Reassessment of Creatine Kinase BB as a Marker for Cancer of the Prostate, Breast, and Lung

Shan S. Wong,1,2 Alan H. B. Wu,1,3 and Herbert A. Fritsche4

We used an enzyme-linked immunoabsorbent assay to measure creatine kinase (CK; EC 2.7.3.2) BB in the sera of 58 cancer patients. A pre-incubation step with an anti-CK-M antibody-coated bead removed M chain components, and CK-BB was quantified with use of an anti-CK-B antibody-coated tube. No cross reactivity was observed with mitochondrial CK or CK-MM; CK-MB cross reacted slightly (1.6%). Macro CK type 1 was measured as CK-BB. Average analytical recovery of purified CK-BB added to serum was 97.7%. Although the enzyme activity of CK-BB is labile, our studies show that this protein is antigenically stable for 12 months when stored frozen. The upper limit of normal for CK-BB concentration was 0.3 μg/L (95th percentile, n = 25). Of the 20 cases of breast cancer of various stages, none showed any increases of serum CK-BB. Only two of 18 patients with prostatic carcinoma (stage D), and two of 10 patients with oat-cell carcinoma of the lung had increased concentrations of CK-BB in the serum. Ten patients with squamous cell cancer of the lung had normal concentration of the enzyme. Thus the CK-BB isoenzyme is not frequently increased in cancers of the prostate, lung, and breast, and it has little apparent value as a tumor marker for these diseases.

Additional Keyphrases: enzyme immunoassay · isoenzymes · tumor markers · macro CK · isoenzyme stability

The creatine kinase (CK; EC 2.7.3.2) BB isoenzyme in serum has been reported to have potential value as a tumor marker for patients with prostatic carcinoma (1–3), breast cancer (4, 5), lung cancer (5, 6), colorectal carcinoma (7), lymphoma (5), and various other malignancies (8, 9). However, the percentage of patients showing abnormal CK-BB concentrations in serum in these malignancies varies considerably from one laboratory to the next. For example, Silverman et al. (1) reported that 89% of untreated prostate carcinoma patients had increased CK-BB in serum, but Zweig and van Steirteghem (10) found only 29% of their patient population to have increased CK-BB. The isoenzyme was measured by radioimmunoassay and electrophoresis in these reports (4, 10, 11), and differences in antisera specificity or test methodology might account for some of the discrepancies in the incidence of increased CK-BB concentration in cancer patients.

To clarify the potential usefulness of CK-BB as a marker, we used a solid-phase enzyme immunoassay that is specific for CK-BB through use of polyclonal antibodies directed against the B polypeptide chain of CK. Using this immunoassay, we investigated the incidence of increased CK-BB concentrations in sera of patients with cancer of the breast, lung, or prostate.

Materials and Methods

Subjects: Sera from 58 patients who had been admitted for evaluation and treatment of cancer of the breast, lung, or prostate at the M.D. Anderson Hospital and Tumor Institute were collected and stored frozen at −20°C until assay. We also assayed blood samples from 25 normal healthy individuals to establish a reference interval for CK-BB, and we used serum specimens containing macro CK types 1 and 2 to assess cross reactivity of these atypical isoenzymes with the CK-BB antiserum.

Reagents: Tubes coated with polyclonal antibody to CK-B, and diluent, washing solution, buffer, substrate chromogen, and the 250 mmol/L H₂SO₄ stopping solution were from the Enzygnost CK-MB kit (Behring Diagnostics, La Jolla, CA 91037). Beads coated with monoclonal antibody to CK-M were from the "Tandem" CK-MB kit (Hybritech, San Diego, CA 92121). Polyclonal anti-CK-B antibody conjugated to horseradish peroxidase (HRP; EC 1.11.1.7) and the free CK-BB standards were from Behring Diagnostics. We used "ID Zone" (Beckman Instruments, Fullerton, CA 92634) as a serum-based quality control material for both the electrophoretic and immunoassay methods. For cross reactivity studies we used purified human CK-MM, CK-MB, and mitochondrial CK, as isolated by the procedure of Roberts and Grace (12).

Procedures: Incubate 200 μL of serum (diluted twofold with CK-MB kit diluent) with a CK-M-antibody-coated bead for 1 h, with constant shaking, to bind CK-MB and CK-MM. Transfer 160 μL of the supernate to a test tube coated with polyclonal anti-CK-B antibody (Behring) and incubate at 37°C for 30 min. Wash twice with 1 to 2 mL of wash solution, then add 200 μL of the HRP-conjugated anti-CK-B antibody to the washed tube and incubate at 37°C for an additional 30 min. Wash the tube three times and add 200 μL of the HRP substrate. After incubating the solution at room temperature for 30 min, quench the reaction with 1 mL of the stopping solution and measure the absorbance at 492 nm (we used a spectrophotometer from Gilford Instruments, Oberlin, OH 44074).

We measured enzymatic activity by using "CK-NAC (UV)" reagent (Boehringer Mannheim Diagnostics, Indianapolis, IN 46250) and a "Multistat III" centrifugal analyzer
Results

Precision: The between- and within-assay precision we obtained with the quality-control samples is shown in Table 1. The concentration of the CK-BB standards ranged from 2.4 to 75 \( \mu \text{g/L} \) (ng/mL).

Analytical recovery: We added various amounts of CK-BB to a serum specimen to determine whether any CK-BB is lost during the two-step immunoassay procedure. The results (Table 2) showed that from 90.9 to 101.1% (average, 97.7%) of immunological activity was accounted for.

Immunological stability of CK-BB in serum. Repeated freezing (at \(-20^\circ \text{C}\)) and thawing (4 \( ^\circ \text{C} \)) of a CK-BB sample up to five times did not cause any significant decrease in the amount of CK-BB measured; moreover, the CK-BB concentration in the sample was stable for at least 12 months when frozen at \(-20^\circ \text{C} \). In contrast, incubation of a sample at 37 \( ^\circ \text{C} \) caused detectable decreases in both enzymatic and immunological activity. When the enzyme activity of one serum sample was decreased by incubation at 37 \( ^\circ \text{C} \), first to 66% and then to 45% of its original activity, the immunological activity of the sample decreased to 92% and 72%, respectively.

Cross reactivity: Purified preparations of mitochondrial CK and CK-MM were not detectable by our assay as CK-BB. Similarly, the immunoassay method detected no CK-BB in samples that showed only CK-MM or macro CK type 2 (13) by electrophoresis. One serum sample that contained macro CK type 1 (immunoglobulin-bound CK-BB) and no CK-BB band on electrophoresis produced a CK-BB concentration of 16.5 \( \mu \text{g/L} \), which suggests that this variant does cross react as CK-BB. When we omitted the pre-incubation with the anti-CK-MM antibody-coated bead, a sample with a CK-MM concentration of 48 \( \mu \text{g/L} \) yielded a CK-BB concentration of 2.4 \( \mu \text{g/L} \) (5% cross reactivity); after pre-incubation with the antibody-coated bead, the apparent CK-BB concentration decreased to 0.77 \( \mu \text{g/L} \), a cross reactivity of 1.6%.

Electrophoresis on agarose gel: We determined the detection limit for the electrophoresis method for CK-BB to be about 4 \( \mu \text{g/L} \); i.e., samples with a CK-BB concentration greater than this demonstrated a clearly discernible band by electrophoresis. To confirm the identity of this band, we observed its absence from samples pre-incubated with CK-BB antiserum. No samples that had normal concentrations (i.e. <0.3 \( \mu \text{g/L} \)) of CK-BB by the enzyme immunoassay showed CK-BB activity by electrophoresis.

Reference interval: The concentration of CK-BB in 25 normal sera ranged from undetectable to 0.3 \( \mu \text{g/L} \), with a mean of 0.14 \( \mu \text{g/L} \) and an upper 95th percentile of 0.3 \( \mu \text{g/L} \). CK-BB concentrations exceeding 0.6 \( \mu \text{g/L} \) (twice the upper limit of normal) were considered abnormal.

Prostatic carcinoma: Serum specimens from 18 patients with prostatic carcinoma (stage D) were analyzed. All of these patients had extensive disease, with metastasis to bone, liver, or lung. Only two had above-normal concentrations of CK-BB (3.1 and 9.2 \( \mu \text{g/L} \)); the remainder had CK-BB concentration ranging between 0.07 and 0.4 \( \mu \text{g/L} \).

Breast cancer: None of the 20 patients with breast cancer showed any increase in concentration of CK-BB in serum (range, 0.1 to 0.5 \( \mu \text{g/L} \)). Eight of the 20 patients had either stage I or stage II disease; the remainder had either progressive or stable stage IV disease.

Lung cancer: Serum specimens from 10 patients with squamous-cell carcinoma of the lung were analyzed; none showed an abnormal concentration of CK-BB (range, 0.04 to 0.2 \( \mu \text{g/L} \)). Only two serum specimens from 10 oat-cell carcinoma patients had significant concentrations of CK-BB (5.1 and 12.0 \( \mu \text{g/L} \); CK-BB in the remaining patients ranged between 0.1 and 0.6 \( \mu \text{g/L} \).

Discussion

The sandwich-type enzyme immunoassay for measuring CK-BB in serum provides an alternative to the radiimmunoassay method previously used to investigate CK-BB in cancer patients (1-10). The suitable analytical precision, specificity, analytical recovery, and low cross reactivity of this independent approach enabled us to re-examine the incidence of abnormal concentrations of CK-BB in serum from cancer patients.

Only four of the 58 cancer patients had significant increases of CK-BB in their serum (Figure 1). CK-BB was confirmed by electrophoresis before and after immunoprecipitation with anti-CK-BB antisera in three of the four samples with increased CK-BB. No CK-BB band was detected in the fourth sample (CK-BB = 3.1 \( \mu \text{g/L} \)) or in the other 54 samples. To substantiate further the absence of CK-BB from these sera, we re-assayed the sera, substituting in the assay system an alkaline phosphatase (EC 3.1.3.1)-conjugated monoclonal antibody to CK-B for the HRP-conjugated polyclonal antibody to CK-B. The same results were obtained (data not shown).

We think it particularly important to note that the low incidence of increased CK-BB is probably not the result of loss of CK-BB immunological reactivity. Although reports
have shown that *enzymatic* activity of CK-BB is easily lost during storage (14), but can be reactivated by adding thiols and chelating agents after collection (15), our experiments suggest that CK-BB is *antigenically* stable for at least 12 months under these conditions.

Soon after the initial report that CK-BB may be useful as a tumor marker (1), the reported incidence of abnormal concentrations of CK-BB in cancer patients has steadily declined. For example, Mercer and Talamo (7) did not find CK-BB useful as part of a tumor marker panel for colorectal cancer; Blick et al. (16) found BB to be a poor predictor for prostate cancer; and Fritsche et al. (17) recently demonstrated that serum CK-BB did not consistently correlate with the clinical status of patients with progressive breast cancer. These results are consistent with the present findings that CK-BB in serum is infrequently found in high concentrations in patients with cancer of the prostate, breast, or lung, even when disease is extensive. Because our assay procedure also detects macro CK type 1, we presume that this variant is also probably not a useful marker of neoplastic diseases.

Earlier reports of increased CK-BB may reflect cross reactivity of the antisera with other CK isoenzymes (e.g., macro CK type 2) in these radioimmunoassays. If such is the case, then macro CK type 2 may be a more useful tumor marker than CK-BB. Several recent reports support this speculation (13, 15). It might be worthwhile to investigate this particular isoenzyme in more detail.

We thank Dr. M. Benjamin Perryman (Baylor College of Medicine) for providing purified CK-MM, -MB, -BB, and monomeric mitochondrial creatine kinase; Dr. Peter Panfilii, Behring Diagnostics, for providing the HRP-conjugated antisera and the CK-B and CK-BB standards; and Dr. Tina Berger, Hybritest, Inc., for providing the alkaline phosphatase-conjugated antisera. This work was supported in part by grant no. 86GF-256 to A.H.B.W. from the American Heart Association, Texas Affiliate.

References