Diagnostic Efficacy of a New Enzyme Immunoassay for Creatine Kinase MB Isoenzyme

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A new commercial enzyme immunoassay kit for quantification of creatine kinase-MB (CK-MB) isoenzyme was compared with its electrophoretic determination with respect to efficacy in diagnosis of acute myocardial infarction. Enzymost™ CK-MB (Behring Diagnostics) is a solid-phase "sandwich"-type enzyme immunoassay with antibodies to the B-subunit coated on plastic tubes and peroxidase-conjugated antibodies to the M-subunit added after incubation with sample. This kit is designed to measure only CK-MB and not CK-MM, CK-BB, adenylylate kinase, or atypical CK molecules. The linear-regression equation comparing the two methods was: Enzymost = 0.98·electrophoresis - 0.72, with a correlation coefficient of \( r = 0.967 \) (n = 143). For 51 patients admitted for diagnosis of possible acute myocardial infarction, the Enzymost kit achieved 100% sensitivity, specificity, and efficiency in predicting the correct diagnosis. Corresponding values for the electrophoretic assay were: 95.5% sensitivity, 93.1% specificity, and 94.1% efficiency. We conclude that this kit method provides an excellent alternative to electrophoresis.

Additional Keyphrases: "kit" methods · electrophoresis on agarose gel · cutoff value · activity vs mass

Creatine kinase (EC 2.7.3.2) plays a central role in energy transfer and is abundant in muscle tissue. Of its three isoenzymes, creatine kinase MM and BB (CK-MM and CK-BB) are found in various tissues, but CK-MB isoenzyme is present in appreciable amounts only in the heart, where it represents 10 to 40% of the total CK activity (1, 2). The low concentration of CK-MB in normal serum and in tissues other than the heart is the basis of its use as a specific indicator in the diagnosis of acute myocardial infarction (AMI) (3, 4).

CK-MB is an excellent indicator for AMI, but not all CK-MB assays measure this isoenzyme with equal specificity and sensitivity. Electrophoresis or column chromatography have been used to separate the isoenzymes physically. However, electrophoresis is susceptible to some artifacts and lacks sensitivity, and chromatography may not completely separate the isoenzymes and may produce false results in the presence of atypical isoenzymes (4–10). Recently devised methods also have drawbacks. Immunoinhibition of the M-subunit activity cannot distinguish CK-MB from CK-BB and may incompletely inhibit M activity in samples with markedly increased CK-MM, also yielding false-positive results (8, 11, 12). Some radioimmunoassays measure only the B subunit and thus cannot distinguish between CK-MB and CK-BB (13, 14). A more promising approach has been the use of anti-CK-M and anti-CK-B antibodies for specific detection of CK-MB by immunoassay (15–18).

The purpose of this study was to evaluate a commercial enzyme immunoassay kit (Enzymost CK-MB, Behring Diagnostics) by comparison with electrophoresis, to determine what possible diagnostic advantage, if any, may be afforded by this method for determination of CK-MB.

Materials and Methods

 Patients and diagnosis. Fifty-six consecutive patients admitted to the hospital as having possible cardiac involvement, and who thus required creatine-kinase isoenzyme monitoring, were the initial subjects in this study. Our review led to unequivocal diagnoses of 44 patients; seven ambiguous cases were referred to a cardiologist, who provided a "most probable" diagnosis. These diagnoses were based on each patient's clinical status and history, electrocardiographic results, and serial CK and lactate dehydrogenase (EC 1.1.1.27) isoenzyme determinations. One patient was deleted from the final calculations because of an unresolvable ambiguity in relation to the final diagnosis, and an additional four patients were not included due to invalid runs of one assay or insufficient sample to fully quantify CK-MB in the Enzymost assay. Thus, the final study included a total of 51 patients.

The enzyme immunoassay data were unknown to the clinician during diagnostic decision making, and clinical information was unavailable to us during either assay. The availability of the electrophoresis results could have biased the diagnosis in favor of this method, but the cardiologist was asked to avoid using CK-MB results as much as feasible in making diagnostic decisions.

To establish a normal range for the Enzymost kit, we randomly selected sera from 56 asymptomatic, ambulatory male and female outpatients being seen for routine physical examinations and determined CK-MB values by the Enzymost method. This group was assumed to be representative of a normal population, and the values obtained for them to be indicative of the normal range for this kit.

Measurement of total CK and CK-MB. Serum samples were collected upon admission and subsequently at 12- to 24-h intervals, depending on each patient's clinical status. All samples were assayed immediately for total CK activity, with use of the Roche "CK-NAC" reagent, in a centrifugal analyzer (Cobas Bio; Roche Biomedical, Nutley, NJ) at a reaction temperature of 37 °C (normal range: men 18–206 U/L; women 2–164 U/L). Electrophoresis and enzyme immunoassay were performed within 24 h after collection, with interim storage at 2 °C.

Electrophoresis: We electrophoretically separated CK isoenzymes on agarose gels (Corning ACl system; Corning Medical, Medfield, MA 02052). The isoenzyme bands were made visible by use of the Corning CPK Isoenzyme Substrate and quantified with a fluorometric scanning densitometer (Model CDS-200; Beckman Instruments, Fullerton, CA 92634).

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**Enzyme immunoassay:** The kit assay was performed according to the manufacturer's direction insert. This assay is a double-antibody, solid-phase enzyme-linked sandwich-type immunoassay. CK-MB and CK-BB in the sample are bound by the anti-CK-B antibodies coated on the inner surface of a test tube. Peroxidase-conjugated antibody to CK-M and CK-MB is added and reacted with retained CK-MB but not with retained CK-BB. Unbound conjugate is removed by washing. A chromogenic substrate is added which, upon reaction, results in the development of a yellow color. CK-MB concentration in the sample is proportional to the color intensity. Results, quantified by comparing sample response with that of CK-MB calibrators of known concentration, are reported in nanograms per milliliter.

**Results**

The Enzygnost assay was plotted point-to-point on log/log graph paper, yielding a calibration curve (Figure 1). The detection limit was taken to be 2.0 ng/mL, the concentration of the lowest calibrator, and the upper limit of the assay was 100 ng/mL.

Initial precision studies yielded the following results: within-assay and between-assay CVs for three control sera distributed across the range of the assay were 6.0–9.8% and 9.3–29.5%, respectively, with the poorest precision being seen at low values of CK-MB. After several kit modifications to improve precision, the following CVs were obtained in a later study involving 10 replicate runs: 4.1% at 10 ng/mL, 3.7% at 16 ng/mL, and 3.0% at 40 ng/mL.

In a small precision study on our electrophoretic technique, we found a between-assay CV of 8.7% at 11.3 U of CK-MB per liter. This refers only to the electrophoretic step. The kinetic total CK has a CV of 11.7% at 128 U/L, so an approximate overall CV of 14.6% could be calculated for the electrophoretic determination of CK-MB at these levels.

A direct method comparison in ng/mL (Enzygnost) and U/L (electrophoresis) for 143 individual samples yielded the following regression equation: Enzygnost = 0.98 · electrophoresis − 0.72. The correlation coefficient (r) was 0.967. For calculation purposes, the designation “trace” by electrophoresis was treated as 0.5% CK-MB, and in the Enzygnost assay, a result of <2.0 ng/mL was treated as zero. Figure 2 is a scattergram of the correlation data.

Table 1 lists the maximum values obtained for all patients by both methods, along with the maximum total CK. Several patients with above-normal total CK were diagnosed as non-AMI; their clinical conditions included burns, ischemia, pulmonary edema, shock, atrial fibrillation, gastroenteritis, and thalamic hemorrhage. The presence of above-normal total CK (up to 3630 U/L at 37 °C) and/or CK-BB (up to 41 U/L at 37 °C) had no apparent effect on results with the Enzygnost assay. The sensitivity, specificity, and efficiency of the two methods in predicting the correct diagnosis for the 51 patients admitted to the study are compared in Table 2. Our usual decision point for electrophoresis (3% CK-MB) is compared with an optimized cutoff of 4.5% in this study; a cutoff of 10 ng/mL in the Enzygnost assay is compared with an optimized cutoff combining a CK-MB >10 ng/mL and >4.6% of the total CK. For all 51 patients, only the peak value of multiple serum collections for each method was used for statistical purposes.

The range of Enzygnost CK-MB values observed in 56 apparently healthy adults was from <2.0 to 7.2 ng/mL, and the cutoff value for “normal” at the 95% confidence limit was 3.0 ng/mL. Among hospitalized non-AMI patients, the range of observed values was much greater: <2.0 to 106 ng/mL. For this group, the 95% confidence limit cutoff for a non-AMI diagnosis was 9.2 ng/mL. All AMI patients had Enzy-
Table 2. Comparative Diagnostic Sensitivity, Specificity, and Efficiency of Enzygnost CK-MB vs. Electrophoresis

<table>
<thead>
<tr>
<th>ASSAY:</th>
<th>ENZYGNOST</th>
<th>ELECTROPHORESIS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;10 ng/mL</td>
<td>&gt;10 ng/mL &amp; ≤4.6%</td>
</tr>
<tr>
<td>True positives (TP)</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>True negatives (TN)</td>
<td>27</td>
<td>29</td>
</tr>
<tr>
<td>False positives (FP)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>False negatives (FN)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
| Sensitivity (TP/TP+FN) | 100% | 100% | 100% | 95.5%
| Specificity (TN/TN+FP) | 93.1% | 100% | 82.8% | 92.1%
| Efficiency (TP+TN/TP+FN+TN+FP) | 96.1% | 100% | 92.2% | 94.1%

gnost CK-MB values exceeding 11 ng/mL, and the observed range of values was from 11 to 246 ng/mL.

Discussion

In considering the results of our study, three points must be emphasized.

First, all of the diagnostic interpretations of CK-MB data involved only the values representing the peak CK-MB in each assay system. It is important to recognize that a single, isolated CK-MB determination will not yield significant diagnostic information.

Secondly, because the definitive decision point in the Enzygnost assay involved reference to the total CK, in order to determine the percentage represented by CK-MB, it must be realized that this critical percentage may vary from laboratory to laboratory. In this study with use of the CK-NAC reagent at 37 °C, the slope of the linear-regression equation was 0.98, which would imply a near-unity relationship between 1 U and 1 µg of CK-MB. However, this relationship will be altered by anything affecting measurement of total CK, such as type of reagent, analysis temperature, or instrument bias. Thus, the decision point of 10 ng/mL alone may continue to be valid in other clinical trials, but the other component of our decision point (4.6% of total CK) can be expected to be valid only if total CK is determined in a similar manner. We recommend that each laboratory wishing to use any assay of this type establish its own diagnostic decision levels.

Finally, as additional studies are undertaken a borderline equivocal zone will probably be noted. Because CK-MB can be above normal in pathological conditions other than AMI (2) and because of methodological imprecisions inherent in any assay system, all CK-MB methods may produce false diagnoses (19). The 100% diagnostic efficiency of the Enzygnost assay in this study is therefore quite impressive. However, others may not be surprised to find a somewhat lower diagnostic efficiency.

The use of a non-isotopic assay, which measures the mass of an enzyme such as creatine kinase-MB rather than the biochemical activity of the molecule, presents a new alternative to the clinical laboratory. In this study, the relative linearity of the calibration curve, the linearity of patient-sample dilutions, and the close correlation of results between the immunoassay and electrophoresis all indicate that measurement of the mass of CK-MB is a valid alternative to the customary activity measurement. The diagnostic utility of the enzyme immunoassay actually surpassed that of electrophoresis, perhaps because of the high specificity of the sandwich-type assay as well as its freedom from the artifacts which frequently appear in electrophoresis. We conclude that the Enzygnost CK-MB assay may be used with confidence in the diagnosis of AMI.

References

6. Pudek MR, Jacobson DE. Falsely negative laboratory diagnosis for myocardial infarction owing to the concurrent presence of macro creatine kinase and macro lactate dehydrogenase. Ibid. 2434–2437 (1982).