Creatine Kinase-MB Activity: Clinical and Laboratory Studies of Specific Immunochemical Technique with Optimized Enzymatic Assay

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ABSTRACT

A centrifugal analyzer method was developed for measuring the MB isoenzyme of creatine kinase (EC 2.7.3.2) in serum by use of a specific immunochemical technique that avoids interference from CK-BB and adenylate kinase. Enzymatic activity was measured kinetically at 30°C with an optimized reagent containing creatine phosphate as substrate. The precision (CV) of the assay was 5 to 12 percent day-to-day (n = 39). The reference interval was 0 to 3.5 U per L (n = 45). Patient samples without detectable CK-MB in a widely-used electrophoretic assay contained up to 12 U per L of CK-MB by the new method. The new test was evaluated carefully in 99 patients consecutively admitted to the coronary care unit. Blood samples were obtained at frequent (four to eight hr) intervals. All patients with acute myocardial infarction (n = 27) had peak CK-MB >7 U per L and >3.5 percent of total CK. The predictive value of this result was 94 percent for the diagnosis of infarction. Abnormal results were documented at the same times (±four hr) following infarction by the electrophoretic and immunochemical techniques. Guidelines were evolved for interpreting the percent of MB (i.e., MB per total CK) at various time points. The test was reliable in documented early recurrent infarctions that occurred in three patients. The method appears to be an attractive alternative to electrophoretic techniques for use in diagnosis of acute myocardial infarction.

Introduction

Various methods have been used to measure the MB isoenzyme of creatine kinase (CK) as an aid in the diagnosis of acute myocardial infarction (AMI). The most widely used procedures involve electrophoresis with colorimetric or fluorimetric detection of the isoenzyme. These techniques have been clinically useful but are only semi-quantitative and are time-consuming. Recently, consid-
erable attention has been focused on quantitative immunochemical approaches to measuring CK isoenzymes including radioimmunoassay and immunoinhibition techniques. Most of these methods exhibit interferences from the BB isoenzyme of CK (CK-1).

An immunoinhibition procedure for CK-MB was described recently which accounted for the interferences from CK-BB by separately measuring this activity. In this method antibodies against M-subunit are used to inhibit the M-subunit activity of CK-MM* and CK-MB. The residual enzymatic activity represents the B-subunit activity of CK-MB but also may include contributions from CK-BB, mitochondrial CK, adenylate kinase, and various “atypical” and “variant” CK's and immune complexes of CK-BB. The activities of CK-BB and the latter enzymes are accounted for in the method by precipitating both CK-MM and CK-MB with anti-M antibodies in a separate tube; the activity in the supernatant includes CK-B, adenylate kinase, etc., and is subtracted from the activity of the immunoinhibited sample. Although this approach provides a very high level of specificity for the MB dimer, it has been used only with a non-optimized assay of enzymatic activity.

The present study was undertaken to develop and characterize a centrifugal analyzer assay of CK-MB that combines the new immunochemical procedure and an optimized reagent for the measurement of enzymatic activity. The use of the optimized assay improved the sensitivity of the assay and avoided “negative” values for CK-MB that were seen when the reagent of Wicks et al was used. The potential clinical uses of this assay have been studied carefully in 99 consecutive patients admitted to a coronary care unit from whom blood samples were obtained at frequent four hr intervals. The new assay appears to provide a reliable test for the early diagnosis of acute myocardial infarction and of early recurrent infarction (“extension”). Guidelines are presented for interpreting the ratio CK-MB total CK in these patients.

**Materials and Methods**

**Materials and General Methods**

The reagents for the immunochemical separation and selective inhibition of the isoenzymes of CK were obtained from Roche.* The reagents consist of goat antiserum against CK-MM and a second antibody suspension consisting of polymer-bound anti-goat immunoglobulin. These reagents and their use have been described and were used with a quality control material from the supplier. The activities of CK-MB and of total CK were measured using an optimized assay reagent based upon that described by Szasz and obtained from Boehringer-Mannheim.† CK-MB was estimated fluorometrically (as negative, trace, or positive) following electrophoresis using the Corning ACI system.‡ Serum samples were stored at 4°C and analyzed within 24 hr of blood collection.

**Immunochemical Assay of CK-MB**

Two hundred µl of patient serum or control are added to each of two tubes. Then 250 µl of goat anti-CK-MM is added to the first tube and the mixture is incubated for 20 min to allow immunoinhibition of CK-M subunits; the residual CK activity in this tube includes the CK-B subunit activity present in CK-MB and CK-BB. To the second or “blank” tube,

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* CK-MB and CK-MM isoenzymes 2 and 3 of CK, respectively.

* Isomune-CK, Roche Diagnostics, Nutley, NJ 07110.
† Bio-Dynamics/bmc, Indianapolis, IN 46250.
‡ Corning Medical, Medfield, MA 02052.
50 μl of goat anti-CK-MM is added, followed by incubation at room temperature for five min, addition of 200 μl of the second antibody, and centrifugation (1000 × g, five min) after an additional five min incubation. The supernatant in this tube serves as a "blank" since the CK-MB and MM are precipitated while the supernatant contains CK-BB and potentially interfering enzyme activities such as adenylate kinase, "variant" CK's, and mitochondrial CK. The CK activities in both tubes are measured at 30°C on a centrifugal analyzer§ using a 50 μl sample from each tube, 100 μl of saline flush, and 400 μl of reagent. The absorbance is determined at 30s intervals for 3.5 min following a lag phase of 120s. The activity in tube two is subtracted from the activity in tube 1. This difference reflects the B-subunit activity of CK-MB (i.e., approximately half of the activity of the CK-MB dimer) and is multiplied by two to provide the estimated CK-MB activity.

Patients

Ninety-nine consecutive patients admitted to the coronary care unit at the University of Virginia were studied prospectively. All patients were admitted within 24 hr of the onset of chest pain. The average age of the patients was 63 ± 13 years (range 31 to 86 years); 56 patients were men and 43 were women. Blood samples were obtained on admission and at four hr to eight hr intervals during the first 24 hrs of hospitalization and at eight hr to 24 hr intervals thereafter for up to 96 hr. Activity of CK-MB was quantitated in all samples with total CK greater than 90 U per L (reference range, 0 to 110 U per L) or if a progressive rise in CK activity was observed in serial samples from the same patient. In patients without other evidence of acute myocardial infarction (AMI), blood sampling was stopped after the first three samples if these samples showed no progressive rise in CK and no CK-MB by the electrophoretic procedure.

The diagnosis of AMI was made by the attending cardiologist based on standard criteria which included clinical history, evolutionary electrocardiographic findings, and serial changes of CK, lactate dehydrogenase isoenzymes and the CK-MB results obtained by the electrophoretic method. The results of the immunochemical assay for CK-MB were not available to the cardiologist for use in making the diagnoses.

Results and Discussion

The present study was undertaken to adopt the immunochemical procedure of Wicks et al17 to use with the optimized assay reagent16 employed in our laboratory for the measurement of total serum creatine kinase at 30°C. This assay reagent contains optimized concentrations of substrate, buffers, salts, and co-factors and includes EDTA to chelate calcium, N-acetyl cysteine as sulfhydryl activator, and adenosine pentaphosphate to inhibit adenylate kinase. Preliminary experiments with the assay reagent of Wicks et al17 at 30°C revealed frequent "negative" activities (decreasing absorbance). A similar experience was reported by Obzansky and Lott10 with an immunoinhibition method for CK-B that used a non-optimized reagent for measurement of the enzymatic activity. With the optimized reagent used in our study, the "negative" activities were avoided and the average activities were increased approximately 1.8-fold compared to the results using the previously described reagent (y = 1.8 x + 6, r = 0.96, n = 45). This improvement in sensitivity appeared useful for the assay of CK-MB in view of the low activity of the isoenzyme in serum.

§ RotoChem IIa/36, American Instrument Co., Silver Spring, MD 20910.
LINEARITY

The linearity of the immunochemical procedure was determined by assay of serial dilutions of serum pools that had elevated total CK activities (figure 1). The assay reagents contained sufficient antibody to provide a linear response to at least 2000 U per L of total CK activity.

PRECISION

The day-to-day precision of the assay was determined by using control material supplied by the manufacturer. At a mean CK-MB activity of 7.7 U per L, the standard deviation was 0.96 U per L (C.V. 12 percent, n = 39).

These results are similar to those reported by others using immunoinhibition techniques to measure CK-B subunit or CK-MB activity (table I). The reported C.V.’s at near-normal activities have ranged between 6.3 percent and 38 percent compared to the present result of 12 percent; at higher activities, the reported C.V.’s are in the range of 2.4 percent to 20 percent compared to 5.0 percent in the present study.

CORRELATION WITH ELECTROPHORESIS

Activity of CK-MB was measured in 200 samples submitted for electrophoretic separation of CK-MB (figure 2). Samples that contained no CK-MB detectable by electrophoresis had CK-MB activities less than 12 U per L with two exceptions. In both of these cases, the electrophoretic result appeared to be in error since both samples were obtained from patients with AMI in whom a sample four hr earlier was positive by the electrophoretic method as was a subsequent sample four hr later; neither patient had evidence of recurrent infarction. In approximately 80 percent of “positive” samples, the CK-MB activity exceeded 12 U per L. Among samples called “trace” by electrophoresis, approximately 95 percent had CK-MB activities between 5 and 15 U per L.

REFERENCE INTERVAL

The reference interval for CK-MB activity was estimated by analyzing sera from 45 ambulatory out-patients sched-
The diagnostic utility of the assay was evaluated in a series of 99 consecutive patients admitted to the coronary care unit on whom serial enzyme determinations were requested. Twenty-seven patients were diagnosed as having had an AMI. Serum CK-MB exceeded 10 U per L in all but two patients with AMI. In these two patients, the peak values were 7.2 U per L and 8.4 U per L, respectively; both had small subendocardial infarctions with peak total CK values of only 142 U per L and 132 U per L, respectively (reference range 10 to 110 U per L).

There was in excess of five percent CK-MB in all but four patients with clinically diagnosed infarction. The clinical findings in these four patients were instructive. One arrived in the coronary care unit after the time of peak CK; in such patients the percentage MB will be low as will be shown. In two patients the low percentage value for CK-MB appeared to be caused by coexistent conditions that produced increased CK-MM or CK-BB (stroke, lung cancer, radiation therapy, and trauma); in such patients, the percentage of MB must be interpreted with caution. In the fourth patient, the CK-MB declined from 18 percent upon admission to 4.0 percent at the time of peak CK, 19 hours after the onset of chest pain. The cause of the disproportionate rise of total CK was not apparent in this 74 year old adult-onset diabetic woman with a history of previous infarction. To investigate this low value, the results were reviewed of other older patients, females, and diabetics following infarction. CK-MB as a percentage of total CK was not influenced by sex (males 8.8 U per L, females 9.0 U per L, n = 16 and 8, respectively), but there was a trend toward lower values in patients with pre-existing diabetes (6.8 percent vs 8.7 percent, n = 5 and 22, respectively), and in patients over 65 years (7.8 vs 9.0 percent, n = 15 and 12, respectively) and in patients with previously documented infarctions (7.8 percent vs 8.6 percent, n = 8 and 19, respectively), but none of these differences in this small series was statistically significant. The findings nonetheless suggest that careful study may be required to define appropriate reference ranges for percent CK-MB within selected groups of patients.

Values of CK-MB exceeded 7 U per L in all patients with AMI and accounted for more than 3.5 percent of the total CK activity in serum at the time of the peak CK-MB. Two of 17 non-AMI patients with CK >90 U per L (reference range:
10-110 U/L) also met these criteria. These criteria resulted in a sensitivity for AMI of 100 percent and specificity of 97 percent. The predictive value of a positive result was 94 percent (29/31), similar to the value of 96 percent reported by Wicks et al17 using this immunochemical method in a study of 83 patients.

**Clinical Comparison to Electrophoretic Results**

The quantitative CK-MB assay compared favorably with the electrophoretic technique for CK-MB in the diagnosis of AMI. Both methods were positive in all AMI patients. However, CK-MB was detected by the electrophoretic procedure in four patients without diagnosed AMI including the two patients without AMI in whom the immunochemical assay was considered to be falsely positive. Moreover, the two falsely positive results by the quantitative method were readily suspected as falsely positive because they were not associated with the characteristic rise and fall of CK-MB activity seen with this method in other patients with infarction and they did not coincide (± four hr) with the peak total CK as was seen in the patients with AMI.

The electrophoretic and immuno-inhibition assays provided laboratory evidence of infarction at about the same times. The first trace or positive electrophoretic result occurred at 4.2 ± 4.0 hr after admission, the first positive immunoinhibition result (CK-MB > 7 U per L) at 5.3 ± 4.6 hr and the first positive electrophoretic result at 5.8 ± 4.4 hr after admission. In 70 percent of patients, the first sample with electrophoretically detectable CK-MB (trace or positive) also contained > 7 U per L of CK-MB measured by the immunochemical method.

**Temporal Pattern of Change in CK-MB Following Acute Myocardial Infarction**

As expected following myocardial infarction12, CK-MB decreased more rapidly than did total CK after peak values were reached. A corollary of the short half-life of CK-MB was a decreasing ratio of CK-MB to total CK following infarction. As shown in table II, this ratio decreased from 8.3 percent to 5.3 percent during the first 24 hrs after the peak CK-MB. Thus, the ratio must be interpreted with caution in patients admitted at late times following the onset of chest pain. Similarly, if samples are drawn at intervals of eight to 24 hrs rather than four hrs, the average percentage of MB at the time of peak CK may be lower than reported here, as will the absolute value of peak CK-MB.

The qualitative assay of CK-MB appeared to provide useful documentation of recurrent myocardial infarction ("extension") because of the rapid decline of serum CK-MB following its initial peak. In three patients, secondary increases of CK-MB activity were observed. In all three cases, re-infarction or extension had been suspected clinically based upon the appearance of new or deeper Q waves following second episodes of chest pain. The second peaks of CK-MB in these three patients occurred at six, nine, and 10 hrs after the second episode of chest pain, suggesting that the peaks were clinically meaningful. Neither the total CK activities nor the electrophoretic MB results had provided a clear indication of the re-infarction, whereas the secondary rise in CK-MB was evident because of

<table>
<thead>
<tr>
<th>Time after peak CK-MB (h)</th>
<th>(n)</th>
<th>CK-MB/Total CK (percent)</th>
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<tbody>
<tr>
<td>0</td>
<td>(27)</td>
<td>8.3 ± 0.6</td>
</tr>
<tr>
<td>4</td>
<td>(22)</td>
<td>7.2 ± 0.7</td>
</tr>
<tr>
<td>12</td>
<td>(27)</td>
<td>6.3 ± 0.6</td>
</tr>
<tr>
<td>24</td>
<td>(19)</td>
<td>5.3 ± 0.6</td>
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*Values are means ± standard errors for 27 patients with acute myocardial infarction. Only 22 and 19 of the patients had samples drawn at 4 and 24 hours, respectively.*
the rapid decline of CK-MB following its initial peak (e.g., figure 3). A prospective study of the immunoinhibition technique in the diagnosis of re-infarction appears warranted in view of the importance of this frequent clinical problem.8

Conclusion

The immunochemical method17 used here merits careful consideration as an alternative to electrophoretic procedures and to other immunoinhibition techniques because of its quantitative results, its freedom from interference by CK-BB, and its analytical sensitivity and precision. The importance of freedom from interference by CK-BB is increasingly evident in view of the frequent recent reports of increased serum CK-BB in conditions such as pregnancy and labor7 and cancer.2,14 The combination of the optimized assay of enzymatic activity with this immunochemical method improves analytical sensitivity and avoids "negative" results. The clinical interpretation of the quantitative results of the new assay will require an increased awareness of factors that affect the absolute and relative amounts of CK-MB in the serum. In particular, the temporal change of the ratio of CK-MB to total CK is emphasized.

References

1. Boone, J., Sampson, E. J., Lewis, S., Whitner, V., McKneally, S., and Houston, B.: An inter-
