Creatine Kinase B-Subunit Activity in Human Sera: Temporal Aspects of Its Sensitivity after Myocardial Infarction

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We assessed the changes in sensitivity and specificity of creatine kinase (CK) isoenzymes at various times after acute myocardial infarction. CK-B was measured by an immunoinhibition technique. CK-MB was measured both by column chromatography on Sephadex DEAE A-50 and by electrophoresis on agarose gel. For CK-B, the sensitivity in detecting infarct varied from 11% in the first 8 h after onset of symptoms to 80% at 32 h after the infarct. Sensitivities for CK-MB by column and by electrophoresis were 25 and 45% for the first 8-h period and 80 and 89% at the 32nd hour, respectively. Comparison of values for CK-B with values for CK-MB by column and by electrophoresis gave correlation coefficients of 0.935 and 0.896, respectively. Patients having macro CK-BB exhibit anomalously high values for CK-B with respect to total CK. Patients having a mild infarct may show no increase in CK-isoenzymes.

The importance of the measurement of the creatine kinase (EC 2.7.3.2) MB (CK-MB) isoenzyme in patients suspected of having acute myocardial infarction (AMI) has been amply documented (e.g., *1–3*). The techniques usually used in determining CK-MB—column chromatography (*4*) and electrophoresis (*5*)—are time consuming and, in the case of chromatographic procedures, require relatively large volumes of sample.

An immunochemical technique has recently been made available that permits measurement of the B-subunit of CK. Gerhardt et al. (*6*) and Ljungdahl et al. (*7*) have reported its use in the diagnosis of AMI; others (*8–11*) have reported sensitivity values for CK-B ranging from 86 to 100%.[1]

Our objectives were (*a*) to examine the temporal sensitivity and specificity of CK-B activity after AMI and (*b*) to compare the results by the immunochemical method with results obtained for the same samples by column chromatography and electrophoresis.

Materials and Methods

Patient Selection

A total of 74 patients suspected of having AMI, who were admitted to the coronary care unit of York Hospital between July 13 and Sept. 16, 1979, were included in the study. These were not consecutive admissions, but no conscious bias was applied in their selection. All patients were informed of the study and consented to be included. Of the 74 patients, 25 were women. One patient died and an autopsy verified the cause of death as AMI.

[1] The sensitivity of a test reflects its positivity in the presence of disease; specificity reflects its negativity in the absence of disease.

Specimen Collection and Handling

The study protocol specified collection of blood at 8-h intervals for a period of 72 h after admission to the hospital. Nine samples were collected from each of 63 patients. Six patients had eight samples each. Two patients had seven and five samples, respectively. Two other patients were discharged after four samples had been collected, and one patient died before the third sample had been obtained. No preservative was added to any sample of serum. Assays for creatine kinase (CK, CK-B, and CK-MB) were done within 24 h of specimen collection.

Clinical Diagnosis

The diagnosis of AMI was made by a panel of three cardiologists who were kept unaware of the CK-B and CK-MB values. Of the following three criteria at least two were required for the diagnosis: (*a*) a history of chest pain consistent with myocardial ischemia; (*b*), a value for total CK of twice the upper reference limit or more, or a characteristic increase of total CK to triple the initial value with a subsequent decrease; (*c*) electrocardiographic changes evolving over a period of up to 72 h, including the appearance of new Q-waves. In the absence of new Q-waves, but with typical pain and serum enzyme changes, alterations of the S-T segment or T-waves were taken to indicate intramural, in contrast to transmural, infarction. By these criteria, 27 patients were classified in the AMI group and 47 in the non-AMI group.

Assay Methods

Total CK. Total CK activity was measured according to the method of Rosalki (*12*) by use of a kit (A-Gent CPK; Abbott Labs., Diagnostics Division, North Chicago, IL 60064). This reagent did not incorporate EDTA as recently recommended (*13*). The assay was done with an Abbott VP bichromatic analyzer at 37 °C.

CK-B subunit activity. CK-B subunit activity was measured by use of the immunoinhibition technique (A-Gent CK-MB; Abbott Labs.). In addition to the standard reagent components for the Rosalki method for CK determination, this kit included antihuman CK antibody prepared in goats, EDTA (2 mmol/L), diadenosine pentaphosphate (10 μmol/L) and N-acetylcysteine (20 mmol/L). We measured CK-B subunit activity at 37 °C with an ABA-100 analyzer, using the two-step procedure recommended by the manufacturer. In the first step, CK-M is inhibited by adding anti-M antibody and residual apparent CK activity (attributable to adenylate kinase, EC 2.7.4.3) is measured in the absence of the substrate phosphocreatine. The CK-catalyzed reaction is initiated in the second step by adding phosphocreatine. The difference in measured activity between the second and first steps is ascribed to CK-B subunit activity and is corrected for residual (adenylate kinase) activity. The manufacturer suggests that samples with total CK activity exceeding 1000 U/L be diluted to avoid saturation of the anti-M antibody.

Column chromatography. A modification of the method of Mercer (*4*) was used to separate CK-MB before its measurement. Columns were prepared from DEAE–Sephadex A-50 in disposable 15-cm Pasteur pipets. The mini-columns
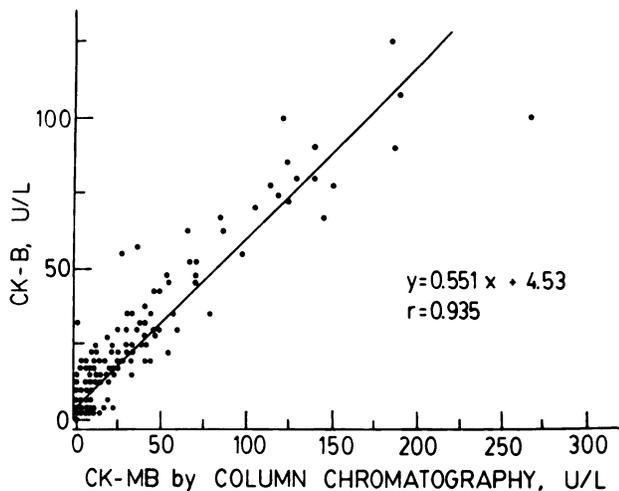
Fig. 1. Results for CK-B by immunoinhibition compared with those for CK-MB by column chromatography (519 samples)

were stored at 2–8 °C under Tris buffer (pH 8.0, 50 mmol/L) containing 100 mmol of NaCl per liter. A 1.0-mL sample of serum was applied to the column and allowed to drain. The elution scheme was modified to incorporate a 4.0-mL wash with 100 mmol/L NaCl to assure complete elution of CK-MM. Three 1.0-mL volumes of the Tris buffer containing 200 mmol of NaCl per liter were successively applied to the column, and eluent fractions 2 and 3 were pooled and assayed for CK-MB activity. Samples having total CK values >2000 U/L were diluted to yield final activities of <2000 U/L and processed as above. Assays were done at 37 °C with the Abbott VP, with use of the same reagent as was used for total CK.

Agarose gel electrophoresis. We separated isoenzymes of creatine kinase on agarose gels by using the Multi-Zone electrophoresis cell and CK isoenzyme agarose gel kits (Beckman Instruments, Inc., Fullerton, CA 92634). The separations were done according to the manufacturer's instructions and the separated isoenzymes were visible by virtue of their fluorescence. A filter-paper overlay technique was used to eliminate the fluorescent artifact that sometimes interferes with CK-BB identification (14). CK-MB was quantitated by fluorometric scanning of the filter paper with a Beckman Model R-112 densitometer.

Reference Intervals

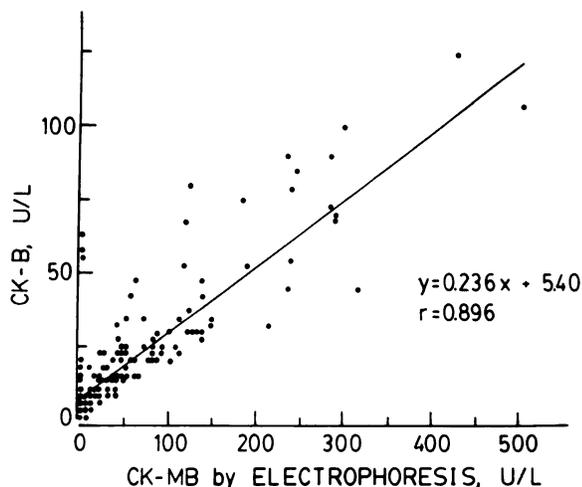Reference intervals for CK and for CK-MB by column

Fig. 2. Results for CK-B by immunoinhibition compared with those for CK-MB by electrophoresis (519 samples)

chromatography were established by nonparametric analysis of data obtained on samples from non-professional blood donors. For CK, the central 95 percentile range is from 25 to 150 U/L. For CK-MB by column chromatography the range is 0–10 U/L.

Analysis of Data

For statistical analysis of the data we used programs from the Statistical Package for the Social Sciences (SPSS) Library (15).

Results

Comparison of CK-B with CK-MB

We compared a total of 519 samples. CK-B values ranged from 0 to 125 U/L. Total CK values ranged from 12 to 3006 U/L.

CK reagent difference. The reagent formulation used for the assay of CK activity in column eluates differed from that used for CK-B activity after immunoinhibition, the latter including EDTA and N-acetylcysteine instead of dithioerythritol (11 mmol/L). A study relating the CK activity difference of these reagent formulations (16) showed that the reagent used in the immunoinhibition assay gave results 28.8% higher than the other reagent.

Column chromatography. We corrected the column-chromatographic data for the activity bias of the reagent used in the assay of eluates, to permit comparison of these results with those of the CK-B assay. Because the antibody inhibits only the M-subunit, the expected slope of a regression line relating CK-B and CK-MB activity is 0.5. The data in Figure 1 show that this value is approached very closely ($y = ax + b$; $a = 0.55$; $b = 4.5$; $r = 0.935$). Use of the uncorrected data results in a regression slope of 0.71.

Electrophoresis. The data plotted in Figure 2 are also corrected for the bias of the CK-B reagent ($y = ax + b$; $a = 0.24$; $b = -5.4$; $r = 0.896$). Hamilton et al. (5) observed similar results on comparing CK-MB activity as measured by column chromatography with that measured by electrophoresis. The uncorrected data yield a regression slope of 0.28.

Sensitivity, Specificity, and Predictive Value

The data (Table 1) have been grouped into 8-h and 16-h periods after the onset of symptoms, to make apparent the changes in sensitivity with time. Data were included only if the onset of symptoms could be ascertained by history with reasonable certainty. For all methods, sensitivity is low in the first 8 h after an AMI. The highest value for CK-B is reached in the second 8 h, while the highest sensitivity for electrophoresis is between 17 and 32 h after infarction. For all tests, the sensitivity declines to <70% after 32 h. The time course for predictive values parallels that for sensitivity and specificity.

Quality Control

CK-B. The between-run precision for CK-B was measured at two activity levels. The standard deviation at 140 U/L was ±9 U/L (n = 31) and at 277 U/L was ±14 U/L (n = 33).

Column chromatography. The between-run standard deviation for Ortho CPK Isoenzyme Control Serum (Lot 7S082) at 50 U/L was ±9 U/L (n = 28).

Electrophoresis. The between-run standard deviation for Ortho CPK Isoenzyme Control Serum (Lot 7S082) at a CK-MB proportion of 15.9% was ±5.0% (n = 33).

Anomalous Results

Atypical CK. One patient in our study showed anomalous results for CK-B and was excluded from the statistical analysis. By electrophoresis, she was demonstrated to have the

Table 1. Sensitivity, Specificity, and Predictive Values of CK Isoenzymes in Myocardial Infarction

	0–8 h [a]		9–16 h		17–32 h		33–48 h	
	Sensy. (PV+)	Specy. (PV−)	Sensy. (PV+)	Specy. (PV−)	Sensy. (PV+)	Specy. (PV−)	Sensy. (PV+)	Specy. (PV−)
				percent				
CK-B	11	100	81	100	80	97	57	97
	(100)[b]	(65)	(100)	(95)	(94)	(90)	(92)	(80)
CK-MB[c]	26	100	88	100	80	98	49	96
	(100)	(66)	(100)	(93)	(95)	(89)	(89)	(76)
CK-MB[d]	45	100	78	98	89	100	68	100
	(100)	(77)	(95)	(90)	(100)	(95)	(100)	(85)

[a] Hours after onset of symptoms.
[b] Numbers in parentheses indicate the predictive value of a positive or negative result.
[c] By column chromatography.
[d] By agarose electrophoresis.

"atypical CK" reported by other investigators (17–20). Only the electrophoretic method was capable of correctly identifying this CK variant.

Intramural infarction. Within our group of 27 confirmed cases of AMI was a subgroup of three patients who were undergoing intramural myocardial infarctions at the time of the study. None of these patients exhibited an increase in CK-MB or CK-B during the first three days after the onset of symptoms. One patient had persistently high values for total CK, between 173 and 334 U/L. However, neither this patient nor the other two had high values for aspartate aminotransferase (EC 2.6.1.1), lactate dehydrogenase (EC 1.1.1.27), or lactate dehydrogenase isoenzymes during the same interval.

The Expected Range for CK-B

We estimated the expected range of values of CK-B in the absence of myocardial infarction from results for a group of 39 patients, none of whom had evidence of myocardial infarction by any of our criteria. However, they were all admitted to the critical care unit. A total of 332 samples was collected from these patients at 8-h intervals and assayed. The values ranged from 1 to 12 U/L, the highest value being observed in two patients. These values are identical with those of Hofvendahl et al. (21). The within-patient variation averaged 3.6 U/L (SD 2.4).

Discussion

The use of CK isoenzymes as a diagnostic tool in patients suspected of having myocardial infarction has been nearly universally accepted. However, column-chromatographic methods for CK isoenzymes are time consuming, and electrophoretic methods are only semiquantitative. When the components are present in widely disparate concentrations, accurate quantification by electrophoresis is impossible: substrate depletion by the greater component causes overestimation of the lesser. The production of antibodies to the M- and B-subunits of CK has stimulated a new mode of testing for CK isoenzymes, based on the immunoinhibition of single subunit activity. This technique, although very effective, must be used with caution because the commercially available antibodies are monospecific, being directed against either the B- or M-subunit. Obzansky and Lott (9) have recently clinically evaluated the Cardiozyme (Dade Division, American Hospital Supply Corp., Miami, FL 33152) immunoinhibition procedure. Because of methodological difficulties, they concluded that electrophoresis is a better clinical method for CK-MB than immunoinhibition. On the other hand, Delahunty and Foreback (8) concluded that the same test is better than electrophoresis, and also more sensitive.

Several investigators (e.g., 1, 2, 22–24) have examined the temporal relationships of enzymes appearing in serum after myocardial infarction. Although the mean values for specific enzymes are different for normal and AMI, the distribution of values within any time period shows overlap into the normal range. Furthermore, the published values for sensitivity and specificity (1–3, 8, 9, 23) are values that may be observed at any time between the onset of symptoms and three to five days later. Others (1, 3) have emphasized the importance of proper timing of collection of serum samples if maximum information is to be obtained from enzyme measurements. Our results show very similar sensitivity and specificity values for CK-MB by electrophoresis or by column chromatography and for CK-B subunit activity during all time periods that we investigated.

However, there are conditions under which the immunoinhibition procedure will yield false-positive results. Because CK-B subunit activity is being measured, any condition resulting in an increase in CK-BB activity in serum may be misinterpreted. The "atypical" CK isoenzyme described by several investigators (9, 17–20) will also appear as apparent CK-B or CK-MB activity in the immunoinhibition and column-chromatographic procedures, respectively. The incidence of the atypical creatine kinase in the population has been estimated by Sax (18) to be as high as 1 to 2%, but our experience in this study and in subsequent use of the test leads us to believe that the incidence is less than 1%. In our experience, the atypical creatine kinase presents as an impossibly high apparent CK-MB. When the CK-B activity exceeds 30% of the total CK, we expect to detect an atypical CK by electrophoresis.

One patient in our study presented with an admission CK of 223 U/L 8 h after onset of symptoms. Her total lactate dehydrogenase and asparate aminotransferase values were within normal limits. Her CK-B subunit activity was 67 U/L (30% of total CK) and her CK-MB by column chromatography was 34 U/L. During the course of her stay, her total CK declined to 82 U/L but her CK-B and CK-MB activities remained nearly constant. Electrophoresis revealed the presence of the atypical CK isoenzyme with a mobility between that of CK-MM and CK-MB. Because of this and other experiences with atypical isoenzymes (25), we believe the activity of CK-B should not be multiplied by 2 as recommended by the manufacturer to give an apparent CK-MB activity, because in many cases, this will result in an apparent MB activity that exceeds the total CK activity!

Clinically mild AMI continues to present a problem in laboratory diagnosis. Three of our patients who presented with typical chest pain and evolving electrocardiographic changes of intramural AMI showed no CK isoenzyme changes during the first three days after the onset of symptoms. Subsequently, while still hospitalized, all three experienced recurrent symptoms with electrocardiographic changes indicative of transmural AMI and a diagnostic increase in serum enzyme

values. Because the level of activity of enzymes in blood is a function of their rate of release into the circulation, slowly evolving or small AMI may fail to elicit diagnostic increases in enzyme activities.

The concept of sensitivity, specificity, and predictive value of positive or negative results has enabled clinicians to choose more effective laboratory testing stratagems in their diagnosis of disease (26). In disorders where laboratory values vary during the course of the disease, the time at which the specimen is obtained may substantially influence the interpretation of the results of that test. In these situations, the temporal aspects of sensitivity, specificity, and predictive value must also be examined.

The sensitivity, specificity, and predictive value of CK-B and other measures of CK isoenzymes change after onset of AMI. The sensitivity for electrophoresis is slightly better than the other methods in the first 8 h after onset of symptoms. At later times, the information obtained by measurement of CK-B activity is equivalent to that obtained by measurement of CK-MB activity, except in cases in which an atypical CK isoenzyme is present.

The times at which CK-B is measured should be predicated upon the onset of symptoms. Samples obtained at admission (to establish a baseline value) and at the time of peak sensitivity for CK-B (8 to 15 h after onset of symptoms) are the most informative in the diagnosis of AMI. Practically, these samples should be collected at 12 and 24 h after onset, to include peak values (24). At times exceeding 32 h, CK-B becomes less sensitive. In mild or slowly evolving AMI, neither CK-B nor any other measure of CK isoenzymes may detect the evolving infarct.

In summary, the immunochemical method for measuring CK-B is faster than chromatography and more quantitative than electrophoresis. It may be substituted for either as a part of the laboratory diagnosis of AMI.

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