Ectopic Production of Creatine Kinase MB: Updated Evaluation by Mass Assays

To the Editor:
There have been several reports of apparently high concentrations of creatine kinase (CK)-MB in the sera of cancer patients [1–4]. In several of these cases, the high activity resulted from the presence of macro CK types 1 and 2 [5]. False-positive results for CK-MB can occur if nonspecific assays such as immunoinhibition are used [6]. These atypical isoenzymes are not inhibited by anti-CK-B antibodies, and high apparent CK-MB activities are produced. Macro CK type 1 (CK-BB bound to IgG) migrates between CK-MM and -MB, whereas type 2 (polymeric mitochondrial CK) migrates cathodic to CK-MM and can be readily differentiated from CK-MB by electrophoresis [7]. However, macro CK-BB linked to IgA can comigrate with CK-MB, and false-positive detection of MB is possible even with electrophoresis [8].

Annesley and McKenna [9] published the one clear case of ectopic CK-MB production by a tumor, by demonstrating high concentrations of CK-MB in homogenized tumor tissue and ruling out the presence of macro CK forms by heat stability tests. We describe a second case in which persistently high concentrations of CK-MB were detected in a patient with metastatic cancer. CK isoenzymes, myoglobin, lactate dehydrogenase isoenzymes, and cardiac troponins T and I were used to rule out the presence of acute myocardial injury.

A 71-year-old white man with a past medical history of coronary artery disease, acute myocardial infarction (AMI), hypertension, congestive heart failure, cerebrovascular accident, carotid stenosis, and atrial fibrillation presented to the Hospital of St. Raphael with a 4-day history of bright red blood per rectum, right lower quadrant pain, and hypotension. He was admitted to the Intensive Care Unit. An upper endoscopy revealed a gastric ulcer that appeared benign on gross inspection. No biopsies were performed. The gastrointestinal hemorrhage subsided with conservative therapy. Although his electrocardiogram was normal, his past history of coronary artery disease prompted testing for cardiac markers. Total CK and CK-MB were persistently increased over the course of this hospitalization with a relative index ranging between 21% and 30% (reference value <2.5%). Total lactate dehydrogenase was also increased, with an isoenzyme pattern containing a predominance of lactate dehydrogenase 5. A diagnosis of AMI was ruled out by the cardiologist. An abdominal CAT scan was obtained to further elucidate the source of his right lower quadrant tenderness. The liver showed marked hepatomegaly with diffuse hypodense nodules consistent with metastatic carcinoma of unknown primary. The patient was discharged to a hospice were he died several days later. Permission for an autopsy was denied.

Total CK and CK-MB (mass assay) were measured at the Hospital of St. Raphael with BM/Hitachi 747® (Boehringer Mannheim) and AXSYM® (Abbott Laboratories) analyzers, respectively. A subset of serum samples were sent to Hartford Hospital for further analysis. CK-MB was assayed on Opus Plus® (Behring Diagnostics), Access® (Sanofi Pasteur Diagnostics), and the ACS:180® (Ciba Corning), and CK isoenzymes and isoforms were assayed with the CardioRep® (Helena Labs.) [10]. Myoglobin and cardiac troponin I were assayed on the Opus [11], and cardiac troponin T (cTnT) was assayed on the ES300® (Boehringer Mannheim) [12]. To determine if heterophile antibodies were present, 20 μL of mouse IgG (Sigma) was added to a 200-μL aliquot of serum and incubated for 4 h at room temperature [13]. The mixture was assayed for CK-MB on the Opus.

Table 1 lists the results of the tests performed on one of several samples tested to further characterize the enzymes and proteins present in these serum samples. Each of the CK-MB mass assays showed increased concentrations exceeding the reference range by at least 10-fold. Because human anti-mouse antibodies can interfere with immunoassays that make use of murine monoclonal antibodies, mouse IgG was added to determine if this would remove the potential interfering agent. Table 1 shows that although a substantially lower residual result was present than was expected after dilution (45.1 μg/mL), the high residual activity suggested that human anti-mouse antibodies could not be responsible for the majority of the apparent CK-MB present. The ratio of cardiac isoforms (MB2/MB3) showed results that were below the reference limit. This ratio may have been falsely low because isoforms were not originally ordered; therefore, the samples had not been properly preserved with EDTA to prevent in vitro isoform conversion before analysis [14]. The measurements were made to verify the presence of two CK-MB isoform bands, which ruled out the presence of a macro CK-BB:IgA complex comigrating with CK-MB (as one band would have been expected). Myoglobin concentration was normal, indicating no active heart, skeletal muscle, or renal disease. Results for both cardiac troponin T and I were below the cutoff concentration, demonstrating the absence of true cardiac injury.

From these results, it is possible that this is another case of ectopic CK-MB production. We were able to better characterize this isoenzyme as being CK-MB because more specific assays are available today for measuring this isoenzyme than at the time of the Annesley report [9]. Moreover, use of new cardiac-specific markers (cardiac troponin T and I) allowed confident exclusion of myocardial injury as a source of increased serum CK-BB (Table 1). The presence of macro CK forms were ruled out because macro CK does not cross-react with either the Conan anti-CK-MB antibody [15] (used in most commercial mass CK-MB assays) or the anti-CK-MB antibody used in the ACS:180 assay (which was developed separately from the Conan antibody). We also selected different mass assays for CK-MB to...
Table 1. Additional cardiac marker studies conducted.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Results</th>
<th>Reference range</th>
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<tbody>
<tr>
<td>CK-MB, Opus</td>
<td>66.2</td>
<td>0–5 µg/L</td>
</tr>
<tr>
<td>CK-MB, Opus + mouse IgG</td>
<td>45.1</td>
<td>0–5 µg/L</td>
</tr>
<tr>
<td>CK-MB, Access</td>
<td>74.1</td>
<td>0–5 µg/L</td>
</tr>
<tr>
<td>CK-MB, ACS:180</td>
<td>51.1</td>
<td>0–5 µg/L</td>
</tr>
<tr>
<td>CK, electrophoresis, %MB</td>
<td>54.0%</td>
<td>0–4%</td>
</tr>
<tr>
<td>CK, isoform electrophoresis, MB2/MB1</td>
<td>0.42</td>
<td>0.55–1.33</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>24.9</td>
<td>0–62 µg/L</td>
</tr>
<tr>
<td>cTnT, ES300</td>
<td>0.01</td>
<td>0–0.1 µg/L</td>
</tr>
<tr>
<td>cTnl, Opus</td>
<td>&lt;0.5</td>
<td>0–0.5 µg/L</td>
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*200-µL sample plus 20 µL of mouse IgG (Sigma).

rule out the presence of an interfering agent that might have interacted with the assay’s detection method. Demonstration of CK-MB in tumor biopsy would have confirmed the presence of ectopic production.

Several other possible explanations for these observations exist. As summarized by Chan et al. [16], CK-MB can be increased by release from nonmyocardial sources (e.g., trauma to skeletal muscles, granulomas, seizures, inflammatory and non-inflammatory myopathies, etc.), cardiac injury other than AMI (e.g., cardiac contusions, myocarditis), and as a result of decreased clearance of serum CK-MB (in patients with hyperthyroidism and hypothyroidism). A high fraction of CK-MB relative to total CK can also be observed after extensive muscle damage or regeneration (such as in marathon runners or patients with Duchenne muscular dystrophy) [17, 18]. We have attempted to eliminate many of these causes of abnormal CK-MB through history review and laboratory testing. The patient did not have a history of extensive skeletal muscle turnover, seizures, skeletal muscle myopathies, or intramuscular injections. Cardiac injuries were ruled out by troponin. Moreover, myoglobin concentrations were within the reference interval, ruling out acute skeletal muscle injury, whereas thyroid function tests indicated no extant thyroid disease. Nevertheless, because we were not able to obtain tumor tissue either before or after death, we cannot confirm the production of CK-MB by the tumor itself.

The clinical importance of ectopic production of CK-MB in a cancer patient is limited, as the incidence of this appears to be exceedingly rare. Such findings did not create much confusion for diagnosis of AMI because serial CK-MB measurements showed that the typical rise and fall pattern for total CK and CK-MB was absent. Testing with other cardiac markers, especially cardiac troponins, was helpful in further ruling out AMI. As more assays for cardiac troponins become commercially available, the use of CK-MB may decline over the ensuing years, further diminishing the importance of finding ectopic CK-MB patients. It will be interesting to see if case reports for ectopic production of cardiac troponin T or I are discovered, as the popularity of this testing increases.

References


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Acute Bacterial Prostatitis Induces Hematogenous Dissemination of Prostate Epithelial Cells

To the editor:
Although not yet a part of clinical chemical practice, molecular techniques are sensitive tools to detect hematogenous spread of solid tumor cells in cancer and may become routine methods in the coming years.