Measuring Creatine Kinase MB Isoenzyme in a Maintenance Hemodialysis Population: Chemiluminometric Immunoassay and Electrophoresis Compared

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In an effort to clarify the issue of potentially false increases in creatine kinase (EC 2.7.3.2) MB isoenzyme (CK-MB) in uremia, we evaluated the CK profile of 84 persons undergoing chronic maintenance hemodialysis. We compared the performance of a new commercial two-site chemiluminometric immunoassay of CK-MB (Magic Lite; Ciba Corning Diagnostics) with that of electrophoresis on agarose gel (CardioTrak-CK; Corning Medical). Results of the new chemiluminometric immunoassay for samples from hemodialysis patients correlated well with those of the electrophoretic method \( (r = 0.86, P < 0.001) \), showing that neither substances in the serum of uremic patients nor CK-MM isoenzyme give false-positive increases in CK-MB isoenzyme. Our evidence suggests that the chemiluminometric method may be more specific than is electrophoresis in establishing absolute CK-MB values in the diagnosis of suspected myocardial injury in this population.

Cardiovascular disease is recognized as the leading cause of death in patients undergoing chronic maintenance hemodialysis (1). Chest-pain syndromes, pulmonary edema, and hypotension are common in this population. Besides physical examination, electrocardiogram, and cardiac ultrasound techniques, diagnostic evaluation of these patients includes quantification of creatine kinase (CK; EC 2.7.3.2) and its isoenzymes. The literature documents that 19% to 47% of uremic patients without evidence of acute myocardial infarction have increased total CK activity in their serum (2–5). Although widely accepted as one of the most sensitive laboratory indicators of myocardial injury (6–8), increases in CK-MB isoenzyme also have been reported for some patients without evidence of acute myocardial injury who were undergoing maintenance dialysis (2, 3, 9, 10). Ill-defined circulating substances in the serum of uremic patients have been thought to be a source of falsely increased CK-MB in some assay systems (3, 10). Thus, in such patients, consistently increased values for apparent CK-MB could complicate the evaluation of chest pain and interfere with the diagnosis of suspected myocardial infarction.

Further to clarify this issue of potentially false increases of CK-MB isoenzyme in uremia, we evaluated the CK profile of a population undergoing chronic maintenance hemodialysis. We compared the performance of a new, relatively fast and simple two-site chemiluminometric immunoassay of CK-MB with that of an assay involving electrophoresis on agarose gel and fluorometric detection. The latter method does not appear to be subject to interference for CK-MB by substances in the serum of uremic patients.

Materials and Methods

Specimen Procurement and Handling

We collected blood from 84 consecutive patients (39 men, 45 women, ages 28–90 years) who were receiving chronic maintenance hemodialysis in our outpatient Artificial Kidney Unit. Clinical diagnoses accounting for chronic renal failure included hypertension, diabetes mellitus, chronic pyelonephritis, collagen vascular disorders, and "chronic glomerulonephritis." None had had known recent episodes of chest pain, pulmonary edema, hypotension, or other events suggesting acute myocardial disease. Blood sampled at the start of a 4-h hemodialysis session was placed on ice and transported to our laboratory, where the serum samples were separated from the clot and stored at \(-20\, ^\circ\text{C}\). Before assay, samples were thawed at room temperature, and all determinations on a given sample were performed on the same day.

Procedures

Total CK activity: An Astra Ideal automated analyzer system (Enzymes Model no. 6860; Beckman Instruments, Inc., Brea, CA) and the Beckman CK Enzyme reagent kit (based on the Rosalki method) were used at \(37\, ^\circ\text{C}\) to determine the total CK activity in each serum specimen. We used Beckman's Triad LINK level 1 and 2 controls. Precision studies showed CVs of 1.5–2.0% and 2.5–3.0% for within-run and between-day assays, respectively. Our laboratory's normal reference intervals for total CK activity in healthy subjects are 2–245 U/L for men and 2–135 U/L for women.

CK-MB by electrophoresis: We assayed each specimen with the CardioTrak-CK electrophoresis system (Corning Medical, Palo Alto, CA; cat. no. 470069) according to the manufacturer's standard procedure. A CK isoenzyme control (Corning Three in One Level II control; cat. no. 470078) was included in each run. After electrophoresis the agarose gel was incubated with the Corning kit substrate and the film was dried and examined under long-wavelength ultraviolet light. Samples having a visibly discernible CK-MB band were scanned fluorometrically with a Cliniscan densitometer (Model 1231; Helena Laboratories, Beaumont, TX), which measured the percentage CK-MB isoenzyme. CVs were 3.5% and 6.8% for within-run and between-day assays, respectively. Our laboratory's normal reference interval for CK-MB in serum of subjects without acute myocardial infarction is 0–3% of the total CK activity, or 0–6 U/L.

CK-MB by two-site chemiluminometric immunoassay (CLIA): We measured the CK-MB concentration in each sample, using the Magic Lite immunoassay system (Ciba Corning Diagnostics Corp., Medfield, MA) according to the manufacturer's instructions. In this procedure, the sample is incubated simultaneously with acridinium ester-labeled mouse antibody to human CK-MB and mouse antibody to human CK-BB bound to paramagnetic particles. The "sandwich" so formed is separated from solution magneti-

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cally and washed. The resulting product is then placed into a Magic Lite Analyzer (cat. no. 472733) where analyzer reagents are injected to activate the acridinium molecules, resulting in photon output. The amount of CK-MB in the sample and the magnitude of the chemiluminescence of the final reaction are directly related, so the Magic Lite Analyzer calculates the CK-MB concentration from the measured photon output. The assay is calibrated to mass units (i.e., µg/L). Corning Magic Lite level 1 and 2 controls (cat. no. 472747) were used. Precision studies showed CVs of 1.9–2.5% and 3.9–4.5% for within-run and between-day assays, respectively. The normal reference interval for CK-MB reported by the manufacturer is 0–7.5 µg/L. In our laboratory, comparison studies with 250 specimens from 150 healthy subjects and patients in the Coronary Care, Cardiopulmonary, and Intensive Care Units gave results that concur with the manufacturer’s reference interval.

The Relative Index of CK-MB, an index directly analogous to the percentage of CK-MB obtained by electrophoresis, is defined as follows:

\[ \text{Relative Index} = \frac{\text{CK-MB (µg/L)}}{\text{total CK (U/L)}} \times 100 \]

The Relative Index is calculated only if total CK activity is increased. In the above-noted comparison studies, we determined that a Relative Index >3 indicated the myocardium as the source of increased total CK activity.

Creatinine: We analyzed for creatinine in a subset of 47 serum specimens, using an alkaline picrate method and a Beckman Synchron CX3 clinical system (Model no. 4429) with Beckman Triad L1NK level 1 and 2 controls. CVs were 0–0.5% and 2.5% for within-run and between-day assays, respectively. Our laboratory’s normal reference interval for creatinine in healthy subjects is 0–13 mg/L.

Results

Total CK activity: The range for total CK activity in the 84 serum specimens was 20–789 U/L, with a mean of 175 U/L (Table 1). Of these values, 21% exceeded the reference interval.

The values for total CK activity and creatinine were compared by the Pearson product moment correlation test, but showed no relationship \( (r = 0.24, P <0.11, n = 47) \). Figure 1 shows a graphic representation and standard linear-regression analysis.

CK-MB by EP and CLIA: No atypical migrating CK bands or CK-BB isoenzyme were identified by electrophoresis in our study samples. There was a strong correlation (Figure 2) between the CK-MB values obtained by electrophoresis and CLIA as demonstrated by the Pearson product moment correlation test \( (r = 0.86, P <0.001, n = 84) \).

The values for CK-MB concentration as measured by CLIA were compared with creatinine concentrations, but showed no relationship \( (r = 0.24, P <0.11, n = 47) \). The regression line is represented by the equation: creatinine (mg/L) = 0.34CK-MB (mg/L) + 103.6.

Table 1 demonstrates that 11% (9 of 84) of the absolute CK-MB values obtained by electrophoresis and 6% (5 of 24) by CLIA fell outside our reference intervals. Table 2 lists complete CK profiles for these specimens. Laboratory criteria suggesting acute myocardial injury were fulfilled in only two patients (A23 and A63), who were identified by both methods.

The first patient (A63), an 84-year-old man with chronic atrial fibrillation and a remotely documented myocardial

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**Table 1. Results of Determinations**

<table>
<thead>
<tr>
<th></th>
<th>Ref. Interval</th>
<th>Range</th>
<th>Mean</th>
<th>No. (and %) of dialysis patients with increased values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine, mg/L</td>
<td>0–13</td>
<td>49–189</td>
<td>117</td>
<td>47 of 47 (100)</td>
</tr>
<tr>
<td>Total CK, U/L</td>
<td>Men: 2–245</td>
<td>20–542</td>
<td>120</td>
<td>4 of 39 (10)</td>
</tr>
<tr>
<td></td>
<td>Women: 2–135</td>
<td>21–789</td>
<td>139</td>
<td>14 of 45 (31)</td>
</tr>
<tr>
<td>CK-MB by electrophoresis, U/L</td>
<td>0–6</td>
<td>0–27</td>
<td>2.7</td>
<td>9 of 84 (11)</td>
</tr>
<tr>
<td>CK-MB by CLIA, µg/L</td>
<td>0–7.5</td>
<td>0.8–20.7</td>
<td>3.9</td>
<td>5 of 84 (6)</td>
</tr>
</tbody>
</table>

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Table 2. CK Profiles of Specimens with increased Absolute CK-MB by Electrophoresis or CLIA

<table>
<thead>
<tr>
<th>Patient</th>
<th>Total CK, U/L</th>
<th>%</th>
<th>U/L</th>
<th>MB by Elect.</th>
<th>MB by CLIA</th>
<th>Relative Index*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A6</td>
<td>542</td>
<td>1.4</td>
<td>8</td>
<td>11.5</td>
<td>2.1</td>
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<tr>
<td>A23</td>
<td>243</td>
<td>3.8</td>
<td>9</td>
<td>12.2</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>A28</td>
<td>557</td>
<td>1.8</td>
<td>10</td>
<td>9.1</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>A33</td>
<td>789</td>
<td>1.8</td>
<td>14</td>
<td>9.1</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>A38</td>
<td>343</td>
<td>2.5</td>
<td>9</td>
<td>6.5</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>A49</td>
<td>408</td>
<td>2.3</td>
<td>9</td>
<td>4.4</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>A63</td>
<td>200</td>
<td>12.9</td>
<td>27</td>
<td>20.7</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>A67</td>
<td>510</td>
<td>1.6</td>
<td>8</td>
<td>6.8</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>A79</td>
<td>490</td>
<td>2.4</td>
<td>12</td>
<td>6.6</td>
<td>1.3</td>
<td></td>
</tr>
</tbody>
</table>

* Calculated only if total CK activity is increased. Patient A63 is a man.

infarction, developed chest pain 1 to 2 h after the start of hemodialysis on the date blood was drawn for this study. Serial electrocardiograms and CK profiles demonstrated acute myocardial infarction. The second patient (A23) was a 70-year-old woman with orthopnea and chronic dyspnea on exertion. Numerous chest roentgenograms documented cardiomegaly and mild vascular congestion, but she had had no documented myocardial infarctions. She was without complaints on the date blood was drawn for this study. Subsequently, she developed two episodes of hemodialysis forearm-graft occlusion, necessitating surgical revision. In the postoperative period of the latter surgery, she became asystolic. Permission for necropsy was not granted.

The sensitivity, specificity, and positive and negative predictive values (all in percent) for absolute CK-MB by electrophoresis and CLIA for acute myocardial injury were 100, 91, 22, 100, and 100, 96, 40, and 100, respectively. However, all of these statistical calculations are 100% for both methods when properly interpreted in conjunction with total CK activity.

Discussion

Our data show that 21% of the specimens from patients undergoing chronic maintenance hemodialysis had increased CK activities, a finding consistent with that of others (2–5). Such values are independent of pre-dialysis creatinine concentrations, as noted in Figure 1 (r = 0.24, P < 0.11). The primary source of CK appears to be skeletal muscle, as reflected by the predominance of CK-MM isoenzyme activity in the electrophotogram, which is in keeping with previously proposed concepts of “uremic myopathy” (4, 11, 12).

We found CK-BB isoenzyme in none of our specimens by electrophoresis, contrary to the findings of others (5, 13, 14). Other studies also have shown that CK-BB is not increased in patients with renal failure; rather, some substance in the serum produces fluorescent interference in the determination of CK-BB by some electrophoretic methods involving fluorescent densitometry (2, 3, 15, 16). Interference was not observed by Homburger et al. (17) or Pascual et al. (18), who used immunological methods to measure CK-BB in hemodialysis patients (17, 18).

In the laboratory evaluation of patients suspected of having acute myocardial injury it is important to quantify both the absolute and relative amounts of CK-MB (7). It is well known that some patients’ serum contains increased activities of CK-MB that are not related to myocardial infarction. In such patients there is usually an increased total CK activity, attributable to damage of tissues other than the heart (e.g., skeletal muscle). The ratio of CK-MB to total CK in other tissues is lower than that for the heart (19). Laboratory criteria that suggest acute myocardial injury include an increased absolute amount of CK-MB when total CK activity is within the reference interval or an increased relative amount of CK-MB in conjunction with an increased total CK activity.

In evaluating the new two-site chemiluminescent immunoassay for CK-MB in this study, we found excellent correlation with our electrophoretic method (r = 0.86, P < 0.001). Both techniques showed 82 of 84 patients to have CK-MB values lower than would suggest myocardial injury. Of the remaining two patients, one had a documented acute myocardial infarction and the other had a clinical course suggesting myocardial injury. Furthermore, CK-MB concentration showed no correlation to predialysis creatinine values (r = 0.24, P < 0.11). Thus, our results demonstrate that uremia alone does not result in significant increases of CK-MB values by either method, and imply that measurement of CK-MB remains a good diagnostic test in the workup of chest pain syndromes in this population. Electrophoresis shows less discrimination of CK-MB activities at the lower end of the scale than does CLIA (Figure 2), reflecting the relative insensitivity inherent in the quantitative application of electrophoresis. This is a theoretical advantage of CLIA, an advantage reflected in our limited data by the selection of only five of 84 patients with increased CK-MB concentration by CLIA compared with nine patients by electrophoresis.

Our study validates the use of the new chemiluminescent immunoassay in hemodialysis patients and shows that neither substances in the serum of uremic patients nor CK-MM isoenzyme interfere with the determination of CK-MB isoenzyme. This assay, with use of established reference intervals and serial determinations of total CK activity and CK-MB, can be used with confidence in the laboratory evaluation of acute myocardial injury.

We are especially grateful to the following individuals (Pacific Presbyterian Medical Center) for their valuable assistance: Voula Sideris and Gloria Rashidi (Clinical Laboratory), Dr. Gary Truex and the entire nursing staff of the Artificial Kidney Unit, Dr. David Farrar (Medical Research Institute), and David Leafer and Deborah Cabebe (Department of Pathology).

References

Lactate Dehydrogenase and Its Isoenzymes in Serum from Patients with Multiple Myeloma

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Concentrations of total lactate dehydrogenase (LDH; EC 1.1.1.27) and LDH isoenzyme patterns were studied in serum of 19 patients with multiple myeloma and in 19 healthy controls. Patients were divided into three groups (pretreatment, nonresponders, and responders to treatment), based on their clinical status at the time of blood sampling for LDH. The LDH values were found to be significantly higher (P < 0.05) in the pretreatment group and in the nonresponders than in the responders and the control group, the mean ± SE values being 445 ± 35 and 532 ± 75 units/mL vs 349 ± 75 and 190 ± 7.1 units/mL, respectively. Compared with responders and healthy controls, newly diagnosed patients and nonresponders had slight diminutions in LDH-1 and LDH-2, but increased LDH-3. We conclude that determination of LDH and its isoenzymes in serum can be of value as prognostic factors in patients with multiple myeloma.

Lactate dehydrogenase (LDH; EC 1.1.1.27), a glycolytic enzyme, is present in various tissues and neoplasms of the human body in multiple molecular forms. This heterogeneity allows electrophoretic fractionation of the enzyme into at least five isoenzymes (1, 2). In recent years the relationship between neoplasia and LDH has been studied with increasing intensity (3, 4). Serum LDH is known to be of prognostic significance in hematological malignancies such as leukemia and malignant lymphoma (5–7). Moreover, in some patients with malignancy, changes in specific LDH isoenzyme patterns in serum correlate with tumor growth or regression; such changes have been used as tumor markers for monitoring therapy or detecting recurrent disease (8, 9). Although the prognostic value of serum LDH in multiple myeloma has been reported (10), LDH isoenzyme patterns have been studied in very few patients with this disorder (11). Thus the distribution of LDH isoenzymes and their possible significance in prognosis of multiple myeloma patients have not yet been established. We undertook this study to determine the value of determining LDH activity and the isoenzyme pattern in serum of patients with multiple myeloma.

Patients and Methods

Serum LDH and its isoenzymes were studied in 19 patients with multiple myeloma: nine men, 10 women, ages 52 ± 3 years (mean ± SE; range 43–73). At the time of serum sampling, 10 patients were newly diagnosed and had received no treatment (pretreatment group). Of the nine patients who had been given treatment, five had responded to therapy (responders), and four had not (nonresponders). Patients with concomitant malignancies were excluded from the study. Venous blood samples obtained from 19 healthy normal people (11 men, eight women, ages 49 ± 3 years; range 28–75) served as controls. Blood samples, allowed to clot for 30 min at room temperature, were centrifuged to separate the serum, and total LDH activity and LDH isoenzyme concentrations were determined that same day. Serum samples with any signs of hemolysis were discarded.

Diagnosis and classification of the patients: The diagnosis of multiple myeloma was based on the fulfillment of at least two of the three following criteria: demonstration of focal or generalized increase in abnormal plasma cells in the bone marrow or other tissues; presence of serum or urinary myeloma proteins, often with an associated reduction of immunoglobulin concentration; and typical roentgenographic changes.

Of the 19 patients with multiple myeloma, 18 had IgG and one had IgA myeloma. The patients were staged according to the method of Durie and Salmon (12): three