Clinical Evaluation of an Immunoinhibition Procedure for Creatine Kinase-MB

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We have clinically evaluated the Dade "Cardiozyme" immunoinhibition procedure for determination of creatine kinase isoenzyme MB (CK-MB) in 71 patients who were suspected of having an acute myocardial infarction. Electrophoresis for CK-MB was also carried out. On the basis of diagnostic sensitivity and specificity for myocardial infarction, we found the Dade procedure for CK-MB to be somewhat inferior to electrophoresis. In 11 patients for whom the time of infarction was known, we observed normal CK-MB results for two of them by both immunoinhibition and electrophoresis during the first 24 h, but subsequently could detect abnormal CK-MB results by both methods. Thus in some patients such data are not helpful for making a diagnosis in the first 24 h. The Dade procedure is easy to perform, but lacks sensitivity in the region of low CK-MB activity, requires a very stable spectrophotometer, is imprecise, and produces negative numerical results in patients without myocardial infarction.

The correct diagnosis of acute myocardial infarction is of paramount importance, because its misdiagnosis can result in a fatal outcome for the patient. Any new diagnostic procedure for it must be at least as good or better than an existing procedure for it to be clinically appropriate. Abnormal serum enzyme activities—in particular AST, CK, LD, and the isoenzymes of CK and LD—have become extremely valuable in the diagnosis of acute myocardial infarction in patients with suspicious clinical signs. This area of diagnostic enzymology has been reviewed in some detail (1–3). Creatine kinase MB isoenzyme (CK-MB) is the most sensitive and specific for acute myocardial infarction of all the commonly done enzyme tests. The average sensitivity and specificity of CK-MB are 98 and 99%, as calculated from data in seven recent reports in which CK-MB was determined electrophoretically (4–10), and 99 and 95%, respectively, where CK-MB was determined chromatographically (11, 12). All of the other enzyme tests—AST, CK, LD, or LD isoenzymes—had average sensitivities and specificities that were less than that of CK-MB for acute myocardial infarction in the above reports. An immunoinhibition method for CK-MB (13) had a sensitivity of 70% and specificity of 95% and therefore was somewhat inferior to the other methods.

The procedure most widely used for CK-MB is electrophoresis (4–12), which is time consuming, semiquantitative at best, depends somewhat on the technique of the operator, and is unsuitable as an emergency procedure. Recently Dade (Dade Division, American Hospital Supply Corp., Miami, FL 33152) marketed their “Cardiozyme” immunoinhibition method for CK-MB. In their procedure, CK activity is measured by the usual Rosalki scheme of reactions (14) in the presence of anti-CK-M antibody. The activity measured is reported to be that of CK-MB. Because the procedure requires only a thermostatted spectrophotometer with 340-nm capability, it is ideally suited for possible automation or for use as an emergency procedure. It could be further enhanced by automated data reduction.

The Dade procedure for CK-MB is disadvantageous, because it also measures CK-BB. Further, it may leave uninhibited the atypical CK that Sax et al. (15) and we (16) have reported. The report of Marchiaio et al. (17) appears to confound the picture of three CK isoenzymes and, if correct, may prove that immunoinhibition procedures are undesirable. However, CK-BB is seen only infrequently after electrophoresis: less than 1% of the time by Lamar et al. (18), and 8% of the time by us (July 1979, 11 of 136 samples had distinct or trace CK-BB band). Further, the CK-BB band is usually faint and represents only a small part of the total CK activity. Thus the error introduced by also measuring CK-BB is negligible in most patients.

We wanted to evaluate the Dade method for possible use in our laboratory. Clearly, a laboratory comparison with electrophoresis is not enough, because it is the diagnostic sensitivity and specificity of any new procedure for acute myocardial infarction that determines its value. Therefore we did a clinical study of the Dade procedure for patients admitted to our cardiac intensive-care unit.

Materials and Methods

Patient group. Seventy-five patients admitted to the coronary care unit during a six-month period were included in the study. The patients were selected without conscious bias from among those admitted to the unit during this time. All the patients we chose had requests for enzyme and isoenzyme studies. Four patients were excluded from further analysis because the samples were hemolyzed, because of incomplete isoenzyme data, or because the patient died before diagnosis. Hemolyzed samples could not be used, because they gave false-positive results for LD isoenzymes and CK-MB by the Dade procedure, possibly owing to interference by adenylate kinase (EC 2.7.4.3) in the latter case. For the remaining 71 patients, we determined CK and LD isoenzymes by the ACI electrophoretic procedures (Corning Medical, Medfield, MA 02052), CK (total) by the Rosalki procedure (14) with a Sigma kit (no. 45-U.V. method; Sigma Chemical Co., St. Louis, MO 63178) and use of a GEMSAB centrifugal analyzer (E.N.I., Fairfield, NJ 07006), and CK-MB was also determined with the Dade "Cardiozyme" kit with a Model 222A spectrophotometer (Gilford Instrument Co., Oberlin, OH 44074) exactly as described in the Dade package insert. CK-MB units by electrophoresis were calculated from the percentage of CK-MB observed after scanning the agarose gels with a fluorometric densitometer and total CK activity as determined with the Sigma method.
In electrophoresis, performed 24 h after admission, CK-MB activity and high CK-MB isoenzymes were available for the diagnosis of acute myocardial infarction or no acute myocardial infarction. The diagnoses were made on the basis of clinical or electrocardiographic findings, CK and LD isoenzymes by electrophoresis, assays for total CK and AST, and technetium scans for some of the patients. The Dade results were not available to the clinicians in making their diagnoses.

Normal ranges. Normal (expected) ranges for CK, CK-MB by electrophoresis, and LD isoenzymes were established by using blood from non-professional blood donors. For CK it is 0–12 Sigma units, central 95th percentile, as obtained from data on 70 men and 30 women. Of 38 adult donors, we saw no CK-MB in 36 and 0.1 and 0.4 Sigma units (1% and 4%) in two presumed normal men; we therefore set the reference range for CK-MB at <0.5 Sigma units. This limit is arbitrary and not completely satisfactory because we have seen values as high as 5 Sigma units of CK-MB with a total CK of 98 Sigma units in swimmers in the course of active physical training. CK and CK-MB must be considered in the context of the physical activity of the individual. For LD isoenzymes, we never saw LD1 greater than LD2 in 50 blood donors with non-hemolyzed blood. For the Dade procedure, we used Dade’s value of less than 4 U/L for CK-MB. A patient was considered to have an abnormal result if any value obtained within the first 48 h after admission to the unit was above the normal range.

Results and Discussion

Patient findings. Of the 71 patients, 28 were diagnosed as having suffered an acute myocardial infarction. Our results are summarized in Table 1. Determination of CK-MB is clearly an excellent test to differentiate acute myocardial infarction from no infarction, and the electrophoretic procedure is somewhat superior to the immunoinhibition procedure. Nevertheless, the Dade method appears to provide useful clinical information. It is somewhat unfair to compare the electrophoretic procedure to the Dade method for CK-MB, because the clinicians saw the former but not the latter in making their diagnoses. One patient with pericarditis and myocarditis, but not acute myocardial infarction, had abnormal results for all four tests (Table 1). Were he removed from the group in Table 1, the specificity of all the tests would improve. It is well known that CK often gives falsely positive results, usually an effect of intramuscular injections.

The four cases with acute myocardial infarction where the Dade results were falsely negative all had 4 or fewer Sigma units of CK-MB, i.e., the Dade kit may lack some sensitivity in the low activity range. However, seven patients of 23, all with acute myocardial infarction and with four or fewer Sigma units, gave a positive result by the Dade method. In this group of 23, the Dade method was positive when electrophoresis showed 11 ± 8 (SD) Sigma units of CK-MB. The regression equation for the 28 acute myocardial infarction patients for the two methods for CK-MB is y (immunoinh.) = 3.52 (electroph.) − 1.92, r = 0.842, std. error of estimate = 20.2, SD of intercept = 5.81, SD of slope = 0.44. A numerical comparison such as this for two entirely different procedures should not be overinterpreted.

Temporal sensitivity of enzyme tests. We attempted to determine the optimum interval after infarction for measuring serum enzymes most usefully. We were able to obtain a good estimate of the time of infarction in 11 patients (Table 2). For all 11 patients the electrophoretic and immunoinhibition procedures provided the same information. Evidently diagnostic judgments of acute myocardial infarction based on enzyme data from the first 24 h may not be correct in some cases.

Negative reaction with Dade method. A disconcerting phenomenon with the Dade method is the negative numerical results for CK-MB in patients without infarction, such as we observed in 35 of 42 such patients. The values ranged from −3 to −40 U of CK-MB per liter, mean −12 ± −8 (SD). Four patients without infarction had results that were zero; the three false-positives of course had positive CK-MB values.

Reduced glutathione always contains some oxidized glutathione, because oxidized glutathione forms during lyophilization of reduced glutathione. In the presence of glutathione reductase, which normally appears in blood, the above reaction consumes NAD(P)H and could lead to a negative absorbance change with time. The net absorbance change seen in the Dade procedure may be the result of competing reactions to produce and consume NAD(P)H. This may be why, in the presence of small amounts of CK-MB, the Dade procedure gives false-negative results and why negative numerical results are seen with non-infarct patients.

Table 1. Data on 71 Patients with Suspected Acute Myocardial Infarction

<table>
<thead>
<tr>
<th>Test</th>
<th>No. tests abnormal in AMI&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. tests normal in AMI</th>
<th>No. tests abnormal in non-infarct</th>
<th>No. tests normal in non-infarct</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK, total</td>
<td>28</td>
<td>0</td>
<td>5</td>
<td>38</td>
<td>100</td>
<td>88</td>
</tr>
<tr>
<td>CK-MB by electrophoresis</td>
<td>27</td>
<td>1</td>
<td>1</td>
<td>42</td>
<td>96</td>
<td>98</td>
</tr>
<tr>
<td>CK-MB by immunoinhibition</td>
<td>24</td>
<td>4</td>
<td>3</td>
<td>40</td>
<td>86</td>
<td>93</td>
</tr>
<tr>
<td>LD isoenzymes</td>
<td>17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11</td>
<td>1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42</td>
<td>61</td>
<td>98</td>
</tr>
</tbody>
</table>

<sup>a</sup> Acute myocardial infarction. <sup>b</sup> LD1 > LD2.

Stable spectrophotometry. Another concern with the Dade procedure is the small absorbance changes per minute that must be used in the calculations. An absorbance change of 0.0006 per min is the upper normal limit. Readings are taken

Table 2. Diagnostic Sensitivity of Enzyme Tests<sup>a</sup> in 11 Patients after Acute Myocardial Infarction

<table>
<thead>
<tr>
<th>Hours after infarct</th>
<th>0–12</th>
<th>12–24</th>
<th>24–36</th>
<th>36–48</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with all normal tests&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Patients with at least 2 abnormal tests&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6</td>
<td>9</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Patients with no data</td>
<td>1</td>
<td>0</td>
<td>2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Same tests as in Table 1. <sup>b</sup> All abnormal in first 24 h.
for 5 min. Clearly, very stable and precise spectrophotometry is required with the Dade method if one is to distinguish normal and diseased.

Dilution study. We wanted to test the effect of dilution on the observed activity in view of Morin's observations (20) that the Dade method gives non-linear dilutions. For an undiluted sample with 17 U/L CK-MB we observed 9 and 7 U/L for a twofold dilution (with isotonic saline), 8, 5, and 5 U/L for a three-fold dilution, and 0 and 4 U/L on a sixfold dilution. The analytical recoveries were imprecise and inaccurate at the higher dilutions.

Quality control results. One operator analyzed in replicate the Dade Cardiozyme controls I and II by the Dade method, with the following results: for I, 6.6 ± 2.5 U/L (SD), n = 28; for II, 27.9 ± 5.6 U/L (SD), n = 37. Control results for electrophoresis for Sigma "CPK Isotrol" done at this time by a variety of operators were: CK-MM, 41.9 ± 3.7% (SD); CK-MB, 35.1 ± 2.3% (SD), and CK-BB, 22.9 ± 3.0% (SD), n = 36.

We conclude that electrophoresis is the better clinical method for CK-MB of the two methods compared here. The Dade method is clearly easier to use in the laboratory and could be automated on a precision spectrophotometer, but it appears to have difficulty detecting low proportions of CK-MB. There may be an interference from oxidized glutathione which could produce the negative numerical results for CK-MB that we observed in samples from patients without acute myocardial infarction. Very stable spectrophotometry is necessary with the Dade method, because a drift of only 0.001 A per 5 min in the decision region between normal and abnormal is intolerable; possibly for this reason our precision with the method and our recovery of diluted samples was poor.

References