Analytical Evaluation of Methods for Serum Creatine Kinase-MB

Electrophoresis, Immunoinhibition and Solid Phase Separation

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ABSTRACT

The purpose of this study was to evaluate immunoassay methods for the measurement of serum cardiac creatine kinase isoenzyme (CK-MB) with respect to sensitivity and specificity. The CK-MB electrophoretic assay (Helena Laboratories) was used as the reference. Two principles of immunoassay were included in the evaluation,—immunoinhibition and solid phase separation. The direct immunoinhibition techniques were from Beckman Instruments (CKMB reagent) and DuPont Medical Products (CKMB). Three solid phase separation techniques were from Abbott Laboratories (IMx CKMB), DuPont (acaPlus MCKMB), and Tosoh Medics Inc. (AIA-Pack CKMB).

The electrophoretic method for separation of the CK isoenzymes has good specificity but lacks sensitivity for CK-MB in low concentrations. The immunoinhibition methods lack specificity and correlate poorly with the electrophoresis method and with the solid phase methods. The solid phase separation techniques are highly sensitive and show an excellent correlation with electrophoresis when based on specificity. The solid phase separation methods correlate well with each other.

Introduction

In the three decades since Dreyfus et al. described the activity of creatine kinase (EC 2.7.3.2;CK) in blood samples of patients with acute myocardial infarction (AMI), numerous methods have been developed to measure this enzyme. The major polymeric forms of creatine kinase (CK) have been well characterized as three cytosolic isoenzymes designated CK-MM, CK-MB, and CK-BB representing the tissues having predominant amounts of each: skeletal muscle, cardiac muscle, and brain, respectively. Methods for the quantitative assays for these isoenzymes have
been reviewed elsewhere. The CK-MB isoenzyme is now recognized as the most sensitive and specific biochemical marker for assessing patients with acute myocardial infarction (AMI) who present to emergency departments with non-diagnostic electrocardiograms. The accurate diagnosis of AMI is critical in all patients regardless of presenting symptoms. This requirement and the recent development of therapeutic intervention for early and rapid myocardial salvage have created the necessity for analytical methods capable of measuring specific serum cardiac markers accurately and rapidly.

Numerous techniques have been used to separate and measure CK-MB. Classic methods include electrophoresis and ion-exchange column chromatography. These are not suitable for rapid testing needed for early diagnosis because they have poor analytical sensitivity, are labor-intensive and time-consuming. A variety of commercial immunochemical methods have gained (and lost) acceptance in the clinical laboratory because of the required criteria for testing, e.g., cardiac specificity, results rapidly available 24-hours per day, and/or analytical specificity and sensitivity. The availability of monoclonal antibodies specific for CK-MB has contributed to the development of assays capable of measuring activity and concentration of this isoenzyme. An important practical advantage of immunochemical assays is that they are adaptable to automated analyzers, and, thus, have the potential of rapid turnaround, analytical reproducibility, and ease of operation.

Immunochemical methods for CK-MB can be classified according to various assay components including the types of antibody(ies) (monoclonal, polyclonal) which determine the specificity of the assay; separation methods for removing free from bound reactants; type of measurement (direct, indirect) and labels such as enzymes, fluorescent molecules, and chemiluminescent compounds used for detection and quantification. Depending on the particular configuration of these components, immunoassays vary in analytical sensitivity (detection limit) and specificity (selectivity for antigen), reproducibility, time of analysis, ease of use, and cost.

The purpose of this study was to evaluate five commercial immunoassays, each based on either of two different principles: (1) direct immunoinhibition with polyclonal antibodies, and (2) two-site immunometric assays with separation on solid phase using either monoclonal-polyclonal or monoclonal-monoclonal combinations of antibodies. The detection systems in these assays were related to catalytic activity of the CK-MB or to the enzyme concentration of CK-MB. The analytical performance of the methods evaluated was based on clinical samples analyzed prospectively in comparison studies. Comparisons were accomplished in two ways: (1) using CK-MB electrophoresis as the reference or comparative assay for the evaluated methods, and (2) using the CK-MB results obtained by each immunoassay for between-assay correlations.

Methods

Testing Phases

This study was performed in two phases as determined by the analytical equipment and techniques for various total CK and CK-MB methods becoming available to our laboratory. Each phase of the study utilized selected samples defined in the following section, Sample Processing and Selection. The samples and sample sizes were different for each phase owing to the availability of blood specimen volume and the shifting patterns of tests requested by physicians at our institution.
The **First Phase** included the following immunoassays (and instrumentation): the ion exchange immunoinhibition method of the DuPont Company* (aca discrete analyzer) and two solid phase separation methods of Abbott Laboratories† (IMx) and Tosoh Medics‡ (AIA-1200) for measuring CK-MB concentration.

The **Second Phase** evolved with the availability to us of the immunoassay systems of two manufacturers: (1) the solid phase separation method of DuPont* for the acaPlus immunoassay processor and the required acaPlus test packs for CK-MB concentration; and (2) the direct immunoinhibition method of Beckman§ for the Synchron CX-7 analyzer. Patient samples included in this phase were also assayed for CK-MB concentration by the Abbott IMx. This facilitated the comparison of the unique solid phase separation methods for CK-MB concentration.

The electrophoretic analysis for CK isoenzymes served as the reference for CK-MB sample selection in both testing phases.

**ASSAYS**

1. **Total CK Activity:** This method is based on the procedure of Oliver* as modified by Rosalki.§ The coupled reaction, which includes N-acetyl cysteine as a CK activator, proceeds sequentially as follows: phosphocreatine in the presence of adenosine diphosphate (ADP) and CK is converted to creatine with formation of adenosine triphosphate (ATP); the ATP is used as a phosphate donor to glucose in the reaction catalyzed by hexokinase with the resulting formation of glucose-6-phosphate (G-6-P) and ADP; finally, in the reaction catalyzed by glucose-6-phosphate dehydrogenase (G-6-PD) in the presence of nicotinamide-adenine dinucleotide (NAD), G-6-P is converted to 6-phosphogluconate with the formation of reduced nicotinamide-adenine dinucleotide (NADH) + (H+). The rate of NADH formation, measured at 340 nm, is proportional to CK activity. The result is expressed in U/L. The IL Monarch‖ and Beckman Synchron CX-7§ were used for the assay of serum CK activity.

2. **Electrophoresis:** CK isoenzymes (CK-BB, CK-MB and CK-MM) were separated on agarose gel (Titan Gel Iso-Dot CK†) in a 2-amino-2 methyl propanol buffer. The ultraviolet visualization of the separated isoenzymes is facilitated by utilizing a substrate with the same catalytic reactions (Titan Gel CK Isoenzyme Reagent‖, catalog no. 3063) as described previously for the total CK activity method. The developed electrophoretogram is scanned densitometrically to determine the amount of NADH fluorescence which is directly proportional to the relative activity of each of the isoenzymes present. The activity (U/L) of each isoenzyme is calculated based on its percentage of the total sample CK activity.

3. **Immunoinhibition:** The Beckman method§ is based on the method of Wurzburg et al.‖ and Gerhardt et al.‖. The intended purpose of the method (Beckman§ CKMB reagent, catalog no. 445375) is to inhibit the CK-MM by addition of an antibody to the CK-M monomer without affecting the catalytic integrity of the CK-MB and CK-BB isoenzymes. The total activity of CK-B subunit of CK-MB (and, if present, CK-BB) is measured

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† Abbott Laboratories, Diagnostics Div., Dept. 49B, Abbott Park, IL 60064.
‡ Tosoh Medics, Inc. 373 Vintage Park Drive, Suite D, Foster City, CA 94404.
§ Beckman Instruments, Inc., 250 S. Kraemer Blvd., Brea, CA 92621.
‖ Instrumentation Laboratories, 113 Hartwell Ave., Lexington, MA 02173-3190.
§ Beckman Instruments, Inc., 250 S. Kraemer Ave., Brea, CA 92621.
‖ Helena Laboratories, P.O. Box 752, Beaumont, TX 77704.
spectrophotometrically by the method of Oliver. The Beckman CK-MB result is expressed in units of U/L. The sensitivity of the assay is 3 U/L.

The DuPont aca CK-MB method (catalog no. 70571901) utilizes a combination of ion-exchange chromatography (IEC) and immunoinhibition. The aca pack with the designated header 'CKMB' is equipped with an IEC column through which the patient sample is passed in order to separate the CK isoenzymes. The CK-MB is eluted into the pack, then antibody to the CK-M monomer is added to inhibit any CK-MM which may be present. The activity of the CK-B subunit of CK-MB (and, if present, CK-BB) is measured spectrophotometrically as previously described. The DuPont aca immunoinhibition result for CK-MB is expressed in units of U/L. The sensitivity of the assay is 3 U/L.

4. Solid Phase Separation: The unique means by which the CK-MB is removed from the biological matrix (serum or plasma) involves a two-site or 'sandwich' enzyme immunoassay. Isolation utilizes an antibody bound to a solid phase to capture the CK-MB. Addition of a second anti-MM antibody conjugated to an enzyme then completes the immune complex “sandwich.” The amount of sample CK-MB is directly proportional to the enzyme activity of the complex and is related to CK-MB standards. The CK-MB concentration is expressed in units of ng/mL or µg/L.

Abbott CK-MB (IMx): This assay is referred to as the microparticle enzyme immunoassay (MEIA). The sample is mixed and incubated with monoclonal anti-CK-MB coated microparticles. A portion of the mixture, containing antigen-antibody-CK-MB complex, is transferred to a glass fiber matrix to which micro particles bind irreversibly. The matrix is washed to remove the unreacted, unbound elements of the sample mixture. A polyclonal anti-CK-MB antibody:alkaline phosphatase (ALP) complex is added to the matrix and reacts with microparticle antibody-bound CK-MB to form the “sandwich.” The substrate, 4-methylumbelliferyl phosphate, is added, incubated, and the enzymatic product, 4-methylumbelliferone, is measured by reflectance fluorometry. The ALP activity is equivalent to the concentration of CK-MB in the original sample. The CK-MB concentration is reported in units of ng/mL or µg/L. The sensitivity of the assay is 0.7 ng/mL or µg/L.

DuPont MCKMB (acaPlus and aca): The acaPlus is an automated device for processing patient samples for immunoassays. The solid phase separation step is accomplished with the use of monoclonal antibody coated chromium dioxide on magnetic particles as the solid support. For the MCKMB (mass CK-MB) assay, the support is coated with antibody specific for the beta subunit of the CK-MB. The serum and a second monoclonal antibody-beta-galactosidase conjugate are added. The second antibody is specific for the entire CK-MB molecule. After a 15 minute incubation and three sequential washes to remove unbound conjugate, a portion of the reaction mixture is transferred to an aca MCKMB analytical test pack which is then transferred to the aca discrete analyzer for measurement of CK-MB. The substrate for the bound beta-galactosidase is chlorophenol red-beta-d-galactopyranoside. The sample CK-MB concentration is equivalent to the concentration of the hydrolytic product, chlorophenol red, and is expressed in units or ng/mL or µg/L. The sensitivity of the assay is 0.5 ng/mL or µg/L.

Tosoh AIA-Pack CKMB (Tosoh AIA-1200): The pack contains magnetic microparticles, the solid phase, to which is bound monoclonal antibody to CK-MB and a second CK-MB monoclonal antibody:ALP complex in the test pack. Sample and buffer are added, and the serum CK-MB is immobilized as a two-
site 'sandwich'. Following a wash cycle to remove extraneous sample constituents and unbound anti-CK-MB antibody conjugate, the substrate, 4-methylumbelliferyl phosphate, is added and the mixture is incubated. The product of the ALP catalytic reaction, 4-methylumbelliferone, is measured fluorimetrically. The ALP activity is equivalent to the concentration of CK-MB in the original sample. Results for CK-MB concentration are expressed in units of ng/mL or μg/L. The sensitivity of the assay is 0.5 ng/mL or μg/L.

CALIBRATION

All CK-MB concentration methods described were calibrated using CK-MB standard concentrations provided by the manufacturers for the respective automated analyses. Based on our experience, analysts are advised to determine the dynamic range and linearity of each methodology and to verify the standard curve for each reagent lot.

ASSAY PRECISION

The intra-assay coefficient of variation (CV) and the inter-assay CV for the immunoassays applied in Phase I (solid phase, Tosoh AIA-Pack CK-MB; immunoinhibition, DuPont aca CK-MB) and in Phase II (immunoinhibition, Beckman CKMB; solid phase, DuPont acaPlus MCKMB; solid phase, Abbott IMx CK-MB) were validated in preliminary experiments in our laboratory. The CVs for these assays were found to be in the range of 3% to 7%, thus consistent with the claims for precision stated in literature published elsewhere for each of these assays.\textsuperscript{12,13,14,15} The inter-assay CV for CK-MB by the electrophoresis comparative method is 12.7% determined from daily quality control data for this assay in four years of clinical use in our laboratory.

SAMPLE PROCESSING AND SELECTION

Patient blood specimens collected without anticoagulant were submitted to our clinical laboratory with the physician-ordered request for serum CK-MB isoenzyme assay and accessioned in the laboratory information system according to routine procedures. The specimens were allowed to clot at room temperature, serum was separated by centrifugation (10 minutes at 2000 relative centrifugal field), and then analyzed for total CK activity and electrophoresis. For those specimens not received within routine working hours of the electrophoresis laboratory, the total CK activity was measured immediately, then aliquots were stored at 4°C for CK-MB isoenzyme assay by electrophoresis and at −70°C. These patient samples were screened for entrance in the study based upon the range of electrophoretic results for CK-MB in order to have expected values within the dynamic range of the immunoassays studied (Table 1).

Those methods employing direct measurement of CK-MB activity (electrophoresis and DuPont and Beckman immunoinhibition) were applied within 24 hours of the day of collection to the aliquots of serum samples stored at 4°C. The CK-MB mass concentration assays were performed on aliquots stored at −70°C. From our previous studies for sample stability these storage conditions (4°C and −70°C) were shown to be adequate for the preservation of CK-MB activity or mass concentration, respectively, since the same value was obtained on assay of the fresh sample and on re-assay following storage. No samples were refrozen and re-assayed.
**TABLE I**

Dynamic Ranges for Immunoassays

<table>
<thead>
<tr>
<th>Methods</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Immunoinhibition</strong></td>
<td></td>
</tr>
<tr>
<td>Beckman</td>
<td>3–150 U/L</td>
</tr>
<tr>
<td>DuPont</td>
<td>0–125 U/L</td>
</tr>
<tr>
<td><strong>Solid Phase</strong></td>
<td></td>
</tr>
<tr>
<td>Abbott</td>
<td>0–300 ng/mL</td>
</tr>
<tr>
<td>DuPont</td>
<td>0–400 ng/mL</td>
</tr>
<tr>
<td>Tosoh</td>
<td>0–600 ng/mL</td>
</tr>
</tbody>
</table>


**STATISTICS AND DEFINITIONS**

Data for assay reproducibility and method comparisons were analyzed by linear regression statistical methods to estimate the relationship between the relevant dependent (y) and independent (x) variables: y-intercept; slope or regression coefficient; and correlation coefficient (R). The variance between pairs of data was calculated as the residual mean square ($S^2_{y/x}$) to estimate the random scatter of data points.

The statistical program used for the analyses applied in this study is published in personal computer software StatView.*

The term "reproducibility" was used in this study to describe the extent of agreement between duplicate analyses, i.e., pairs of results, on each patient serum specimen. The term “n” is defined as a sample of the patient population studied based on the description and criteria stated in the previous section, Sample Processing and Selection. In the results described, the n value for each experiment is the number of patient serum specimens included.

Analytical sensitivity refers to the ability of a method to detect small quantities of the analyte of interest. It is defined as the lowest measurable concentration of an analyte that can be reproducibly distinguished from zero. Analytical specificity refers to the ability of a method to determine only the analyte of interest in a complex matrix without analytical interference by closely related or unrelated substances.

**Results and Discussion**

**REPRODUCIBILITY OF PATIENT SAMPLE RESULTS**

In the comparisons of duplicate analyses of patient samples using linear regression analysis, the electrophoretic and immunoinhibition methods demonstrate acceptable reproducibility (table II). The DuPont acaPlus assay shows relatively low random scatter ($S^2_{y/x} = 1.3$) interpreted as excellent reproducibility compared with the Tosoh AIA method.

TABLE II
Reproducibility of Methods for Serum Creatine Kinase MB

<table>
<thead>
<tr>
<th>Method</th>
<th>n</th>
<th>Intercept</th>
<th>Slope</th>
<th>R</th>
<th>$S^2_{y/x}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrophoresis</td>
<td>40</td>
<td>2.443</td>
<td>0.928</td>
<td>0.994</td>
<td>7.2</td>
</tr>
<tr>
<td>Beckman CX-7c</td>
<td>40</td>
<td>-0.296</td>
<td>1.015</td>
<td>1.000</td>
<td>0.4</td>
</tr>
<tr>
<td>DuPont acac</td>
<td>40</td>
<td>0.046</td>
<td>0.960</td>
<td>0.989</td>
<td>2.1</td>
</tr>
<tr>
<td>Abbott IMx</td>
<td>72</td>
<td>1.689</td>
<td>0.967</td>
<td>0.991</td>
<td>8.9</td>
</tr>
<tr>
<td>DuPont acPlusd</td>
<td>72</td>
<td>-0.081</td>
<td>0.999</td>
<td>1.000</td>
<td>1.3</td>
</tr>
<tr>
<td>Tosoh AIA-1200d</td>
<td>42</td>
<td>0.713</td>
<td>0.993</td>
<td>0.995</td>
<td>4.7</td>
</tr>
</tbody>
</table>

* Duplicate assays of each specimen were performed in random order and in different runs. For each method, there were either 40 or 72 pairs of results. The first versus the second member in the pairs of results for all specimens were compared by linear regression analysis.

b The term "n" is the number of patient specimens included in these experiments.

c Immunoinhibition immunoassay.

d Solid phase separation immunoassay.

which demonstrates somewhat greater scatter for duplicate assays ($S^2_{y/x} = 4.7$) interpreted as good reproducibility. The Abbott IMx method is less reproducible ($S^2_{y/x} = 8.9$) than the other mass concentration assays studied here. This corroborates the observations of other investigators.16

The use of CK-MB measurements to assist in the diagnosis of AMI is well established. Early detection of AMI has been greatly improved with the development and availability of immunoassays for CK-MB.17,18 However, any imprecision of these methods could result in inconsistent detection of borderline increases of CK-MB levels. The low random scatter between duplicate analyses ($S^2_{y/x}$) observed for the immunoinhibition assays is primarily due to the automation of these methods and not necessarily to their accuracy.

METHOD COMPARISONS

Comparison data for the CK-MB methods studied in the First Phase are found in table III. Single assays were performed on each specimen by each method. The electrophoretic and Tosoh

TABLE III
Method Comparisons for Creatine Kinase MB in First Phase

<table>
<thead>
<tr>
<th>X</th>
<th>Y</th>
<th>n</th>
<th>Intercept</th>
<th>Slope</th>
<th>R</th>
<th>$S^2_{y/x}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrophoresis</td>
<td>DuPonta</td>
<td>64</td>
<td>6.693</td>
<td>0.374</td>
<td>0.708</td>
<td>19.2</td>
</tr>
<tr>
<td>Electrophoresis</td>
<td>Abbottb</td>
<td>64</td>
<td>5.837</td>
<td>0.844</td>
<td>0.918</td>
<td>18.9</td>
</tr>
<tr>
<td>Electrophoresis</td>
<td>Tosohb</td>
<td>64</td>
<td>2.685</td>
<td>1.060</td>
<td>0.925</td>
<td>22.5</td>
</tr>
<tr>
<td>Abbott</td>
<td>DuPort</td>
<td>64</td>
<td>3.404</td>
<td>0.461</td>
<td>0.802</td>
<td>16.3</td>
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<tr>
<td>Tosoh</td>
<td>DuPort</td>
<td>64</td>
<td>4.859</td>
<td>0.373</td>
<td>0.809</td>
<td>16.0</td>
</tr>
<tr>
<td>Abbott</td>
<td>Tosoh</td>
<td>64</td>
<td>-4.001</td>
<td>1.239</td>
<td>0.994</td>
<td>6.6</td>
</tr>
</tbody>
</table>

a Immunoinhibition immunoassay.
b Solid phase separation immunoassay.
AIA methods correlate well (R = 0.925) but vary in the magnitude of differences between the results (S²y/x), this being attributed to the comparison of relative activity to concentration. Similarly, the correlation between the electrophoretic and the Abbott IMx methods is acceptable (R = 0.918). The DuPont aca immunoinhibition method does not correlate well with electrophoresis (R = 0.708). The results by the DuPont aca immunoinhibition method are consistently lower than those by electrophoresis (slope = 0.374). This variance is probably due to lack of elution of the CK-MB species by ion-exchange chromatography and/or to lack of specificity of the polyclonal CK-M antibody in the reagent pack.

The solid phase separation methods by Abbott (R = 0.802) and Tosoh (R = 0.809) do not correlate well with the DuPont aca immunoinhibition method. The Abbott and Tosoh methods correlate well (R = 0.994) but do differ in the magnitude of analytical results (slope = 1.239).

None of the methods studied in the First Phase are deemed to be directly transferable as indicated by the slopes of the respective comparisons.

The comparative data for the methods studied in the Second Phase are shown in Table IV. Single assays were performed on each specimen by each method. The electrophoretic method exhibits poor correlation with the Beckman immunoinhibition method (R = 0.355) but compares favorably with the solid phase separation assays of Abbott (R = 0.897) and DuPont (R = 0.868). The Beckman immunoinhibition assay correlates poorly with the solid phase separation methods of Abbott and DuPont.

The Abbott and DuPont solid phase separation methods correlate well with each other. The observed variance (S²y/x = 6.2) is due to the reproducibility of the Abbott method as previously stated.

**SPECIFICITY AND SENSITIVITY**

To assess the analytical specificity for CK-MB of the Beckman immunoinhibition assay and the two concentration assays versus electrophoresis (table V), eight samples were selected prospectively based on the electrophoretic activity detected for the three isoenzymes of CK (MM, MB, and BB) and an atypical form, macro CK. The macro CK is considered to be CK-BB complexed with immunoglobulin. Single assays were performed on each sample by each method. The fractional activity (U/L) of each component was derived from the

### Table IV

**Method Comparisons for Creatine Kinase MB in Second Phase**

<table>
<thead>
<tr>
<th>Method</th>
<th>Method</th>
<th>n</th>
<th>Intercept</th>
<th>Slope</th>
<th>R</th>
<th>S²y/x</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrophoresis</td>
<td>Beckman</td>
<td>92</td>
<td>14.809</td>
<td>0.174</td>
<td>0.355</td>
<td>26.0</td>
</tr>
<tr>
<td>Electrophoresis</td>
<td>Abbott</td>
<td>92</td>
<td>4.004</td>
<td>0.517</td>
<td>0.897</td>
<td>14.5</td>
</tr>
<tr>
<td>Electrophoresis</td>
<td>DuPont</td>
<td>92</td>
<td>4.141</td>
<td>0.519</td>
<td>0.868</td>
<td>16.8</td>
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<tr>
<td>Abbott</td>
<td>Beckman</td>
<td>92</td>
<td>14.002</td>
<td>0.316</td>
<td>0.372</td>
<td>25.8</td>
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<tr>
<td>DuPont</td>
<td>Beckman</td>
<td>92</td>
<td>14.167</td>
<td>0.308</td>
<td>0.374</td>
<td>25.7</td>
</tr>
<tr>
<td>Abbott</td>
<td>DuPont</td>
<td>92</td>
<td>-0.283</td>
<td>1.020</td>
<td>0.983</td>
<td>6.2</td>
</tr>
</tbody>
</table>

* Immunoinhibition immunoassay.
* Solid phase separation immunoassays.
relative percentage of total CK activity. Four samples having no detectable MB activity by electrophoresis demonstrated CK-MB concentrations below the reference range (<5 ng/L). The CK-MB values for three of these samples by the immuno­inhibition assay fell within the reference range (<9.5 U/L) but one exceeded (56 U/L) this range. Of these eight samples, six contained increased BB activity (from 4 to 95 U/L) two of which also had elevated macro CK activity (80 and 96 U/L). These six samples demonstrated a wide range of CK-MB activity (7 to 164 U/L) by the immunoinhibition assay. These results by the immunoinhibition method disagreed strikingly with the electrophoretic and the concentration assays for CK-MB. In contrast, the results for the same six samples by the IMx and acaPlus methods fell within the comparable range for CK-MB by the electrophoretic method.

Ten patient samples were selected for comparison of the analytical sensitivity for CK-MB by the Beckman immunoinhibition or the concentration assays versus electrophoresis (table VI). Single assays were performed on each sample by each method. Four of these samples had an undetectable amount of CK-MB activity (0 U/L) by electrophoresis, but measurable and concordant concentrations of CK-MB by the IMx (0.9 to 4.6 ng/mL) and the acaPlus (0.7 to 3.8 ng/mL) assays. The activity measured by immunoinhibition varied widely (2 to 56 U/L) and discordantly compared with the results obtained by electrophoresis or by the concentration assays. Samples with CK-MB in trace amounts (1 to 3 U/L) and higher by electrophoresis demonstrated concordance with the comparative values by the immunoinhibition and the concentration methods.

These observations (tables V and VI) are consistent with previous reports for the analytical specificity and sensitivity of some of these methods and support the following conclusions: (1) electrophoresis demonstrates high analytical specificity but relatively low sensitivity for CK-MB; (2) the analytical sensitivity of the concentration assays exceeds that
of the immunoinhibition methods; and, (3) the immunoinhibition method has poor analytical specificity, as shown by the lack of differentiation between CK-MB, CK-BB, and the atypical form.

Summary

Electrophoresis currently is the reference method for assay of serum CK-MB. While it has excellent specificity, its sensitivity is relatively low at decision levels for the isoenzyme. It is time-consuming thus, should be considered unsuitable as an emergency procedure.

The ion-exchange chromatography technique, while having demonstrated specificity for isolation of the CK isoenzymes, is somewhat cumbersome and time-consuming. This technique would not be suitable for assisting in rapid clinical differential in suspected acute myocardial infarction.

Immunoinhibition methods do not employ separation techniques that may be applied to automated analytical systems which may provide rapid turnaround time. These methods in principle use anti-CK-M antibodies to inhibit the CK-MM isoenzyme and the CK-M subunit of CK-MB present. The activity of the uninhibited CK-B subunit is measured. The lack of specificity of these methods can be due to CK-BB in the sample or to the presence of atypical and uninhibited sample CK. The immunoinhibition methods are rapid in turnaround and should be amenable to timely differential diagnosis. However, these assays, when applied for low or within reference range patient values in particular, would be inaccurate owing to uninhibited non-CK-MB forms which may exist in a sample. This could lead to spurious results, and perhaps, clinical misinformation.

Analytical methods using the principle of solid phase show excellent specificity and sensitivity. The specificity of these methods is directly related to the clonal characteristic of the antibodies used for separation and isolation of the CK-MB from the sample. The DuPont acaPlus and Tosoh AIA-1200 assays use a monoclonal-monoclonal “sandwich,” whereas Abbott utilizes a monoclonal-
polyclonal approach. This may account for the relative imprecision of the Abbott IMx method. These methods are relatively rapid (DuPont, approximately 30 minutes; Abbott and Tosoh, approximately 40 minutes) thus combined with specificity and sensitivity would contribute meaningful objective information to the differential diagnosis of acute myocardial infarction.

Solid phase separation employing monoclonal-monoclonal "sandwich" assay for the analysis of patient serum CK-MB is our recommendation.

Acknowledgements

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