Measurement of plasma cardiac troponin I concentration by use of a point-of-care analyzer in clinically normal horses and horses with experimentally induced cardiac disease

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Objective—To compare cardiac troponin I (cTnI) concentrations determined by use of a point-of-care analyzer with values determined by use of a bench-top immunoassay in plasma samples obtained from clinically normal horses with and without experimentally induced cardiac disease, and to establish a reference range for plasma equine cTnI concentration determined by use of the point-of-care analyzer.

Animals—83 clinically normal horses, 6 of which were administered monensin to induce cardiac disease.

Procedures—A blood sample was collected from each of the 83 clinically normal horses to provide plasma for analysis by use of the point-of-care analyzer; some of the same samples were also analyzed by use of the immunoassay. All 83 samples were used to establish an analyzer-specific reference range for plasma cTnI concentration in clinically normal horses. In 6 horses, blood samples were also collected at various time points after administration of a single dose of monensin (1.0 to 1.5 mg/kg) via nasogastric intubation; plasma cTnI concentration in those samples was assessed by use of both methods.

Results—The analyzer-specific reference range for plasma cTnI concentration in clinically normal horses was 0.0 to 0.06 ng/mL. Following monensin treatment in 5 horses, increases in plasma cTnI concentration determined by use of the 2 methods were highly correlated (Pearson correlation, 0.83). Peak analyzer-determined plasma cTnI concentrations in monensin-treated horses ranged from 0.08 to 3.68 ng/mL.

Conclusions and Clinical Relevance—In horses with and without experimentally induced cardiac disease, the point-of-care analyzer and bench-top immunoassay provided similar values of plasma cTnI concentration. (*Am J Vet Res* 2010;71:55–59)

Cardiac troponin is a highly sensitive and specific biomarker of myocardial injury in humans. In patients with myocardial infarction, sepsis, or traumatic myocardial injury, assessment of circulating cTnI concentration is used to aid diagnosis and provide prognostic information.[1–3] Blood concentrations of cTnI in clinically normal cats and dogs have been reported as well as cTnI concentrations in cats and dogs with gastric dilatation and volvulus,[4] congestive heart failure,[5] neoplasia (and receiving chemotherapy),[6] heatstroke,[7] cardiomyopathy, and pericardial disease.[8–10,a] Data regarding circulating cTnI concentrations in horses are limited except for reports of assessments made in Thoroughbreds in training or on pasture[11–13] and in a few cases of myocarditis,[14] cantharidin toxicosis,[15] ruptured aortic jet lesions,[16] snake envenomation,[b] airway obstruction,[17] babesiosis,[18] electrical cardioconversion,[19] sepsis,[20] and monensin intoxication.[21] Various bench-top methods were used for the analysis of cTnI concentrations in those studies. More recently, a point-of-care analyzer[c] has become available and was validated for measurement of whole blood cTnI concentration in humans.[22] However, it is unknown whether this point-of-care analyzer would accurately determine circulating cTnI concentrations in horses. The purpose of the study reported here was to compare cTnI concentrations determined by use of a point-of-care analyzer with values determined by use of a bench-top immunoassay[d] in plasma samples obtained from clinically normal horses and horses with experimentally induced cardiac disease. Values in clinically normal horses were acquired to establish a reference range for plasma equine cTnI concentration determined by use of the point-of-care

Abbreviation

cTnI	Cardiac troponin I

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analyzer. If the point-of-care analyzer could be used to detect changes in plasma cTnI concentration in sick horses, we would anticipate that its application would be of benefit in the evaluation of myocardial injury in horses in field settings.

Materials and Methods

Horses—Eighty-three clinically normal horses that were either part of a teaching herd at Cornell University's Equine Research Park or admitted for performance evaluation at the equine performance testing center were included in the study. The study protocol and care of the horses were approved by the Cornell University College of Veterinary Medicine Institutional Animal Care and Use Committee.

The 83 horses included both sexes (53 females and 30 males [10 geldings]). The horses were 1 to 31 years old; 40 horses were 1 to 10 years old, 33 horses were 11 to 20 years old, and 10 horses were ≥ 20 years old. There were 19 mixed-breed horses, 12 Quarter Horses, 4 Standardbreds, 26 Thoroughbreds, and 22 warmbloods. From each of the 83 horses, a blood sample (4 mL) was collected to obtain plasma; cTnI concentration in each plasma sample was determined by use of a point-of-care analyzer. In 21 of the samples, the cTnI value was measured a second time at 10 minutes after the first measurement to determine repeatability. Cardiac troponin I concentrations in the plasma samples obtained from 5 horses treated with monensin[e] were also determined by use of a bench-top immunoassay. Physical examination revealed no abnormal findings in any horse at the time of the initial blood sample collection.

A subset of 6 horses (4 geldings and 2 females; age range, 5 to 12 years) that were no longer required for use in the university's teaching and research programs was selected to undergo experimental induction of cardiac disease after the initial blood sample collection. Each of the 6 horses was administered a single dose of monensin (1 to 1.5 mg/kg) via nasogastric tube. Monensin was administered in different dosages and vehicles (corn oil[f] or water) to horses that were fed (n = 2) or from which food was withheld (4) so that a wide range of plasma cTnI concentrations would be assessed, thereby ensuring that the point-of-care analyzer data were accurate at both low and high cTnI concentrations, compared with results of bench-top testing. From each of the monensin-treated horses, a blood sample (4 mL) was collected immediately before (0 hours) and at 0.5, 1, 3, 6, 12, 24, 36, 48, 72, and 96 hours after monensin administration. Further sample collection was performed in horses that had high heart rates at 96 hours until the heart rate returned to the pretreatment rate. Throughout the 96-hour study period following administration of monensin, each horse was monitored for any abnormal clinical signs, and the heart rate was determined at 6-hour intervals. As part of the study design, horses that developed any signs of fulminant heart failure (coughing, jugular pulses, edema, or dyspnea at rest) were euthanatized that day via IV injection of pentobarbital sodium,[g] and a complete necropsy was performed.

Plasma cTnI analysis—Via jugular venipuncture, blood samples were collected in tubes containing lithium heparin; samples were immediately centrifuged for 5 minutes, and plasma was frozen at –70°C for storage prior to measurement of cTnI concentration. The samples were analyzed in batches within 1 month after completion of the experimental procedures. Plasma cTnI concentrations were determined by use of the point-of-care analyzer, and results were compared with concentrations determined by use of a bench-top immunoassay. The immunoassay involved 2 murine monoclonal antibodies against human cTnI and had a lower limit of detection of 0.01 ng/mL. The point-of-care analyzer used a 2-site ELISA with 2 monoclonal antibodies (1 caprine and 1 murine) against human cTnI and had an analytic sensitivity of 0.02 ng/mL and a reportable range of 0.0 to 50 ng/mL (greater values are reported as > 50 ng/mL). Use of the analyzer followed the technical guidelines of the manufacturer. The point-of-care analyzer required 16 to 22 µL of plasma/analysis.

Data and statistical analysis—The values of plasma cTnI concentration obtained by use of the 2 different methods for the 67 samples collected from 5 of the 6 monensin-treated horses were compared. Samples from the horse administered the highest dose of monensin (1.5 mg/kg) could not be used because plasma cTnI concentration increased to > 50 ng/mL as determined by use of the point-of-care analyzer. Because the exact concentration was not known, the data could not be compared with the bench-top immunoassay data. The concentrations were determined for samples collected immediately before and at intervals after monensin administration.

A reference range for plasma cTnI concentration determined by use of the point-of-care analyzer was established from samples obtained from 83 clinically normal horses; the reference range was based on the range of concentrations for the entire group. The repeatability was determined by obtaining 2 consecutive readings of cTnI concentration in each sample from each of 21 horses; data were assessed by use of a paired *t* test.

Calculation of Pearson correlation coefficients was performed to assess the correlation between plasma cTnI concentrations determined by use of the point-of-care and bench-top methods in samples obtained from the monensin-treated horses. The same statistical method was used to evaluate the correlation between cTnI concentration and the age of the clinically normal horses as a group. The impact of the sex and breed of the horse on the analyzer-determined concentration measurement was evaluated by use of a Kruskal-Wallis 1-way ANOVA. Computer software was used to perform all the statistical analyses.[h] For all analyses, a value of $P < 0.05$ was considered significant.

Results

Blood samples were successfully collected from each of the 83 horses and from the subset of 6 horses before and at all scheduled time points after monensin administration. Cardiac troponin I concentration was measurable in plasma samples by use of both the point-of-care analyzer and bench-top immunoassay.

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The plasma samples obtained from the monensin-treated horses were assessed by use of the point-of-care analyzer and the bench-top immunoassay, and correlation between concentration measurements derived by use of the 2 methods was high ($r = 0.83$; $P < 0.001$; **Figure 1**). Frequency distribution of plasma cTnI values was the same for both methods, although the absolute plasma cTnI values were not always the same. In the 83 clinically normal horses, the range of plasma cTnI concentrations determined by use of the point-of-care analyzer was 0.0 to 0.06 ng/mL; 92% of the cTnI values ranged from 0.0 to 0.02 ng/mL, and 95% of the values ranged from 0.0 to 0.03 ng/mL (**Figure 2**). The mean ± SD plasma cTnI concentration was 0.0 ± 0.01 ng/mL. The Pearson analysis of data for all 83 horses revealed no correlation of plasma cTnI concentration with age ($R = -0.098$, which for all practical purposes was considered zero). By use of a Kruskal-Wallis 1-way ANOVA for nonparametric data, plasma cTnI concentrations did not differ by breed ($P = 0.73$) or sex ($P = 0.71$).

In plasma samples obtained from 21 of the 83 horses, the repeatability of the point-of-care analyzer testing was assessed as the difference between 2 consecutive analyzer-determined plasma cTnI concentration measurements. If the test was 100% repeatable, the difference between the 2 readings for any plasma sample should be zero. This hypothesis was tested by use of a paired *t* test, and the resultant value was $P = 0.72$. Plasma cTnI concentrations in 70% of the 21 samples for which repeated measurements were available differed by only 0.01 ng/mL (**Figure 3**).

In the subset of 6 monensin-treated horses, plasma cTnI concentrations immediately before monensin administration were < 0.05 ng/mL, and values became abnormally elevated in 5 of the horses between 24 and 72 hours after monensin administration, indicating that monensin caused myocardial injury in these horses. One horse died suddenly 83 to 84 hours following monensin administration. Because of an increase in plasma cTnI concentration (> 50 ng/mL) as determined by use of the point-of-care analyzer within 24 hours after monensin administration, data from this horse could not be used for comparison with data obtained by use of the bench-top immunoassay. Two horses had minimal increases in plasma cTnI concentration (0.08 and 0.11 ng/mL) and remained clinically normal. The peak plasma cTnI concentrations from the 5 horses used to compare the point-of-care analyzer and bench-top immunoassay values ranged from 0.08 to 3.68 ng/mL (50 ng/mL being the maximum numerical value displayed by the point-of-care analyzer). Signs of cardiac disease varied among the 6 horses that received monensin. In 4 of the 6 monensin-treated horses, an increase in heart rate developed, compared with pretreatment rates. These 4 horses had initial increases in plasma cTnI concentration within 24 to 60 hours after monensin administration. Two horses were euthanatized 5 days and 7 months, respectively, following monensin administration because of cardiac dysfunction and clostridial diarrhea in 1 horse (present at study termination) and because of cardiac dysfunction in the other horse. At the time of euthanasia, plasma cTnI concentrations remained high in these 2 horses. At necropsy, myocyte necrosis was detected in the 3 horses that died or were euthanatized. Another horse had tachycardia for 9 days but subsequently recovered clinically.

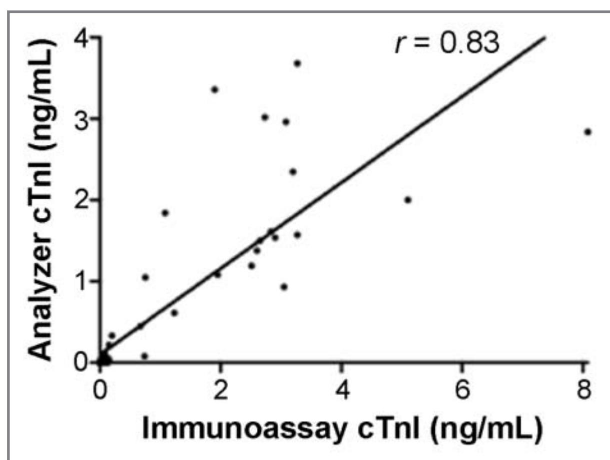

Figure 1—Correlation between cTnI concentration in plasma samples obtained from 5 horses before and after monensin administration by use of a point-of-care analyzer and a bench-top immunoassay. Correlation between the 2 methods was high ($r = 0.83$; $P < 0.001$).

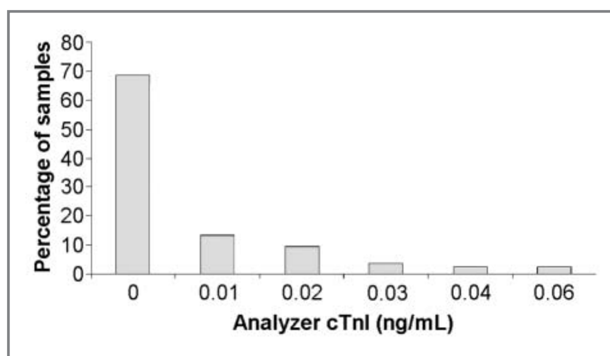

Figure 2—Distribution (%) of cTnI concentrations in plasma samples obtained from 83 clinically normal horses determined by use of a point-of-care analyzer. In 92% of samples, the cTnI concentration was 0.0 to 0.02 ng/mL; in 95% of samples, the cTnI concentration was 0.0 to 0.03 ng/mL.

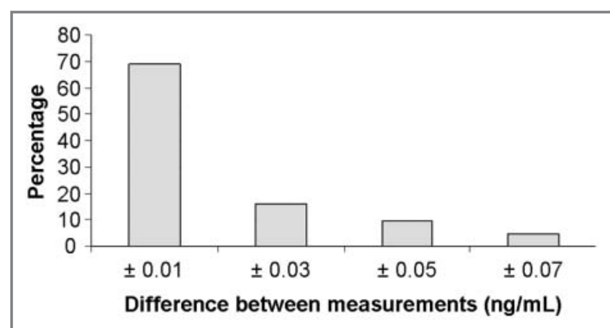

Figure 3—Data regarding the repeatability of the point-of-care analyzer for determination of cTnI concentrations on 2 occasions in plasma samples obtained from 21 clinically normal horses (42 measurements). In 70% of the samples, the repeated measures of cTnI concentration differed by only 0.01 ng/mL; variation between consecutive measurements obtained by use of the analyzer was considered minimal.

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Discussion

On the basis of results of the present study, the point-of-care analyzer appears to be a quick and convenient method for measurement of plasma cTnI concentration in horses, and analyzer-determined values are comparable to those obtained by use of a bench-top immunoassay. Although both assessment methods were initially developed for use in humans, it was not surprising that they were useful for assessment of plasma cTnI concentrations in horses. The amino acid sequence of equine cTnI has > 90% similarity to the sequences of human, canine, and feline cTnI proteins, and the epitopes detected by commercial analyzers are highly conserved among the latter 3 species.[10,23,24]

In clinically normal horses, age, breed, and sex had no effect on plasma cTnI concentration measurements. Frozen heparinized samples of plasma were analyzed in the present study. It is known that cTnI or cardiac troponin T is stable for at least 1 year in serum samples stored at –70°C.[25] The use of other anticoagulants such as EDTA, oxalate, or citrate interferes with the assay, resulting in falsely low cTnI concentration measurements. The point-of-care analyzer has been validated for measurement of cTnI concentration in fresh whole blood samples from humans[22] (ie, measurement within 1 to 2 minutes after acquiring the sample), and results are available within 10 minutes, which may be most applicable to clinical practice. In addition to the cTnI assay, the point-of-care analyzer can be used to measure circulating concentrations of electrolytes, glucose, and biochemical analytes and to assess blood gas variables and Hct.

The performance characteristics of the point-of-care analyzer for analysis of human plasma samples and heparinized samples of whole blood spiked with human cTnI have been previously determined (according to the manufacturer's instrument specifications). The purpose of our study was to determine whether the analyzer could be used to measure equine plasma cTnI concentration in a predictable manner and detect concentration increases following experimentally induced acute cardiac damage in clinically normal horses. To assess reliability of the analyzer assay for use in horses, measures of repeatability (precision testing) were performed. There was good agreement between repeated measurements of samples in our study. We did not attempt to correlate plasma cTnI concentrations with severity of clinical disease (eg, arrhythmias, cardiac function, and heart size). However, in monensin-treated horses, plasma cTnI concentration increased early after monensin administration and remained elevated, at least until heart rate returned to pretreatment values, which was consistent with acute myocardial injury. Further studies are needed to assess the effect of renal function or other commonly used drugs on plasma cTnI concentration in horses.

Although the results of the present study indicated that the point-of-care analyzer detects cTnI in equine plasma samples, the exact epitope that it targets is not known because that is proprietary information. Differences in absolute value determined by use of the point-of-care analyzer and the bench-top immunoassay might be attributable to a difference in targeted amino acid sequences, the lack of mass standardization, and heterogeneity in the cross-reactivity of antibodies against various troponin forms.[26–28] The results of the present study cannot be extrapolated to other available cTnI assays. Thus, it remains a concern when a clinician attempts to compare results from 1 laboratory or assay with those from another.

Horses in the present study were administered monensin to establish that cardiac muscle necrosis was associated with increased plasma cTnI concentration and that increases in cTnI concentration were detectable by use of the point-of-care analyzer, and to compare the 2 testing methods over a wide range of cTnI concentrations. The development of severe myocyte necrosis and even clinical heart failure in 3 of the horses was unexpected because monensin was administered at a dose equivalent to only 50% of the reported LD_{50}.[29] Procedural differences between the present study and that investigation to determine the LD_{50} of monensin in horses may be the reason that those horses became severely affected at this dose. In the present study, the vehicle for monensin administration was vegetable oil and food was withheld from 4 of the 6 horses prior to treatment; in the LD_{50} investigation, the vehicle for monensin administration was mineral oil and food was not withheld from the horses prior to treatment. In our study, no effort was made to evaluate prognosis in relation to plasma cTnI concentration. On the basis of our limited clinical experience with assessment of plasma cTnI concentrations in critically ill hospitalized horses, we believe that change in cTnI concentration during hospitalization is of greater prognostic value than is magnitude of cTnI concentration at the time of admission. This is similar to the prognostic value of circulating lactate concentration in critically ill hospitalized horses.[i]

Overall, the results of the present study indicated that the point-of-care analyzer allows rapid, portable, and accurate detection of cTnI in equine plasma samples. Stall-side assessment of this cardiac biomarker in horses should enhance the ability of practicing clinicians to detect myocardial damage and aid in the management and treatment of horses with cardiac disease.

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