Creatine Kinase B Subunit as Measured with a Radioimmunoassay Kit in Detection of Acute Myocardial Infarction

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Results with a commercial radioimmunoassay (RIA) reagent kit for quantification of the creatine kinase B subunit (CK-B) (Nuclear-Medical Laboratories, Irving, TX 75061) were compared with results obtained by electrophoresis for patients consecutively admitted to our coronary care unit for suspected acute myocardial infarction. Analytical sensitivity, precision, and specificity of the RIA were satisfactory. Its clinical efficacy was assessed in 97 patients suspected of having had an acute myocardial infarction. Of 30 patients who had had an acute myocardial infarction, increased CK-B was detected by RIA in 30 and by electrophoresis in 27. The temporal relationship between CK-B by RIA and CK-MB by electrophoresis was similar. Of 66 admissions where infarction was not established, CK-B was negligibly increased in samples from four patients by RIA, and from one by electrophoresis. Although not abnormally increased (>5 U/L), CK-MB was detected by electrophoresis in samples from another five of these 66 patients. We conclude that estimation of CK-B by this RIA is an excellent alternative to estimation of CK-MB by electrophoresis in patients suspected of having had an acute myocardial infarction.

The most useful laboratory marker for acute myocardial infarction (AMI) has been the detection and quantification of the MB isoenzyme of creatine kinase (EC 2.7.3.2; CK-MB) in blood (1–5). Physical separation of the CK isoenzymes is followed by reaction with a suitable substrate, and the enzymatic activity is estimated (6). Recently, RIAs involving antisera directed against the B subunit of CK have been described (7,8). We have examined the analytical characteristics and clinical efficacy of a commercially available RIA kit based on such an approach to the measurement of CK-B.

Materials and Methods

Patients and Diagnosis

We studied 97 patients admitted consecutively to the coronary care unit of our hospital for suspected AMI. One patient experienced two separate myocardial infarctions during the same admission. Two patients were admitted twice during the study period. Four patients were excluded because of insufficient clinical information and (or) incomplete diagnostic testing so that data were available for 93 patients and 96 episodes of suspected AMI. Blood was sampled from 73 patients for estimation of CK within 24 h of symptom onset. Another 23 patients experienced initial symptoms longer than 24 h before presenting themselves for admission so that initial testing was delayed at least this much. Blood specimens were routinely obtained shortly after admission and again after 12, 24, and 48 h. Additional specimens were obtained when clinically indicated.

CK-MB was measured by electrophoresis and CK-B was estimated by RIA in all samples. Electrocardiograms were obtained on admission and daily for several days. The clinical and laboratory findings of all patients were reviewed.

CK-B was also measured by RIA in sera obtained from 58 ambulatory outpatients free of active cardiac disease who were referred for thyroid function testing.

A diagnosis of myocardial infarction was made by cardiologists responsible for the patients' hospital care, based on each patient's clinical presentation and course, admission and subsequent electrocardiographic findings, pyrophosphate, thallium or gated blood pool imaging, and serum myocardial enzyme measurements. A serum CK-MB activity of >5 U/L (derived from the total CK activity × the percentage MB from electrophoresis) was generally considered consistent with myocardial damage. Exceptions included those patients with markedly increased total CK activity and a low (<5%) proportion of isoenzyme MB and patients with a normal value for total CK and an increased MB percentage (apparent CK-MB concentration 5–10 U/L). In these patients, the diagnosis was more frequently based on other studies. We made no effort to separate subendocardial from transmural damage, but included both in the AMI group. CK-B measurements by RIA were not reported to the clinicians and therefore did not influence the diagnosis, but we did correlate them with the clinical and laboratory data.

Measurements of CK

Serum, separated from erythrocytes shortly after collection, was stored at 4 °C until electrophoretic separation and quantification of CK isoenzymes, done the same day. If RIA was not done on the same day, serum samples were frozen until assay (within two days of collection).

The stability of immunoreactive CK-B stored at −20 °C or subjected to repeated freeze–thaw cycles was evaluated for 20 samples (10 with increased CK-MB, 10 with normal content). CK-B was measured by RIA on the day of collection and the samples were stored at −20 °C for five months or eight months. After each interval, 10 samples were thawed and assayed by RIA. All 20 samples were again frozen, thawed after one additional week of storage at −20 °C, and assayed a third time.

Radioimmunoassay. We measured CK-B with RIA reagent kits from Nuclear Medical Laboratories, Inc., Irving, TX 75061. The Nuclear Medical Laboratories, Inc. system is a homologous system with iodinated CK-BB for tracer, CK-BB for calibration, and rabbit antiserum raised against CK-BB derived from human brain. After sequential addition of unlabeled ligand, antibody, and then labeled ligand, with two 1-h incubations, the antibody-bound fraction was separated from the free fraction with goat IgG directed against rabbit IgG and polyethylene glycol. We used for assay

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2. Nonstandard abbreviations: AMI, acute myocardial infarction; CK, creatine kinase; B, M, subunits of CK; NSB, nonspecific binding.
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control three serum pools (L1, L2, L3) provided by the manufacturer. Within- (10 replicates) and between-assay (means of replicates, 15 assays) precision (CV) was assessed. Analytical recoveries of CK-BB (6-47.5 ng) added to a human serum pool containing a low concentration of CK-MB were calculated as the percentage of the total CK-BB added that was accounted for (measured value minus endogenous value). To evaluate cross reactivity, we examined the displacement of radiolabeled CK-BB from antibody by CK-BB, CK-MB, and CK-MM. Dilutions of 12 patients' sera with above-normal CK-MB were prepared with isotonic saline and tested in the same assay with undiluted serum samples. Nonspecific binding (NSB; apparent binding in the absence of primary antibody) was measured in about 10% of all patients' sera studied.

The standard dose-response curve ranged from 0 to 45 μg/L and was expressed as logit percent [(bound activity minus NSB)/(bound activity at zero concentration minus NSB)] vs log calibrator concentration (μg/L). A computer program involving a weighted least-squares regression was used to estimate control or patients' serum CK-MB concentrations.

Electrophoresis. CK isoenzymes in serum samples were separated by electrophoresis on agarose (Corning ACI; Corning Medical, Medfield, MA 02052). After incubation with substrate, isoenzymes were identified under ultraviolet light and quantified with a densitometer (CD5-200; Beckman Instruments, Fullerton, CA 92634). We estimated total serum CK activity by measuring NADH production at 340 nm with a batch analyzer (Baker Instrument Corp., Bethlehem, PA, 18001; Impact 400's, Gilford Instrument Labs., Oberlin, OH 44074) and Worthington reagents (Statzyme CPK-n-1; Worthington Diagnostics, Freehold, NJ 07728).

Results

Radioimmunoassay

Standard (calibration) curves for CK-B were similar to those illustrated in the manufacturer's product insert. Zero standard (maximum) binding was 53% to 61% when corrected for NSB. Logit/log transformation adequately linearized the calibration curve for concentrations between 5 μg/L and 35 μg/L. Sensitivity (B/Bo = 90%) was 5 μg/L, and NSB was 4.5 to 6.5% of the total activity, both in standards and in patients' sera. Within-assay and between-assay precision for three control pools were 1.6-9.4% and 7.3-13%, respectively.

Recoveries of 6.0, 11.9, and 23.8 ng of CK-BB calibrator added to a human serum pool with a low initial concentration of CK-B were 100, 92, and 93%, respectively.

Cross reactivity with CK-MB and CK-MM is shown in Figure 1.

Fig. 2. Representative displacement observed with dilutions of patients' sera containing above-normal concentrations of CK-B

Dilutions with isotonic saline of 12 patients' sera gave results that were slightly nonparallel to the standard curve. The B/Bo values for the lowest dilutions were less than predicted by measurements in undiluted sera. However, subsequent dilutions more nearly paralleled the standard curve, as shown in Figure 2, and apparent CK-B concentrations based on diluted specimens were higher than those for the undiluted serum.

Although we observed no significant differences in apparent CK-B concentrations (as measured by RIA) when patients' sera were stored frozen for as long as eight months and subjected to repeated freeze-thaw cycles (data not shown), results did vary slightly more for samples stored frozen for eight months than for those stored frozen for five months.

Patients' Samples

Figure 3 shows the frequency distribution of CK-B concentrations in ambulatory individuals (single samples) and

Fig. 3. Apparent CK-B concentrations observed in serum from noninfarcted ambulatory persons and hospitalized patients

The mean CK-B for samples from 56 ambulatory noncardiac outpatients was 3.5 μg/L (range <2.5 to 5.2 μg/L). CCU, coronary care unit
was above normal (11 μg/L), but CK-BB and CK-MB were not detected by electrophoresis. By RIA, 62 non-AMI patients exhibited peak CK-B RIA concentrations of <7.0 μg/L. The three remaining patients had peak CK-B RIA concentrations of 7.0, 7.0, and 7.5 μg/L. The mean peak CK-B concentration in these 65 patients was 5.0 μg/L (range, 2.7 to 7.5 μg/L).

CK-MB was detected by electrophoresis for six of the 66 non-AMI patients in at least one of their samples. In one, CK-MB was >5 U/L; in two, it was <5 U/L; and in the remaining three, it was only "detectable."

**Patients with AMI.** Of the 30 patients diagnosed as having had an AMI, one was a three-month-old infant with endocardial fibroelastosis with necrosis. CK-B in a serum sample from this patient was 26 μg/L by RIA, and CK-MB was 74 U/L by electrophoresis. Twenty-six of the remaining patients were tested for CK-B within 24 h after onset of symptoms. Only one patient was tested more than 48 h after onset of symptoms, and this one had had symptoms for two weeks before admission; the diagnosis for this patient was recent subendocardial infarction on the basis of a positive pyrophosphate scan, for which results were normal on re-study five days later. CK-MB was not detected by electrophoresis, and the peak CK-B was 7.0 μg/L by RIA in this patient. In the other 28 patients diagnosed as having had an AMI, CK-B concentrations by RIA were >7.0 μg/L within 48 h after onset of symptoms (range, 7.0 to 59.0 μg/L). By electrophoresis, 27 of the 30 patients had maximum CK-MB concentrations exceeding 5 U/L.

CK-B measurements by RIA and CK-MB measurements by electrophoresis were similar in patients with carcinoma, renal insufficiency, and in all but the one patient described above with cerebrovascular insufficiency. Fourteen of the 93 patients had a history of malignant disease—four actively present, one with metastatic prostatic carcinoma. Seven patients had significant renal failure (creatinine >30 mg/L); three were being chronically dialyzed. Nine patients had significant cerebrovascular disease, either old cerebrovascular accidents (six) or concurrent cerebrovascular insufficiency (three).

**Discussion**

Methods for CK-MB quantification based on estimation of enzymatic activity have been most widely applied in the detection of AMI (5, 6). These methods require measurement soon after specimen procurement, physical separation of the CK isoenzymes, and a separate measurement of total creatine kinase activity. Interest in methods for measurement of CK-MB directly without isoenzyme separation led to the development of radioimmunoassays (7, 8). Many RIAs utilize antisera raised in animals against CK-BB derived from human brain tissue and do not necessarily require that the detected isoenzyme or subunit be enzymatically active. Such assays probably detect CK-MB by recognition of the CK-B subunit in the intact CK-MB molecule. It has been suggested that some RIAs may recognize enzymatically inactive B subunit of CK or even CK-BB present in the blood after AMI (9–11). Despite uncertainty regarding the identity of the analyte detected, these RIAs appear to discriminate AMI from non-AMI patients (8).

Recently, RIA assay systems for the detection of CK-B or CK-MB have become available commercially (12). This report describes our experiences with one commercially available RIA kit developed to detect CK-B after AMI. This RIA utilizes rabbit antibody to the B subunit of CK, and CK-BB is used for both radioiodination and calibration. Labeled CK-BB is displaced from antibody in normal serum, resulting in apparent CK-B concentrations in ambulatory persons.
and in hospitalized patients with diseases other than AMI, as shown in Figure 3. Thus a decision threshold must be chosen to differentiate infarcted from noninfarcted patients. We were able to choose a decision threshold that resulted in increased results in all patients we studied with AMI and yet minimized falsely positive results (Figure 4). In a different study, we found above-normal results in a greater proportion of non-AMI patients when we used two other RIA assay systems (12).

The mean result for ambulatory subjects (3.5 μg/L) was lower than that seen in the noninfarcted coronary-care unit patients (5.0 μg/L) (Figure 3). This discrepancy may be attributed to several factors: (a) Ambulatory subjects had a single sample collection and determination; non-AMI patients are represented by values from several determinations. (b) The slightly higher mean CK-B concentration in the non-AMI coronary care unit patients may reflect myocardial necrosis in at least some of these patients, which is not detected by electrophoresis and currently is not clinically recognized.

CK-B as measured by the RIA was unaltered by storage for five to eight months at −20 °C, nor did repeated freezing and thawing change the measured concentration. This assay has adequate sensitivity and satisfactory precision for clinical application. Analytical recovery of CK-BB calibrator added to serum was satisfactory. Based on the known cross-reactivity with CK-MB (Figure 1), CK-MB added to patients’s sera would not be expected to yield quantitative recoveries. Failure to demonstrate parallelism on dilution (Figure 2) is not surprising, because the antisem used in this assay is directed against CK-BB and does not have the same affinity for CK-BB and CK-MB (Figure 1) (7, 8). Because of this nonparallelism, estimates of CK-B concentration based on measurements made on diluted sera are apparently higher than those based on undiluted sera. We think it unlikely that any diagnostic or prognostic information is lost because of this nonparallelism of dilutions.

With few exceptions, the present RIA produced results for both infarcted and noninfarcted coronary care unit patients that were similar to those by electrophoresis (Figures 4 and 5). The temporal sequence of CK concentrations after AMI were the same by both methods; after myocardial infarction, we were not able to demonstrate CK-B in the blood earlier by RIA than CK-MB by electrophoresis. When the clinical sensitivity was 100% for RIA, specificity was 94%. We did not observe a persistent increase in CK-B with this RIA as we did with some other commercially available RIAs (12). Although the presence of CK-B in the blood associated with malignant disease poses a potential problem (9, 13–18), we did not observe inappropriate results in the patients we studied. Acute neurological disease also may result in detectable serum CK-B (19). In one of our patients, an increased CK-B as measured by RIA accompanied severe cerebral anoxia in a patient thought not to have had an AMI. Renal failure did not increase CK-B concentrations in serum.

We have demonstrated in this study that the clinical efficacy of CK-B measurements by the Nuclear Medical Laboratories, Inc. RIA kit is similar to that of CK-MB measurements by electrophoresis in patients suspected of having had an acute myocardial infarction. This kit offers the advantages of less technical time and manipulation, sample stability, and quantitative results. The need to separately determine total CK activity is eliminated. We conclude that this CK-B radioimmunoassay kit offers a preferred alternative to CK-MB estimation by electrophoresis in patients suspected of having had AMI.

References