tivity would be the hope of clinical as well as laboratory hematologists provided specificity is also acceptable. But until such investigations are performed, such conclusions would be premature.

Although the results of these studies are intriguing, they cannot yet be considered definitive. I hope that the group from Sweden and/or other investigators will pursue these leads. If the results are as anticipated, laboratories and manufacturers can consider filling future vacuum blood collection tubes with optimal amounts of isocitrate rather than the time-honored citrate anticoagulant.

References

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Metastatic Alveolar
Rhabdomyosarcoma with Increased
Serum Creatine Kinase MB and
Cardiac Troponin T and Normal
Cardiac Troponin I

To the Editor:

We describe a 53-year-old man with metastatic alveolar rhabdomyosarcoma who had a massively increased creatine kinase MB (CK-MB) mass and index. The CK-MB increase was initially interpreted as evidence of myocardial infarction (MI), but the CK-MB remained increased. The patient had increased serum concentrations of cardiac troponin T (cTnT) and normal cardiac troponin I (cTnI). We theorize that tumor anaplasia caused malignant myocytes to reexpress CK-MB and cTnT isoforms. Rhabdomyosarcoma release of both CK-MB and cTnT has not been described previously.

Serum CK and lactate dehydrogenase (LD) activities were assayed at 37 °C with the Hitachi 911 automated analyzer [Boehringer Mannheim (BM)]. CK-MB mass was measured with the Access (Beckman Instruments) immunoenzymatic assay. Serum cTnT was evaluated using a second-generation Elecsys 1010 immunoenzymatic assay (BM). Serum cTnI was analyzed using both the AxSYM (Abbott) microparticle enzyme immunoassay and the Opus Plus (Dade Behring) fluorogenic two-site immunoassay.

The patient presented to a community hospital with acute, bilateral leg weakness. He had a 3-week history of worsening thoracic back pain and lower extremity paresthesias, and 3 months earlier he had discovered a subcutaneous mass of his left foot. Foot x-ray at that time was unremarkable. The patient’s neurologic examination revealed that lower extremity motor power was decreased to 2+/5. He was unable to walk. His deep tendon reflexes were diminished, and his plantar responses were abnormal bilaterally. The patient was continent of urine but had anal sphincter laxity. He had decreased sensation below the nipples. Spinal cord compression was diagnosed. Computerized tomography scan revealed a soft tissue mass that permeated the T2 vertebral body and impinged on the spinal cord. A left foot mass was also confirmed, and computerized tomography scan revealed a circumscribed soft tissue tumor, measuring 4 × 3.9 × 3.8 cm that surrounded the lateral fifth metatarsal. Core biopsies of both tumors revealed similar, round-cell neoplasms that were confirmed to be alveolar rhabdomyosarcomas by immunohistochemical and ultrastructural studies, with positive staining for vimentin, desmin, and muscle-specific actin. The patient underwent thoracic laminectomy. Postoperative neurologic function was unchanged. The patient was treated with three cycles of doxorubicin and cis-platinum and then with spinal radiotherapy. Six months after his initial presentation, the left foot mass began to grow rapidly. The foot tumor was treated with radiotherapy, but within 1 month, the patient developed left inguinal lymph node metastases and deep venous thrombosis. He was readmitted to hospital where he developed an episode of atypical chest pain. An electrocardiogram revealed only nonspecific T-wave abnormalities. His serum CK was 336 U/L [reference interval (RI), 45–220 U/L]. His CK-MB mass was 150 µg/L (RI, 0.0–5.0 µg/L) with a CK-MB index of 45 (RI, 0.0–2.0), a cTnT of 0.95 µg/L (RI, 0.00–0.10 µg/L) and a cTnI of <0.5 µg/L on the Opus Plus (reference value, <0.5 µg/L) and 1.2 µg/L on the AxSYM (RI, 0.0–2.0 µg/L). There was no biochemical evidence of hepatic or renal failure. CK electrophoresis revealed increases of all CK isoenzymes (Table 1). Atypical CK variants were not present. A diagnosis of MI was maintained until serum cardiac enzyme levels, measured 3 days later, showed sustained increases. At this time, the patient had a CK of 334 U/L, a CK-MB mass of 195 µg/L, a CK-MB index of 58, and a cTnT of 0.80 µg/L, and a cTnI of <0.5 µg/L (Opus Plus) and 0.8 µg/L (AxSYM). Serum LD was increased to 570 U/L (RI, 95–195 U/L). Considering the history of atypical chest pain, the equivocal electrocardiogram changes, and the sustained and dramatically increased CK-MB mass and index, a recent MI

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**Table 1. CK and LD isoenzyme electrophoretic fractionation.**

<table>
<thead>
<tr>
<th>CK isoenzymes (RI), µg/L</th>
<th>LD isoenzymes (RI), µg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-MM 130 (0–170)</td>
<td>LD5 334 (&lt;60)</td>
</tr>
<tr>
<td>CK-MB 106 (0–14)</td>
<td>LD5 758 (&lt;70)</td>
</tr>
<tr>
<td>CK-BB 25 (ND)*</td>
<td>LD5 318 (&lt;45)</td>
</tr>
<tr>
<td></td>
<td>LD6 45 (&lt;30)</td>
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<tr>
<td></td>
<td>LD6 45 (&lt;30)</td>
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Total CK 261
Total LD 1500

* ND, usually not detected.
was considered unlikely. The aberrant cardiac markers were thought to be secondary to tumor expression of CK-MB and cTnT.

The patient returned 2 weeks after discharge with new spinal cord compression. Postradiotherapy analysis revealed a CK of 1364 U/L, a LD of 1500 U/L, a CK-MB mass of 1047 μg/L, a CK-MB index of 77, a cTnT of 2.49 μg/L, and a cTnI of <0.5 μg/L (Opus Plus) and 1.1 μg/L (AxSYM). LD electrophoresis revealed predominant increases of LD4, LD2, and LD3 (Table 1). The LD4-to-LD2 ratio was 0.44. There were no further clinical episodes compatible with MI, nor did the patient have congestive heart failure. The patient died 11 months after his initial presentation. No postmortem examination was performed.

Rhabdomyolysis, Duchenne muscular dystrophy, polymyositis, viral myositis, and various other myopathies may cause false-positive CK-MB tests for MI (1). Malignancies have also been associated with increased serum CK-MB and CK-BB (1–3). Usually the pattern of CK-MB increases is sustained, with CK-MB indexes ranging from 40 to 60, beyond values usually observed with MI. Features consistent with CK-MB release from a non-cardiac source in our patient included a sustained increase of serum CK-MB mass that fluctuated little over a 3-day period, CK-MB indexes of 45 and 58, and a marked increase of serum CK-MB after radiotherapy. CK electrophoresis confirmed the absence of CK variants. The increase of all LD isoenzyme fractions is seen with neoplasms (4). Cardiospecific cTnI values remained within the RI s in two different immunoassays, effectively dismissing ongoing myocardial ischemia. In addition to CK-MB isoenzyme expression, our patient’s rhabdomyosarcoma also likely expressed cTnT.

Rhabdomyosarcoma expression of both CK-MB and cTnT can be explained by tumor anaplasia and concurrent expression of fetal phenotypes. Certain tumors, in particular lung carcinomas, develop an ability to synthesize peptide hormones secondary to tumor anaplasia and altered gene expression (5). Maturing chick embryo skeletal muscle shows progression of CK isoenzyme content from CK-BB to CK-MB to CK-MM (6). Fetal human skeletal muscle is known to express cTnT isoforms (7). With skeletal muscle maturation, there is increased expression of skeletal TnT isoforms and concurrent down-regulation of cTnT isoforms (7–8). Adult rat skeletal muscle has shown re-expression of cTnT isoforms after denervation injury (9). Re-expression of fetal genes is also thought to occur in the diseased skeletal muscle of Duchenne muscular dystrophy and polymyositis. The cTnI isoform, unlike cTnT, is not expressed by skeletal muscle at any point during muscle maturation, and therefore, diseased skeletal muscle shows a lack of cTnI expression (10).

Although our conclusions are speculative, the cTnT increases clearly were not associated with renal or congestive heart failure. The major limitation of our investigation was the lack of direct tumor analysis by either immunohistochemical or genetic techniques. Isoforms of cTnT have been identified in diseased skeletal muscle of chronic renal failure patients by Western blots and highly specific M7 and M11.7 monoclonal antibodies for cTnT (11, 12). These monoclonal antibodies are the same ones that are used in the BM second-generation cTnT immunoassay. Although cTnT isoform expression occurs in the skeletal muscle of chronic renal failure patients, it apparently does not produce false positives in second-generation BM cTnT tests because of differential detection of cTnT epitopes by M7 and M11.7 antibodies (11). The cTnT isoforms expressed in skeletal muscle neoplasms may have epitope combinations that produce false-positive cTnT tests, even with second-generation immunoassays. Haller et al. (13) were unable to demonstrate cTnT mRNA expression in skeletal muscle from five patients with end-stage renal failure. To our knowledge, no studies have investigated rhabdomyosarcomas at the mRNA level to determine their expression of troponins. Further rhabdomyosarcoma genetic studies are required to elucidate their protein expression. We theorize that rhabdomyosarcoma anaplasia leads to an immature skeletal muscle phenotype that may cause false-positive biochemical testing for MI, secondary to expression of CK-MB and cTnT.

We thank the technologists at the Ottawa Hospital for technical assistance.

References
Mass Spectrometry of Nucleic Acids

To the Editor:

We read with interest the review by Kricka (1) on nucleic acid detection technologies, in which he mentioned that nucleic acids do not have any intrinsic properties for direct detection. In response to this, we would like to point out the determination of intrinsic molecular weights of nucleic acids using mass spectrometry (MS) has been widely accepted as one of the most accurate methods to detect nucleic acids (2). Using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS, a mass resolution of 1 per 1000 and the detection of low femt mole quantities of DNA can be achieved routinely (3). Nucleic acids ranging from 2 to 2000 nucleotides can be detected by using MALDI-TOF MS (4). Because of the mass differences of the nucleobases, MS can also be used to analyze mixtures of different nucleic acid fragments without the use of any label (5). Furthermore, in most cases, the separation of the fragments before MS measurements is not required. The minimum sample volume required for MALDI-TOF MS is only a few nanoliters (3). MS can, therefore, be easily linked to any miniaturization of sample processing. Typically, each mass spectroscopic measurement including acquisition and interpretation of mass spectrum takes <10 s. With the availability of automatic high-throughput MS systems that include sample preparation (6), the cost-effectiveness of using MS to analyze nucleic acids has become comparable to other analytical techniques. Currently, the size of a MALDI-TOF mass spectrometer is similar to an immunoassay analyzer. However, as stated in a recent report (7), the size of mass spectrometers can be substantially reduced. Together with the continued development of software for automated interpretation of mass spectra, MS has a great potential to become one of the most important analytical tools for clinical laboratories. Some of the current clinical applications of MS are (a) DNA sequencing (8); (b) detection of genetic variations such as single-nucleotide polymorphisms (9), microsatellites (10), short tandem repeats (11), and small insertions/deletions; and (c) gene expression.

References