Early Diagnosis of Acute Myocardial Infarction by Rapid Analysis of Creatine Kinase Isoenzyme-3 (CK-MM) Sub-Types

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We compared the clinical sensitivity, specificity, and diagnostic efficiency of measuring creatine kinase-3 (MM) isoenzyme sub-types (CK, EC 2.7.3.2) with the measurement of CK-2 (MB) isoenzymes for the diagnosis of acute myocardial infarction. Serial blood collections at 3-h intervals from 35 patients with acute myocardial infarction were examined. In attempts to reperfuse their coronary arteries, some of these patients were treated with pharmacological thrombolysis (streptokinase, tissue plasminogen activator), with or without coronary angioplasty. The infarction patients were divided into two groups: patients who were successfully treated with thrombolytic agents (i.e., they achieved coronary reperfusion), and patients who were treated unsuccessfully or who were not treated acutely. We also examined blood from 34 non-infarction patients. We measured CK-3 sub-types by both anion-exchange liquid chromatography and a modified high-voltage electrophoresis method, and CK-2 by immunoprecipitation. Our results show that during the first few critical 3 to 9 h after onset of chest pain, measurement of CK-3 sub-types has the highest diagnostic efficiency; in contrast, CK-2 has the highest efficiency during the 10- to 21-h time intervals. Thus early diagnosis of acute myocardial infarction can be based on rapid assays of CK-3 sub-types.

Additional Keyphrases: streptokinase thrombolysis coronary reperfusion chromatography anion-exchange electrophoresis cellulose acetate electrophoresis, high-voltage

Acute coronary thrombosis is thought to be the etiologic agent in many cases of myocardial infarction (MI).1 To minimize the extent of myocardial necrosis, cardiologists are now evaluating methods for revascularizing occluded arteries. Thrombolytic agents such as streptokinase and tissue plasminogen activator (TPA), and methods such as percutaneous coronary angioplasty, have the greatest chance of success when administered to the patient as soon as possible after the onset of chest pain (1, 2). It is therefore important to differentiate between MI and non-MI patients so that the former can be considered for thrombolytic therapy, and the latter transferred out of the coronary-care unit. Currently, in the emergency room, the decision of whether or not to treat each patient is made only on the basis of electrocardiographic findings and clinical history. Early biochemical markers for cardiac necrosis are useful to rule-in acute MI and to justify immediate thrombolytic therapy. Absence of this evidence, however, does not contra-indicate acute treatment, because the infarction may still be at an early reversible stage. Although creatine kinase(CK)-2 and lactate dehydrogenase(LD)-1 isoenzymes are most often cited as markers for acute MI, other proteins have been evaluated. The concentration of, e.g., myoglobin in serum is increased over reference limits within 2 to 4 h after acute MI, peaking at 9–12 h (3, 4). Myosin light chains are also useful in the diagnosis of acute MI, abnormal concentrations of them having been observed within 5 to 6 h after onset of chest pain (5, 6). For the early diagnosis of MI, both of these markers offer some advantage over CK-2, which, in cases not treated with thrombolytic agents, does not peak until 18–24 h after the onset of chest pain. Unfortunately, methods for myoglobin and myosin light chains require radioimmunoassay or enzyme-linked immunoassay, so that much of the advantage afforded by the earlier appearance of these proteins in serum is negated by the time required to obtain results. Additionally, the clinical specificity for these markers is considerably less than that for CK-2 (4, 5).

We have therefore investigated the utility of measuring the three sub-types of CK-3 (MM), the focus of several investigations (7–13). The most cathodic sub-type, CK-3a, is the gene-encoded form produced in tissues, which is converted (by serum carboxypeptidase) first to CK-3b and then to CK-3c, the latter being the form detected in serum (6). In controlled studies of MI in dogs, CK-3 sub-types were released within 1 h after the onset of an induced infarction, well before total CK rose above the upper limit of normal (9, 10). Studies in humans have also shown earlier release patterns of CK-3 sub-types than of CK-2 (11). Because existing methods for CK-3 sub-types are based on time-consuming isoelectric focusing and extended-time electrophoresis, we examined the measurement of the sub-types by anion-exchange liquid chromatography (LC) and high-voltage electrophoresis (HVE), which yield results in <30 min. We assessed clinical sensitivity and specificity at various decision limits by plotting receiver-operating characteristic (ROC) curves for results from patients in a coronary care unit.

1 Nonstandard abbreviations: MI, myocardial infarction; TPA, tissue plasminogen activator; CK, creatine kinase (EC 2.7.3.2); LD, lactate dehydrogenase (EC 1.1.1.27); LC, liquid chromatography; HVE, high-voltage electrophoresis; ROC, receiver–operating characteristic curves.

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Materials and Methods

Subjects

We selected 69 patients who had been admitted to the coronary-care unit for evaluation of acute chest pain: 35 patients with acute MI (ages 35–82 years, 30 men, five women) and 34 in whom this diagnosis was ruled out (ages 18–90 years, 19 men, 15 women). All patients were enrolled under a protocol reviewed and approved by the Committee for the Protection of Human Subjects at the University of Texas Health Science Center at Houston and Hermann Hospital. Written consent was obtained from all patients. The diagnosis was made by the attending physicians on the basis of clinical history, electrocardiographic recordings, changes in serum enzyme activities (total CK, CK-2, total LD, LD-1) and coronary angiography, when available. The results for CK-3 sub-type analysis were not considered in the clinical diagnosis. We also analyzed sera collected from 27 apparently healthy individuals, to establish a range to compare CK-3 sub-type results.

Thrombolytic therapy: For those patients deemed suitable for treatment with streptokinase, the protocol was stabilization by lidocaine, sublingual nitroglycerin, and nifedipine; cardiac catheterization; coronary angiography; and treatment with intercoronary nitroglycerin and streptokinase (2000–4000 USP units/min for 90 min). Immediate post-treatment assessment was made by 301T imaging. Later assessment was made by repeat arteriography, left ventriculography, and gated imaging of pooled cardiac blood.

For patients treated with TPA, a 1.0 mg/mL solution of recombinant human TPA (Genentech Inc., South San Francisco, CA 94080) was administered intravenously at a constant rate, usually for 90 min. Percutaneous coronary angioplasty was administered through cardiac catheterization according to a method described by Gruntzig et al. (14).

Specimens: Whenever possible, blood from patients with acute MI was drawn at 3-h intervals for the first 24 h after chest pain, and at 6-h intervals from 24 to 48 h afterwards. For non-MI patients, we took a single blood sample each day for two days. All blood was collected by venipuncture into evacuated serum-separator tubes (Becton Dickinson, Rutherford, NJ 07070), centrifuged, and the serum stored at −20°C until analysis.

Methods for Total CK and CK-2

Total CK activity was measured in a centrifugal analyzer (Multistat III; Instrumentation Laboratory, Lexington, MA 02173) with "CK-NAC" reagents (Boehringer Mannheim Diagnostics, Houston, TX 77063). CK-2 was measured by immunoprecipitation (15) (with an Isomune CK-MB kit, Roche Diagnostics, Nutley, NJ 07110). Both assays were performed at 37°C.

CK-3 sub-form assay: Patient's samples and controls for CK-3 sub-types were measured by LC as described elsewhere (13), and by a modified electrophoretic procedure on cellulose acetate (CPK-US; Helena Labs., Beaumont, TX 77704). In the electrophoretic procedure, we changed the buffer pH from 8.8 to 8.4; increased the applied voltage from 300 to 800 mV, the electrophoresis time from 10 to 12 min, and the incubation temperature from 37°C to 45°C; and decreased the incubation time with substrate from 25 to 8 min. All of the other conditions were as described by the manufacturer. The acetate plates were scanned at 366 nm in a microcomputer-controlled densitometer (EDC; Helena Labs.). The turnaround time for eight samples was <30 min.

Statistical analysis: The programs used for plotting ROC curves have been described (16). Differences between means were assessed by Student's t-test.

Sub-Type Nomenclature:

We have adopted a convention consistent with that of the International Union of Pure and Applied Chemistry (17), and as suggested by others (7, 11, 13, 18, 19), whereby the most anodic sub-type is designated with the lowest Arabic numeric subscripts. Additionally, we have adopted the convention whereby the most anodic of the isoenzymes (MM, MB, and BB) is designated with the lowest Arabic numeric suffix. Thus, for the MM sub-types, the serum form is designated as CK-3 and the tissue form as CK-3a.

Results

CK-3 sub-types in a normal population: Table 1 shows the range of values (mean ± SD) obtained for the sub-types determined by both analysis methods for 27 apparently healthy individuals. The electrophoresis method has lower values than does the ratio of CK-3a/CK-3 to anion-exchange chromatography.

Typical results in MI: Typical LC chromatograms for CK-3 sub-types, in patients with acute MI, have been presented elsewhere (15). Figure 1 shows a cellulose gel and corresponding quantitative densitometric scans (Table 2) obtained with HVE for a patient with acute MI. In the earliest hours after acute MI (5 h), the CK-3 sub-type pattern resembles that observed in normal sera; at 9 h, the predominant sub-type has shifted from CK-3a to CK-3b, corresponding to the acute release of the tissue form. At 12 h, CK-3b

Table 1. CK-3 Sub-types in a Normal Population

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<tr>
<th>Sub-type</th>
<th>Percent of total CK as measured by</th>
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<tr>
<td></td>
<td>LC</td>
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<tr>
<td>CK-3_3</td>
<td>10.2 ±25.5</td>
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<tr>
<td>CK-3_2</td>
<td>21.6 ±52.9</td>
</tr>
<tr>
<td>CK-3_a</td>
<td>31.8 ±67.5</td>
</tr>
<tr>
<td>CK-3b/CK-3_1</td>
<td>0.19 ±0.71</td>
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<td>n = 27 subjects.</td>
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Fig. 1. Typical results from HVE of CK-3 sub-types for samples from patient with acute MI, 5–25 h after onset of chest pain (numerical values given in Table 2).

All scans were normalized to the largest fluorescent band.
begins to be converted to CK-3\textsubscript{2}, and by 25 h the predominant form is again CK-3\textsubscript{1}.

**Comparison of LC and HVE methods**: Figure 2 shows the analytical correlation of the ratio of CK-3 sub-types as measured by LC and HVE ($y \ = \ 0.406x - 0.16, r = 0.892$). Although the slope is not unity, there is adequate correlation to suggest that these methods are comparable.

**Appearance of CK-2 and CK-3 vs time after onset**: We divided the 35 MI patients into two groups, depending on the choice of treatment and its success: (a) 25 patients treated with streptokinase, TPA, and (or) angioplasty for which there was successful reperfusion of the affected coronary artery, as determined by cardiac catheterization and coronary angiography; (b) 10 patients either treated with the above, but for whom reperfusion did not occur (five cases), or for whom acute intervention was not attempted (i.e., they received conventional treatment with antianginal drugs and anticoagulants, five cases). Figure 3 shows the peak time after the onset of chest pain for total CK and CK-2 and CK-3 sub-types for each group. For total CK, the reperfusion group (group 1) showed significantly earlier release (15.9 vs 22.3 h, $p <0.001$) and at higher enzyme activities ($x \ = \ 2610 \pm 1860$ vs $1590 \pm 780$ U/L) than for the non-reperfused and non-treated MI group. The recanalization of a previously occluded coronary artery produces the *washout* phenomenon of cardiac enzymes that has been described by Kwong et al. (20), who studied patients successfully treated with streptokinase. Figure 3 also shows the time at peak concentrations for CK-3 sub-types as compared with CK-2. In both groups, the ratio of CK-3\textsubscript{2} to CK-3\textsubscript{1} peaked significantly earlier ($p <0.001$) than for CK-2 (a difference of 4 h for group 1, 8 h for group 2). The difference between the reperfused and non-reperfused groups for CK-3 sub-types, however, was not as great (10.8 vs 12.2 h) as what was observed for total CK.

The release of CK-3 sub-types as compared with CK-2 for a typical patient is shown in Figure 4. As illustrated in this case, the CK-3 sub-types first becomes increased above the

<table>
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<th>Table 2. Typical Results by HVE for CK-3 Sub-types, CK-2, and Total CK after MI</th>
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<tr>
<td>Time (h) after onset of pain</td>
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<td>5</td>
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<td>9</td>
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<td>12</td>
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<td>18</td>
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<td>25</td>
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*Quantified by CK-NAC, Boehringer Mannheim Diagnostics. *Quantified by Isomune CK, Roche Diagnostics. *Quantified by HVE (example shown in Fig. 1). *Sample with highest CK-3\textsubscript{3}/CK-3\textsubscript{1}. *Peak total CK at 21 h.

reference limit 7 h after the onset, as compared with 10 h for CK-2. In addition, the greatest increase, i.e., the sample with the best potential for discriminating MI from non-MI, is that taken at 10 and 14 h, respectively. These data suggest that CK-3 sub-types are useful for early MI detection. After the acute release of the CK-3\textsubscript{1} sub-type, it is rapidly converted in the blood to the forms typical of serum, and the ratio of sub-types returns to normal limits sooner than for CK-2 (Figure 4).

**Clinical sensitivity and specificity**: Figure 5A shows the receiver-operating characteristic curves for CK-2 and CK-3 sub-types at the critical 3–9 h time interval after the onset of chest pain. The curves obtained for CK-3 sub-types by LC
(curve a) and HVE (curve b) are similar. Both of these curves demonstrate that CK-3 sub-types are superior in diagnostic efficiency to the CK-2 (curve c) at this time interval. In contrast, Figure 5B shows the ROC curves at the 10–18 h time interval, which shows that the efficiency for the diagnosis of MI has shifted in favor of measurement of the CK-2 isoenzyme.

Table 3 summarizes these results at a single decision limit. In order to compare clinical sensitivity between the various methods, we chose a decision limit such that clinical specificity for each method was the same (96%). As shown, the optimum diagnostic time-“window” for CK-3 sub-types is 7–9 h. Thereafter, the efficiency begins to diminish. In contrast, the efficiency for CK-2 progressively increases from 7 to 9 h, is equivalent to CK-3 at 10 to 12 h, and reaches a maximum at 19 to 21 h.

When comparing clinical specificity, we found that the measurement of total CK-2 isoenzymes was superior to the measurement of CK-3 sub-types. False-positive concentrations of CK-3 sub-types can be observed in acute muscle disease, although this is usually not part of the differential diagnosis for acute MI.

Discussion

Many investigators believe that stat determination of CK-2 is unnecessary for routine diagnosis of acute MI because definitive diagnosis is best made with serial isoenzyme measurements of CK and LD coupled with clinical history and electrocardiographic findings (15, 21, 22). For those MI patients who are to be treated conventionally—i.e., no attempts to recanalize the coronary circulation—this remains true. However, if use of thrombolytic agents is contemplated, they should be initiated as soon after the onset of chest pain as possible for optimal salvage of the jeopardized myocardium. Early and successful treatment of evolving acute MI by thrombolytic agents can reverse acute ischemia in advance of significant injury and prevent the release of cardiac enzymes into the circulation (23). But, because streptokinase can produce bleeding and coronary angioplasty requires cardiac catheterization, early and definitive diagnosis is desirable so that thrombolysis is not attempted on patients who do not have occluded coronary arteries. Clinical specificity can be sacrificed somewhat under these conditions so that MI can be diagnosed early.

Our data demonstrate the diagnostic advantage of CK-3 sub-type analysis for early MI diagnosis. Neither CK-2 or CK-3 subtypes are as diagnostically efficient at 3–9 h after the onset of MI as CK-2 is after 10–21 h. Thus, it is important that physicians using this information be aware of the limitations of CK-3 sub-types, when considering thrombolytic therapy. In addition, measurement of these sub-types cannot replace CK-2 for documenting myocardial necrosis. CK-2 together with LD-1 are still the best overall markers for acute MI, with diagnostic efficiencies at optimum time windows approaching 100%. CK-3 sub-types may have a role early, however, as we can now identify three time windows for the optimum use of cardiac isoenzymes: an early time window (10–21 h) for which CK-2 is most useful, a late window (16–30 h) for which LD-1 is most useful (24), and a very early window (3–9 h) for which CK-3 sub-types are most useful. A complete cardiac isoenzyme profile may involve a combination of all three.

We have developed and evaluated rapid methods for the analysis of CK-3 sub-types based on anion-exchange LC and HVE on cellulose acetate. Although other methods such as
isoelectric focusing may have better resolution, they are not useful for emergency testing because of their complexity and long turnaround time. Both LC or HVE can produce results within 30 min.

When comparing LC and HVE for sub-type analysis, we found that both are roughly equivalent in terms of analytical sensitivity and specificity, cost, and clinical efficiency for early MI diagnosis. HVE, however, has a higher throughput, because multiple samples can be analyzed concurrently. This advantage is of lesser importance if individual samples are to be measured on an urgent ("stat") basis. For routine sub-type analysis, either a liquid chromatograph or a cellulose acetate electrophoresis system is required. Because many laboratories in the United States routinely use electrophoresis, the HVE method, which requires only minor modifications, is probably most generally available.

In our investigation we did not study the role of CK-2 subtypes in the diagnosis of MI. The current LC assay is not optimized for CK-2 sub-types, which would require modifications of assay variables. The HVE method can detect CK-2 sub-types but, because the proportion of total CK-2 is only 5 to 10% of total CK, the sensitivity must be improved if these sub-types are to be routinely detected, especially in MI-cases where total CK is within the normal reference interval or is only marginally increased (25). Simultaneous determination of CK-2 and CK-3 sub-types, if sufficiently sensitive, may provide high clinical specificity while maintaining the advantage of early MI detection.

This was a retrospective study; i.e., the treatment of the MI patients was selected without knowledge of the CK-3 sub-types results. Indeed, many of these patients were treated with intravenous TPA in the emergency room before any serum-enzyme results were known. To accurately assess the impact that these determinations may have on early diagnosis and treatment, a prospective randomized study would be necessary that would make results available on an emergency basis. Through such a study, the health benefits as compared with the assay costs could be evaluated.

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References