of 955 patients with chest pain, 119 (12.5%) had infarction identified by use of CK-MB mass, and 203 (21%) had unstable angina. CK-MB subforms were most sensitive and specific (91% and 89%) within 6 hours of onset, followed by myoglobin (78% and 89%). For late diagnosis, total CK-MB activity (derived from subforms) was the most sensitive and specific (96% and 98%) at 10 hours from onset, followed by troponin I (96% and 93%), but not until 18 hours, and troponin T (87% and 93% at 10 hours). In unstable angina, CK-MB subforms were increased in 29.5%, myoglobin in 23.7%, troponin I in 19.7%, and troponin T in 14.8%. All markers were increased in 99 patients. With each marker as the diagnostic standard, CK-MB subforms and myoglobin remained the most sensitive for early diagnosis.

Conclusions—The CK-MB subform assay alone or in combination with a troponin reliably triages patients with chest pain and should lead to improved therapy and reduced cost. (Circulation. 1999;99:1671-1677.)

Key Words: creatine kinase ■ troponin T ■ troponin I ■ myoglobin ■ myocardial infarction
Methods

Study Design
A multicenter, prospective, double-blind study was performed involving Baylor College of Medicine and the University of Texas Medical School. Enrollment was consecutive 24 hours a day, 7 days per week, at 4 affiliated teaching hospitals in Houston, Tex: Ben Taub General Hospital, Hermann Hospital, The Methodist Hospital, and Veterans Affairs Medical Center. Data and statistical analyses were performed by the independent biostatistics core in the Department of Public Health at the University of Texas Health Science Center in Houston. The protocol was approved by the Institutional Review boards of the medical schools and hospitals, and signed informed consent was required for participation. Eligible enrollees had to be 21 years of age with chest pain ≥15 minutes suspected to be myocardial in origin and occurring within 24 hours of presentation.

Blood samples were obtained on arrival, 1 hour after arrival, every 2 hours up to 6 hours from onset of chest pain, and then every 4 hours thereafter for 24 hours. Samples were placed on ice for transport to the core laboratory. A study coordinator collected the clinical data. Results were not available to laboratory personnel. Management and disposition of the patient were determined by the attending physician. Attending physicians and investigators did not have access to results of the cardiac markers of the core laboratory. ECGs were interpreted by 2 cardiologists unaware of any results.

Assays for Cardiac Markers
Blood for CK-MB subforms was collected in EDTA,14 and the plasma was recovered; for all other markers, blood was clotted and serum was recovered and stored at 2°C to 8°C if assayed the same day or frozen at −20°C and assayed later. Standard controls were assayed routinely. Total CK activity was quantified by use of a spectrophoto-

Criteria for the Diagnosis of Infarction and Other End Points
The upper limit for an early diagnosis was arbitrarily defined as 6 hours from onset of chest pain; late diagnosis was defined as any subsequent time. The diagnostic standard for myocardial infarction was a CK-MB mass ≥7 ng/mL and CK-MB index (CK-MB mass/CK) ≥2.5% determined by the results of the core laboratory in ≥2 samples obtained in the first 24 hours after hospital arrival or in 1 sample if only 1 sample was available for analysis. Unstable angina was a clinical diagnosis determined by the investigator as chest pain occurring at rest or with increased frequency or severity within the prior 24 hours. Myocardial infarction was classified as Q-wave or non–Q-wave infarction on the basis of any ECG obtained in the first 48 hours as follows: new or presumably new pathological Q wave ≥40 ms in ≥2 contiguous ECG leads; R wave ≥40 ms in V1; and R/S ratio ≥1 in V1; or loss of ≥25% R-wave amplitude in ≥2 contiguous leads compared with a previous ECG or documented on 2 serial ECGs. Although the criteria established prospectively to confirm the diagnosis of myocardial infarction were based on CK-MB detected by the mass assay, analysis was also performed in which each marker was used as the diagnostic standard and the relative sensitivity and specificity of the remaining markers were determined.

Statistical Analyses
Differences in age and the proportion of men in each group were tested with 1-way ANOVA and the χ2 test, respectively. Sensitivity and specificity were obtained cross-sectionally (at time period displayed ≥0.5 hours). Exact 95% CIs for binomial proportions were calculated for sensitivity and specificity. Differences in sensitivity between the MB subforms and myoglobin performed on the same samples were analyzed by McNemar’s test. A value of P=0.05 was considered significant. Receiver-operator characteristic (ROC) curves were constructed for each marker, and the area under the curve was calculated.12

Results
Of the 955 patients enrolled, myocardial infarction was confirmed by CK-MB mass criteria in 119 (12.5%). Clinical characteristics of the patients are shown in Table 1. In the 119 patients with acute myocardial infarction confirmed by elevated plasma CK-MB mass, the ECG was diagnostic (ST-segment elevation) in only 45%. There were 36 patients (30.3%) who had Q-wave infarcts; the remaining 83 had non–Q-wave infarction. Although not prospectively planned, it is of note that the attending physicians (blinded to the core laboratory results) made a diagnosis of myocardial infarction based on clinical features and ECG findings in 128 patients, 38 of whom were not confirmed by CK-MB mass criteria. In 6 of these 38 patients, CK-MB mass was increased but in only 1 sample, which did not meet the prospective criteria for infarction (≥2 samples); all 6 also had increased troponin T, troponin I, CK-MB subforms, and total CK-MB activity. Of the remaining 32 patients, 25% had increased troponin T, 34.3% had increased troponin I, 40.6% had increased myoglobin, and 65.6% had increased CK-MB subform activity. On admission, ECG findings in these 32 patients were T-wave inversion (65.7%), ST-segment depression (7.8%), Q waves (32.1%), and ST-segment elevation (47.3%). Of the 119 patients with confirmed myocardial infarction, the attending physician diagnosed 29 as having unstable angina.
The ECG findings in these patients were ST-segment depression (34.5%), T-wave inversion (44.8%), and ST-segment elevation (17.2%). In the 119 patients with infarction, reperfusion therapy was administered to 34.4%: thrombolytic therapy to 30 patients (25.2%) and primary PTCA to 11 (9.2%).

In Table 2, the diagnostic sensitivity and specificity of each marker are compared with CK-MB mass as the diagnostic standard at selected intervals from onset of chest pain. The markers for early diagnosis, CK-MB subforms and myoglobin, exhibited sensitivities of 91% and 78%, respectively, at 6 hours from onset, with similar specificities of ~89%. In contrast, total CK-MB mass, total CK-MB activity, and troponin I and T had sensitivities of only 66.0%, 74.5%, 57.5%, and 61.7%, respectively. The sensitivity and specificity of CK-MB subforms from the first sample were 48.7% and 87.6%, respectively, which were similar to those of myoglobin, 48.7% and 87.7%. The sensitivity and specificity of CK-MB subforms from the combined first and second samples were 81.6% and 87.6%, respectively, which were similar to those of myoglobin, 48.7% and 87.7%. The sensitivity and specificity of CK-MB subforms decreased significantly after 10 hours from onset of symptoms. The sensitivity and specificity of each marker for late diagnosis with CK-MB subforms, myoglobin, troponin T or I, or total CK-MB activity as the diagnostic standard are shown in Table 4. There were 99 patients in

![Figure 1](https://example.com/hypothetical_url)  
**Figure 1.** Overall sensitivity and specificity, together with confidence limits for each marker at 6 hours from onset of symptoms. TN indicates troponin.

Values are percentages.
whom all markers were increased. It is noteworthy that all patients presenting with infarction and ST-segment elevation exhibited an increased in all markers. With CK-MB mass as the diagnostic standard, 119 patients were identified as having an infarction compared with 260 identified with CK-MB subforms, 170 with CK-MB activity, 231 with troponin I, 166 with troponin T, and 276 with myoglobin. Most of these patients had a clinical diagnosis of unstable angina in which CK-MB mass was not increased but other markers were. In 512 patients, none of the markers was increased, and none of these patients was diagnosed as having an infarction by the study criteria. The specificity for total CK-MB activity, troponin I, troponin T, or CK-MB mass was virtually identical, varying from 95% to 99% with each of the other markers used as the standard. The specificity of the CK-MB subforms or myoglobin was slightly less at 93% and 90%, respectively. The total area enclosed by each ROC curve for each marker is included in Table 5.

The diagnosis of unstable angina was made in 203 (21.3%) patients. Pertinent clinical characteristics are given in Table 1. A proportion of the unstable angina population exhibited an increase in ≥1 marker: CK-MB subforms were increased in 29.5%, myoglobin in 23.7%, troponin I in 19.7%, and troponin T in 14.8% (Table 6). ECG was abnormal in 67%, consisting of T-wave inversion (69.9%), ST-segment depression (21.3%), ST-segment elevation (27.2%), and left bundle-branch block (0.06%) (Table 1).

Discussion
In this multicenter, double-blind, prospective study with consecutive enrollment of 955 patients, 12.5% (119) had myocardial infarction and 21% (203) had unstable angina. The most sensitive early marker for myocardial infarction (6 hours after onset) was CK-MB subforms (91%), followed by myoglobin (78%). CK-MB subform analysis of the first sample and a second sample obtained 1 hour later accurately detected 80% of the patients subsequently confirmed to have myocardial infarction. The negative predictive value of CK-MB subforms at 6 hours was 97%, which is very important given that the probability of infarction in this population is only ≈10%. The prospective diagnostic standard for myocardial infarction was CK-MB mass, but with each marker used as the gold standard, CK-MB subforms remained the most sensitive marker for early diagnosis. Thus, a normal CK-MB subform at 6 hours reliably excludes infarction. Total CK-MB (activity or mass), troponin I, and troponin T were reliable late markers, with CK-MB activity having a sensitivity and specificity of 96% and 98%, respectively, at 10 hours from onset; for troponin I, sensitivity and specificity were 96% and 93%, but not until 18 hours. In unstable angina, the markers were increased in 15% to 30% of patients, with CK-MB subforms increased most frequently.

This is the first study to compare the diagnostic accuracy of all the markers. Because laboratory personnel were blinded to clinical data and the investigators were blinded to the results of the markers, the analysis provides an objective comparison. The patient population was enrolled consecutively and

![Figure 2. Overall sensitivity and specificity, together with confidence limits for each marker at 18 hours from onset of symptoms. TN indicates troponin.](image-url)
appears reflective of the population with chest pain observed nationally given the frequency of infarction (12.5%). CK-MB mass was selected as the diagnostic standard because it has been the diagnostic standard worldwide for >2 decades and because extensive clinical and experimental evidence indicate increased plasma CK-MB reflects infarction. The most recent study performed in the conscious dog indicates that irreversible myocardial injury induced by complete coronary occlusion (15 to 20 minutes) and confirmed histologically by electron microscopy 72 hours later is associated with increased serum CK, whereas severe myocardial ischemia (glycogen depletion and cell swelling) induced by 10 to 15 minutes of coronary occlusion was not associated with increased serum CK. Neither serum CK-MB activity nor CK-MB subforms are increased with exercise-induced reversible myocardial ischemia detected by thallium. Despite claims that the cardiac troponins may be released with ischemia, experimental studies to determine whether their release reflects myocardial ischemia, necrosis, or both are lacking.

There are no published studies in which all the markers are compared in relation to onset of symptoms. Other studies have evaluated selected markers, but usually in select populations, in relation to time of presentation, and frequently with inadequate sample size. Our results showing that CK-MB subforms are a reliable marker for triaging patients in the first 6 hours after onset of symptoms are virtually identical to those of a previously reported large, prospective study. Other studies have consistently shown CK-MB subforms to be the most sensitive and specific marker within 6 hours of onset of symptoms. The addition of myoglobin to CK-MB subforms did not increase diagnostic sensitivity. In 309 patients, de Winter et al noted myoglobin to be a sensitive early marker of myocardial infarction, whereas troponin T was a late marker, similar to total CK-MB. Brogan et al showed that troponin I and CK-MB had similar sensitivities and specificities in 171 patients.

In the GUSTO IIA study of 801 patients with unstable angina, troponin T was increased in 36%, and the 30-day mortality rate was 11.8% compared with 3.9% in patients with normal levels. In TIMI IIIB, a study of 1404 patients with unstable angina and non–Q-wave infarction, troponin I was increased in 41%, with a mortality rate of 3.7% compared with 1.0% in the group with normal levels. A recent study of just unstable angina showed that troponin T and I were increased in 24%. In TIMI IIA (622 patients

### TABLE 4. Sensitivity and Specificity of Each Marker at 14 Hours After Onset With Different Diagnostic Standards

<table>
<thead>
<tr>
<th>Marker</th>
<th>Subform</th>
<th>Myoglobin</th>
<th>Troponin T</th>
<th>Troponin I</th>
<th>CK-MB Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subform</td>
<td>76.0</td>
<td>59.6</td>
<td>79.7</td>
<td>62.2</td>
<td>82.9</td>
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<tr>
<td>Myoglobin</td>
<td>52.0</td>
<td>63.5</td>
<td>55.1</td>
<td>40.8</td>
<td>57.1</td>
</tr>
<tr>
<td>Troponin T</td>
<td>50.0</td>
<td>44.2</td>
<td>81.7</td>
<td>50.0</td>
<td>68.6</td>
</tr>
<tr>
<td>Troponin I</td>
<td>59.0</td>
<td>49.0</td>
<td>78.1</td>
<td>70.0</td>
<td>74.3</td>
</tr>
<tr>
<td>CK-MB activity</td>
<td>49.0</td>
<td>52.9</td>
<td>73.9</td>
<td>55.1</td>
<td>90.0</td>
</tr>
<tr>
<td>CK-MB mass</td>
<td>58.0</td>
<td>45.2</td>
<td>68.1</td>
<td>51.0</td>
<td>71.4</td>
</tr>
<tr>
<td>Specificity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subform</td>
<td>100.0</td>
<td>93.9</td>
<td>92.1</td>
<td>93.6</td>
<td>93.2</td>
</tr>
<tr>
<td>Myoglobin</td>
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<td>100.0</td>
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<td>89.0</td>
<td>90.1</td>
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<tr>
<td>Troponin T</td>
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<td>96.1</td>
<td>100.0</td>
<td>97.0</td>
<td>97.0</td>
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<tr>
<td>Troponin I</td>
<td>95.3</td>
<td>94.7</td>
<td>94.0</td>
<td>100.0</td>
<td>93.2</td>
</tr>
<tr>
<td>CK-MB activity</td>
<td>97.9</td>
<td>96.5</td>
<td>95.4</td>
<td>96.2</td>
<td>100.0</td>
</tr>
<tr>
<td>CK-MB mass</td>
<td>99.1</td>
<td>98.3</td>
<td>98.5</td>
<td>99.6</td>
<td>99.6</td>
</tr>
</tbody>
</table>

Values are percentages.

### TABLE 5. Estimated Area Enclosed by the ROC Curve for Each Marker at 6 and 14 Hours After Onset of Symptoms

<table>
<thead>
<tr>
<th>Marker</th>
<th>6 h</th>
<th>14 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK subform</td>
<td>0.950</td>
<td>0.941</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>0.920</td>
<td>0.836</td>
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<tr>
<td>Troponin I</td>
<td>0.886</td>
<td>0.969</td>
</tr>
<tr>
<td>Troponin T</td>
<td>0.949</td>
<td>0.910</td>
</tr>
<tr>
<td>CK-MB activity</td>
<td>0.944</td>
<td>0.988</td>
</tr>
</tbody>
</table>

### TABLE 6. Proportion of Patients With Clinical Diagnosis of Unstable Angina Without Increased CK-MB Mass but With an Increase in Other Markers

<table>
<thead>
<tr>
<th>Diagnostic Marker</th>
<th>Frequency of Increased Serum Marker, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-MB subforms</td>
<td>29.5</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>23.7</td>
</tr>
<tr>
<td>Troponin I</td>
<td>19.7</td>
</tr>
<tr>
<td>Troponin T</td>
<td>14.8</td>
</tr>
<tr>
<td>Total CK-MB activity</td>
<td>15.3</td>
</tr>
</tbody>
</table>

n=203.
with unstable angina. troponin T was increased in 19.5%, similar to our results. The sensitivity of CK-MB subforms was greater, being increased in 29.5%. It remains a conundrum as to whether a minor increase in ±1 marker should be diagnosed as myocardial infarction. There was a core of 99 patients in whom all markers other than CK-MB mass were increased. Myoglobin was increased in 276, and troponin I was increased in 166. Most additional patients had a clinical diagnosis of unstable angina. Thus, the CK-MB mass assay is less sensitive than CK-MB activity, CK-MB subforms, or the troponins. CK-MB activity and troponin T were virtually identical as diagnostic standards, with 170 patients with increased CK-MB activity and 166 with increased troponin T having an infarction. CK-MB subforms and troponin I were also similar, with 260 with elevated CK-MB subforms and 231 with elevated troponin I having an infarction. These results indicate that CK-MB enzymatic activity is a more appropriate assay than CK-MB mass.

We propose the diagnosis of myocardial infarction rather than unstable angina when associated with an increased CK-MB because the data suggest that myocardial necrosis, while controversial, does have significant prognostic and therapeutic potential. Patients with increased total CK-MB, troponin T, or troponin I consistently exhibit an increased risk of clinical events, including death. Even after PTCA, those with minimal increased total CK-MB exhibited increased risk over those without increased CK-MB. Patients with circumflex coronary obstruction seldom present with ST-segment elevation and should benefit from fibrinolytic therapy; however, given that the ECG findings are nonspecific and the risk of stroke with fibrinolitics is substantial, infarction needs to be confirmed before therapy. In TIMI IIb, patients with unstable angina receiving thrombolytic therapy had increased death and infarction, but patients with non–Q-wave infarction had similar outcomes whether they received thrombolitics or not. However, the mean time from onset of symptoms to therapy was 9 hours, which is probably too late to be effective. An early diagnosis (80% within 1 hour after admission) can now be made in these individuals, and an appropriate trial with fibrinolytic therapy should be performed to test the hypothesis that thrombolysis is beneficial in non–Q-wave myocardial infarction.

The importance of triaging patients with chest pain in the emergency room, in addition to the diagnostic and therapeutic implications, is cost-effectiveness. We have shown that early triaging based on CK-MB subforms could potentially save billions of dollars through the avoidance of unnecessary hospital admissions. A pilot study recently performed in 1314 patients with chest pain triaged on the basis of CK-MB subforms reduced the cost per patient by 35%. These results applied nationally would represent billions of dollars saved. The assays for each marker are automated, have similar costs, and require 25 minutes. In the selection of a single assay, CK-MB subforms provide the earliest diagnosis; total CK-MB activity, derived from it, is the most sensitive for late diagnosis. If one prefers a combination of assays, then CK-MB subforms, in combination with troponin I or T, are recommended. Sampling for 24 hours provides a baseline for subsequent procedures, detection of early reinfarction, and a rough estimate of infarct size from the peak serum activity. Early reinfarction (accounting for 40% of deaths after thrombolysis) is best detected by the CK-MB subforms because total CK-MB mass or activity remains increased for 48 hours and the troponins for ±10 days, whereas CK-MB subforms return to normal in 18 to 24 hours.

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