Concentration of cardiac troponin I in a horse with a ruptured aortic regurgitation jet lesion and ventricular tachycardia

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A 18-year-old Thoroughbred gelding was referred to our veterinary medical teaching hospital for evaluation of apparent colic and tachycardia (100 to 140 beats/min) that developed after a bout of exercise. The horse had been examined 3 years previously after a heart murmur had been detected during a routine physical examination. Evaluation at that time revealed a heart rate of 36 beats/min with a normal cardiac rhythm, and auscultation revealed a grade 3/6 diastolic decrescendo murmur with point of maximal intensity at the heart base on the left side. A clinical diagnosis of aortic regurgitation was made. Since that time, the horse had continued to be used for sporting events and had successfully completed a jumping event earlier on the day of admission. About 30 minutes after that bout of exercise, the horse began to have signs consistent with colic (sweating, pawing, and rolling). The horse was sedated (0.5 mg of xylazine hydrochloride/kg of body weight, IV) by the attending veterinarian and promptly referred to our facility because of the magnitude of tachycardia.

On admission (day 1), the horse appeared distressed, and its shouldered were wet with sweat. It would intermittently paw violently and had to be removed from the examination stocks because it was attempting to become recumbent. Examination revealed severe tachycardia (180 beats/min), and auscultation revealed a grade 2/6 holosystolic murmur at the heart base on the left side. Mucous membranes in the oral cavity were tacky and dark red and had a refill time of 3 seconds. Quality of pulses at peripheral locations was poor, and distal aspects of the extremities were cold. The jugular veins were moderately distended (ventral to a point in the middle of the neck), but peripheral edema was not detected. The respiratory rate also was high (72 breaths/min), and an increased intensity of vesicular sounds was ausculted throughout all lung fields.

Borborygmi were diminished, but the abdomen was not distended, and abnormalities were not detected on per rectal palpation. Tachycardia and jugular distention suggested a primary cardiovascular problem, rather than an abdominal disorder, and analysis of a base-apex ECG revealed ventricular tachycardia.

During the initial evaluation, heart rate increased to 220 beats/min, and the horse became more agitated. Xylazine (0.5 mg/kg, IV) and butorphanol tartrate (0.05 mg/kg, IV) were administered in an attempt to calm the horse, but shortly thereafter, the horse collapsed. Subsequent administration of lidocaine hydrochloride (total of 2 g, IV, as a combination of bolus injections and mixed with other fluids for IV infusion) resulted in conversion to atrial fibrillation and a decrease in heart rate to 60 beats/min. The horse promptly became less anxious, and approximately 5 minutes later, the horse stood. At that time, jugular distention was not apparent. Additional treatment included administration of fluids (10 L/h) and dexamethasone sodium phosphate (0.2 mg/kg, IV).

Initial laboratory data included results for venous blood gas analyses (all values within reference ranges), CBC, and serum biochemical analyses. Abnormalities included an increase in hematocrit (32%; reference range, 32 to 48%); hyperglycemia (275 mg/dl; reference range, 75 to 119 mg/dl); increases in serum total bilirubin (3.3 mg/dl; reference range, 0.1 to 2.1 mg/dl) and creatine (3.2 mg/dl; reference range, 0.8 to 1.8 mg/dl); concentrations; and increases in muscle enzyme activity (creatine kinase [CK], 1,647 U/L; reference range, 160 to 420 U/L; aspartate transaminase, 403 U/L; reference range 210 to 380 U/L). Additional diagnostic evaluations included abdominocentesis and evaluation of a sample of abdominal fluid (serosanguinous fluid recovered with a typical cell count of 244 cells/µl but a high protein concentration of 5.5 g/dl) and thoracic radiography (nothing abnormal detected).

An echocardiographic examination then was performed. The long-axis view revealed a tract-like echoluent area in the interventricular septum starting at the origin of the aortic root (approx 4 cm ventral to the valve leaflets) and extending dorsally into the septum toward the right atrium (Fig 1). Although the aortic valve leaflets appeared normal on the short-axis view, an echogenic structure could be intermittently imaged below the septal leaflets. This structure was suspected to be a thrombus. During real-time echocardiography, the septal portion of the aortic root appeared unstable.

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In human beings, increased serum concentrations of cardiac troponins provide a sensitive and specific marker of myocardial damage.
Assessment of concentrations of cardiac troponins may be a useful tool in the evaluation of horses with suspected myocardial disease.
and would deviate into the aortic lumen during systole, consistent with a loss of support adjacent to the septum. The abnormal echolucent area also could be seen during M-mode echocardiography, giving the septum a double-layered appearance (Fig 2). When considered in combination, these findings were consistent with a defect or fistula in the ventricular myocardium below the aortic valve and a possible subvalvular thrombus.

The horse was initially placed in a padded stall, and heart rate and rhythm were monitored continuously, using a telemetric ECG. Intravenous administration of fluids (supplemented with calcium borogluconate; final Ca\(^{2+}\) concentration of 12.8 mEq/L) was continued at a rate of 1.5 L/h. Fifty liters were administered during the first 24 hours of hospitalization. The horse passed several piles of normal feces and remained quiet during the night. Throughout the night, heart rate varied between 60 and 80 beats/min with persistent atrial fibrillation. The horse was fed hay on the morning of day 2. The horse’s appetite at that time was good. Additional treatment administered at that time included a second dose of dexamethasone sodium phosphate (0.2 mg/kg, IV).

When hay was fed again later during day 2, ventricular tachycardia returned (heart 140 beats/min), and the horse became agitated (pawing and displaying signs of abdominal pain or colic). Boluses of lidocaine (400 mg, IV) were administered at 5-minute intervals. After administration of the third dose (ie, 1,200 mg), the rhythm converted to atrial fibrillation with intermittent paroxysms of ventricular tachycardia. Persistent ventricular tachycardia returned within 30 minutes and subsequently was treated by IV administration of a bolus (400 mg) of quinidine gluconate followed by additional quinidine boluses (320 mg) administered IV at 10-minute intervals. After administration of 2,000 mg of quinidine gluconate, the rhythm converted to normal sinus rhythm with intermittent premature ven-
tricular depolarizations. An infusion of lidocaine (1.2 g/h, IV) was initiated, and feed was withheld.

The horse remained comfortable and had a normal sinus rhythm during the night of day 2. Feed was introduced without problems on day 3, and the lidocaine infusion was discontinued 24 hours later. Auscultation at that time revealed a grade 1/6 holosystolic murmur and a grade 3/6 decrescendo diastolic murmur over the area of the aortic valve. The double-layered appearance of the septum was evident during follow-up echocardiography, and Doppler ultrasonography revealed only mild aortic regurgitation. Administration of dexamethasone sodium phosphate was continued on a decreasing dosage schedule (120, 50, 30, and 20 mg/d on days 3 to 6, respectively), and treatment with prednisone (2.0 mg/kg, PO, q 24 h) was initiated on day 7. Additional hematologic and serum biochemical analyses on day 5 revealed lymphopenia (620 cells/µl, attributed to treatment with corticosteroids), an increase in serum total bilirubin concentration (3.8 mg/dl), and increases in muscle and hepatic enzyme activities (aspartate transaminase, 773 U/L; alkaline phosphatase, 313 U/L; reference range, 90 to 295 U/L; and l-iditol dehydrogenase, 16.6 U/L; reference range, 1.8 to 13.1 U/L).

Abdominocentesis on day 5 yielded fluid with a normal gross appearance (clear with a yellow tinge), and total protein concentration was estimated at < 2.5 g/dl, as determined on the basis of refractive index.

Intravenous administration of fluids was discontinued on day 4, and brief periods of controlled walking were allowed beginning on day 6. Analysis of telemetric ECG recordings revealed that these walks on day 6 were initially accompanied by transient episodes of ventricular tachycardia (heart rate, 130 to 140 beats/min), which returned to sinus rhythm (60 to 80 beats/min) within the first 5 minutes after the horse began to walk. On day 7 and subsequent days, the cardiac rhythm remained relatively stable (normal sinus rhythm with intermittent isolated premature ventricular depolarizations). On day 9, a treadmill exercise test (walk and trot) was attempted to determine whether it would be safe to transport the horse to the owner’s stable. Within 30 seconds after it started to walk on the treadmill, the horse developed ventricular tachycardia, and it collapsed shortly after removal from the treadmill. Although conversion to normal sinus rhythm was successful after IV administration of several boluses of lidocaine (total amount administered, 1,800 mg), failure of the horse to successfully complete the treadmill test supported a guarded to poor prognosis for return to performance. Consequently, on day 12, the owner requested that the horse be euthanatized.

Postmortem examination was limited to examination of the heart, which was subjectively assessed to be of normal size. All cardiac chambers and valves were normal, except for an endocardial defect located below the aortic root on the septal wall of the left ventricle. This defect appeared to be a rupture of a thick, yellow endocardial jet lesion (7 cm in length X 1.5 cm in width that was associated with long-standing aortic regurgitation), which continued dorsally as a tract (3 cm in length X 0.5 cm in width) into the septum and almost extended into the right atrium (Fig 3).

Furthermore, the rupture had produced a 3 X 1-cm flap of thick (2 mm) myocardium that could freely move to a subvalvular location. We believed this lesion was interpreted to be a thrombus during initial echocardiographic examination.

Histologic examination revealed focal areas of hemorrhage and profound fibrinocellular exudate along the edges of the tract. Beneath this exudate was a mix of mononuclear cell infiltration and areas of proliferating fibrous connective tissue and mineralization. Multifocal areas of acute myocardial necrosis, characterized by hyperesinophilia and loss of cross striations in myocardial fibers, were evident in the underlying myocardium.

Several times during the course of hospitalization, serum samples were obtained from the affected horse and several healthy horses. Some of the samples were frozen for subsequent analysis. Fresh and frozen-thawed serum samples were assayed for concentrations of the myocardial isoenzyme of CK (CK-MB) and cardiac troponin-I (cTnI). Commercially available ELISA kits were used to measure concentrations of cTnI and CK-MB; kits were used in accordance with the manufacturer’s instructions. The CK-MB assay had a limit of detection of 0.7 ng/ml in serum, and between-day and within-assay coefficients of variation (CV) for a CK-MB standard of 4 ng/ml were 10.3 and 6.0%, respectively, for the laboratory conducting the assays. According to the literature provided with the kit, a serum CK-MB concentration > 2.0 ng/ml in serum is considered to represent increased myocardial damage associated with acute myocardial infarction.
spiked with 2.5, 5.0, or 10 ng of human cardiac troponin I/ml.

Table 1—Recovery of human cardiac troponin I from equine serum

<table>
<thead>
<tr>
<th>Expected concentration (ng/ml)</th>
<th>Measured concentration (ng/ml)</th>
<th>Recovery (%)</th>
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<tbody>
<tr>
<td></td>
<td>Range</td>
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<tr>
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<td>3.2-4.3</td>
<td>3.7</td>
</tr>
<tr>
<td>10</td>
<td>7.6-8.5</td>
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Values reported represent data for serum samples obtained from 3 healthy horses.

Regurgitation are common in older horses,34 we are not aware of any reports in which rupture of an endocardial jet lesion and tract formation into the interventricular septum have led to ventricular tachycardia. In retrospect, the double-layered echocardiographic appearance of the interventricular septum was a result of the tract being filled with blood. Similarly, movement of the myocardial flap to a subvalvular location during ejection gave the appearance of a subvalvular thrombus. Medical history, clinical signs, and echocardiographic findings in the horse reported here were similar to those reported for horses with aortocardiac fistulas.

Rupture of the right aortic sinus in horses with aortocardiac fistulas can produce a dissecting tract or fistula into the right atrium, right or left ventricle, the interventricular septum, or a combination of these areas. Aortocardiac fistulas have been most commonly found in middle-aged or old stallions and geldings. Acute distress, monomorphic ventricular tachycardia, and loud (grade 3/6 to 6/6) continuous right-sided murmurs are detected in most affected horses. Similar cardiac lesions have been reported in middle-aged men and are presumed to be the result of a congenital aortic anomaly that leads to formation of an aneurysm in the right aortic sinus.5,30 Subsequent rupture leads to development of a dissecting tract into or through the interventricular septum. Formation of an aneurysm in the right aortic sinus and development of aortocardiac fistulas also has been reported in horses. However, it is most common that predisposing cardiac lesions have not been detected in horses with aortocardiac fistulas. Furthermore, a history of aortic regurgitation was found in only 1 previously reported aortocardiac fistula.7 After restoration of normal sinus rhythm in the horse reported here, the lack of a systolic or continuous murmur was surprising, because the endocardial flap should have produced turbulence. Interestingly, postmortem examination in another horse with an aortocardiac fistula in which only a low-grade murmur was detected revealed a dissecting tract that communicated with the right atrium and left ventricle.7

Ventricular tachycardia can develop as a result of electrolyte disturbances, sepsis or toxemia, or an underlying cardiomyopathy, and horses with multi- or rapid monomorphic ventricular tachycardia (heart rate > 100 beats/min) reportedly have a poor prognosis for survival.13,12 Increases in serum CK-MB activity have been reported as a method to document myocardial damage in horses with ventricular tachycardia.15 However, only 1.3% of the total CK activity in an equine heart is attributable to CK-MB,16 in contrast to up to 20% in a human heart17; therefore, it is a relatively insensitive marker of myocardial damage in horses. Thus, it is not surprising that serum CK-MB concentrations of the horse with ventricular tachycardia reported here, as well as several healthy horses, were less than the limit of detection for the assay, although a lack of specificity of the human assay for equine CK-MB was not ruled out. Furthermore, interpretation of serum CK-MB activity can be complicated by increases in total CK activity as well as reexpression of CK-MB in situations with damaged skeletal muscle or chronic renal disease.18,19 Also, a relatively short half-

myocardial infarction in humans. Initially, assays of a frozen (−20 C) serum sample obtained at admission and a fresh serum sample (stored at 4 C) obtained on day 5 had CK-MB concentrations of < 0.7 ng/ml for both samples, and cTnI concentrations were 5.9 and 4.3 ng/ml for samples obtained at admission and on day 5, respectively. A second assay was performed with frozen serum samples obtained at admission and on day 5 and a fresh serum sample obtained on day 9 (4 hours after the treadmill test) as well as fresh serum samples obtained from 2 healthy horses. Again, CK-MB concentrations were < 0.7 ng/ml for all samples, and concentration of cTnI was 5.3, 4.4, and 5.8 ng/ml for samples obtained at admission and on days 5 and 9, respectively. In contrast, cTnI concentrations were < 0.3 ng/ml in sera of both healthy horses. In a third assay, serum concentration of cTnI was measured in samples obtained from an additional 5 healthy horses (including a serum sample obtained 12 hours after one of the horses exercised by running on a treadmill), and all were < 0.3 ng/ml, except for 1 sample (not the postexercise sample) that had a cTnI concentration of 1.0 ng/ml. Analysis of these results suggested that cTnI concentrations were consistently high in our horse with myocardial necrosis, whereas increases in serum concentrations of CK-MB did not happen or were not detectable with the assay used. To our knowledge, this is the first report of an increase in cTnI concentration in a horse with cardiac lesions.

Because the cTnI assay was not validated for use on equine sera, except for concurrent assay of sera from healthy horses described here, serum samples collected from 3 healthy horses were spiked with varying amounts of human cTnI (assay standard). Recovery ranged from 64 to 88%, indicating that the assay accurately detected cTnI (Table 1).1 Because of a lack of purified equine cTnI, absolute assay specificity could not be established; however, cTnI appears to be highly conserved among mammalian species (96.4% DNA sequence homology between human and bovine cTnI).

Furthermore, a similar assay, conducted by use of a murine antibody raised against human cTnI, detected a cTnI-like compound in equine cardiac tissue.7 In that report, cross-reactivity with equine skeletal muscle homogenates was < 1%. When considered in combination, the spiking-recovery data reported here and information contained in the other report1 provide strong support that the assay used for this report detected a human cTnI-like compound that, in all likelihood, was equine cTnI.

Although diastolic murmurs associated with aortic
life for increased CK activity (including CK-MB activity), which usually peaks within 24 hours after an injury and returns to baseline values within 2 to 3 days, results in a narrow window of opportunity for use of this diagnostic test.

In a search for a sensitive and specific marker for myocardial damage that has a broad diagnostic window, recent interest has focused on the cardiac troponins.13–22 Troponin-C, I, and T are components of the tropomyosin-troponin complex associated with the actin filaments in striated (cardiac and skeletal) muscles. However, cTnl and cardiac troponin T (cTnT) have differing amino acid sequences at their N-terminus, compared with that for skeletal muscle isoforms. Thus, use of immunoassays directed at the N-terminus can differentiate between cardiac and skeletal muscle troponin isoforms.14,15 A small fraction of the cardiac isoforms also found free in the cytosol of cardiac myocytes (cTnl, 3 to 5%; cTnI, 6 to 8%); consequently, myocardial damage will result in an early increase in serum concentrations of cTnl and cTnI (release of cytosolic fraction), which can persist for several days as a result of additional release from degenerating myofibers. Recently, studies in human myocardial damage, as determined on the basis of ECG and echocardiographic changes, in comparison with CK-MB activity or CK-MB concentration.14,16–21 The lack of an increase in serum cTnl concentration in human athletes following marathon runs (ie, at a time when serum CK activity is increased) provides further support that skeletal muscle damage does not result in an increase in serum cTnl concentration.15,16

Considerable homogeneity of the N-terminal gene sequence for cTnl among mammalian species has been documented.21–23 In fact, homogeneity of cTnl of dogs and large animals (cattle, horses, and pigs) is greater with human cTnl than for cTnl of laboratory animals. In a recent study in which investigators used an immunoassay similar to the one used by the laboratory that conducted the testing in our report, equine cardiac tissue had a cTnl content of 9.1 ± 0.1 mg/g of heart tissue (150% the value for human myocardial tissue).2 Combined with the cTnl results reported here, analysis of these findings supports a potential for use of human cTnl immunoassays as a diagnostic aid in assessment of myocardial damage in horses.

References