Discordance Between Results for Serum Troponin T and Troponin I in Renal Disease

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Two patients were investigated for unexplained increases in troponin T. In the first patient, who had rhabdomyolysis and acute renal failure, troponin T reached a peak value of 13.50 μg/L (67.5-fold the upper reference limit). The second patient had chronic renal failure and the troponin T peak value was 2.85 μg/L (14.3-fold the upper reference limit). Clinical investigations indicated no evidence of myocardial damage. Serum or plasma specimens were analyzed for total creatine kinase (CK), CK-2 mass, CK-2 isoenzyme ratio, myoglobin, troponin T, troponin I, and myosin light chains; all except troponin I were at above-normal concentrations. We also investigated six additional renal patients with above-normal troponin T; troponin I was slightly increased in only one of these six patients. Our findings demonstrate discordance between results for troponin T and troponin I in renal patients.

Indexing Terms: creatine kinase/isoforms/isoenzymes/myosin light chains/myocardial infarction/rhabdomyolysis

Several new biochemical markers have been investigated for the early and accurate diagnosis of myocardial infarction (MI) (1–6). Of the commercially available assays, myoglobin and isoenzymes of creatine kinase-2 (CK; EC 2.7.3.2) are regarded as the earliest markers of MI (4, 6). However, the clinical specificity of both is questionable, false positives having been reported in patients with renal failure and skeletal muscle damage (7). Cardiac troponin T, in contrast, has been reported as the most specific biochemical marker of MI (3). The initial increase of troponin T in blood after MI is similar to that of CK-2 mass but troponin T remains increased for as long as 10 days after MI. In the cardiac troponin T assay (Boehringer Mannheim, Mannheim, Germany), two specific monoclonal antibodies are used, which gives a little cross-reactivity with the skeletal troponin T (8). Although most early investigations showed almost absolute specificity of troponin T for cardiac damage (8), the problem of increased concentrations of troponin T in renal disease was not addressed.

Troponin I has also been reported to have a very high specificity for cardiac disease (5). A recent study showed no increase of troponin I in patients with acute or chronic skeletal muscle disease or chronic renal failure (9). Troponin I concentration is above the reference range within 6 h after the onset of chest pain, peaks at 12 h, and remains increased for at least 144 h after the onset of symptoms (10, 11). Because of its very high cardiac specificity, troponin I has been shown to be an excellent marker for the diagnosis of perioperative MI (12).

Here we present two cases of renal disease, in the absence of myocardial damage, with unexplained high concentrations of troponin T but normal values for troponin I. Serum specimens from these patients were also analyzed for CK, CK-2 mass, CK-2 isoenzymes, myoglobin, and myosin light chains-1 (MLC-1). We subsequently confirmed these findings by evaluating another six renal patients with increased troponin T.

Materials and Methods

Materials. We used Immulon 4 microtiter plate removable strips (Dynatech Labs., Chantilly, VA), SuperBlock™ blocking and assay buffer and streptavidin–horseradish peroxidase (HRP) conjugate (Pierce Chemical Co., Rockford, IL), and o-phenylenediamine dihydrochloride (OPD, Sigma Chemical Co., St. Louis, MO); all other chemicals were from Fisher Scientific Ltd. (Unionville, Ontario, Canada). Antibodies to MLC-1 and troponin I were obtained from Spectral Diagnostics (Toronto, Ontario, Canada).

Reagents. Coating buffer was either carbonate buffer (100 mmol/L, pH 9.6) or phosphate buffer (0.2 mol/L, pH 6.5). The general assay buffer contained 10 mmol/L K2HPO4/KH2PO4, pH 7.3, 1.5 g/L bovine serum albumin, 0.5 g/L Tween 20 surfactant, and 150 mmol/L sodium chloride. The assay buffer for the troponin I assay was SuperBlock plus 0.5 g/L Tween 20. The incubation buffer was 50 mmol/L K2HPO4/KH2PO4, pH 7.3, 10 mmol/L EDTA, 10 g/L digested casein, 1 mol/L NaCl, 0.5 g/L Tween 20, and 70 mmol/L 2-mercaptoethanol. The substrate buffer was a mixture of 48.5 mL of 0.2 mol/L citric acid and 51.5 mL of 0.2 mol/L Na2HPO4 buffer. The OPD substrate mixture consisted of 12.5 mL of substrate buffer, 16 mmol/L OPD, and 5.9 mmol/L H2O2. The wash solution was 20 mmol/L K2HPO4/KH2PO4, pH 7.3, 300 mmol/L sodium chloride.
and 0.5 g/L Tween 20. The biotinylation procedure is described elsewhere (13).

**Specimens.** Blood samples were drawn on admission and at 24–48-h intervals thereafter, for 7–10 days. Serum specimens were used for all biochemical analyses except that CK-2 isoforms were determined in plasma. We used only blood specimens that were sent for routine biochemical investigations; thus, no additional blood was drawn from patients solely for the purpose of this study. Informed consent, usually required in clinical trials using human subjects, was therefore deemed unnecessary.

**Assays.** Serum creatine kinase, urea, and creatinine were determined with the Synchron CX5® analyzer (Beckman Instruments, Brea, CA). CK-2 mass and serum myoglobin were determined with the Stratus II analyzer (Baxter Diagnostics, Deerfield, IL). Troponin T was determined by the Enzymun Test™ system with the ES 300 automated immunoanalyzer (both from Boehringer Mannheim). CK-2 isoforms were determined on Cardio-Rep™ (Helena Labs., Beaumont, TX) electrophoresis system.

Serum MLC-1 was determined by an ELISA on a 96-well microtiter plate. The monoclonal antibody (93PTG011-I, supplied by Spectral Diagnostics), 5 mg/L, was immobilized on the wells of a microtiter plate by incubating at 4°C overnight in the coating buffer. Any remaining binding sites on the plate were blocked with 200 μL of SuperBlock per well at room temperature for 1 h. To the coated and blocked plate, we added 50 μL of standards, controls, or serum specimen and incubated for 1 h at room temperature with shaking on a shaker. After washing the plate, we added 100 μL of the diluted detection antibody (93DAP170-J) in general assay buffer (see above) and incubated for another 1 h at room temperature with shaking. The plate was washed again and 100 μL of the goat anti-chicken IgY–HRP conjugate was added to each well. After a 30-min incubation, the plate was washed and 100 μL of the OPD substrate mixture was added. The reaction was stopped after 30 min by adding 50 μL of 2 mol/L H₂SO₄. The absorbance of each well was read at 490 nm on a Multiplan MCC/840 Mark II microtiter plate reader (Flow Labs, McLean, VA).

Troponin I was also determined by an ELISA on a 96-well microtiter plate (14). The monoclonal antibody (93PTG127B, supplied by Spectral Diagnostics; Kᵣ 4 × 10⁻⁸ mol/L), 7.5 mg/L, in phosphate coating buffer was immobilized on the wells of a microtiter plate by incubation at 4°C overnight. The plates were blocked with 200 μL of SuperBlock per well at room temperature for 1 h. To the blocked wells we added 50 μL of the incubation buffer and 100 μL of standards, controls, or serum specimen. The plate was incubated at room temperature on a shaker for 1 h. After washing the plate with the wash solution, we added 100 μL of the diluted detection antibody (chicken IgY biotinylated 93DAP163-J; Kᵣ 4 × 10⁻⁹ mol/L) in SuperBlock assay buffer per well and incubated the plate for 1 h at 37°C. After this we washed the plate, added 100 μL of the diluted streptavidin–HRP conjugate per well, and incubated for 30 min. The plate was then washed and the reaction with OPD substrate was carried out as described above.

**Results**

The assay of MLC-1 is linear to 100 μg/L and the analytical sensitivity (detection limit) is 0.5 μg/L. The total imprecision is <5%. There is no interference from hemolysis, icterus, or lipemia. Cross-reactivity with skeletal MLC-1 is <12%. Increased levels of MLC-1 are detected in the blood of patients with skeletal muscle damage.

The analytical precision of the cardiac troponin I assay was determined by using pooled sera. Within-run precision was 3.9% (n = 10) at a mean concentration of 27.4 μg/L. Between-run precision over a period of 1 week was 7.0% (n = 7) at a mean concentration of 12.5 μg/L. The analytical linearity was determined by serial dilution of the stock standard solution (1000 μg/L). As shown in Fig. 1A, the assay is linear to at least 25 μg/L.

The background absorbance did not exceed 0.10 at 490 nm. The minimum detection limit, calculated as the concentration of troponin I at 2 SD above the mean absorbance of zero standard, was 0.2 μg/L; therefore, the analytical range is 0.2–25 μg/L. The analytical recovery of exogenously added troponin I in three separate serum samples, from three different patients, was 102% ± 5% (Table 1). There was no interference from hemoglobin (up to 13.7 g/L); however, both icterus and lipemia may show slight interference (Table 2).

There is no cross-reactivity with the skeletal troponin I at concentrations ≤10 000 μg/L (14). No increase in cardiac troponin I values was detected in patients with acute skeletal muscle disorder, e.g., rhabdomyolysis (see Case Histories). Changes in serum concentrations of CK-2 mass and concentrations of troponin T and I, compared with those in a patient with a non-Q-wave infarction, showed similar initial increases (Fig. 2).

However, the total increase above the baseline was higher for troponin T and I than for CK-2 mass. In addition, the concentrations of troponin T and I in
serum stayed above normal for a longer time than CK-2 mass did (Fig. 2).

Case Histories

Case 1. A 42-year-old man was admitted with a complaint of a massive circumferential painful bruising of the left thigh and an anterior compartment syndrome of the lower left leg resulting from a fall; he was noted to be intoxicated (serum ethanol 53 mmol/L). On examination the pulse was 90 beats/min and regular, blood pressure was 130/80 mmHg, and heart sounds were normal. A few crackles were heard in the right lower chest, the abdomen was soft and nontender, and there was no significant organomegaly. Within a few hours of admission he developed acute renal failure due to rhabdomyolysis: serum myoglobin 14 890 µg/L (reference 0–110 µg/L), serum urea 28.3 mmol/L (2.9–9.6 mmol/L), and serum creatinine 676 µmol/L (71–141 µmol/L). Fig. 3 summarizes the behavior of selected biochemical markers; CK-2 isozymes were also determined. Note the prolonged increase of troponin T, which remained high for >250 h from the time of admission, while all other cardiac markers, with the exception of MLC-1, had returned to baseline. Throughout this period concentrations of troponin I remained within the reference range. Urinalysis showed abnormal numbers of hyaline casts, erythrocytes, and leukocytes. Urine protein was 5 g/L. Several electrocardiogram traces showed no abnormalities. Results of two-dimensional echo and radionuclide (scintigraphy) investigations were also negative.
The patient's past medical history included a sex-change (female to male) operation, hormonal therapy, and complications arising from the construction of an unsuccessful penile prosthesis including the insertion of a urinary catheter. Medications prior to and during hospitalization included clonazepam, perphenazine, lorazepam, and multivitamin supplements. This acute renal failure was treated by hemodialysis and the painful swelling eventually resolved, although the anterior compartment syndrome required acute surgical decompression. He was discharged 3 weeks after admission with improving renal function.

Case 2. A 69-year-old man admitted with a complaint of acute low back pain requiring a morphine infusion to control the pain. He had a 14-year history of chronic renal failure (requiring hemodialysis four times weekly), diabetes mellitus with associated retinopathy and neuropathy [requiring daily soluble and (neutral pro- tamine Hagedorn) insulin injections], chronic low back pain (requiring regular use of analgesics), hypertension, atrial fibrillation, depression, and chronic nausea. Other medications included lorazepam, ranitidine, ibuprofen, naloxone, erythropoietin, multivitamin supplements, and calcitrol.

The cause of his acute low back pain was an epidermal abscess requiring surgical excision; otherwise, the results of physical examination was unremarkable. Several electrocardiogram traces showed no changes other than evidence of atrial fibrillation. A summary of selected biochemical markers is shown in Fig. 4; CK-2 isoforms were also determined. The increase of troponin T was prolonged, and plateaued.

Additional cases. Six more patients who had acute or chronic renal disease and increased concentrations of troponin T were investigated (Table 3). Troponin I values were within the reference range except for one patient. Two patients (in addition to Case 1) had acute renal failure and four had chronic renal failure (in addition to Case 2).

Discussion

The troponins, present as a group of three subunits in the troponin complex on the thin filament of muscle myofibrils, are involved in the regulation of muscle contraction. Troponin T is the tropomyosin-binding subunit, troponin I is the actomyosin ATPase-inhibiting subunit, and troponin C is the calcium-binding subunit (1, 2). Each of the troponins has different isoforms in different muscle types, and the distribution of each varies (15). Cardiac troponin C is also expressed in adult skeletal muscle; therefore, it is not a potential cardiac specific marker for myocardial injury (16). Cardiac troponin T and I, which are expressed in cardiac muscle and not in healthy adult skeletal muscle or other tissue types, possess very high cardiac specificity (15). The expression of troponin T in developing and regenerating skeletal muscle is nonspecific (17); nevertheless, this unique tissue specificity of cardiac troponins T and I has led to their use as markers of cardiac damage. At present, an assay of troponin T is commercially available from Boehringer Mannheim, and the troponin I assay is expected soon to be commercially available from both Baxter Diagnostics (10) and Sanofi Diagnostics Pasteur (Chaska, MN) (11).

Unfortunately, the specificity of troponin T has not been fully delineated in noncardiac patients (8). Initial observations indicate that troponin T is not increased in patients undergoing cardiac or noncardiac surgery unless there is associated perioperative MI (18). In addition, no increase of serum troponin T is observed in marathon runners or after heavy exercise (19). Furthermore, the cross-reactivity of the cardiac troponin T assay antibody with the skeletal troponin T has been reported to be 3.6% (8). An increase in troponin T reported in patients with polymyositis/dermatomyositis without cardiac involvement is probably due to this cross-reactivity (20). In a more recent study, Wu et al. (21) documented that troponin T had lower specificity than CK-2 for myocardial damage. By comparison, the specificity of troponin I has been studied in more detail in noncardiac patients (9). In the absence of myocardial injury, troponin I remains within the reference range in acute muscle injury (trauma, rhabdomyolysis, or postrace marathon runners), in chronic myopathy (myositis or Duchenne muscular dystrophy), in patients with chronic renal failure requiring regular hemodialysis (9), and in patients undergoing noncardiac surgery (12).

In the present report, we describe two patients with
unexplained increases in troponin T; Figs. 3 and 4 depict changes in their serum concentrations of total CK, CK-2 mass, myoglobin, MLC-1, troponin T, and troponin I from the time of admission. As shown, concentrations of all these analytes were increased except troponin I. The peak concentration of troponin T was 13.50 μg/L (67.5-fold the upper reference limit) in case 1 and 2.85 μg/L (14.3-fold the upper reference limit) in case 2. The CK-2 isoform ratios (MB2/MB1) were positive in both patients: 4.1 in case 1 and 3.4 in case 2 at one occasion (upper reference limit = 1.0). Serum urea and creatinine were increased in both patients. Interestingly, troponin I was within the reference range in both patients (0–0.5 μg/L), which was consistent with all clinical observations and cardiac investigations. It is of interest to compare the profile of the concentrations of troponin T in these patients with the profile of troponin T after MI (Fig. 2).

Great amounts of CK and myoglobin are present in skeletal and cardiac muscle and both are released into the blood after acute or chronic muscle damage. CK-2, by comparison, is present only in trace amounts (<1%) of the total CK activity in healthy skeletal muscle, but constitutes 20–30% in myocardium. However, injured skeletal muscle, undergoing regeneration, may produce increased amounts of CK-2 to the extent that its proportion is similar to that in myocardium; this renders an increased blood concentration of CK-2 nonspecific for myocardial damage. Cardiac MLC-1 has considerable amino acid homology with skeletal MLC-1. Consequently, none of the assays so far described for MLC-1 has absolute specificity for cardiac muscle. We believe that high concentrations of MLC-1 in the two cases presented are the result of cross-reactivity with skeletal MLC-1. Similarly, CK-2 isoforms have also been recently shown to be nonspecific for myocardial damage, being increased in blood after skeletal muscle damage or heavy physical damage (7).

Are these two isolated cases of discordance between troponin T and I, or is it a common occurrence? To answer this, we investigated six more patients with acute or chronic renal disease and with increased concentrations of troponin T (Table 3). Interestingly, troponin I was within the reference range for all but one.

Table 3. Comparison of troponin T and I values in six patients with renal disease.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Troponin T</th>
<th>Troponin I</th>
<th>Clinical diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22.85</td>
<td>0.98</td>
<td>Acute renal failure, rhabdomyolysis</td>
</tr>
<tr>
<td>2</td>
<td>26.70</td>
<td>0.94</td>
<td>Chronic renal failure, gastrointestinal bleeding</td>
</tr>
<tr>
<td>3</td>
<td>32.70</td>
<td>1.54</td>
<td>Chronic renal failure</td>
</tr>
<tr>
<td>4</td>
<td>5.20</td>
<td>0.40</td>
<td>Chronic renal failure</td>
</tr>
<tr>
<td>5</td>
<td>3.65</td>
<td>0.72</td>
<td>Chronic renal failure</td>
</tr>
<tr>
<td>6</td>
<td>4.10</td>
<td>0.40</td>
<td>Acute renal failure, rhabdomyolysis</td>
</tr>
</tbody>
</table>

To the best of our knowledge, this is the first documentation of discordance between troponin T and I results. Why is troponin T increased in patients with rhabdomyolysis and chronic renal failure? Three possible mechanisms suggest themselves: massive release of skeletal troponin T detected due to the assay's cross-reactivity, skeletal muscle gene expression of cardiac troponin T, or the existence of minimal cardiac damage (microinfarction) (22–24). Cardiac troponin T expression is suppressed in skeletal muscle during ontogenic development, but there may be reexpression during regeneration of the atrophied muscle (17). Unlike troponin T, cardiac troponin I is never produced in skeletal muscle during fetal development, being synthesized only in the myocardium. Therefore, cardiac troponin I is not expressed in regenerating adult skeletal muscle and it is not present in the circulation except after myocardial damage. Chronic renal failure is generally associated with a mild myopathy (uremic polynuropathy); therefore, the above explanation may be valid for case 2, whereas assay cross-reactivity is the probable explanation in case 1. Finally, the existence of minimal cardiac damage is difficult to rule in or rule out because of a lack of a diagnostic gold standard for this phenomenon.

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References

Note added in proof: After this manuscript was accepted, a Letter to the Editor was published in this journal first describing the discordance between troponin T and I results in chronic renal failure subsequently reported in this Case Report (Hafner et al. Cardiac troponins in serum in chronic renal failure [Letter]. Clin Chem 1994;40:1790–1).