The Importance of Identification of the Myocardial-Specific Isoenzyme of Creatine Phosphokinase (MB Form) in the Diagnosis of Acute Myocardial Infarction

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SUMMARY

Serial plasma determinations of the isoenzymes of CPK were performed in all patients (376) admitted to a coronary care unit during a 12-month period with diagnosis of possible acute myocardial infarction. Results were compared with data from other enzyme studies and from the electrocardiogram. An attempt was made to determine the incidence of falsely positive CPK-MB (myocardial-specific form). "No acute infarction" was diagnosed in all patients in whom neither total CPK nor the isoenzymes of LDH indicated myocardial necrosis, and in whom there were no QRS changes on ECG. Incidence of falsely negative CPK isoenzyme data was also determined. All patients, in whom total CPK was transiently elevated, and LDH exceeded LDH2, and new QRS changes occurred, were termed "definite" acute infarction. CPK-MB form was present in all 55 of these (0% false negative). Therefore, determination of the isoenzymes of CPK by this method provides both a sensitive and specific indication of acute myocardial infarction.

Additional Indexing Words:
Acute myocardial infarction
Enzymology
Isoenzymes

Creatine phosphokinase: MB form

The early and accurate identification of myocardial necrosis in patients experiencing symptoms suggestive of acute coronary insufficiency is a common and important clinical challenge. The diagnosis of myocardial infarction depends upon the interpretation of historical, electrocardiographic, and serum enzyme data. Each of these variables, however, is known to lack precision with respect to sensitivity, specificity, or both. A history of severe, prolonged substernal chest pain is often absent in patients with infarction, and, when present, it may be the result of events other than myocardial infarction. New "Q wave" changes on the electrocardiogram are quite specific for acute infarction, but are absent in approximately 30% of autopsy-proven cases. Alterations of the S-T and T waves occur in nearly all patients with acute myocardial infarction, but identical changes may be seen during episodes of ischemia without infarction, and even with noncoronary problems.

Because of the above, most physicians rely heavily on serial changes of enzyme levels in serum to define or exclude the diagnosis of acute myocardial infarction. Alterations of the serum enzymes occur in nearly all cases of proven infarction. However, even the most sensitive of those in routine use, creatine phosphokinase (CPK), can also be elevated by strenuous exercise, chronic alcoholism, convulsions, pulmonary disease, cardioversion, cerebrovascular disease, and intramuscular injections. Thus, while the routine determination of serum CPK is a sensitive index of myocardial necrosis, the enzyme lacks specificity and many false-positive elevations are observed.

In a previous report, a method was described for the identification and quantitation in serum or plasma of the CPK-MB isoenzyme of creatine phosphokinase. This hybrid form is known to be in highest concentration in cardiac muscle. The purpose of this study is to describe the value of the CPK-MB isoenzyme in our clinical experience with
a large series of patients with suspected acute myocardial infarction.

Methods

Sample Handling. Blood samples were withdrawn by the vacutainer technic, using a tube containing EDTA as anticoagulant. Plasma was immediately separated from the cells by centrifugation and was removed by pipette. Plasma samples were kept cold but not frozen until enzyme and isoenzyme determinations were made within the subsequent 24-hour period.

Laboratory Procedures. Plasma CPK was determined by the method of Rosalki,14 using the modified reagent substrate prepared by Eskalab. Lactic dehydrogenase (LHD) activity was obtained by the method of Henry.15 All determinations were carried out on the Eskalab Clinical Chemistry System, using the Eskalab alpha-dual beam spectrophotometer and flow-cell system.

Plasma samples were electrophoresed on a supporting medium of 1% ionagar. The electrophoretic equipment and isoenzyme detection methods with quantitation are those of Nerenberg16 for LDH and of Roe17 for CPK isoenzymes and their quantitation. The detection reactions for both LDH and CPK isoenzymes were the same as used in determination of the total enzyme activities. The LDH isoenzymes were visualized by precipitation of formazan, while the fluorescent CPK forms were detected under ultraviolet light due to the production of NADPH during the course of the reaction. Quantitation of the CPK forms was accomplished by fluorometric scanning of electropherograms, using the Turner-III fluorometer with a thin layer scanning door coupled to Photovolt-552 densitometer and automatic integrator.

Homogenates of human autopsied specimens of various tissues have been assayed for type of CPK activity. Results from brain, skeletal muscle, and cardiac muscle are presented in figure 1.

Results

Quantitation and Temporal Relationships

Plasma levels of the activities of LDH, CPK, and CPK-MB were determined on admission to the Coronary Care Unit and every 4 hours thereafter, from 10 consecutive patients who had definite evidence of acute myocardial infarction on the ECG. In six of these, observation of the complete curves of both total and CPK-MB was possible. The remaining four patients either entered that unit after enzyme rise had begun or expired prior to the time of return of normal plasma levels.

Figure 2 shows the time course of rise of both total CPK and CPK-MB after the acute onset of symptoms. The only data included in this illustration are the average times after acute onset of symptoms and plasma levels of the first abnormal result, the maximally abnormal result, and the final abnormal result. The time from onset of symptoms to the initial elevation of total CPK as well as the

*Smith-Kline Instruments Inc., Palo Alto, California.

![Figure 1](image-url)

**Figure 1**

Organ assay of isoenzymes of CPK. Homogenates of autopsy specimens of human tissues are assayed for type of CPK activity. Light gray areas represent areas of isoenzyme localization. Skeletal muscle in slot 1 has MM form only. Heart muscle in slots 2 and 5 has both MM and MB forms. Brain in slot 7 has BB form only. (Slots 3, 4, and 6 are not used.)
appearance of the heart band varied from 2 to 12 hours. The CPK-MB form was detectable in the plasma for no less than 24 hours and no more than 72 hours after its initial appearance. Total CPK remained elevated for an average of 60 hours, and for an average of 24 hours following the disappearance of CPK-MB. These findings emphasize the importance of proper timing in evaluation of the significance of enzyme and isoenzyme determinations. Absence of CPK-MB in patients who are more than 24 hours remote from the onset of their acute episode may not be used as evidence for exclusion of the diagnosis of acute myocardial infarction.

Maximum total CPK activity varied from 390 to 3423 IU (upper limit of normal is 130 IU). This peak was attained between 16 and 20 hours after the onset of symptoms. The portion of the total CPK activity attributable to the CPK-MB form varied from 12% to 38%. Thus, the major form of CPK released from myocardial cells is the MM form (muscle band), which is the isoenzyme form normally seen in serum and plasma samples. Also, it is apparent from the temporal relationships of total and CPK-MB that the MM form persists in the plasma for a longer period of time. Thus, the heart-specific isoenzyme is the minor and more transiently observed fraction of CPK released from damaged myocardial cells.

Figure 3 presents the total and MB form in one patient in this series. The secondary peak of total CPK which occurred at 8 AM of Day 2 was accompanied by increased CPK-MB. This occurred after a bout of recurrent pain and probably indicated extension of infarction.

**Sensitivity and Specificity**

During the calendar year 1971, 376 consecutive patients were admitted to the Coronary Care Unit because of a history of chest pain suggesting myocardial infarction. Patients were eliminated from this study if they had concurrent chronic myocarditis, recent cardiac surgery, or cardiac arrest with prolonged periods of closed-chest cardiac massage. In addition, patients who had their onset of symptoms more than 24 hours prior to admission, or who died less than 24 hours after admission, were excluded.

Each of the 328 patients who remained in the study group had a history of chest pain compatible with acute myocardial infarction. They were further characterized by the presence or absence of (1)
ECG changes of infarction, (2) elevation of total CPK, (3) a ratio of LDH1:LDH2 of >1, and (4) detectable CPK-MB on agar gel electrophoresis. Use of these four descriptors provided 16 potential subgroups of the 328 patients as noted in table 1.

For purposes of definition: (1) the electrocardiogram was considered positive when new QRS changes indicative of infarction were observed or, in the absence of prior tracings, when transient S-T and T wave changes indicative of epicardial injury accompanied the QRS appearance of infarction; (2) the total CPK was considered abnormal when plasma levels transiently exceeded 130 IU; (3) LDH isoenzymes were indicative of myocardial infarction when LDH1 clearly exceeded LDH2 in the absence of any indication of hemolysis; and (4) CPK isoenzymes were positive when the MB form was identified in plasma.

Our first objective was to identify the incidence of a falsely negative ECG in patients with "definite infarction." A definite diagnosis of acute infarction was made in 84 patients (groups 1 and 2) without reference to the ECG. These patients had typical chest pain, a positive total CPK, reversed LDH1:LDH2 ratio, and positive CPK-MB. Fifty-five of these (group 1) had a positive ECG. However, 29 patients (group 2) failed to show definite positive ECG changes, either because a concurrent intraventricular conduction disturbance precluded the appearance of QRS changes or because S-T and T wave changes, but no alteration of the QRS complex, occurred. None of these patients maintained a normal ECG throughout the 5-day monitoring period. No patient had a positive ECG without at least one positive enzyme parameter to confirm the impression of acute infarction (group 12). These observations confirm the generally accepted view that the QRS changes characteristic of myocardial infarction are, indeed, a highly specific index. These data also show, however, that these rigid ECG criteria are absent in a significant number of patients with definite acute myocardial infarction (i.e., 34% in this series). The limited sensitivity of the ECG in the diagnosis of acute infarction emphasizes the importance of identifying enzyme criteria which are equally specific but of greater sensitivity than the ECG.

In recent years, the quantitation of total CPK in serum has become routine in many laboratories, and the method has become useful for identification of acute myocardial infarction. However, because of the high incidence of CPK elevation due to many noncardiac problems, as noted above, it is important to examine the present data with respect to the incidence of falsely elevated levels. A diagnosis of acute myocardial infarction was definitely excluded in 213 of our 328 patients without reference to total CPK (groups 13 and 16). These patients had a history suggesting infarction, but no QRS changes on ECG, no excess of LDH1 over LDH2, and the CPK-MB isoenzyme was absent. Of these 213 patients (group 13), 32 had elevations of total CPK compatible with acute infarction. Thus the incidence of falsely positive total CPK was 15%. However, of the 56 patients with definite myocardial infarction proven by ECG and by characteristic LDH and CPK isoenzyme patterns (groups 1 and 3), 55 (group 1) had a positive total CPK. Thus an elevated total CPK is a highly sensitive index of infarction, but there are significant numbers of patients (15%) in whom an elevated total CPK is misleading.

Total CPK is a sensitive index of infarction, and the CPK-MB form is found in significant concentration only in heart muscle. The CPK-MB isoenzyme was detected in each of 55 patients with acute myocardial infarction proven by history, ECG, LDH isoenzymes, and total CPK (groups 1 and 5). Of 182 patients in whom the diagnosis of myocardial infarction was excluded by ECG, iso-LDH, iso-LDH, iso-LDH.

Table 1

<table>
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<tr>
<th>Group</th>
<th>Pts in group (no.)</th>
<th>ECG</th>
<th>Total CPK</th>
<th>LDH1:LDH2</th>
<th>CPK-MB</th>
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<tr>
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<td>181</td>
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*All 16 potential diagnostic subgroups possible by use of four parameters (ECG, total CPK, iso-LDH, and iso-CPK) are shown. However, all patients could be stratified into 12 groups, and five of these had only one or two patients. Of the remaining groups, 1, 2, and 4 were considered definite infarction; groups 13, 14, and 16 were called definitely no infarction, and group 7 remained unknown.
and total CPK (groups 15 and 16), only one (0.6%, group 15) had a falsely positive CPK isoenzyme analysis. Thus, when patients are seen within the first 24 hours after the onset of symptoms, the plasma assay for heart band iso-CPK, by the technics noted above, approaches 100% accuracy for either the diagnosis or exclusion of acute myocardial infarction.

In our series of patients with acute myocardial infarction proven by history, ECG, and both total CPK and isoenzyme analysis (groups 1 and 4), there was a 10% incidence (group 4) of falsely negative isoenzymes of LDH (LDH1 > LDH2). Of patients in whom the diagnosis of acute myocardial infarction was excluded by the above criteria (groups 14 and 16), there were 5% (group 14) who had a falsely positive LDH1 : LDH2 ratio. Thus LDH isoenzymes were relatively sensitive and specific, but not as accurate as CPK isoenzymes for either defining or excluding the diagnosis of acute infarction. However, isoenzymes of LDH are of particular value in patients who present more than 24 hours after the onset of symptoms. The CPK-MB isoenzyme disappears 24–48 hours after acute infarction, but an LDH1 : LDH2 ratio of greater than one may persist for 5–10 days, thereby indicating the likelihood of recent infarction.

A summary of the sensitivity and specificity of each of the four diagnostic parameters is presented in Table 2.

One might hypothesize that patients with an "enzyme-proven" acute myocardial infarction, without Q waves on the ECG, would be in a particularly low-risk subgroup. In our series, however, hospital mortality was 22% (14 of 62) when the ECG was diagnostic, and 21% (six of 29) when only S-T and T-wave changes were present. It was not possible to identify any particularly high- or low-risk subgroup of patients on the basis of these diagnostic variables.

Further insight into the specificity of the CPK-MB isoenzyme was obtained by the analysis of plasma enzymes in 50 consecutive patients who underwent coronary angiography using the Judkins technic. Electrocardiograms were obtained daily, before and after the angiogram; none showed definite evidence of acute myocardial infarction. All enzymes (total CPK, MB-CPK, and LDH1 : LDH2) were normal prior to the procedure. Total CPK became elevated in 22 of these patients within 24 hours after the angiography. In one patient the CPK-MB form was detected and was accompanied by LDH1 : LDH2 elevation, prolonged pain, and S-T and T-wave changes, confirming the diagnosis of acute infarction. Thus, in 46% of the patients, an elevation of total CPK occurred after coronary angiography. The MB form was present in only one. These observations emphasize the specificity of this isoenzyme and indicate a noncardiac source of the total CPK, probably skeletal muscle at the site of the percutaneous approach.

**Discussion**

In the mid-1950s Wroblewski, LaDue, and coworkers17–20 established both the experimental evidence and clinical significance of the relationship between acute myocardial infarction and an elevation of enzymes in serum. They noted a correlation between the concentration of these enzymes in serum and the size of myocardial infarction. They also found that transient coronary insufficiency, which produced ST-T wave changes but no Q waves on the ECG, also failed to produce other evidence of tissue necrosis or elevation of enzyme concentrations in plasma. The enzymes they studied (SGOT and LDH), however, were not specific for myocardial necrosis. In the mid-1960s Hess21 and others22–24 reported the use of total CPK in the clinical diagnosis of myocardial infarction. This enzyme was reported to have some advantage over SGOT and LDH because its relative increase in

**Table 2**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sensitivity (%)</th>
<th>False-negative (%)</th>
<th>False-positive (%)</th>
<th>Specificity (%)</th>
</tr>
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<tbody>
<tr>
<td>ECG</td>
<td>66</td>
<td>34</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Total CPK</td>
<td>98</td>
<td>2</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>LDH1:LDH2</td>
<td>90</td>
<td>10</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>CPK-MB</td>
<td>100</td>
<td>0</td>
<td>1</td>
<td>99</td>
</tr>
</tbody>
</table>

*The incidence of both falsely positive and falsely negative diagnostic parameters is shown. A parameter was considered falsely positive when all others (three) were negative. Likewise, a parameter was considered falsely negative when all others (three) were positive. Lack of false positives is indicative of a specific parameter and lack of false negatives indicates high sensitivity.
plasma was greater and earlier following the myocardial necrosis. Sobel and his co-workers investigated the kinetics of both the appearance and subsequent clearance of total CPK activity from plasma following myocardial infarction in experimental animals. A knowledge of these kinetics potentially has led to a noninvasive technique for estimating the size of a myocardial infarction. It should be noted, however, that the high incidence of falsely elevated total CPK reported by others and confirmed in this report, imposes serious limitations on the use of the total CPK as a diagnostic aid. It also seems very probable that, in man, extrapolation of infarct size from measurements of total CPK will be subject to numerous errors and possible misinterpretations. Earlier, Karliner and Sobel had investigated the use of glyceraldehyde phosphate dehydrogenase because of its rapid and transient appearance in plasma after acute infarction on experimental animals. This enzyme offered a capability for detection of extensions of myocardial necrosis but was also found, like total CPK, to lack specificity. The MB isoenzyme of CPK would seem to offer a reasonable solution to these problems. It is both a sensitive and specific indicator of myocardial necrosis. Its appearance and disappearance are rapid enough that it should be a sensitive index of subsequent extensions of infarction. The concepts developed by Sobel and his colleagues for extrapolating infarct size from total CPK should be applied to the analysis of the MB fraction of CPK, and the results of such an analysis might provide an accurate technique for estimating the size of myocardial infarction.

The usefulness of serial changes of the total CPK in the diagnosis of acute myocardial infarction would appear from previous results and our own to have two significant limitations. First, CPK is present in significant quantity in both heart and skeletal muscle leading to a high incidence of falsely elevated total CPK. This problem could be solved almost totally by the identification of the isoenzymes of CPK. A second limitation to the use of CPK in the diagnosis of infarction is due to persistence of the elevation for only 2–3 days after the onset of infarction. This problem is accentuated with determination of the isoenzymes of CPK, since the MB form disappears approximately 24 hours before total CPK returns to normal. Thus, while the isoenzymes of CPK are both specific and sensitive for myocardial injury, their diagnostic value will be applicable only to those patients who are seen early after the onset of symptoms. It is of interest to note that reversal of the normal LDH1 : LDH2 ratio is both relatively sensitive and specific for myocardial infarction. The finding of an abnormal LDH1 : LDH2 ratio adds certainty to the diagnosis of infarction, and this abnormal ratio persists for several days, thus making it more useful in the patient who is seen more than 24–48 hours after the onset of symptoms.

The technic described in this report provides a reproducible, relatively uncomplicated, and inexpensive approach to the diagnosis of myocardial necrosis in patients who present symptoms that suggest acute myocardial infarction. The results of our analysis indicate that the determination of the CPK-MB fraction in plasma provides both a sensitive and specific indicator of myocardial necrosis. Its early appearance in plasma after the onset of symptoms in patients who have suffered myocardial infarction makes an early diagnosis possible. The rapid return of CPK-MB levels to the normal range makes this enzyme particularly useful in the recognition of extensions of infarction which occur subsequent to the initial episode. The ability to quantitate the CPK-MB fraction, its sensitivity and specificity, and its rapid appearance and disappearance make this enzyme ideal for testing feasibility of quantitating infarct size and for evaluating therapeutic interventions which are aimed at protection of the ischemic myocardium.

**Acknowledgment**

The authors wish to acknowledge the technical assistance of Mr. Larry Patterson and Mr. Fred Frizzelle, and the secretarial aid of Mrs. Bess Cebe and Miss Virginia Hicks.

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_Circulation, Volume XLVII, February 1973_
ISOENZYMES IN ACUTE MI


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Circulation. 1973;47:263-269
doi: 10.1161/01.CIR.47.2.263

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