Immunoglobulin-Bound Creatine Kinase BB in Serum with Electrophoretic Migration between MM and MB Isoenzymes

To the Editor:

An atypical creatine kinase (CK, EC 2.7.3.2) electrophoretic band with migration between isoenzymes CK-MM and CK-MB, as reported by many authors (1–6), can be erroneously classified as CK-MB, or a band containing CK-MB activity.

The atypical CK isoenzyme pattern was first reported, on cellulose acetate electrophoresis, by Velluti et al. (1), the unusual band being attributed to a lipoprotein-bound CK-MM. Urdal and Landaa (3) were the first to suggest that the atypical electrophoretic band was CK-BB complexed with IgG, a postulate supported by recent “rocket” immunoelectrophoresis studies in which the presence of IgG in the artifactual band was described (4). Others (5, 6), using column chromatography with subsequent analysis of eluates by enzymic methods (5) and radioimmunoassay (RIA) (6), reported the presence of CK-BB in the intermediate electrophoretic band.

We tested for the presence of an antibody-bound CK-BB moiety in the sera of three patients, using the following independent experiments:

1. Removal of the artifactual immunoglobulin-bound CK-BB electrophoretic band by use of antihuman IgG together with polyethylene glycol as an immunoglobulin precipitating agent.

2. Demonstration of increased CK-BB by RIA in sera in which the artifact was demonstrable, with no concomitant evidence of CK-BB or CK-MB on electrophoresis.

3. Demonstration of increased binding of 125I-labeled CK-BB in serum containing the artifact.

4. Creation of the CK electrophoretic artifact by addition of CK-BB antiserum to a human serum sample containing endogenous CK-BB.

Initially, we examined the presence of the electrophoretic fluorescent artifact in serum from three patients who had no apparent common symptomatology. We applied 10 μL of serum to a cellulose acetate plate (Titan III Iso-Flur, cat. no. 3506; Helena Laboratories, Beaumont, TX 77704) that had been presoaked in Trias/barbitual buffer (pH 8.8; ionic strength 0.029) for 30 min. After electrophoresis in the same buffer for 10 min at 300 mA, the plate was treated with CK substrate (Helena Laboratories, cat. no. 5130). All three patients’ specimens had the atypical migrating band in their electropherograms. We were able to remove the atypical electrophoretic band by immunoprecipitation, which involved treating 200 μL of serum from each of the three patients with 200 μL of antihuman IgG, incubating for 15 min at 37 °C, precipitating the resulting antibody complex with 1 mL of polyethylene glycol solution (PEG, 250 g/L), and centrifuging (20 min, 2700 × g). The identical experiment was repeated except that antihuman IgA and IgM were used.

Electrophoresis of the resulting supernatants showed no atypical CK band with the antihuman-IgG-treated specimen (Figure 1), whereas specimens treated with antihuman-IgA and IgM were unchanged. Evidently IgG was involved in the atypical CK band seen in the serum electropherograms of these patients.

We then tested the three patients’ specimens for immunoreactive CK-BB by RIA (Mallinckrodt, Inc., St. Louis, MO 63134). We treated 100 μL of patient’s serum with 100 μL of CK-BB antiserum, incubated for 2 h at 5 °C, added 100 μL of 125I-labeled CK-BB, and incubated the mixture for 2 h at 5 °C. Antibody-bound CK-BB was separated by adding 1 mL of a second antibody–PEG solution (PEG 60 g/L), followed by centrifugation at 1500 × g. Radioactivity in the resulting pellet was then measured. CK-BB values by RIA were 41.4, 35.0, and 13.5 μg/L for the three specimens, all of which exceeded our normal range, 0.5 to 5.9 μg/L, based on serum CK-BB data collected from 40 normal healthy volunteers. None of these three specimens had electrophoretically detectable CK-BB (in the usual electrophoretic migrating position) or CK-MB. Nevertheless, immunoreactive CK-BB was present in these three patients’ sera.

To demonstrate increased uptake of radioactively labeled CK-BB in samples with the intermediately migrating electrophoretic artifact, we first had to displace endogenously bound CK. Because urea (8 mol/L) reportedly is effective in dissociating antibody-bound ligands (7), we added 5 mL of 8 mol/L urea to (a) 100 μL of patient’s serum in which the atypical CK band had been detected, (b) 100 μL of a serum control sample containing no atypical CK band, and (c) 100 μL of a serum control sample with no atypical band but treated with 10 μL of CK-BB antiserum (Mallinckrodt). These serum/urea solutions were incubated for 25 h at room temperature, after which we added 100 μL of 125I-labeled CK-BB. Each reaction mixture was then transferred to dialysis tubing (exclusion limit, 1500 daltons; American Scientific Products, McGaw Park, IL 60085) and dialyzed against water for 4 h with water changes every 30 min. Complete removal of urea was confirmed by urea nitrogen measurement in the dialysate. Subsequently, we added 400 μL of dialysate to 400 μL of phosphate buffer (10 mmol/L, pH 7.0) and incubated for 30 min at room temperature. The antibody-bound labeled CK-BB complex was precipitated by adding 1 mL of polyethylene glycol (60 g/L) and centrifuging for 20 min at 2700 × g. Radioactivity was measured in the resulting precipitates. The patient’s specimen with the atypical electrophoretic band had 4.8-fold more radioactivity than the serum control sample, supporting the idea that a specific immunoglobulin binder of labeled CK-BB was present in the patient’s serum. Antibody-bound radioactivity (cpm) was as follows: control serum, 1409; control serum treated with antibody, 3778; and patient’s serum containing the atypical electrophoretic band, 6774. We performed a similar procedure on one of the patient’s serum containing the atypical band, using a dextran/charcoal slurry (8, 9) to remove the endogenous CK-BB from the antibody before adding the isotopically labeled CK-BB. The resulting antibody-bound radioactivity was no more than
for the normal control material, suggesting a high binding affinity for the immunoglobulin/CK-BB complex.

In an effort to reproduce the atypical electrophoretic CK band in vitro from endogenous CK-BB, we added 600 μL of CK-BB antisera to 200 μL of patient's serum containing an appreciable amount of CK-BB, probably from a small-cell carcinoma of the lung. After incubation of this mixture for 30 min at room temperature, electrophoresis revealed an intermediately migrating CK band similar to the ones observed in the three patients' specimens already described (Figure 2). This suggests that binding immunoglobulin IgG to CK-BB retards the electrophoretic migration of the latter such that it will appear between CK-MM and CK-BB on the electropherogram.

We conclude that the intermediately migrating CK band detected in the sera of our three patients contains a CK-BB entity with both CK enzymic activity and CK-BB immunoreactivity, and with retarded electrophoretic migration because of binding by IgG.

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References


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Amniotic Fluid Squalene and Gestational Age

To the Editor:

Accurate assessment of gestational age near term is often difficult when the date of the last menstrual period is uncertain and no ultrasonographic data are available.

Study of the cytological and biochemical composition of the amniotic fluid (AF) can give information on gestational age and fetal maturity, but not many attempts have been made to so establish whether the true chronological term has been reached or passed.

Recently, however, Wysocki et al. (1) reported that squalene, a hydrocarbon originating mainly in fetal sebaceous glands, markedly increases in the AF when pregnancy reaches the 39th to 40th week. The squalene/cholesterol ratio progressively increases near and after term, so that it is possible to assess whether a pregnancy is at the 38th, 40th, or 42nd week and beyond. False-negative and false-positive results are extremely rare.

Since this promising study no other report on squalene assay in the AF near term has appeared.

We collected 104 samples of AF by transabdominal amniocentesis or at the moment of amniotomy, from 81 pregnant women whose gestation was either normal or slightly complicated by modest Rh-immunization, class A diabetes, or suspected intra-uterine growth retardation. Gestational age was estimated both by maternal history (date of last menstrual period, of quickening, and by ultrasonic evaluation before the 24th week of pregnancy) and by physical and neurological examination of the newborns.

In five cases the AF specimen was stained with meconium and in one with blood. Squalene and cholesterol were determined according to Wysocki et al. (1).

Briefly, AF (5-10 mL) was centrifuged (1000 × g, 10 min) and the resulting pellet was washed three times with 8-mL portions of isotonic saline. Each time after the supernate or the washing solution was removed, the interior of the test tube was carefully wiped with tissue paper. The pellet was then suspended in 2 mL of distilled water/acetone (1/1 by vol), vortex-mixed for 1 min, re-extracted with 2 mL of ethyl acetate, and the water phase aspirated. The organic phase was dried over anhydrous sodium sulfate and finally evaporated to dryness under a stream of nitrogen. The dry residue was incubated for 30 min at 56 °C in the presence of 0.1 mL of bis(trimethylsilyl) trifluoroacetamide (Merck, Darmstadt, F.R.G.), and 2 μL of this solution was chromatographed on a 45 cm × 2 mm (i.d.) glass column packed with SE-30, 3% on Chromosorb W, DMCS (C. Erba, Milano, Italy). The column temperature was 345 °C, the carrier (N2) flow rate 50 mL/min. The instrument (Fractovap; C. Erba) was equipped with a flame ionization detector.

Peak height was measured and the results were expressed as squalene/cholesterol molar ratio. The results are presented in Figure 1.

The ratio before the 40th week was <0.4 in 66 of 70 cases. At the 40th week and after, the ratio exceeded 0.4 in 28 of 34 cases. In three of these latter six cases in which the ratio did not exceed 0.4, the AF was stained with meconium.

Not many cases at or beyond week 42 have been examined; in those measured, however, the squalene/cholesterol ratio is usually >1. Several tests giving indications of the maturity of various fetal organs and functions have been performed on AF to evaluate gestational age and fetal maturity. Determinations of bilirubin, creatinine, and orange cells generally allow one to assess whether a pregnancy has reached the 37th week, but no significant change during the following weeks have been reported. Lecithin assay, the lecithin/sphingomyelin ratio, and the palmitic/stearic fatty acid ratio give important indications of fetal lung maturity and consequently of the probability of the newborn's developing hyaline membrane disease, but these tests may show "mature" values five or even more weeks before term. Again, no major change of values occurs after