Cardiac Specific Creatine Phosphokinase Isoenzyme in the Diagnosis of Acute Myocardial Infarction

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SUMMARY
The specificity and sensitivity of serum creatine phosphokinase cardiac specific isoenzyme (MB) in the diagnosis of acute myocardial infarction (AMI) was evaluated. An ion-exchange chromatographic technique was used to isolate MB. Sera layered on mini-columns of DEAE-Sephadex were eluted with Tris-buffered sodium chloride. Quantification of isolated MB was performed by creatine phosphokinase (CPK) assay (Rosalki method) of column effluents. MB was expressed as a percentage of the simultaneously determined total serum CPK. MB was determined in 100 consecutive admissions to the Coronary Care Unit. Acute myocardial infarction was diagnosed by accepted criteria. In 47 patients with proven AMI, including three with normal total CPK, peak MB was greater than 4% of total CPK. In 49 patients without AMI, including 15 with elevated total CPK (due to trauma, injections, cardioversion), peak MB was less than 2% of total CPK. MB was elevated, but did not peak, in four patients without AMI but with chronic atrial fibrillation.

Isolation and quantification of MB by this technique is rapidly and easily performed and provides a specific and extremely sensitive tool for the diagnosis of AMI.

THE MEASUREMENT OF CREATINE PHOSPHOKINASE (CPK) in the serum has proved to be a useful tool in the diagnosis of acute myocardial infarction. In a large number of patients, however, an elevated CPK value adds little information because of the presence of concomitant skeletal muscle damage. Resolution of this problem has been advanced by the development of techniques that separate CPK into its three isoenzymes (MM, MB, and BB). Separation and quantification of MB isoenzyme, which is found almost exclusively in heart muscle, provides a more specific indicator of acute myocardial infarction than total CPK alone. The clinical usefulness of this procedure has been attested to in several reports, including one large series of unselected patients admitted to a Coronary Care Unit. However, the quantification techniques in use up to this time are probably too laborious and time-consuming for most clinical laboratories.

In the present study a new technique of MB isolation and quantification that is both rapid and sensitive was utilized in the diagnosis of acute myocardial infarction in a large series of unselected patients consecutively admitted to the Coronary Care Unit. In addition to specificity and reliability, the sensitivity of the technique was evaluated to determine its utility in the diagnosis of myocardial necrosis so limited that total serum CPK activity remains within the normal range.

Methods
Serum for CPK isoenzyme quantification was obtained from 100 consecutive patients admitted to the Coronary Care Unit of Montefiore Hospital during March and April, 1974. (Three other patients admitted during that period died before adequate samples could be obtained.) Samples were obtained on admission and daily thereafter. Patients were classified as having acute myocardial infarction on the basis of typical, prolonged chest pain plus diagnostic Q waves and/or typical rise and fall of total CPK. In the absence of diagnostic Q waves, nontransmural infarctions were diagnosed on the basis of symmetrically inverted T waves, 5 mm or more in depth, persisting for four days, or new, ischemic-type, ST-segment depression of 2 mm or more persisting for 24 hours. CPK isoenzyme quantification was also carried out on samples obtained from 24 patients without known cardiac disease in whom the serum total CPK was elevated. In addition, samples from 24 healthy laboratory technicians were quantified.

Total CPK and cardiac-specific CPK (MB isoenzyme) determinations were performed immediately after centripugation of the sample. Total serum CPK was determined on the Abbott ABA-100 (Abbott Laboratories) with an ultraviolet kinetic test kit (Smith-Kline Instruments) based on the method of Rosalki. Cardiac-specific CPK isoenzyme was determined by the ion-exchange column chromatographic technique of Mercer.
Figure 1 is a diagrammatic representation of the chromatographic process. A 1 ml serum sample was applied on mini-columns (0.5 × 6.0 cm) of DEAE-Sephadex-A-50 and sample effluent was collected in the first vial. After the mini-column had drained the column was transferred to a second vial. Subsequent elution was stepwise with Tris-hydrochloride buffer (0.05 M, pH 8.0) containing 0.10 M and 0.20 M sodium chloride. Four 1-ml fractions of the 0.10 M sodium chloride buffer were collected in vials 2, 3, 4, and 5 and four 1-ml fractions of the 0.20 M buffer in vials 6, 7, 8, and 9. Total elution time for a single sample was about 15 minutes. Simultaneous fractionation of 40 samples required about 75 minutes.

Figure 2 depicts the ion exchange chromatographic behavior of the MM and MB isoenzymes. CPK activity of column fractions was quantified with partially reconstituted Smith-Kline CPK reagent (36 ml of water to a 50 ml bottle). Aliquots of column fractions (0.100 ml) were added to the concentrated CPK reagent (0.250 ml). Sample addition resulted in dilution of the concentrated CPK reagent to the prescribed and optimal reagent concentration. Linearity of the CPK reaction up to 1,000 I.U. and ten minutes at 37°C was observed. Specificity of the CPK reagent system for CPK activity was confirmed when the omission of creatine phosphate from the reagent resulted in the disappearance of activity. Rate of activity was measured at a 5 min interval with the Abbott ABA-100. The Abbott instrument was set to maximum sensitivity in order to take advantage of its ability to detect absorbance changes as low as 0.00015. Routinely, only the MB isoenzyme-containing fractions (6, 7, 8, and 9) were assayed. Activity in fraction 6 was used to check the possibility of MM isoenzyme carryover. Normally, fraction 6 was without significant activity unless CPK samples with activity near 3,000 I.U. were applied to the column. Activity in fractions 7, 8, and 9 was summed and expressed as a percentage of the total serum CPK.

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\text{Activity in Vials 7, 8, and 9 (I.U.)} \times 100 = \% \text{MB isoenzyme}
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Total serum CPK Activity (I.U.)

Results were returned to the Coronary Care Unit on a same day basis, usually within six hours.

Results

Method reliability for the detection of MB isoenzyme by the chromatographic procedure was followed by daily analysis of a serum pool (frozen) collected from patients with myocardial infarction. The mean (N = 19), standard deviation, and coefficient of variation for the determination of MB isoenzyme in the abnormal pool was 56.6 I.U., 5.7 I.U., and 10.0%, respectively. Expressed as a percentage of total CPK, a value of 11.3 ± 0.25% (2 sd) was calculated. The mean (N = 21), standard deviation, and coefficient of variation for the determination of MB isoenzyme in a normal serum pool (frozen) was 0.79 I.U., 0.17 I.U., and 21.1%, respectively. A value of 1.6 ± 0.67% (2 sd) was calculated when MB isoenzyme was expressed as a percentage of total CPK. No loss of MB activity was observed in the frozen pools (normal and abnormal) for a one month period. Moreover, sera stored at 4°C for three days showed no loss of MB isoenzyme.

Table 1 lists the raw values obtained for total CPK and MB isoenzymes and expresses the latter as a percentage of the former in the Coronary Care Unit patients with and without infarction and in the two control groups. In the healthy laboratory technicians, MB in the serum was either absent or present in very small amounts, up to a maximum of 2% of the total CPK. In the 24 hospital patients without evidence of cardiac disease and with elevation of total CPK, there was a wider range of raw values of MB up to 8 I.U. in one case. However, as in the other control group, MB represented only a small percent of the total CPK (up to 1.1%).

Of the 100 patients consecutively admitted to the Coronary Care Unit, 47 had proven acute myocardial infarction. In these patients, the peak MB expressed as a percentage of total CPK was elevated, ranging from 4% to 22%. Among these 47, there were three patients...
in whom the total CPK was never elevated. There were eight patients with other potential causes for elevation of total CPK. In the entire group the raw values for MB isoenzyme ranged from 3.4 I.U. to 333 I.U. There were four patients in whom MB peaked a second time, following a second episode of chest pain, despite equivocal changes in the total CPK. The time course for percent MB in a typical patient with acute myocardial infarction is illustrated in figure 3.

In 34 of 53 patients without AMI the total CPK was within the normal range (0-110 I.U.), and the MB level was never greater than 1.8 I.U. (0-1.8 I.U.). There were 15 patients in this group without acute infarction in whom the total CPK was elevated, presumably secondary to noncardiac causes (de-fibrillation, intramuscular injections, surgery, accidental falls). As would be expected (in view of the very high total CPK values), raw values for peak MB varied over a broad range, (0.1-9.5 I.U.). When MB was expressed as a percentage of total CPK, however, peak MB never exceeded 1.9%.

Four patients without acute myocardial infarction presented a typical pattern. They had MB values ranging from 3.4 I.U. to 8.7 I.U. As would be expected with normal CPK levels, the percent MB was elevated (4-16%). The values remained elevated for the five days they were measured and in none of these patients could an MB peak be identified (fig. 4). Interestingly, all four patients had atrial fibrillation, three of them on a chronic basis and one for most of his stay in the Coronary Care Unit. A similar situation developed in two patients with acute myocardial infarction and atrial fibrillation. In these two cases the MB was elevated on admission and then rose further to a peak. The values then fell to a baseline that remained above the upper limits of normal for several more days.

Discussion

There have been several studies on the role of MB quantification in the diagnosis of acute myocardial infarction. These have all utilized electrophoresis and fluorescent staining. Such techniques are time-consuming and require well-trained laboratory technicians. In the present study an entirely different approach is used, the isoenzymes being isolated by ion-exchange chromatography, utilizing mini-columns. The effluents containing isolated MB

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**Table 1**

<table>
<thead>
<tr>
<th>Summary of Results</th>
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<tbody>
<tr>
<td>Number of patients</td>
</tr>
<tr>
<td>AMI</td>
</tr>
<tr>
<td>↑ CPK</td>
</tr>
<tr>
<td>N1 CPK</td>
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<tr>
<td>No AMI</td>
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<tr>
<td>↑ CPK</td>
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<tr>
<td>N1 CPK</td>
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<tr>
<td>No AMI, atrial fib</td>
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<tr>
<td>*Hospitalized patients</td>
</tr>
<tr>
<td>†Controls</td>
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</tbody>
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*Hospitalized patients (not in the CCU) with elevated total CPK and no evidence of AMI.
†Healthy laboratory technicians.

Abbreviations: I.U. = International Units; AMI = acute myocardial infarction; ↑CPK = total creatine phosphokinase greater than 130 I.U.; N1 = normal; atrial fib = atrial fibrillation; Peak total CPK = range of the single highest CPK value in each patient; Peak MB = range of the single highest MB isoenzyme in each patient in I.U.; Peak MB (%) = range of the single highest MB isoenzyme expressed as a percentage of the simultaneously determined total CPK in each patient.
are quantified by the usual method of CPK determination. With this technique MB and total CPK of each sample can be determined utilizing the same technique and the same laboratory apparatus.

This new method for MB quantification is rapid, reproducible, and easily performed on a same-day basis. Determination of isolated MB and total CPK by the same technique allows the former to be reported as a percentage of the latter. This is probably more meaningful in a clinical setting than the raw numbers themselves.

The value of isolating and quantifying the cardiac-specific CPK isoenzyme in the diagnosis of acute myocardial infarction has been demonstrated previously and is confirmed in the present study. In this series of 100 patients, 23 had elevations of total CPK that could easily have been attributed to the many types of skeletal muscle injuries that occur in Coronary Care Unit patients. These include cardioversion and defibrillation, multiple intramuscular injections for pain or vomiting, postoperative chest pain, trauma secondary to falls from infarction-related arrhythmias, hypotension and prolonged use of rotating tourniquets. When MB isoenzyme is determined in International Units and as a percentage of total CPK, this very large group of bewildering patients can be easily clarified.

Consideration of percent MB is required for accurate diagnosis because, although the heart is richest in the MB isoenzyme, it is not the only source of it. Skeletal muscle contains small amounts of MB which become apparent only when analyzing large amounts of tissue extract. Since the total serum CPK pool in normal subjects is probably derived from all skeletal muscle, MB should be present in normal serum. In fact MB has been demonstrated in normal serum, and was found in trace amounts (up to 2%) in a majority of the 24 normal control subjects in the present study. Serum samples with increased total CPK activity due to skeletal muscle damage would therefore also have increased MB, but percent MB would not be increased. Thus in the 15 patients without acute myocardial infarction but with elevated total serum CPK that could be attributed to skeletal muscle necrosis, peak percent MB never exceeded 1.9%, even though there were some elevated raw MB values (table 1), and remained well below the 4.0% that represents the lowest peak percent MB found in any of the patients with acute myocardial infarction.

In the present study of unselected, consecutively admitted patients to a Coronary Care Unit, there was excellent separation of patients with acute myocardial infarction, diagnosed on fairly rigid clinical grounds, and those without acute infarction. There was no overlap between the two groups in 96 out of 100 cases when a cut-off of 4% MB was used. There were no false-negative cases in the 47 patients with clinically diagnosed acute infarction. Of the 53 patients who did not have the electrocardiographic changes required to diagnose acute myocardial infarction, percent MB remained below 3.3% in 49. Although definitive autopsy confirmation is lacking, it appears highly probable that the MB in the serum of these 49 patients is derived from skeletal muscle. The remaining four patients, representing false-positive cases, did have characteristics, described below, which could help identify them and clarify their diagnosis.

The four patients with false-positive elevations of MB are of great interest. Such patients should be able to be separated out with little difficulty in proper classification, since in no case was there an identifiable MB peak, the values remaining at about the same level throughout the sampling period (fig. 4). Moreover, this phenomenon was noted only in patients with fairly prolonged atrial tachyarrhythmias. The genesis of the elevated MB in these cases is not clear, although the occurrence of tachycardia in patients with coronary artery disease has been reported to increase total CPK in the serum. Additional support for this hypothesis comes from a recent report of pacing-induced ischemia in patients with coronary artery disease, leading to elevated levels of coronary sinus CPK and depletion of myocardial inorganic phosphate.

MB quantification is also of distinct benefit in diagnosing an extension of the original myocardial infarction. In seven patients in the present study, prolonged chest pain recurred within a few days of admission. In all seven, the total CPK continued to fall from the previously elevated value, but in four patients there was a second sharp peak in MB. These results cannot be considered unequivocal because of
the inconstant electrocardiographic changes, but they do suggest another area in which quantification of MB is of value.

The sensitivity of the present technique is demonstrated by the detection of elevated MB isoenzyme in serum samples with total CPK activity within the normal range. Although recent further refinements in the electrophoretic technique provide a similar sensitivity, the refined method becomes even more cumbersome for clinical use. In the present study, quantification of MB successfully identified the three patients with acute myocardial infarction and normal values for serum total CPK. The increased sensitivity this illustrates, combined with the high degree of specificity noted above, renders the present technique of MB quantification invaluable in the diagnosis of patients with chest pain and equivocal (or no) electrocardiographic change. In such patients, elevation of the percent MB almost certainly indicates myocardial necrosis. An MB of less than 2%, on the other hand, effectively rules out the diagnosis of acute myocardial infarction.

References
Cardiac specific creatine phosphokinase isoenzyme in the diagnosis of acute myocardial infarction.

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