Increased Creatine Kinase Isoenzyme MB Values in Patients without Myocardial Infarct

Leslie M. Shaw and David A. Newman

Six of 13 randomly selected patients in a medical intensive-care unit with above-normal creatine kinase MB activities had diagnoses other than myocardial infarction. These data, which indicate the need for further study, were obtained during evaluation of a commercially available column procedure (Biodynamics/bmc).

We report our recent experience with a commercial column procedure for determination of the MB isoenzyme fraction of creatine kinase (EC 2.7.3.2), an assay of well-documented value (1–3). Chromatographic separation by stepwise salt elution from columns of DEAE-Sephadex A-50 is now a practical clinical laboratory procedure for measuring CK MB activity (2, 4, 5). The MB isoenzyme is considered to be cardiac-specific because its activity in this tissue far exceeds that in other tissues (6). The diagnostic specificity of this laboratory test in a coronary-care unit was recently documented (1). Here, we report our findings in an initial clinical evaluation of the column procedure marketed by Biodynamics/bmc, Indianapolis, Ind., with which we determined the total CK and CK MB activities in sera from 39 patients admitted to the medical intensive-care unit.

Before the clinical evaluation, we assessed several analytical characteristics of the method. CK activities were determined by the BMC-NAC method (7). We confirmed the manufacturer’s claim that carryover of MM isoenzyme into the MB fraction is insignificant. We were satisfied that the second of the two 5-ml aliquots of MM buffer contained very little CK activity as compared to the first for sera with total CK values up to 2200 U/liter. This is illustrated in greater detail in Figure 1, in which the MM fraction was collected in five 2-ml aliquots and assayed for CK activity. The CK MM activity for this serum was 1586 U/liter. The results indicate the virtual absence of MM activity in fractions 4 and 5. A further indication of minimal carryover of the MM isoenzyme into the MB fraction is illustrated in Figure 2, A and B. Four pools were prepared by mixing a pool of normal serum with serum prepared from cadaver blood in the following proportions: I—normal serum alone; II—two volumes of normal serum plus one volume of cadaver serum; III—one volume of normal serum plus two volumes of cadaver serum; and IV—cadaver serum alone. Aliquots of each pool were stored frozen. We made a total of five determinations of the MM and MB activities in each pool during two weeks. Each data point in Figure 2 is the mean of these five determinations; the brackets indicate one standard deviation. Cadaver blood is a convenient human source of high CK MB activity. In this experiment, the cadaver serum had a mean total CK activity of 35 246 U/liter, CK MM of 29 870 U/liter, and CK MB of 518 U/liter. The total CK activity of the normal pool was 114 U/liter with MM activity of 107 U/liter and no detectable MB activity. Figure 2 shows very good agreement of the experimentally determined MM and MB activities in the four pools with the predicted linear relationship between activity and pool number. If there were carryover of MM, the experimentally determined activities would show curvature. That is, the MM values would tend to fall off from the theoretical line as MM activity increased, while the MB activities would tend to increase above the theoretical line as MM activity increased. Our data show that this does not occur, thus serving further to substantiate that carryover of the MM fraction into the MB fraction is minimal.

Day-to-day precision was evaluated during two weeks by using two human serum pools, stored frozen, which contained above-normal CK MB activity. A total of 15 determinations of MB activity were made for each pool, with the following results:

Pool 1, \( \bar{x} = 43.4 \) U/liter, SD = 3.0, CV = 6.9%

Pool 2, \( \bar{x} = 74.5 \) U/liter, SD = 1.96, CV = 2.6%

These data indicate reasonable precision for this method. MB activities were determined in the sera of 43 patients with normal total CK activity and who did not have a diagnosis of myocardial infarction. The range of values calculated from the mean ± two standard deviations was \(-2.07-9.4 \) U/liter; the range obtained after eliminating all values below and above the 2.5 and 97.5 percentiles was 0.0–10.4 U/liter.

We compared the column procedure with cellulose acetate
Table 1. Patients with Increases in Either Total CK, or CK MB, or Both

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>CK activity, U/liter</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Subendocardial M.I. *—48 h after onset of chest pain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.I.—72 h after onset of chest pain</td>
<td>101</td>
<td>10.4</td>
<td>10.3</td>
</tr>
<tr>
<td>CABG and peri-operative M.I.</td>
<td>2207</td>
<td>43.4</td>
<td>2.0</td>
</tr>
<tr>
<td>Seizures, M.I.—24 h after onset of chest pain</td>
<td>2593</td>
<td>61.0</td>
<td>2.4</td>
</tr>
<tr>
<td>Probable M.I.—24 h after onset of chest pain</td>
<td>93</td>
<td>14.8</td>
<td>15.9</td>
</tr>
<tr>
<td>Inferior wall M.I.—24 h after onset of chest pain</td>
<td>495</td>
<td>14.8</td>
<td>3.0</td>
</tr>
<tr>
<td>Inferior wall M.I.—24 h after onset of chest pain</td>
<td>1693</td>
<td>194.5</td>
<td>11.5</td>
</tr>
<tr>
<td>48 h later</td>
<td>1023</td>
<td>57.3</td>
<td>5.6</td>
</tr>
<tr>
<td>72 h later</td>
<td>409</td>
<td>7.8</td>
<td>1.9</td>
</tr>
<tr>
<td>24 h after triple CABG</td>
<td>1615</td>
<td>83.3</td>
<td>5.2</td>
</tr>
<tr>
<td>96 h after triple CABG</td>
<td>284</td>
<td>10.4</td>
<td>3.7</td>
</tr>
<tr>
<td>168 h after triple CABG</td>
<td>89</td>
<td>5.2</td>
<td>5.8</td>
</tr>
<tr>
<td>Cardiac arrest</td>
<td>504</td>
<td>13.0</td>
<td>2.6</td>
</tr>
<tr>
<td>24 h later</td>
<td>1398</td>
<td>15.6</td>
<td>1.1</td>
</tr>
<tr>
<td>Cardiopulmonary arrest</td>
<td>394</td>
<td>11.3</td>
<td>2.9</td>
</tr>
<tr>
<td>Pacemaker implant</td>
<td>167</td>
<td>13.9</td>
<td>8.3</td>
</tr>
<tr>
<td>Adenocarcinoma of the lung; dermatomyositis</td>
<td>5773</td>
<td>46.9</td>
<td>0.8</td>
</tr>
<tr>
<td>Parkinson’s disease, coma, T-wave abnormalities</td>
<td>7814</td>
<td>26.0</td>
<td>0.33</td>
</tr>
<tr>
<td>Chronic atrial fibrillation</td>
<td>294</td>
<td>3.5</td>
<td>1.2</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>428</td>
<td>8.7</td>
<td>2.0</td>
</tr>
<tr>
<td>Hypertensive crisis; pulmonary edema</td>
<td>262</td>
<td>6.9</td>
<td>2.6</td>
</tr>
</tbody>
</table>

* Abbreviations: M.I., myocardial infarct; CABG, coronary artery bypass graft.

Electrophoresis (8) for a series of 15 medical intensive care unit patients. In nine of the sera, an MB band was detected by electrophoresis. All nine had increased MB activities by the column method (ranging from 18.0 to 84.0 U/liter). Four sera that were negative by cellulose acetate electrophoresis had MB activities ranging from 10.4 to 15.6 U/liter. The limited sensitivity of the electrophoretic technique is the probable reason for these differences, because Yasmineh and Hanson (9) have shown that 11.5 U/liter is the limit of sensitivity of the cellulose acetate electrophoresis method. With the CK assay system used in our study, the limit of sensitivity lies between 15.6 and 18.0 U/liter. The reason for this limit being higher than they determined is that the CK assay method in our study is significantly more optimal (7) than theirs. The remaining two sera that were negative for MB by electrophoresis had MB-by-column values of 9.6 and 6.9 U/liter. In two of the 15 sera,

![Fig. 1. Creatine kinase activity in five 2-ml fractions](image1)

![Fig. 2. Enzyme activity vs. fraction number](image2)

Fig. 1. Creatine kinase activity in five 2-ml fractions

Fig. 2. Enzyme activity vs. fraction number

Four pools were prepared from normal serum and cadaver serum. Five determinations of CK MM and CK MB activities in each pool were made during two weeks. Mean values for MM and MB activity are displayed in A and B, respectively; brackets indicate one SD.
the BB isoenzyme was detected by electrophoresis. Because the column method does not include a buffer for the BB isoenzyme, we did not evaluate BB isoenzyme activity by this procedure.

We also compared the BMC column with the modified Mercer (4) procedure marketed by Worthington Biochemical Corp., Freehold, N. J. 07728. MB values (ranging from 2 to 61 U/liter by the BMC assay) were determined for 47 randomly chosen sera from patients in the medical intensive-care unit. Linear regression analysis of this data defined the equation y = 0.62 x - 0.18 (x: BMC values; y: modified Mercer values). The correlation coefficient of 0.91 indicates a good correlation between results by the two methods.

In the initial clinical evaluation of the BMC column, sera from 39 patients admitted to the medical intensive-care unit were assayed for total CK and CK MB activities. The clinical histories of all patients with above-normal values for either total CK activity or CK MB activity (10.4 U/liter or greater), or both, were reviewed. Table 1 summarizes these data. A total of 16 patients had either increased total CK activity, increased MB activity, or both. Of these, 13 had MB values ≥ 10.4. In the latter group of patients, six were diagnosed as having a myocardial infarct and one was diagnosed as a probable infarct. The remaining six had other diagnoses. One of these was a patient who had coronary artery bypass graft surgery, a procedure known to release the MB isoenzyme into the circulation (3). Two of the patients had suffered cardiac arrests, a condition for which others have reported increased MB values (10). The patient who had a pacemaker implant had a high normal total CK activity of 167 U/liter with an MB value of 13.9 U/liter one day after surgery. In this patient, a right ventricular tracer was inserted via the right cephalic vein. According to the medical history, this individual made good progress after the implant, with no complications. We have not seen any data in the literature documenting CK MB values in patients with pacemaker implants with no clinical evidence of myocardial infarction. Possibly, in this case, the tip of the pacemaker catheter entered myocardium, producing injury to a small portion of tissue and subsequent release of CK. Only further study will establish whether or not elevated CK MB activity is a regular finding in pacemaker-implant patients. The patient whose total CK and CK MB activities were 5773 and 46.9 U/liter, respectively, had dermatomyositis secondary to carcinoma of the lung. These data are consistent with the fact that patients with dermatomyositis frequently have significantly increased CK activity and, in one study, three of eight patients with this disorder had increased MB activities (11), and the authors suggested that the diseased tissue was the source of the MB isoenzyme. In the parkinsonian patient, total CK activity was 7814 U/liter and MB activity was 26 U/liter. This patient had no previous history of cardiac problems. He entered the hospital after a fall in which he hit his head. During his hospital stay, he became comatose. Electrocardiograms consistently showed pre- ventricular contractions but no other evidence of ischemia. The markedly increased total CK activity undoubtedly originated from skeletal muscle, possibly due to parkinsonian tremor.

We believe that the CK MB activity in this patient's serum originated from skeletal muscle, because CK MB is present in skeletal muscle homogenates when total CK activity is extremely high (12). In this patient, the MB activity was 0.59% of the total activity, the lowest such percentage observed in this series of patients. The other three patients in this study had slightly increased total CK activities but normal CK MB activities. Their diagnoses included chronic atrial fibrillation, congestive heart failure, and hypertensive crisis with acute pulmonary edema secondary to that. In all three cases, myoccardial infarction was ruled out on the basis of clinical and electrocardiographic results.

In this preliminary survey of randomly selected patients in the medical intensive-care unit, six of 13 patients (46%) with increased MB activities (10.4 U/liter or greater) had diagnoses or conditions other than acute myocardial infarction, although increased MB activity is the most specific enzyme test for acute myocardial infarction in a coronary-care unit (1). The value of this test in establishing this diagnosis is unquestioned. However, it is important to recognize conditions or diseases one would expect to find in a general hospital population or in patients in medical intensive-care units in which serum CK MB activity is increased. Roberts and Sobel (3) emphasize the value of MB determination in differentiating myocardial ischemia and myocardial necrosis, in the diagnosis of peri-operative myocardial infarction in non-cardiac surgery, and the availability of a rapid CK MB procedure which allows for the prompt exclusion of myocardial infarction (3). They point out, however, that MB determination is of little value in cardiac surgery such as coronary artery bypass grafting or valve replacement, because these procedures themselves cause the release of significant MB into the circulation, and the data on our two such patients agree with this. The increased MB values in our patient with dermatomyositis and in the patient with a pacemaker implant emphasize the need for further study of serum MB activity in such patients, to assist both the clinician and the laboratorian in properly selecting patients for MB determination and correctly interpreting data on MB.

We found the BMC column technique to be reasonably precise, with minimal carryover of the MM fraction into the MB fraction. The column is easy to set up and can provide MB results within 20 min of the time that the sample is applied to a column. According to the manufacturer, the salt (MgCl₂) concentration in the MB buffer, 150 mmol/liter, results in efficient removal of the MB isoenzyme from the column, but under these conditions the BB isoenzyme is partly removed. Thus, this method, like immunological methods using anti-BB antisera, will overestimate MB activity in sera containing the BB isoenzyme. The significance of this is not now clear because (a) BB isoenzyme activity, when present, is usually very low, and (b) the available data in the literature show that increases in BB usually occur in association with increases in MB. Clearly, further investigation of the extent and clinical significance of increased BB is needed.

References

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Gas–Liquid Chromatographic Microdetermination of Underivatized Ethosuximide (α-Ethyl-α-Methyl Succinimide) in Plasma or Serum

A. J. Fellenberg and A. C. Pollard

A gas–liquid chromatographic procedure for the microdetermination of ethosuximide is described. Ethosuximide is extracted from acidified plasma or serum into chloroform containing an internal standard α,α-dimethyl-β-methyl succinimide. Part of the initial chloroform extract is gently evaporated, the residue redissolved in n-heptane at 60 °C, and an aliquot analyzed by gas–liquid chromatography. The procedure is rapid, reliable, sensitive, and specific. It requires a 25–50 μl sample for a single estimation, has a detection threshold of less than 10 μmol/liter, and is suitable for routine clinical use.

Although several gas–liquid chromatographic procedures for the determination of underivatized ethosuximide (EMS) are available (1–6), each possesses one or more undesirable features. These include lengthy and tedious isolation techniques (1–3), detector or column unsuitable for estimation of valproic acid as well as EMS (4, 6), emulsion formation during extraction (5) and the unnecessary complication of requiring different isolation procedures for macro and micro samples (2, 6), and relatively large sample volumes (0.5–2.0 ml)—a decided disadvantage for pediatric use. Further, since in all of these procedures the EMS is ultimately concentrated in a relatively polar solvent, the material analyzed by gas-liquid chromatography is likely to be contaminated by other drugs, lipids, and polar substances, which in our experience (7, 9–11) and that of others (1, 8) is detrimental to prolonged column life and the rapid and convenient analysis of multiple samples.

In the procedure described below the above undesirable features are eliminated or minimized.

Materials and Methods

Reagents and Glassware

Citrate–phosphaté buffer, pH 3.0: Titrate 200 ml of saturated NaH2PO4 (Merck No. 6346; Merck Darmstadt, G.F.R.) with saturated citric acid (Merck No. 244), approximately 8 ml, to pH 3.0.

Chloroform, spectroscopic grade (Merck No. 2447).

n-Heptane, spectroscopic grade (Merck No. 4366).

Stock internal standard, α,α-dimethyl-β-methyl succinimide (Aldrich No. 16, 350-13; Aldrich Chemical Co., Milwaukee, Wisc. 53233) 5 mmol/liter (750.9 mg/liter) in chloroform.

Working internal standard, 500 μmol/liter in chloroform: Dilute 1 ml of stock solution to 10 ml with chloroform.

Stock α-ethyl-α-methyl succinimide (EMS) solution, (kindly supplied by Parke Davis and Co., Sydney, N.S.W., Australia), 10 mmol/liter, in pooled drug-free human serum: Dissolve 141.2 mg in 5 ml of water and dilute to 100 ml with serum. Store in 1.0-ml aliquots at −15 °C.

Working EMS solution, 750 μmol/liter in pooled human serum: Dilute 750 μl of stock solution to 10 ml with drug-free pooled human serum. Store in 0.2-ml aliquots at −15 °C.

Quality-control serum (pooled human serum containing added EMS): Rapidly stir 100 ml of serum and slowly add about 7 mg of EMS. Continue stirring the mixture for 1 h and store in 0.2-ml aliquots at −15 °C.

Glass vials, 0.3 ml, complete with screw caps (with Teflon-faced cap discs; Pierce “Reactivial“No. 13220; Pierce Chemical Co., Rockford, Ill. 61105).

Extraction Procedures

To an 0.3-ml “Reactivial” add 50 μl of plasma or serum. For 25-μl samples add a further 25 μl of drug-free pooled human serum. At the same time prepare control and standard vials. To the control add 50 μl of quality-control serum, and to the standard, 50-μl of working EMS solution.

To all vials add 10 μl of the pH 3.0 buffer and immediately vortex-mix for 5 s. To each vial add 50 μl of working internal standard solution, cap securely, and vortex-mix for 30 s; centrifuge at 2000 × g for 10 s. Gently aspirate and discard the aqueous (upper) phase, leaving the protein layer intact. With a small spatula, push the protein layer aside and transfer 25 μl of the chloroform extract to another vial, and evaporate it ambient temperature, aided by a gentle stream of nitrogen. Add 25 μl of n-heptane, cap the vial securely, and incubate at