

Concordance of Creatine Kinase-MB Activity and Mass

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The recent availability of monoclonal antibodies that are highly specific for creatine kinase (CK; EC 2.7.3.2) MB isoenzyme should allow for the development of rapid, sensitive, and specific assays of CK-MB mass and activity. However, the relationship between the mass concentration of CK-MB and its activity in plasma has previously been thought by some to be variable. To determine the extent to which discrepancies of potential clinical significance might arise between measurements of activity and mass in plasma, we compared CK-MB activity and concentration in 1298 samples obtained from 226 patients admitted to the cardiac-care unit. CK-MB activity concentration was determined with an immunoadsorption assay, and mass concentration was measured by an automated "sandwich" assay (Magic Lite; Ciba Corning Diagnostics). Both of these assays are based on specific monoclonal antibodies for CK-MB. Values obtained with these assays correlated well ($r = 0.94$). Normal and abnormal values with the two assays were concordant in 96% of the samples. In all but three instances, differences occurred late after myocardial infarction and were characterized by minimal increases as determined by one method vs values at the upper limit of normal as determined with the other. Thus, measurements of CK-MB mass and activity concentrations in plasma with assays based on these specific monoclonal antibodies are comparable for the detection or exclusion of acute myocardial infarction.

Measurement of the activity of the MB isoenzyme of creatine kinase (CK; EC 2.7.3.2) in plasma is recognized as the method of choice for the diagnosis or exclusion of acute myocardial infarction (1, 2). However, the activity of CK in plasma may be influenced by inhibitors (3, 4), interference from other enzymes (5, 6) and drugs (7), prolonged storage or inadequate preservation (8-10), pH and ionic concentrations used in the analyses (11, 12), and assay temperature (13). Consequently, assay of the mass concentration of the enzyme protein may be more reliable. Previous results of comparisons of CK-MB activity with the results of determinations of mass based on antibodies specific for the B subunit of CK have found the relationship between CK-MB activity and B subunit concentration to be variable. Some report concordance (14, 15); others have found discrepancies (16, 17). B subunit concentration has been observed by some to be increased despite normal values for total CK and CK-MB activity (16, 17). Such discrepancies may in some instances be ascribable to the use of antibodies that recognize the B subunit of CK-BB, in addition to that of CK-MB, because CK-BB may be increased in patients with cancer (2), chronic renal failure (18), and neurological damage (2) such as that occurring after cardiac or respiratory arrest (19, 20). Under other circumstances, it is possible that enzyme activity has decreased despite the continuing presence of immunoreactivity (21). Clarification of these discrepancies is essential to the proper interpretation of the results of

assays of CK-MB concentration, given that most criteria for the diagnosis of myocardial infarction are based on measurements of CK-MB activity.

The recent development of monoclonal antibodies specific for the CK-MB isoenzyme has led to immunoassays that are specific for detection of the concentrations of CK-MB activity and mass. To determine the extent to which discrepancies of potential clinical significance might arise between measurements of activity and mass in plasma, we compared the concentrations of CK-MB activity and mass early and late after myocardial infarction, and in the absence of infarction, to determine the diagnostic sensitivity and specificity of each measure.

Materials and Methods

Blood Samples

Samples of blood ($n = 1298$) for determination of CK-MB activity and mass concentration were acquired from 226 patients on admission to the Cardiac Care Unit of Barnes Hospital and thereafter at least every 12 h for at least 10 days. Additional samples were obtained from patients ($n = 16$) being treated with fibrinolytic agents for acute myocardial infarction. Thirty-one patients had chronic renal insufficiency, as evidenced by concentrations of serum creatinine >20 mg/L. The mean age of the patients studied was 64.0 (SE 0.8) years. Samples were acquired 11.6 (SE 1.3) h after onset of symptoms, when this could be determined.

Samples for CK-MB were collected into tubes containing 5.7 mmol of ethylene glycol-bis(β -aminoethyl ether)- N,N,N',N' -tetraacetic acid per liter (final concentration). Samples were centrifuged immediately, the plasma was separated, and 2-mercaptoethanol was added to give a final concentration of 11.4 mmol/L. Activity was analyzed immediately in most instances, or on occasion, after storage at -20 °C for no longer than 18 h. Aliquots of each plasma sample were stored at -20 °C for later measurement of the mass concentration of CK-MB.

Activity Concentration of CK-MB in Plasma

The activity of CK-MB in plasma was measured with an immunoadsorbant assay based on a monoclonal antibody that recognizes the CK-MB but not the CK-BB or CK-MM isoenzymes (provided by J. Ladenson, Washington University School of Medicine) (22). Hexokinase, NAD^+ , ADP, glucose-6-phosphate dehydrogenase, and creatine phosphate were purchased from U.S. Biochemical Corp., Cleveland, OH. All other reagents were obtained from Sigma Chemical Co., St. Louis, MO. We incubated, for 1 h at room temperature, 0.1 mL of plasma with monoclonal-antibody-coated $\frac{1}{4}$ -in. (diameter) polystyrene beads (Sigma). The beads were then washed three times with 50- μ L portions of pH 7.4 wash buffer (per liter, 50 mmol of Tris HCl, 150 mmol of NaCl, and 10 g of bovine serum albumin). The activity of the CK-MB captured by the antibody-coated beads was assayed by incubating each bead with 0.1 mL of a coupled substrate solution [per liter, 1 mmol of ADP, 2 mmol of NAD^+ , 2500 U of hexokinase, 1650 U of glucose-6-phosphate dehydrogenase, 2 mmol of Mg acetate, 21 mmol of bis(2-hydroxyethyl)-

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Received November 14, 1988; accepted December 28, 1988.

imino-tris(hydroxymethyl)methane; 2-bis(2-hydroxyethyl)-amino-2-(hydroxymethyl)-1,3-propanediol, 1.9 mmol of AMP, 3.6 mmol of D-glucose, and 330 mmol of creatine phosphate, pH 6.8] at 37 °C. The reduction of NAD⁺ by glucose-6-phosphate dehydrogenase was measured by adding 0.25 mL of a solution containing, per liter, 1.5 U of diaphorase and 800 mg of *p*-iodonitrotetrazolium violet. The reduction of *p*-iodonitrotetrazolium in the solution was catalyzed by the presence of diaphorase and NADH, producing the purple dye formazan. The concentration of formazan was based on its measured absorbance at 492 nm. The activity of CK-MB in the sample was determined by comparison with standards prepared by adding purified human CK-MB to heat-inactivated plasma (22). Measurements and concentrations were linearly related for CK-MB activity concentrations of 5 to 150 U/L. Samples found to have greater than 90 U of CK-MB activity per liter were diluted with normal pooled plasma inactivated by heating at 56 °C and re-assayed. For control plasma, the intra-assay CV for this method is 3.7% (SD = 3.7%; n = 157), the intra-assay CV is 17.8%. The upper limit of normal activity concentrations of CK-MB is 12 U/L.

Mass Concentration of CK-MB in Plasma

The mass concentration of CK-MB in plasma was determined with a "sandwich"-type assay based on a specific monoclonal antibody for CK-MB (Magic Lite CK-MB assay; Ciba Corning Diagnostics Corp., Walpole, MA). We incubated 10 µL of plasma with a suspension of magnetic particles containing a monoclonal antibody specific for the B subunit of CK and a second monoclonal antibody (specific for CK-MB) coupled to an acridinium ester. After washing the particles, we used a magnetic test tube rack to separate the particles from the supernate. Monoclonal antibody bound to CK-MB was detected after two washes by addition of reagents that develop chemiluminescence in reaction to the antibody-coupled acridinium ester, exactly as described in the package insert. An automated analyzer quantified the luminescence. We determined the concentration of CK-MB by comparison with a 10-point standard curve prepared by the manufacturer and calibrated for each assay with two standards (23). The range of mass concentration of CK-MB measured by this assay without diluting the plasma was 1 to 526 µg/L. We confirmed that the standard curve was linear for CK-MB values of 1 to 200 µg/L. For control plasma, intra-assay CV was 4.8% (SD = 0.3%; n = 298), the interassay CV 11.1%. The upper limit of normal for the concentration of CK-MB in plasma is 7 µg/L. The specificity of this assay for CK-MB was confirmed by the lack of change in the measured concentration of CK-MB when we added to normal pooled plasma either CK-BB, 1000 µg/L, or CK-MM, 5000 µg/L.

We also evaluated a more rapid assay procedure in which the incubation interval for plasma, antibodies, and particles was shortened to 15 min.

Statistical Analysis

Data were analyzed by Spearman's correlation coefficient and linear regression. Data are presented as the mean ± SD except where noted.

Results

Diagnosis of Myocardial Infarction

The mass and activity concentrations of CK-MB in plasma correlated closely ($r = 0.94$, Figure 1). Results of the two

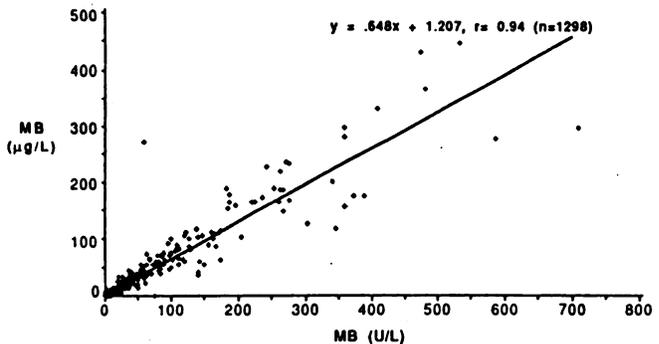


Fig. 1. CK-MB activity concentration and CK-MB mass concentration in plasma compared

Samples (n = 1298) were obtained from 226 patients admitted to the cardiac care unit. The data were analyzed by linear regression as shown. Upper limit of normal for the activity assay is 12 U/L, for the mass assay 7 µg/L. The results of the two assays are closely correlated ($r = 0.94$)

assays were concordant for the increase or lack of increase of CK-MB above the normal range in 93% (220/226) of patients and 97% (1254/1298) of the samples. They were similarly concordant in 90% of patients (28/31) with renal dysfunction.

The mass concentration of CK-MB in plasma was increased in 88 patients (normal <7.0 µg/L) and activity was increased in 79 (normal <12 U/L). In 12 patients, CK-MB activity was in the upper portion of the normal range, while concentration was minimally increased (≤ 10 µg/L in each instance). In eight patients, myocardial infarction was very probable, based on other clinical information (e.g., positive CK-MB activity before admission to the coronary care unit, or cardiac catheterization with presumed new regional wall motion abnormalities) (Table 1). In the remaining four patients, clinical criteria for myocardial infarction were not present (patients 4, 6, 8, and 10; Table 1). The activity

Table 1. Characteristics of Patients with Discordant Results for CK-MB Activity Compared with CK-MB Mass

CK-MB		Clinical history
Activity, U/L	Mass, µg/L	
<i>Patients with increased mass of CK-MB (>7 µg/L) but normal activity (<12 U/L)</i>		
3	10	Cardiac catheterization consistent with MI
10	9	CK-MB activity increased before CCU admission
9	9	Cardiac catheterization consistent with MI
5	8	Pulmonary embolism, no evidence for MI
11	9	Cardiac catheterization consistent with MI
6	8	Cardiac arrest, but no evidence for MI
7	9	Cardiac catheterization consistent with MI
9	8	Severe CAD but no wall motion abnormalities
9	9	Cardiac arrest, probable MI
9	9	Clinical information not available
8	8	CK-MB activity increased before CCU admission
10	8	CK-MB activity increased before CCU admission
<i>Patients with increased activity of CK-MB (>12 U/L) but normal mass (≤ 7 µg/L)</i>		
17	7	MI, sample obtained late
13	5	MI before transfer, late sample
12	7	MI, small non-Q-wave infarction

MI = myocardial infarction, CCU = coronary care unit, CAD = coronary artery disease. Results of cardiac catheterization were considered consistent with the diagnosis of acute MI when presumed new wall-motion abnormalities were present.

concentration of CK-MB was only slightly increased in three patients, but the mass concentration was at the upper limit of the normal range. All three had clinical characteristics highly suggestive of acute myocardial infarction (Table 1). In all but one instance in which there was a lack of concordance of results of the two assays and a myocardial infarction was confirmed, the diagnosis was one of small non-Q-wave myocardial infarction.

Activity Concentration vs Mass Concentration of CK-MB Early during the Evolution of Acute Infarction

Serial blood samples were obtained from 34 patients before concentration of CK-MB mass or activity peaked in the plasma. The mass concentration and activity concentration of CK-MB in plasma correlated closely ($r = 0.92$) on the upslope of the time-activity and mass-activity curves. This was also the case after administration of fibrinolytic agents, when the rates of change in mass and activity concentration were similar (Figure 2). At the very high values achieved after reperfusion, greater differences between mass and activity concentration were observed (Figure 2), but they were not of diagnostic significance. This may reflect the nonlinearity of the assays when very high concentrations of CK-MB are measured by both methods.

Activity Concentration vs Mass Concentration of CK-MB Late during the Evolution of Acute Infarction

The rates of decrease in the concentrations of mass and activity of CK-MB after their peak values were comparable in plasma samples from patients with myocardial infarction. The concentration of activity and mass correlated closely on the downslope of the CK-MB time-activity concentration and time-mass concentration curves ($r = 0.9$, Figure 3a). However, in 13 patients the mass concentration of CK-MB remained abnormal at a time when the activity concentration had returned to normal limits. In 12 others, the activity concentration of CK-MB was minimally increased at a time when mass concentration had returned to normal limits (Figure 3b).

Results of a More Rapid Assay Procedure for Measurement of the Mass Concentration of CK-MB

The mass concentration of CK-MB measured after an initial 15-min incubation of the plasma with the antibody-coated magnetic particles and that after the recommended 30-min incubation were comparable ($r = 0.97$, $n = 50$). Increased mass concentrations of CK-MB in plasma, consistent with acute infarction, were detected in 48 of the 50

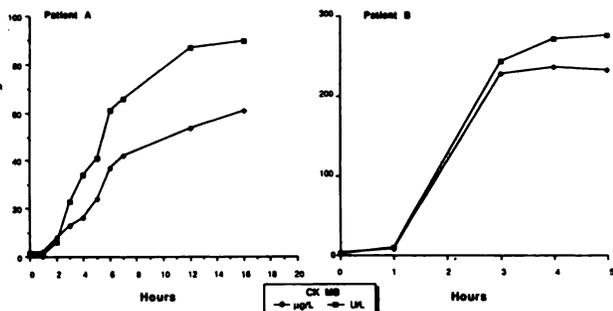


Fig. 2. Example of the time-activity and time-mass concentration curves for two patients (A and B) with acute myocardial infarction and receiving fibrinolytic agents
The rate of increase of CK-MB is similar as measured by both types of assays

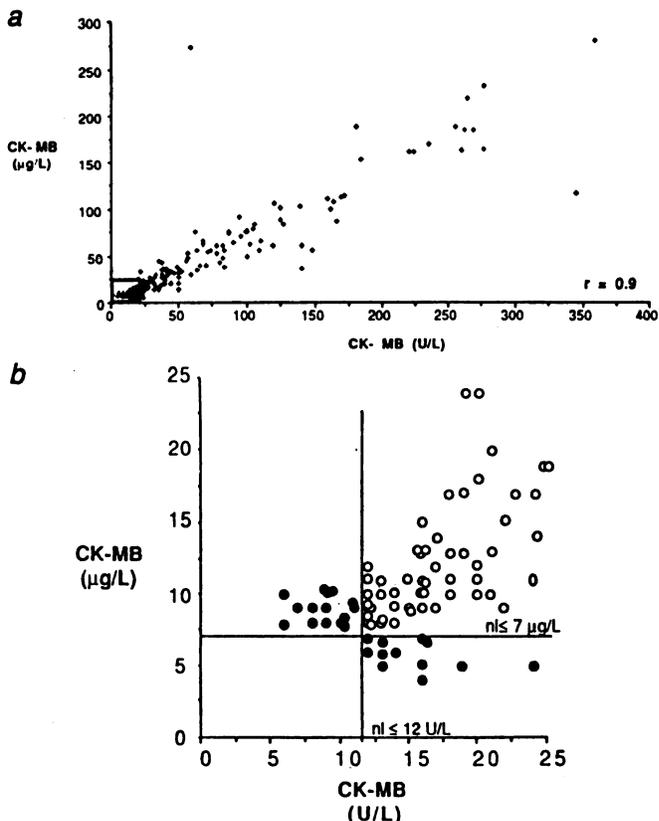


Fig. 3. (a) Comparison of the mass concentration of CK-MB with activity concentration in plasma from patients in whom blood samples were obtained late after myocardial infarction, at a time when the mass and activity concentrations of CK-MB were decreasing but had not yet returned to the normal range ($n = 180$ samples); (b) Detailed view of the outlined area in a: samples in which either the mass of CK-MB or its activity, but not both, had decreased to the normal range
The lines indicate the upper limit of normal (n) for each assay. Discordant results of the two assays of CK-MB occurred only in samples with borderline increases above the normal reference interval

samples for which values were similarly increased when measured after a 30-min initial incubation. In the two instances in which a minimally increased mass concentration of CK-MB was measured after the longer initial incubation, the mass concentration as measured with the more rapid procedure was at the upper limit of normal.

Discussion

Highly specific monoclonal antibodies for CK-MB will allow for the development of rapid, sensitive, and specific assays of CK-MB mass and activity concentrations in plasma. Such assays should be more reliable and less technically demanding as well as more sensitive, rapid, and specific than previous ones. The potential variability of measurement of enzyme activity due to the presence or absence of inhibitors; reagent differences; or dependence on pH, ionic concentrations, or incubation temperature may favor the use of assays of CK-MB mass concentration. Our data indicate that criteria for the diagnosis or exclusion of acute myocardial infarction based on the concentration of activity or mass have comparable sensitivity and specificity. The use of fibrinolytic agents or the presence of renal dysfunction does not change this relationship. Accordingly, assays based on mass and activity concentration can be used interchangeably to optimize the utilization of resources in individual

laboratories without fear that diagnostic efficiency will be significantly altered. Shorter incubation intervals that allow for faster analyses also may be used without significant loss of diagnostic sensitivity.

Our data are consistent with those from previous studies at our institution comparing activity concentration with mass concentration values obtained by the use of a radioimmunoassay of the CK-B subunit (14). They indicate that the specific activity of the CK-MB isoenzyme does not change in vivo during the evolution of acute myocardial infarction, and that the decreasing CK-MB activity concentration reflected on the downslope of the CK-MB time-activity curve in plasma is ascribable to renal clearance of the isoenzyme rather than to a loss of activity. Others have found that the concentration of the CK-B subunit is more persistently increased than activity concentration, especially during the downslope of the CK-MB time-activity curve (15, 16). These disparate results may reflect differences in patient selection or different specificities of the antibodies used. In this same context, the extent to which results obtained with other assays of mass concentration will correlate with CK-MB activity concentration also will depend on the specificity of the antibodies used and characteristics of the assay, and must be confirmed by each laboratory before implementation.

In addition to the novel approach to measurement of the mass concentration of MB-CK with this assay (e.g., chemiluminescent reaction and magnetic particles), other new technologies also are being developed to facilitate rapid testing. The findings reported here indicate that assays of the activity or mass concentration of CK-MB in plasma, based on monoclonal antibodies specific for CK-MB, should be of comparable value.

This work was supported by SCOR in Ischemic Heart Disease HL-17646. We thank Ciba Corning Diagnostics for providing assay reagents; Mary Jane Eichenseer, Janice Gualdoni, and Connie Large for the excellent technical assistance; and Sue Payne for secretarial help.

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