

Creatine Kinase-MB Protein Mass Is a Better Indicator for the Assessment of Acute Myocardial Infarction in the Lower Range of Creatine Kinase Level

Hiromi SEO, M.D.,* Shunichi MIYAZAKI, M.D.,
Takashi FURUNO, M.D.,* Hiroshi NONOGI, M.D.,
Kazuo HAZE, M.D.,
and Katsuhiko HIRAMORI, M.D.**

SUMMARY

The theoretical and clinical validity of immunochemiluminometric assay of creatine kinase (CK)-MB protein mass was assessed in patients with acute myocardial infarction and the results were compared with those of immunoinhibition assay of CK activity. Serial changes of both CK-MB protein mass and CK-MB activity were analyzed in 20 consecutive patients. In all 312 samples from 20 patients, protein mass and activity of CK-MB showed good correlation. The exponential fitting of the time-value curve of CK-MB protein mass showed a better correlation coefficient than that of CK-MB activity (0.97 ± 0.02 vs 0.93 ± 0.07 , $p < 0.05$), indicating that the CK-MB level measured by the immunochemiluminometric assay was less scattered than that measured by the immunoinhibition method. This finding was most evident at lower CK-MB values (< 500 IU/l). The rate of disappearance from serum of CK-MB protein mass was faster than that of CK-MB activity (0.54 ± 0.23 hr⁻¹ vs 0.28 ± 0.13 hr⁻¹, $p < 0.001$). This may indicate that some amount of the CK-MB activity may be inactivated in the early phase of the release into the serum from the necrotic myocardium.

Thus, the immunochemiluminometric assay of CK-MB protein mass has superiority in the diagnosis of acute myocardial infarction compared with the immunoinhibition method, especially when the measured CK-MB level is low. This feature may be useful to distinguish a small myocardial infarction from severe ischemia without myocardial necrosis. (*Jpn Heart J* **34**: 717-727, 1993)

Key Words:

Acute myocardial infarction Creatine phosphokinase Myocardial necrosis

From the Division of Cardiology, National Cardiovascular Center, Suita, Japan.

Present address: * Kochi Medical School, Okohcho, Nangoku, Kochi 783, Japan, and ** the Second Department of Medicine, Iwate Medical University, School of Medicine, 19-1 Uchimarui, Morioka, Iwate, 020, Japan.

Address for correspondence: Shunichi Miyazaki, M.D., Division of Cardiology, National Cardiovascular Center, 5-7-1 Fujishirodai, Suita, Osaka 565, Japan.

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ALTHOUGH there are several diagnostic indicators of acute myocardial infarction, such as typical chest pain of long duration, abnormal ST elevation in the electrocardiogram, abnormal uptake of technetium-pyrophosphate in radioisotope imaging, diminished wall motion in echocardiography or ventriculography and enzyme studies, a definite diagnosis of acute myocardial infarction is made by the abnormal elevation of creatine kinase-MB isoenzyme (CK-MB) released from necrotic myocardial tissue.¹⁾⁻³⁾ However, the activity of CK-MB in the serum can be influenced by the presence of other enzymes,⁴⁾ temperature, pH, ions used in the assays,^{5),6)} and by prolonged storage.^{7),8)} These factors sometimes make it difficult to diagnose acute myocardial infarction when the elevated serum CK-MB activity is low. Recently the Ciba Corning Magic Lite CK-MB immunoassay, which is an immunochemiluminometric assay using a mouse monoclonal anti-CK-MB antibody labelled with acridinium ester and a mouse monoclonal anti-CK-BB immobilized on paramagnetic particles, has become available for use in the clinical setting for the determination of CK-MB protein mass.⁹⁾ However, its validity in acute myocardial infarction has not yet been established in clinical settings.

The present study was designed to evaluate the theoretical and clinical validity of the immunochemiluminometric assay of CK-MB isoenzymes in patients with acute myocardial infarction by a comparison of the characteristics of the time-value curves.

METHODS

This project was approved by the Committee on Human Research in this institute, and informed consent was given by all the patients who agreed to be enrolled in this study.

Study Patients

Twenty consecutive patients with acute myocardial infarction who were admitted to the coronary care unit of this institute within 24 hours after the onset of symptoms were enrolled in this study. The diagnosis of acute myocardial infarction was based on the presence of at least two of the following three standard criteria: 1) typical chest pain persisting for at least 30 minutes; 2) ST-segment elevation lasting more than 30 minutes in at least 2 leads of the standard 12-lead electrocardiogram; and 3) serial elevation of serum glutamic oxaloacetic transaminase, lactic dehydrogenase, and total CK conforming to the pattern typically seen in patients with acute myocardial infarction. There were 15 males and 5 females with a mean age of 62 years (range 49-73 years). There were 11 anterior, 3 lateral and 3 inferior myocardial infarctions. In 3 patients, the site of

myocardial infarction was not known. Eight of the 20 patients were treated with intracoronary thrombolytic therapy.

Specimen collection

Blood samples were collected serially, without any anticoagulant, from all patients on admission and thereafter every 3 hours during the first 24 hours after the onset of myocardial infarction, and then every 6 hours until total CK activity returned to near-normal levels (approximately 160 IU/l). Total CK activity, CK-MB activity and CK-MB protein mass were measured in each blood sample. Samples were centrifuged immediately and serum was separated. Total CK and CK-MB activity were analyzed immediately. Aliquots of each serum sample were frozen at -20°C for later measurement of CK-MB protein mass.

Total CK Assay

Total CK activity was measured spectrophotometrically at 340 nm and 37°C as described by Rosalki.¹⁰⁾

Immunoinhibition Assay

CK-MB activity was measured by the immunoinhibition assay with anti-M antibodies to inhibit the activity of CK-MM and half the activity of CK-MB.¹¹⁾ The CK-MB activity of the sample was twice the residual activity, since the M-subunit of CK-MB was also inactivated.

Immunochemiluminometric Assay

The CK-MB protein mass in the serum was determined with the "Magic Lite CK-MB Immunoassay" and "Magic Lite Analyzer" by labeling monoclonal anti-CK-MB with acridinium ester and immobilizing anti-CK-BB on paramagnetic particles.⁹⁾ In the analysis using the chemiluminometric analyzer, the acridinium ester was hydrolyzed and oxidized, producing light, the intensity of which was proportional to the CK-MB protein mass in the sample.

Mitochondrial CK

In 4 patients, mitochondrial CK was measured using the electrophoretical method as reported by Kanemitsu et al.¹²⁾ In brief, CK isoenzymes were separated electrophoretically on agar plates. To observe conversion in the electrophoretic pattern, the enzyme was incubated in urea (2 mol/l) at 26°C for 1 hour after the patient's serum had been treated with anti CK-MM antibodies. The creatine kinase of mitochondrial origin was determined by the migrating pattern of CK.

Exponential model fitting for CK-MB time-value curve

Because CK-MB activity in the serum has been used as a standard to diagnose and to assess the size of acute myocardial infarction, we incorporated the theoretical model of the time-value curve, which should be exponential,¹³⁾ and estimated the fit of the time value curve obtained from CK-MB activity and protein mass to this model. In order to calculate the disappearance rate (Kd) and the cumulative CK released (CKr) in estimating infarct size, the following equation proposed by Sobel et al¹⁴⁾ was used;

$$\text{CKr} = E(t) - Kd \int E(t) dt$$

where, E(t) equals serum CK value at time t and Kd is the disappearance rate of CK from the serum. In this equation, the time-value curve fits closely the exponential model. Therefore, when the values of CK-MB protein mass and activity are plotted against time on a semi-logarithmic graph from the peak down to normal levels (assumed to be 7.5 ng/ml of CK-MB protein mass and 20 IU/l of activity in our laboratory) the correlation coefficient (r) in linear regression between time and the logarithm of the CK-MB value would be close to 1. We evaluated the theoretical fit of the decline of the time-value curve to the exponential model by calculating and comparing these correlation coefficients. We also examined the fit of the time-value curve of CK-MB protein mass and activity to the mono-exponential model over a range of less than 500 IU/l and less than 1000 IU/l of CK-MB activity to assess the small scattering of CK-MB protein mass at a lower level.

Statistical analysis

A paired t-test was used to compare the correlation coefficient (r) in fitting to the mono-exponential model and the disappearance rate (Kd) measured by the protein mass to that measured by activity of CK-MB. All values are expressed as mean \pm SD.

RESULTS

Correlation between CK-MB protein mass and activity

In all 312 samples in this investigation, the protein mass and activity of CK-MB showed good correlation (r=0.94, Fig. 1). Some plots showed discrepancies between the two assays. Serial plotting always presented smooth curves in immunochemiluminometric assays and some unexplained far-apart plots in immunoinhibition assays.

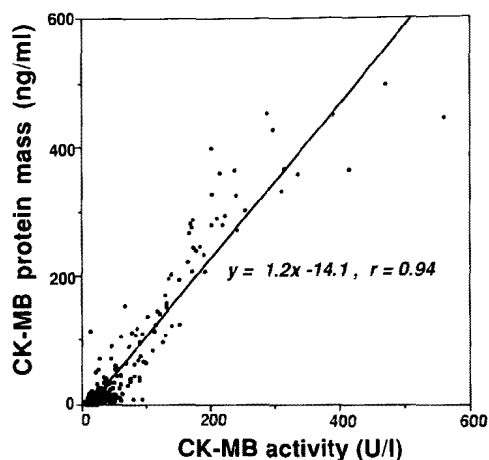


Fig. 1. Correlation of CK-MB values measured by immunochemiluminometric assay and by immunoinhibition assay. Linear regression of these plots shows good correlation ($r=0.94$) with a few discrepancies. CK=creatine kinase.

Time to peak value

In 11 of the 20 patients, CK-MB protein mass and activity peaked after admission. There was no significant difference between time to the peak value of protein mass (9.2 ± 4.5 hr) and CK-MB activity (10.5 ± 3.9 hr), obtained from time-value curves in these patients. In the remaining 9 patients, CK-MB values had peaked at or before admission, so that serial values measured for these patients showed only decreasing patterns.

Mono-exponential fitting of time-value curve in decay

The representative original time-value curves of CK-MB protein mass and activity are shown in Figure 2. When the logarithms of CK-MB protein mass and activity are plotted against time from the peak value down to the normal value, correlation coefficients in linear regression, between time and the logarithm of the value in decay are closer to 1.0 ($p < 0.05$) in CK-MB protein mass (0.97 ± 0.02 hr⁻¹) than in CK-MB activity (0.93 ± 0.07 hr⁻¹) (Fig. 3A). As shown in Figure 4A, the correlation coefficient in CK-MB activity decreased to 0.82 ± 0.2 when the degree of fit was examined in the lower range of values (less than 500 IU/l), while the correlation coefficient in CK-MB protein mass remained constant (0.96 ± 0.05 hr⁻¹). This finding was also observed in the range of less than 1000 IU/l (Fig. 5). These results indicate that the immunochemiluminometric assay would provide more accurate CK-MB values than the immunoinhibition assay does, especially in the lower range of CK-MB values.

In 3 of 4 patients in whom measurement of mitochondrial CK was performed, the appearance of mitochondrial CK was observed (Fig. 6).

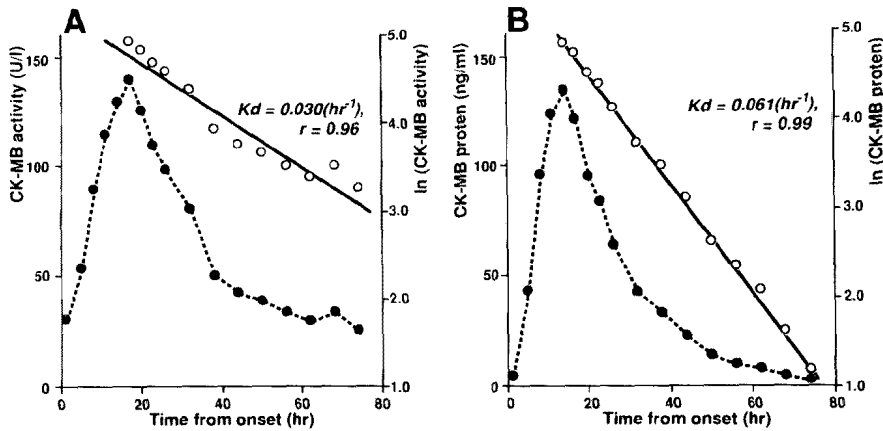


Fig. 2. Examples of time-value curves of CK-MB activity (left panel, A) and CK-MB protein mass (right panel, B) from a 53-year-old male with acute lateral myocardial infarction. Serum CK-MB values were plotted (closed circle) on a time-value curve and the logarithms of these values were plotted (open circle) from the peak down to normal. The disappearance rate (K_d) was calculated as the absolute slope of linear regression of logarithmic plots. CK=creatine kinase.

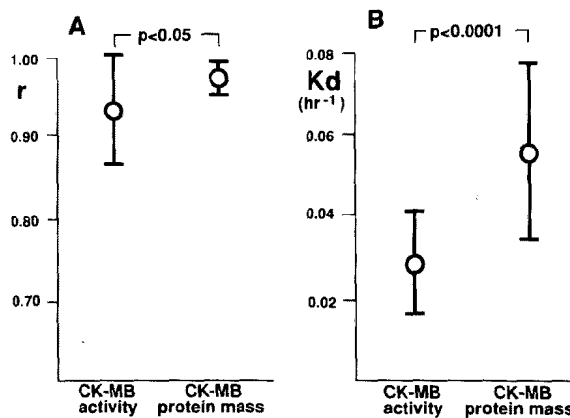


Fig. 3. Comparison of decay properties of CK-MB protein mass and activity. A: Correlation coefficients (r) in fitting the decay of time-value curves to the mono-exponential model are closer to 1.0 ($p < 0.05$) and smaller in variance in CK-MB protein mass than in CK-MB activity, suggesting that the decay of CK-MB protein mass is better fitted to a mono-exponential model than is CK-MB activity. B: Comparison between the disappearance rate (K_d) of CK-MB protein mass and activity. Disappearance rate (K_d) is faster in CK-MB protein mass than in CK-MB activity ($p < 0.0001$). CK=creatine kinase.

Fractional disappearance rate (K_d)

In all 20 patients, the disappearance rate (K_d) was faster for CK-MB protein mass (0.54 ± 0.23) than for CK-MB activity (0.28 ± 0.13) (Fig. 3B). Creatine

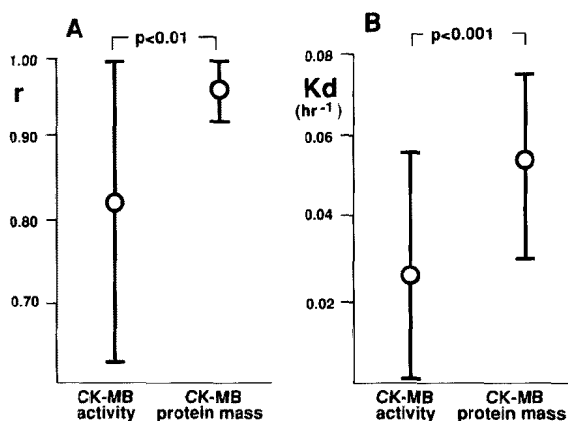


Fig. 4. Comparison of decay properties between CK-MB protein mass and activity in the range less than 500 IU/l of CK-MB. Abbreviations are the same as in Figure 3. Note that the correlation coefficient of CK-MB activity is lower than that in Figure 3 with a larger standard deviation.

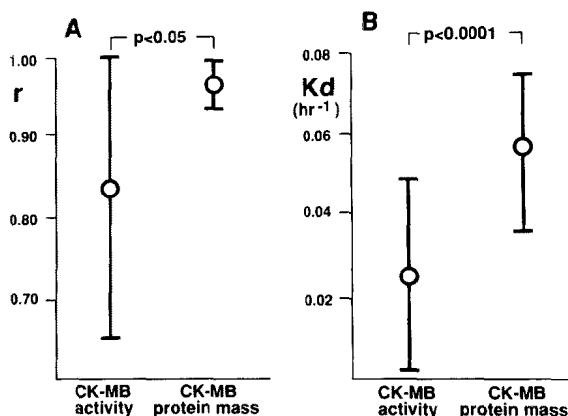


Fig. 5. Comparison of decay properties of CK-MB protein mass and activity at less than 1000 IU/l of CK-MB. Abbreviations are the same as in Figure 3. The phenomenon indicated in Figure 4 was also observed in the range of less than 1000 IU/l.

phosphokinase-MB protein mass disappeared more rapidly from the serum in immunochemiluminometric assays than did CK-MB activity in immunoinhibition assays.

DISCUSSION

In this study, we have shown in the clinical setting that the time-value curve of the CK-MB protein mass obtained by immunochemiluminometric assay fits the theoretical exponential model better than that obtained from the CK-MB

activity curve, especially in the lower range of CK-MB values, and that the rate of decline of CK-MB protein mass is faster than that of CK-MB activity.

Previous investigations have shown that the immunochemiluminometric assay is a fast and sensitive CK-MB assay⁹⁾ and that it interferes less with macro-CK and adenylate kinase.¹¹⁾ In these studies the immunochemiluminometric assay was found to be an accurate method from the technical point of view. In the clinical setting, Eisenberg et al¹⁵⁾ reported that CK-MB values of 1298 samples from patients with acute myocardial infarction, measured as CK-MB activity by immunoabsorption assay and as CK-MB protein mass by immunochemiluminometric assay, correlated well and were concordant in 96% of the samples. However, they measured samples for only 12 hours after the onset of infarction, which would be insufficient time to obtain enough samples to draw precise time-value curves of CK-MB protein mass and CK-MB activity. Although they did not show the superiority of the measurement of CK-MB protein mass, we incorporated the theoretical model of the decline of CK-MB as a standard, and proved the better fit of CK-MB protein mass than that of CK-MB activity, especially at lower CK-MB values. This finding indicates that interference with CK-MB activity by several factors¹¹⁾ was enhanced in the lower range, whereas that with CK-MB protein mass was minimal. As shown in Figure 6, mitochondrial CK peaked 20 hours after the onset, while both CK-MB protein mass and CK-MB activity are already decreasing. In this case, the slower decline of CK-MB activity could be enhanced by a delayed increase in mitochondrial CK. The other 2 patients showed similar appearance of mitochondrial CK during decay of CK-MB values. Delayed appearance of mitochondrial CK would cause a slow decline of CK-MB activity measured by immunoinhibition assay. Thus, the CK-MB protein mass should be a better indicator in the diagnosis of small areas of myocardial necrosis.

We demonstrated that CK-MB protein mass disappeared from the serum more rapidly than does CK-MB activity, as shown in Figure 3. The site and mechanism of enzyme inactivation and/or removal have been unclear and little is known about the distribution spaces and clearance rates of enzymes in humans.^{16,17)} Only 15–30% of CK lost from necrotic myocardium is released into the serum, and the remainder undergoes local degeneration.¹⁸⁾ Because the rate of decline of CK-MB should be determined by the difference between the enzyme extracted from the heart and the enzyme released endogenously, the faster disappearance of CK-MB protein mass than that of CK-MB activity would indicate that inactivation of CK-MB activity occurs early during release from necrotic tissue which would cause a relatively low peak of CK-MB activity during the early phase, hence continuing release of CK-MB would cause a slower disappearance rate during the late phase of acute myocardial infarction. There are

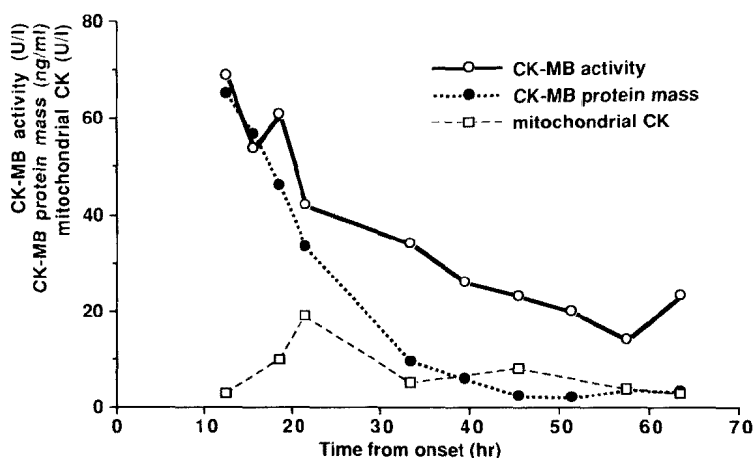


Fig. 6. Time-value plot of CK-MB activity (open circle), CK-MB protein mass (closed circle) and mitochondrial CK (open square) in a 68 year old male (case 5 in Table I) with acute inferior myocardial infarction. Mitochondrial CK showed delayed appearance and peaked when CK-MB protein mass had already been in decline.

Table I. Patient Profile and CK-MB Data

No.	Age/ Gender	Site of Infarct	Intervention (result)	Peak value (time from onset; hr)		Disappearance rate (Kd)	
				CK-MB activity	CK-MB protein	CK-MB activity	CK-MB protein
1	61/M	ANT	ICT (unsuccessful)	560 (13)	498 (16)	0.068	0.097
2	66/M	ANT	NO	* *	* *	0.016	0.024
3	49/M	ANT	ICT(successful)	222 (14)	398 (11)	0.035	0.083
4	66/F	LAT	NO	* *	* *	0.011	0.061
5	68/M	INF	ICT+PTCA (successful)	* *	* *	0.032	0.093
6	68/M	INF	NO	177 (6)	244 (3)	0.034	0.058
7	65/F	Unknown	NO	42 (14)	33 (11)	0.032	0.078
8	53/M	LAT	NO	140 (17)	135 (14)	0.030	0.061
9	55/M	ANT	ICT+PTCA (successful)	314 (7)	429 (10)	0.026	0.069
10	72/M	ANT	PTCA (successful)	* *	* *	0.022	0.026
11	54/M	LAT	NO	* *	* *	0.010	0.024
12	60/M	ANT	NO	14 (10)	17 (7)	0.012	0.039
13	68/F	ANT	NO	12 (12)	17 (9)	0.022	0.030
14	73/F	ANT	NO	* *	* *	0.019	0.030
15	56/M	ANT	ICT (successful)	254 (6)	326 (3)	0.041	0.067
16	53/M	ANT	NO	310 (8)	454 (5)	0.030	0.057
17	52/M	ANT	ICT(unsuccessful)	* *	* *	0.033	0.064
18	64/M	INF	ICT(successful)	202 (12)	280 (12)	0.037	0.048
19	73/F	Unknown	NO	* *	* *	0.035	0.046
20	73/F	Unknown	NO	* *	* *	0.018	0.045

*indicates a patient whose CK-MB values had peaked at or before admission. CK=creatine kinase; M=male, F=female; ANT=anterior myocardial infarction; LAT=lateral myocardial infarction; INF=inferior myocardial infarction; ICT=intracoronary thrombolysis; PTCA=percutaneous transluminal coronary angioplasty.

other possible explanations for the faster disappearance rate of CK-MB protein mass than of CK-MB activity: 1) Immunochemiluminometric assay measures both active and inactive enzymes. These may be different properties of degradation in the reticuloendothelial system. On the assumption that the degradation of inactivated CK-MB precedes that of active CK-MB, the disappearance rate of the mixture of active and inactive CK-MB may be larger than that of active CK-MB alone. 2) The false increase in CK-MB activity in immunoinhibition assays during the late phase of acute myocardial infarction may affect the disappearance rate more slowly than in immunochemiluminometric assays. Immunoinhibition involves the use of anti-M antibodies to inhibit the activity of MM and of half of MB. Residual "non-M" isoenzyme activity is then measured as CK-MB activity. Therefore, in the presence of atypical isoenzyme (i.e., macro-CK), a false increase may occur. 3) Different reperfusion status may also affect the disappearance rate, because early coronary reperfusion is well known to increase peak CK-MB.¹⁹⁾ However, in the present study, comparison was made between Kd by CK-MB protein and that by CK-MB activity in individual patients, and the Kd by CK-MB activity always showed smaller values than that by CK-MB protein mass (Table I). Therefore, the difference in reperfusion status does not basically affect the results.

Clinical implication

Precise assessment of abnormal elevation of CK-MB is important not only for the diagnosis of acute myocardial infarction but also for the estimation of infarct size. When the area of necrotic myocardium is small, scattered levels of CK-MB in the serial time-value curve might cause inappropriate assessment of the area of necrotic myocardium. Immunochemiluminometric assay is an accurate method, allowing the precise assessment of acute myocardial infarction, even when the area of myocardial necrosis is small.

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