Comparison of Enzyme Immunoassay and Immunoprecipitation for Creatine Kinase MB in Diagnosis of Acute Myocardial Infarction

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We compared the clinical performance of measuring creatine kinase (EC 2.7.3.2) isoenzyme MB by use of an enzyme immunoassay (Enzygnost CK-MB™, Behring Diagnostics) with an immunoprecipitation method (Isomune-CK™, Roche Diagnostics) for the diagnosis of acute myocardial infarction. Sera from 80 patients admitted to the coronary care unit because of chest pain were examined: 40 who had this diagnosis of myocardial infarction, and 40 in whom it was ruled out. In addition, sera from 40 apparently healthy individuals were examined. The clinical sensitivity and specificity of these methods were evaluated by use of receiver operating characteristic curves. We conclude that for clinical efficiency, this enzyme immunoassay is slightly superior to the immunoprecipitation assay we used, because of its greater analytical sensitivity and precision for measuring the mass of the isoenzyme.

Additional Keyphrases: heart disease • isoenzymes • cutoff (decision) value • sensitivity • specificity • receiver-operating characteristic curves

Measuring creatine kinase (CK; EC 2.7.3.2) isoenzyme MB is important for the diagnosis of acute myocardial infarction (MI), and numerous different commercial methods for this have been developed.1 Immunological assays for CK-MB are the newest method, the first of these being immunoinhibition, which is based on the selective inhibition of M subunits and the measurement of residual B subunits (1–3). Immunoinhibition methods led to the discovery and characterization of atypical isoenzyme forms such as macro CK-BB. When these isoenzymes are present in the sera, values for MB are falsely high. Immunoprecipitation (IPPT) combines immunoinhibition with use of a second antibody, which effectively blanks out the contribution of these abnormal forms from the calculation (3, 4), thus resulting in a more specific method for CK-MB.

Both of these immunochromatographic methods measure CK-MB indirectly by a difference calculation. Direct measurement of CK-MB requires the use of both M- and B-specific antibodies, such as the immunoradiometric method (5), which measures mass concentration of the enzyme instead of its activity concentration. Most recently, enzyme immunoassays (EIA) have been developed to eliminate the problems associated with radiolabels. A review of each of these methods has been published (6). We evaluated the performance of one of these new diagnostic kits for CK-MB (Enzygnost CK-MB; Behring Diagnostics, La Jolla, CA 92037), and compared results with those obtained with the immunoprecipitation technique (Isomune CK-MB; Roche Diagnostics, Nutley, NJ 07110) in a clinical study of patients admitted to the coronary care unit for chest pain.

Materials and Methods

Subjects: We selected 80 patients admitted to the coronary care unit in whom the diagnosis of MI was suspected because of chest pain and (or) other objective criteria. Of these patients, 40 subsequently were diagnosed as having an MI, on the basis of having fulfilled two of the following three criteria: a clinical history including chest pain, new Q waves or S-T segment changes on electrocardiographic recordings, and abnormal activity for serially obtained enzyme values for CK, lactate dehydrogenase, aspartate aminotransferase, and isoenzymes CK-MB and lactate dehydrogenase 1. Their ages ranged from 31 to 82 years (28 men, 12 women). The remaining 40 patients were diagnosed as not having had an MI (ages 25 to 96 years, 26 men, 14 women). The values (by IPPT) for the enzymic activity of CK-MB were used in the diagnosis, but the results of the EIA for CK-MB were not made known to the attending physicians.

In addition, 40 apparently healthy individuals—outpatients undergoing routine physical examinations—were selected to represent a control population (ages 30–79 years, 19 men, 21 women).

Specimens: Blood samples were drawn from both groups of patients at regular intervals after admission. For each patient with myocardial infarction, we selected at least four serial determinations from each of four time intervals after the onset of chest pain: 6–12 h, 12–24 h, 24–36 h, and 36–48 h. If blood from an MI patient was not available at each of these intervals, e.g., because of admission 12 h after the onset of reported chest pain, their results were not used in this study.

For the non-MI group, we determined CK-MB from two blood samplings: on the day of admission and on the day after. Although other blood samples were drawn to help in ruling out the diagnosis, their enzyme results were not used in the study once it was established that these patients were non-MI. For the control group of normal subjects, only a single determination was made. We stopped collecting samples when we had 40 for each group.

Blood collected by venipuncture into evacuated serum separator tubes (Becton Dickinson, Rutherford, NJ 07070) was centrifuged and the serum was stored at 4 °C. Total CK and CK-MB activity were assayed within 24 h after collection. For enzyme mass concentrations, the serum was stored at −20 °C until analysis within one week of collection.

Reagents and apparatus: To determine total CK activity, we used the Isomune-CK kit (Roche Diagnostics), for which the normal reference interval was 8–132 U/L; for CK-MB activity, we used the Isomune CK-MB kit (Roche Diagnostics) (4). Both assays were performed in a centrifugal analyzer (Centrifichem 400; Baker Instruments, Pleasantville, NY 10870) at 37 °C.
For measuring mass concentrations, we used the Enzygnost CK-MB kit (Behring Diagnostics). The principle of this assay is described elsewhere (7). Absorbances were measured in a spectrophotometer (Gilford Instrument Labs., Oberlin, OH 44074). Total CK was also measured at 30 °C with "CK-NAC" reagents from Behring Diagnostics, in a centrifugal analyzer (Multistat III; Instrumentation Laboratory, Lexington, MA 02173). We followed the recommendations of the manufacturer for each of these kits.

For statistical analysis we used a package developed in-house for use with UNIX® (version 7, Model 3220 Processor; Perkin-Elmer Computer Systems Div., Oceanport, NJ 07757). Programs for receiver-operating characteristic (ROC) curves were written according to accepted methods (8).

Control materials: For the IPPT method, we used an in-house CK-MB control prepared from human heart (9). For the EIA method, we used "L.D. Zone" control material (Beckman Instruments, Fullerton, CA 92634). As control materials for total CK, we used "Monitrol ES" (Levels 1 and 2; American Dade, Miami, FL 33152) and "Maxitrol" (Behring Diagnostics).

Results

Distribution of Results

Figure 1 shows the values obtained by EIA (in µg/L) and by IPPT (in U/L), for the three groups of patients studied. For the non-MI and MI groups, only peak values are shown. The reference interval for CK-MB was determined to be 0–5 µg/L (EIA) and 0–8 U/L (IPPT), in terms of 95 percentiles of results for the control population. The ranges of CK-MB values for the non-MI group were 0–136 µg/L and 0–140 U/L for EIA and IPPT, respectively, and 25–1028 µg/L and 20–540 U/L, respectively, for the MI group.

Discordant cases: We examined the case records for the patients whose peak enzyme values by either method did not match their clinical diagnosis. In the five cases of false-positive test results, all were from patients with ischemic heart disease. Indeed, many of them may have suffered some degree of myocardial necrosis and have had sera that contained abnormal concentrations of CK-MB. Neverthe-

less, an MI was ruled out in these patients by the lack of acute electrocardiographic and enzyme changes.

The six cases of false-negative results were all from women at least 60 years old, all of whom had lower than average values for peak CK and CK-MB: total CK averaged 300 U/L for the false-negative group vs 2400 U/L for the true-positive group. When we compared their values for percent CK-MB/total CK, however, three of four patients by EIA and five of six patients by IPPT were MI-positive by the criteria of 7% and 5% of total CK, respectively. We conclude that these women have small muscle masses and that the detection of MI in this population requires lower decision limits for CK-MB.

Analytical Variables

Precision: We determined the within-run and day-to-day precision for CK-MB as measured with EIA and IPPT. The CVs for EIA were 6 and 16% at 6 µg/L for within-run (n = 7) and day-to-day (n = 18) precision, respectively, and 5 and 6% at 125 µg/L, respectively. The CVs for IPPT for a separate high control were 3 and 9% at 140 U/L for within-run (n = 7) and day-to-day (n = 18) precision, respectively. We were unable to determine the precision of the low control used in the EIA assay because values were below the detection limit of the IPPT assay (expected value <3 U/L).

The analytical sensitivity of the EIA method is set at the concentration of the lowest calibrator, typically 2 µg/L. The sensitivity of the IPPT with the CentrifChem is 4 U/L. Although it is not possible to convert concentration values to enzyme activity directly, ruling out statements of statistical significance of any comparison of results, the EIA method demonstrates a distinct advantage over IPPT.

Analytical correlation: As shown in Figure 2, we plotted the analytical results for 160 patients' specimens (four each from 40 different patients with acute MI) by EIA (in µg/L) vs those by IPPT (in U/L). We used results only from MI-positive patients so that the low CK-MB concentrations are not over-represented in the correlation. The regression equation was: EIA = 1.90 IPPT – 1.8 µg/L; r = 0.994. This correlation was obtained by using a robust linear regression analysis, which minimizes the influence of outliers (10).
Curves for CK-MB vs Time after MI

To obtain more-extensive curves for heart isoenzyme release vs time for CK-MB, we extended the comparison of EIA vs IPPT from 48 to >60 h in seven of the 40 MI-positive patients. Samples were drawn about every 10 h. As shown in Figure 3 for two typical patients, results by the EIA and IPPT methods are nearly identical in terms of the rate of CK-MB release, the time at which the peak value for CK-MB is obtained, and the rate of elimination of the isoenzyme. These results imply equivalence between the release of enzymically active and immunologically reactive forms of CK-MB: i.e., that measurement of mass as is valid as measurement of activity.

ROC Curves

ROC curves for CK-MB concentrations of mass and activity: Figure 4 compares the ROC curves obtained for EIA vs IPPT at different time intervals. To calculate clinical sensitivity, we determined the number of true positives and false negatives in the 40 MI-positive patients for each of the two above-specified time intervals at various decision limits. We varied these limits from 0 to 250 μg/L (or U/L), in increments of 5. For clinical specificity, we determined the number of true negatives and false positives in the 40 MI-negative patients at these same decision limits and we combined the results for two collections from the same patient (n = 80). Data from normal individuals were used to establish the reference intervals but were not used in the ROC curves. In comparing methods, we observed slight improvements in diagnostic efficiency in the 6- to 12-h interval for EIA (Figure 4A), with little or no differences at the other intervals.

ROC curves by %CK-MB/total CK values: Figure 5 is representative of the ROC curves obtained when both the quantitative value of CK-MB (in mass and activity units) and its percent of the total CK are plotted together at a given time interval. For the latter case, we varied the decision limits from 0 to 20% in increments of 1%. In all cases, the μg/L and U/L criteria were superior in diagnostic efficiency to the percentage criterion, for both EIA and IPPT.

Comparison of ROC curves within a single method: Figure 6 shows all four ROC curves plotted together for EIA. The results for IPPT are similar. As expected, the 12- to 24-h curve exhibited the highest clinical efficiency because CK-MB attained its peak value most commonly (70%) during this interval. The efficiencies gradually decrease for the intervals of 24–36, 6–12, and 36–48 h.

Optimum Clinical Sensitivity and Specificity

Using the results obtained from the ROC curves, we selected a single CK-MB decision limit and computed the clinical sensitivity and specificity for EIA and IPPT. Table 1 shows the results for each time interval. In addition, we determined the specificity of the two methods when both the quantitative value for CK-MB and its percentage limits of total CK for acute MI are used in making the diagnosis. The specificity was 94% for both EIA and IPPT, when the decision limits for CK-MB were set to >55 μg/L and 7% for EIA, and >35 U/L and 5% for IPPT. The clinical sensitivity remained unchanged, at 90% and 85%, thus improving the clinical efficiency to 92 and 91% for EIA and IPPT, respectively, at 12–24 h after the suspected infarct.

Discussion

Measurement of CK-MB is accepted as the most specific and sensitive method for diagnosis of acute MI of any laboratory test. Our data demonstrate that, for most patients, EIA results will compare well with those obtained by an accepted IPPT method.

New treatments methods, e.g., use of intracoronary artery streptokinase, are placing increased demands on laboratory tests to provide MI diagnosis before peak concentrations of CK-MB in blood are reached. For this, measurement of subbands of CK-MM (11) may rival assays of serum myoglobin (12) as markers for early MI detection. More sensitive and precise analytical methods for CK-MB can also provide earlier detection of MI than can older methods that lack sensitivity (e.g., electrophoresis) by being able to distinguish values that exceed sharper and more precise discrimination limits (5). In addition, our limited data suggest that such methods would decrease the proportion of false-negative diagnoses, particularly in elderly patients, who can suffer small infarcts without increases in total CK (13, 14).

In our study, the EIA method has greater analytical sensitivity than the IPPT assay for CK-MB. One of the weaknesses of the IPPT method is that negative values are often obtained at low CK-MB activity because the results are calculated by difference in the activity in two tubes (3). Conversely, the increased analytical sensitivity of the EIA method may explain the slightly higher clinical sensitivity observed in the early 6- to 12-h sampling of suspected MI patients (Figure 4A), particularly in elderly women. Little difference in clinical efficiency is shown at the other time intervals (Figure 4, B–D), because the activities are usually much greater than the decision limit and are not limited by analytical sensitivity. The disadvantages of EIA methods are that they are more costly and time consuming to perform than immuno inhibition and IPPT methods.

The clinical sensitivity and specificity of the Enzymost EIA method is similar to that in other reports for enzyme mass measurements (5, 7). In general, however, comparisons of the clinical sensitivity and specificity of various
Fig. 4. ROC curves for CK-MB with EIA (□) and IPPT (○) vs time intervals after onset of pain: (A) 6–12 h, (B) 12–24 h, (C) 24–36 h, (D) 36–48 h

Fig. 5. Representative ROC curves for EIA with absolute CK-MB (□) vs % CK-MB/total CK (○) as criteria for MI (12–24 h time interval selected)

Fig. 6. ROC curves for CK-MB by EIA at various time intervals after onset of chest pain: 6–12 h (□), 12–24 h (○), 24–36 h (△), 36–48 h (＋)
methods in published reports are difficult because of sampling variations. In addition, in most reports the decision limits are based on peak enzyme values, which is unrealistic in a prospective diagnostic situation. We and others (15) have chosen to evaluate decision limits at different time intervals. The best decision limits and the manner of reporting results must be independently determined by each user because of differences in population and needs of each laboratory. Nevertheless, as shown in Table 1 and Figure 6, the proper timing of collecting specimens in relation to the onset of chest pain remains the most critical variable affecting the success of CK-MB measurements.

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References