Creatine Kinase Isoforms in Ischemic Heart Disease

Alan H. B. Wu

The MM and MB isoenzymes of creatine kinase exist in serum as a collection of at least three major MM and two major MB isoforms. Each of these are derived from single tissue MM and MB isoforms, which are converted to these other forms by carboxypeptidase N after their release from necrotic skeletal and myocardial tissue. Measurement of the MM isoforms in ischemic heart disease is useful for early diagnosis of acute myocardial infarction and for the noninvasive determination of coronary artery reperfusion for infarction patients receiving thrombolytic therapy. Because MM is also released in acute skeletal-muscle disease, MB isoform measurements may have the highest clinical sensitivity. These determinations are important for providing objective information to cardiologists who need to make critical decisions concerning the management of these patients. I review the procedures for treating patients with myocardial infarction, the potential role of CK isoforms, and the methods currently available for isoform analysis, including high-resolution electrophoresis, isoelectric focusing, and liquid chromatography. Rapid and highly sensitive methods are needed for implementation of CK-MM and MB isoforms for prospective emergency determinations for patients with acute myocardial infarction.

Additional Keyphrases: post-synthetic modifications · myocardial infarction · thrombosis · thrombolytic therapy · coronary artery reperfusion · streptokinase · tissue plasminogen activator · coronary angioplasty · high-voltage electrophoresis · isoelectric focusing · liquid chromatography

Thrombolytic Therapy in Acute Myocardial Infarction

Many cases of acute myocardial infarction are caused by a clot forming within a coronary artery that has been narrowed by atherosclerosis. Some investigators now believe that rupture of atherosclerotic plaques with thrombus formation and vasospasms is the underlying pathogenesis of coronary occlusion (1). The goal of prospective thrombolytic therapy is to restore coronary circulation by clearing thrombi via activation of the natural fibrinolytic system (2). Among the thrombolytic agents currently in use or under investigation are streptokinase, tissue plasminogen activator (TPA), urokinase, and pro-urokinase (3, 4). In addition, methods such as percutaneous transluminal coronary angioplasty have been extensively used to reperfuse coronary blood flow (5). Treatment by any of these methods provides a less radical alternative to acute surgery for coronary artery bypass.

Selection and use of these therapeutic modalities is guided by the clinical history, coagulation status, age of the patient, and the facilities available to the physician. Objective biochemical data, although desirable, are not currently available to be a part of the decision making process. Figure 1 summarizes the various procedures available for acute management of myocardial infarction. No single approach is applicable to all patients. For example, if the patient is older or has a history of bleeding, a conservative regimen of medications may be the best approach. If thrombolytic therapy is selected, either streptokinase or TPA can be administered intravenously. If cardiac catheterization is available on an urgent ("stat") basis, either intracoronary thrombolytic therapy or coronary angioplasty, or both, can be given. Angioplasty works best with sub-occluded arteries, whereas the most effective regimen for many patients may be to treat first with intravenous streptokinase or TPA, and then with angioplasty (3). The decision to acutely catheterize the patient may depend on the success of intravenous therapy in restoring coronary circulation (see Figure 1). Definitive assessment of patency is best achieved by coronary angiography, which requires cardiac catheterization. Patients with successful reperfusion, however, would not have otherwise needed to be catheterized. Therefore, definitive methods for the non-invasive determination of reperfusion are desirable. Current non-invasive measures of reperfusion include the cessation of chest pain, the detection of acute changes by

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Fig. 1. Available therapeutic options for acutely treating myocardial infarction

The non-invasive determination of reperfusion is particularly important for patients treated with intravenous thrombolytic therapy (shown in bold), because angioplasty or bypass surgery may be acutely necessary if such treatment is unsuccessful. Patients who are treated through cardiac catheterization can be immediately evaluated by angiography and given angioplasty if necessary. (Figure modified from Chatterjee [3])

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1 Nonstandard abbreviations: CK, creatine kinase (ATP: creatine N-phosphotransferase, EC 2.7.3.2); MI, myocardial infarction; IEF, isoelectric focusing; and TPA, tissue plasminogen activator.
electrocardiography, and the presence of reperfusion arrhythmias. These are not objective criteria and are of limited accuracy (6, 7).

Optimum use of thrombolytic therapy requires that agents be administered as soon after the onset of chest pain as possible. Data from the Thrombolysis in Myocardial Infarction (TIMI) Study have shown that, irrespective of the agent or route of administration used, the success rate of recanalization decreases with increasing time of onset to treatment (8, 9). Moreover, studies on mortality 21 days after acute MI have shown that early treatment with streptokinase will reduce the relative risk of death by 26% (P = 0.0005) and 20% (P = 0.03), when administered 0–3 and 3–6 h after onset of pain, respectively (10). These findings correlate with studies done in dogs demonstrating that irreversible myocardial damage proceeds 3–6 h after coronary occlusion—"the wavefront phenomenon" (11). Many cardiologists apparently now accept that, when possible, thrombolytic therapy should be given to patients with acute myocardial infarction within 4 h after chest pain (12, 13). Because of this need to treat early and the lack of available markers for early MI diagnosis, these agents are often given when only a presumptive diagnosis of MI has been rendered. This is an undesirable situation, because if thrombolytic therapy is given to the non-MI patient, adverse effects may unnecessarily result. Some of the reported complications of thrombolytic therapy include bleeding (ranging in severity from puncture-site hemorrhage to intracranial bleeding) (9, 14), allergic reactions (in relation to streptokinase) (10), cardiac arrhythmias (14), and other more isolated effects. New biochemical markers are therefore needed that not only can detect early myocardial infarction but for which rapid analytical methods are available for reporting results on a stat basis. Creatine kinase (CK) isoforms have been studied both as a non-invasive marker for coronary artery reperfusion, and for early diagnosis of acute MI.

Creatine Kinase Isoforms

Biochemical Considerations

Unlike atypical isoenzymes such as macro CK types 1 (CK-BB bound to immunoglobulin) and 2 (polymeric mitochondrial CK), which only appear in serum on rare occasions (15, 16), serum isoforms to CK-MM and CK-MB are part of the normal clearance process for CK and are present in all human sera. They should not be considered as variants. Moreover, their existence does not alter the interpretation of CK-MB isoenzyme determinations.

The biochemical characterization of CK isoforms have been reviewed by Panteghini (17). Examination of human myocardial and skeletal muscles reveals the presence of single CK-MM and -MB isoforms (18). These native or pure gene products are post-synthetically modified upon release into the circulation to produce two additional MM and one extra MB isoforms (five total MM and MB isoforms) (19).

The nature of these modifications has been extensively studied by hybridization experiments, which demonstrated the existence of two separate M subunits monomers (20, 21), designated in this paper as the tissue (M_T) and serum (M_S) subunits. The conversion of M_T to M_S is catalyzed by carboxypeptidase N (22, 23). Combinations of M_T and M_S, together with the CK-B subunit, make up the five dimeric CK-MM and MB isoforms that are observed in serum. Figure 2 summarizes the unidirectional, enzyme-catalyzed reactions on tissue CK isoforms, and defines the nomenclature that will be used throughout this work. Isoforms are listed with consecutive Arabic numbers, beginning with the band having the fastest mobility toward the anode by zonal electrophoresis.

CK Isoforms in Ischemic Heart Disease

**CK-MM isoforms in dogs:** The potential for MM isoform measurements in man can be assessed by inducing acute myocardial infarctions in dogs under controlled conditions. The dog is an excellent model for studying the myocardial release of MM after acute MI because (a) the time of onset of infarction can be precisely controlled by occluding specific coronary arteries with a balloon cuff, (b) the duration of the occlusion and subsequent reperfusion is controlled, and (c) the canine myocardium contains predominately MM (95%). The balloon occluder is inserted after aseptic left thoracotomy. Experimental myocardial infarction is induced after a one- or two-week recovery period or when values for total CK in serum have returned to baseline. Although the kinetics of release and clearance are accelerated in the canine model, the MM isoforms show a parallel relationship to what is observed in man; the myocardium contains a single isoform with the highest electrophoretic migration toward the cathode, which converts to two other isoforms of decreasing isoelectric points at parallel rates in vivo and in vitro (24).

Further studies in dogs have shown that the site at which MM isoform conversion occurs is the serum and not the necrotic myocardium or lymph (25). Thus measurement of
MM1 can be theoretically useful in predicting the time of onset of enzyme release into the blood. Examination of the rate of CK-MM isofrom release and conversion in the canine model can provide justification for examining MM isoforms for early detection of MI in man. In studies in which blood is collected at hourly intervals from dogs after artificial coronary artery occlusion, definitive detection of MI is possible as early as 1 h after coronary occlusion and before the concentration of total CK rises above the reference limit (26). Although simulated myocardial infarction experiments cannot be done in man, studies have shown that volunteers who undergo a series of skeletal-muscle contractions will have measurable increases in MM3 without significant increase in total CK by 2 h after exercise, reflecting recent skeletal muscle necrosis (27). These findings suggest that isofrom measurements should theoretically be able to detect myocardial necrosis within the 4-h time window that is essential for thrombolytic therapy.

Early diagnosis of acute myocardial infarction in man: From the earliest reports of CK-MM isoforms, specific changes have been described in the serum of patients with acute myocardial infarction (19). Figure 3 shows a typical example of isofrom release and conversion after MI. Because irreversible myocardial damage after infarction is characterized by the "bolus-like" release of tissue enzymes and proteins, the concentration of MM3 in serum should be high if the blood is sampled before there is substantial amount of conversion by carboxypeptidase. Samples collected later show increasing concentration of MM2 and MM1 as they are converted. Concentrations of MM3 reach their maximum in the circulation well before peak concentrations of total MB appear. In one study, the average time for peak enzyme activity of eight patients averaged 23 ± 4 h for total CK, 25 ± 5 h for MB, and 16 ± 4 h for MM3 (28). The ratio of MM3/MM1 appears to be the best indicator for early MI detection, with the peak ratio occurring at 10.6 ± 2 h after acute onset, 14 h before peak CK-MB.

The clinical sensitivity for CK-MM isoforms and CK-MB can be directly compared at different time intervals after the onset of MI by adjusting the decision limit so that the clinical specificity is identical for both methods. As shown in Table 1, the sensitivity for MI when the MM3/MM1 ratio is used is highest during the first 3–9 h after MI, whereas for MB the sensitivity is highest at 9–21 h (29). The effective "window" for use of MM isoforms is similar to other early MI markers such as myoglobin (30) and myosein light chains (31) (see Table 2). Studies determining the clinical sensitivity of MM isoforms within the critical 4 h after onset have been limited. In one study, 24 of 28 MI patients (86%) were positive by the ratio of MM3/MM1 in samples collected on the average of 3.9 ± 0.4 h after onset of chest pain (32). Total CK and CK-MB concentrations were within the normal limits in many of these patients. In another, clinical sensitivities of 74% were found when the first sample from the emergency room was analyzed (33). As promising as these preliminary results appear, the clinical specificity for MM isoforms remains an important limitation (as discussed in the next section), and measuring of MB isoforms must be further examined.

MM isoforms for determination of reperfusion: Measurement of total CK and CK-MB can be used for the determination of successful reperfusion. Patients who have been successfully treated with streptokinase exhibit an earlier release pattern and higher enzyme activities (the "washout" phenomenon) than do MI patients for whom reperfusion is unsuccessful or MI patients not treated acutely (34). The manner in which thrombolysis is effected is not important, because similar results are obtained when revascularization is achieved by either streptokinase or TPA.

The analysis of CK-MM isoforms is an earlier indicator and better discriminator of coronary reperfusion than either total CK or MB. Studies in dogs have shown that when the rate of %MM3 increase is used as a marker, successful reperfusion can be demonstrated within 30–60 min after the release of the occlusion, in contrast to the 3–5 h needed for total CK (35). Similar results have been obtained in human studies of acute MI treated with thrombolytic agents (34, 36–38). With the MM3/MM1 ratio as the criterion, successful reperfusion is characterized by peak ratios of the MM isoforms 4–6 h after acute onset (Table 3). Patients for whom thrombolysis was unsuccessful or MI patients not treated with these agents show peak MM concentrations 10–12 h after onset. The absolute magnitude of the MM3/MM1 ratio can also be used to distinguish between these two groups (39). Furthermore, differences in the rate of MM3 decline can be used to determine successful reperfusion (40). A rate of decline that is >3.1%/h is indicative of reperfusion.

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**Table 1. Clinical Sensitivity for CK-MM isoforms vs MB**

<table>
<thead>
<tr>
<th>Time after onset, h</th>
<th>MM3/MM1</th>
<th>Total CK-MB</th>
</tr>
</thead>
<tbody>
<tr>
<td>3–6</td>
<td>58</td>
<td>38</td>
</tr>
<tr>
<td>6–9</td>
<td>91</td>
<td>79</td>
</tr>
<tr>
<td>9–12</td>
<td>86</td>
<td>87</td>
</tr>
<tr>
<td>12–15</td>
<td>84</td>
<td>90</td>
</tr>
<tr>
<td>15–18</td>
<td>60</td>
<td>90</td>
</tr>
<tr>
<td>18–21</td>
<td>32</td>
<td>96</td>
</tr>
<tr>
<td>21–24</td>
<td>10</td>
<td>87</td>
</tr>
</tbody>
</table>

a Data from Wu et al. (29). b Based on the number of positive test results in 24 MI patients. Decision limits were set so that clinical specificity was 86% for both methods in 34 non-MI patients. c Measured by electrophoresis (equipment: Helena Labs, Beaumont, TX 77704). d Measured by immunoprecipitation (equipment: Roche Diagnostics, Nutley, NJ 07110).

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**Table 2. Early and Late Markers for Acute Myocardial Infarction**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Time window, h</th>
<th>Use in acute MI</th>
<th>Efficiency, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myoglobin and CK-MM</td>
<td>10–24</td>
<td>Early marker</td>
<td>95</td>
</tr>
<tr>
<td>Isoforms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatine kinase-MB</td>
<td>10–24</td>
<td>Early marker</td>
<td>95</td>
</tr>
<tr>
<td>Lactate dehydrogenase-1</td>
<td>18–36</td>
<td>Late marker</td>
<td>90</td>
</tr>
</tbody>
</table>

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**Fig. 3. Typical densitometric scans of CK-MM isoforms in serum of a patient with acute myocardial infarction after assay by electrophoresis on cellulose acetate**

The time indicates the hours after the reported onset of chest pain. At 4 h, the MM isoform pattern shows minimal elevations in MM2. At 6 h, the activity of MM3 becomes more significant. At 9 h, MM3 is at peak concentration. At 12 h, some of the MM4 has converted to MM5, and at 15 h, the relative activities of the MM isoforms return to normal.
Table 3. CK Isoenzymes and Isoforms after Acute Myocardial Infarction in Published Reports

<table>
<thead>
<tr>
<th>Measured enzyme</th>
<th>Reperpusion group</th>
<th>Nonreperfusion group</th>
<th>Ref. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total CK</td>
<td>12.1 ± 0.1</td>
<td>20.7 ± 8.6</td>
<td>36</td>
</tr>
<tr>
<td>MB</td>
<td>8.6 ± 2.1</td>
<td>18.5 ± 6.7</td>
<td></td>
</tr>
<tr>
<td>MM₂/MM₁</td>
<td>5.5 ± 1.0</td>
<td>10.7 ± 3.8</td>
<td></td>
</tr>
<tr>
<td>Total CK</td>
<td>15.9 ± 5.6</td>
<td>22.3 ± 5.0</td>
<td>29</td>
</tr>
<tr>
<td>MB</td>
<td>14.8 ± 8.4</td>
<td>20.3 ± 4.3</td>
<td></td>
</tr>
<tr>
<td>MM₂/MM₁</td>
<td>10.8 ± 5.6</td>
<td>12.2 ± 4.8</td>
<td></td>
</tr>
<tr>
<td>Total CK</td>
<td>12.0 ± 3.1</td>
<td>20.8 ± 3.8</td>
<td>37</td>
</tr>
<tr>
<td>MB</td>
<td>11.0 ± 2.3</td>
<td>20.1 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>MM₂/MM₁</td>
<td>4.2 ± 1.8</td>
<td>11.8 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>Total CK</td>
<td>10.8</td>
<td>23.1</td>
<td>38</td>
</tr>
<tr>
<td>MB</td>
<td>10.4</td>
<td>23.1</td>
<td></td>
</tr>
<tr>
<td>MM₂/MM₁</td>
<td>5.2</td>
<td>9.9</td>
<td></td>
</tr>
</tbody>
</table>

whereas a rate of <3.1%/h is indicative of unsuccessful treatment. For clinical use, however, information on the rate of decline is not as useful as peak measurements because it requires more sampling and takes longer to compute. Early determination of reperfusion is important for cardiologists when other therapeutic options are being considered. The earliest indicator of success may be following the rate at which enzyme is released from the myocardium into the circulation. As yet, no such studies have been described.

In summary, although enzyme methods cannot replace coronary angiography for the quantitative assessment of reperfusion, they may be useful together with clinical signs as a qualitative indicator, which may preclude the need for acute catheterization.

MM Isoforms in Non-Myocardial Disease

Skeletal muscle disease: As compared with results for CK-MB, the major limitation of MM isoform measurement for patients with acute MI is lesser clinical specificity (25). Because skeletal muscle consists largely of MM₃, it is not surprising to find sera that contain high concentrations of the tissue CK isoforms from patients with skeletal-muscle diseases, reflecting active muscle necrosis. However, if the rate of CK release from tissues is slower than the rate of conversion in serum by carboxypeptidase, as would be expected in chronic muscle disease (e.g., Duchenne muscular dystrophy), MM₁ should be at a higher concentration than MM₃ (44), because MM₃ has roughly a twofold shorter biological half-life than MM₁ (21). Results for MI will be falsely positive if the rate of release is faster than the rate of conversion. Annesley et al. (42) reported that patients with stable polymyositis typically have ratios of tissue to serum MM isoforms that are >1, which, taken alone, might suggest acute MI. Fortunately, these patients do not usually present with symptoms of acute MI, and polymyositis is not one of the diagnoses considered in differentiating the cause of acute chest pain. Moreover, the concurrently measured value for CK-MB would be expected to be low, and this can provide a way to distinguish falsely high MM₃/MM₁ results.

Skeletal muscle trauma and exercise: High concentrations of MM₃ are also to be expected in the blood of patients with acute muscle trauma or individuals who undergo extensive exercise. The concentration of MM₃ increases sharply in runners after a long-distance race (43). Thus individuals with acute skeletal muscle damage cannot be assessed by MM isoform analysis for concurrent MI if the suspicious symptoms begin within 24 h after trauma or exercise. In such cases, however, the interpretation of CK-MB is also usually very difficult because of MB release from skeletal muscles (44). After 24 h, much of the MM₃ initially released will have been converted to MM₂ and MM₁, and detection of myocardial necrosis would be possible, even in the presence of a high concentration of total CK, if the absolute concentration of MM₃ is used as the diagnostic criterion.

Analytical Methods

Electrophoresis

CK isoforms can be measured by a modification of the zone electrophoresis technique that is used in many clinical laboratories (e.g., Beckman's Paragon and Helena's Titan Gel) by either increasing the duration of the electrophoresis or the voltage applied. Prolonging the time, typically from 30 to 90 min (and thereby decreasing the throughput), will allow MM isoform separation without requiring additional apparatus. The bands can be made visible by overlaying a blot soaked with CK reagents and substrates as currently done in most, if not all, commercial CK assays. An alternative method (immunoblotting) is to overlay a blot containing antisera specific to the CK-M subunit (45). A second radiolabeled antibody is then added that recognizes the first, and the mass concentration is determined by measuring the radioactivity of bands excised from the gel. Immunoblotting has high analytical sensitivity, but it is impractical for routine use, because the process requires two days to complete.

Increasing the applied voltage during the electrophoresis step results in good electrophoretic resolution with increased analytical throughput over extended-time electrophoresis. Electrophoresis time can be as little as 12 min when the voltage is increased from 100 V to 800 V (46). Under such conditions, the overall analysis time is less than 30 min. Further increases in applied voltage can shorten electrophoresis time to 5 min, but additional apparatus is needed, including a stronger power supply and cooling plates to minimize local heating—otherwise, extra unexplained cathodic and anodic bands are produced. Figure 4 shows typical results obtained with a voltage of 1700 V applied for 5 min.

![Fig. 4. Electrophoretic pattern of CK-MM and MB isoforms after high-voltage electrophoresis on agarose gel, showing the presence of additional MM isoforms](image-url)

Lane 1: quality control sample denoting migration of MM, MB, and BB. Lanes 2–6: different patients in various stages of acute myocardial infarction. Lane 7: A non-MI patient
Isoelectric Focusing

CK isoforms can be well resolved by isoelectric focusing (IEF). The isoelectric points for MM isoforms, as determined by various investigators, have been summarized by Penteghini and range from 6 to 7 (17). Despite the differences in specific analytical variables used by the different investigators, results are in general agreement. As with high-voltage electrophoresis, however, extra MM bands are observed with this technique. Although the nature of these extra bands is unknown, it has been postulated that the isoforms migrating cathodic to MM are precursors to the tissue isoform, whereas those migrating anodic to MM are further degradation products (46). As many as 14 bands have been observed in serum, with some of the minor bands migrating between the major isoforms (47).

Extra isoforms have also been reported when tissue extracts are examined by IEF (23, 43, 48–50). Although MM is most prominent, cationic and anodic isoforms have been reported. Indeed, as many as 21 different isoforms of MM have been identified in human, rabbit, and bovine skeletal muscle (51). Examination of tissue isoforms is important for fundamental biochemical studies, but their measurement will not likely play a role in clinical diagnosis.

Chromatofocusing Chromatography

This technique is similar in principle to IEF (21, 24, 52), separating isoforms on the basis of their isoelectric points. Samples are injected onto a packed column and are eluted by a pH gradient that is varied from 9 to 7. Individual isoforms are collected and assayed for CK activity. Although this technique does not give the resolution of isoelectric focusing, it can be adapted for rapid on-line assay by use of either micro-bore glass (52) or HPLC columns (53). In addition, preparative chromatofocusing can be used to purify CK isoforms for use in validation studies.

High-Pressure Liquid Chromatography

CK isoenzymes can be separated and measured by HPLC on anion-exchange columns. The separated isoenzymes are detected by monitoring the fluorescence of NADH produced by reacting the isoenzymes with substrates added through a post-column reagent pump (54). Sensitivity and specificity can be increased when the isoenzymes are coupled to luciferin and luciferase, with monitoring of the bioluminescence (55).

Isoforms can also be separated by HPLC when more exacting conditions and higher-efficiency anion-exchange columns are used (56). Adjusting the pH of samples to 7.80 and decreasing the flow rate to 0.5 mL/min can resolve MM isoforms better than many of the other methods. Post-column addition of CK reagents and detection are as described above. However, MB isoforms are not detectable under these conditions, because they are retained on the guard column. The turnaround time is ~30 min.

Summary of Analytical Methods

Important criteria of analytical performance for any clinical laboratory method generally include precision, sensitivity, specificity, accuracy, sample throughput, and costs. Resolution is also important, because it directly affects interpretation of results. I consider most of the methods described above to be deficient in one or more of these criteria. In terms of accuracy, e.g., it is surprising that few data are available on validation studies performed with purified isoforms. Part of the difficulty in performing such studies was the lack of commercially available reference materials. Hitherto, analytical-recovery studies required that the investigator first purify myocardial or skeletal tissue, convert tissue isoforms, and re-isolate post-synthetic products. Purified isoforms are now available, so future validation studies should be easier to perform. Table 4 compares the composition of commercially available purified CK isoforms with that of quality-control materials that can be used for validating new analytical assays for isoforms.

Data on analytical precision are also notably absent from many reports. Because isoelectric focusing and liquid chromatography (with either on-line detection or collection and analysis of fractions) are so complex, zonal electrophoresis probably will be best reproducible. Typical within-run CVs range from 3% to 5% (20). Electrophoresis also has the shortest analysis time when high voltages are used, and appears to be the most practicable method for use in routine clinical analysis. Electrophoresis is also the least costly technique, because the electrophoresis apparatus is already available in most large hospitals and reference laboratories. Costs for isoelectric focusing, in contrast, are typically high, both for equipment and for preparing or purchasing the focusing gels. Some pathologists and laboratory scientists consider IEF to be more a research tool (57).

Analytical sensitivity varies considerably among the available methods (17). Electrophoresis may be the most sensitive: roughly 5 U/L for single isoform bands. These detection limits, however, are not sufficient for the low

Table 4. CK Isoform Analysis of Commercial CK Materials as Assayed by High-Voltage Electrophoresis

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Product</th>
<th>Matrix</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified materials</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aalto</td>
<td>CK-MM3</td>
<td>Tris buffer</td>
<td>MM3</td>
</tr>
<tr>
<td>Aalto</td>
<td>CK-MM2</td>
<td>Tris buffer</td>
<td>MM2</td>
</tr>
<tr>
<td>Aalto</td>
<td>CK-MM1</td>
<td>Tris buffer</td>
<td>MM1</td>
</tr>
<tr>
<td>Aalto</td>
<td>CK-MB2</td>
<td>Tris buffer</td>
<td>MB2</td>
</tr>
<tr>
<td>Aalto</td>
<td>CK-MB1</td>
<td>Tris buffer</td>
<td>MB1</td>
</tr>
</tbody>
</table>

Controls for CK-MM

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Product</th>
<th>Matrix</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beckman</td>
<td>ID Zone</td>
<td>Human albumin</td>
<td>MM3, MB2, BB</td>
</tr>
<tr>
<td>Helena</td>
<td>Titan gel</td>
<td>Human serum</td>
<td>MM1, MB1, BB</td>
</tr>
<tr>
<td>Roche</td>
<td>Cardiotor</td>
<td>Human serum</td>
<td>MM3, MB2, BB</td>
</tr>
<tr>
<td>Cala</td>
<td>CKMB isoenzyme</td>
<td>Human serum</td>
<td>MM1, MB2</td>
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</tbody>
</table>

Controls for total CK

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Product</th>
<th>Matrix</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>American Dade</td>
<td>Moni-trol ES</td>
<td>Human serum</td>
<td>MM, 9</td>
</tr>
</tbody>
</table>

*Escondido, CA. **Brea, CA. ***Beaumont, TX. ****Nutley, NJ. *****Los Osos, CA. 1Miami, FL. *In addition, a band migrating anodic to MM is present.  #Unsure
activities of MB isoforms that are expected during early acute MI, because the MB isoform content typically is <10% that of MM. The differences in analytical specificity between these methods are also considerable. Both IEF and high-voltage electrophoresis produce extra anodic and cathodic bands not observed when identical samples are assayed by liquid- or chromatofocusing-chromatography. Some investigators have suggested that these additional MM isoforms may be denaturing artifacts resulting from interactions with carrier ampholytes during IEF or sample heating during electrophoresis (24). If these isoforms do occur naturally, they cannot be explained by the model of two different M subunits. As yet, there is no satisfactory explanation for these additional forms, nor have the minor isoforms been used in any clinical studies. Clearly, considerably more comparative work is needed before a single method is generally accepted for clinical use.

Discussion

The most promising applications for the known major CK isoforms are for patients with acute MI. For early diagnosis, analytical methods must be available that can produce results on an urgent ("stat") basis. Although several methods have been developed with 30-min turnaround times, including HPLC, chromatofocusing chromatography, and high-voltage electrophoresis, none is suitable for stat analysis at all hours in a routine hospital laboratory, because they are too labor intensive and specialized. Recently, however, a high-voltage electrophoresis analyzer has been introduced (Rep; Helena Laboratories, Beaumont, TX) that is rapid and sufficiently automated to permit stat CK isoform measurements (58). The electrophoresis voltage is set to 1800 V, producing isoform separations within 5 min with a total analysis time of <15 min.

In addition to the availability of stat methods, one must be able to use isoforms to detect MI within 4 h after onset of chest pain if thrombolytic therapy is to be selected. Although studies in dogs suggest that this should be possible, measurements in evolving human MI have not assisted in this diagnosis with the same degree of efficacy that is currently enjoyed for CK-MB.

Early diagnosis will require measurement of samples with total CK activity that are still within the reference range, so increased analytical sensitivity for MM isoforms is essential. Achieving this goal will require experimentation with different reagent formulations for staining and visualization, or sample concentration before analysis, or both. Increased sensitivity would also be expected with immunoassay methods. Monoclonal antibodies to MM and MB isoforms are being developed. Coupling such antibodies to the immunoconcentration format (59) may produce the fastest and most sensitive method for isoforms. Antibodies specific to MM$_1$ (60) and MM$_2$ (Isorm-MM; International Immunoassay Laboratories, Inc., Santa Clara, CA) have been raised and could provide the basis for a new generation of CK immunoassay tests.

For MI patients who are being treated with intravenous thrombolytic agents, measurement of MM isoforms provides a non-invasive measure of therapeutic success. Prospective analysis is important in management decisions, particularly for MI patients for whom thrombolysis was unsuccessful. In this case, additional therapy may be required, such as angioplasty or bypass surgery. Frequent and rapid analysis of isoforms may improve the overall prognosis of MI patients, because angiography may not be available at all hours and important case-management decisions might be delayed. Isoforms are currently not being evaluated because of a lack of acceptance among cardiologists and laboratory personnel, and the lack of a suitable stat analysis method.

Because they are more tissue specific, CK-MB isoforms have the greatest potential for applications in assessing myocardial disease. Although clinical studies have shown that MM isoforms appear earlier in the serum after MI than does MB, this may simply reflect the lack of analytical sensitivity for detecting MB, and not a retarded release of MB relative to necrotic myocardial tissue. If MB isoforms indeed first appear in the serum at the same time as MM, the improvements in analytical sensitivity will produce a clinically more efficient marker for MI. In this instance, the ratio of MB$_3$ to MB$_1$ would be the most discriminating factor.

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References
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