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Biochemical Markers of Cardiac Injury in Normal, Surviving Septic, or Nonsurviving Septic Neonatal Foals

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The cardiac biomarkers cardiac troponin T (cTnT) and I (cTnI) and the cardiac isoenzyme of creatine kinase (CKMB) are used extensively in human medicine to diagnose and provide valuable prognostic information in patients with ischemic, traumatic, and septic myocardial injury. We designed a study to establish normal values for these markers in healthy, neonatal foals and to compare them with values obtained from septic neonates in a referral hospital population. The 25th, 50th, 75th, and 95th percentiles for cTnI and CKMB in the healthy-foal population were 0.08, 0.14, 0.25, 0.49 ng/mL and 1.4, 2.3, 4.0, 7.4 ng/mL, respectively. The values obtained for cTnT were frequently (43/52 foals; 83%) below the lower limit of detection of the assay (0.009 ng/mL), but the median and range were 0.009 and 0.009–0.041 ng/mL, respectively. In the septic foal population, the 25th, 50th, 75th, and 95th percentile values for cTnI and CKMB were 0.05, 0.12, 0.22, and 1.10 ng/mL and 2.0, 4.4, 7.8, and 24 ng/mL, respectively. The values obtained for cTnT were less frequently below the lower limit of detection (23/38 foals; 60%) compared with the healthy foal population, and the median and range were 0.009 and 0.009–0.20 ng/mL, respectively. Significantly higher values were observed for cTnT and CKMB in septic foals compared with the healthy neonatal foal population, but there were no differences among septic foals in survivors compared with nonsurvivors. These findings suggest that myocardial injury occurs during septicemia in neonatal foals but that the injury is not associated with survival among septic foals.

Key words: Cardiac troponin I; Cardiac troponin T; CKMB; Myocardial injury; Sepsis.

Biochemical markers of myocardial injury in humans are commonly used diagnostic and prognostic tools in the evaluation of patients with acute myocardial injury associated with ischemic damage due to infarction.[1–3] Similarly, certain cardiac biomarkers (particularly cardiac troponin I [cTnI]) have been demonstrated to become elevated during septic shock in both adults and children.[4,5] In veterinary medicine, commercial assays designed to detect human cardiac troponin have been used to establish normal cTnI ranges for dogs and cats[6] as well as cTnI and cardiac troponin T (cTnT) values for dogs with congestive heart failure, asymptomatic cardiomyopathy, or undergoing doxirubicin therapy[7] and cats with hypertrophic cardiomyopathy.[8] Investigation of cTnI and cTnT elevations associated with infectious disease has been limited to a single study analyzing these cardiac biomarkers in canine babesiois.[9] Investigation of cardiac biomarkers in the horse have been limited, although recently, Phillips et al[10] established a normal reference range for cTnI in normal adult Thoroughbreds in training or at pasture. Case reports documenting cTnI elevations in a horse with myocarditis[11] and in a horse with a ruptured aortic jet lesion[12] have also been published. Cardiac troponin I levels were also measured and found to be similar to healthy controls in 2 horses undergoing electrical cardioversion for atrial fibrillation.[13] However, there is variation in the commercial assays used in these studies, and although these assays have not been validated for use in the horse due to the lack of isolated and purified equine cardiac troponin, the molecular structure of cTnI is highly conserved across mammalian species.[14] There are no reports of cTnT values in the horse and no reports of either cTnT or cTnI values in neonatal foals. Similarly, there is little information regarding the myocardial isoenzyme of creatine kinase (CKMB) in adult horses, although it increases in cases of myocardial injury associated with monensin toxicosis[15,16] and has been documented to increase in cases of aortic root disease.[17]

Neonatal septicemia is the leading cause of death in foals during the first week of life and, although survival rates within referral hospitals have increased over the last few decades, there is still much about the pathophysiology of the systemic inflammatory response syndrome that occurs during sepsis in foals that is not known. Despite aggressive treatment, up to 50% of foals with sepsis still die and satisfactory pathophysiologic explanations for the cause of death in some of these foals are not always forthcoming. A better physiologic understanding of the multiple-organ dysfunction encountered in many nonsurviving septic neonates alongside earlier detection of cardiovascular and cardiopulmonary dysregulation might assist clinicians both therapeutically and prognostically. As a consequence, we designed a prospective study to establish normal reference limits for the cardiac biomarkers CKMB, cTnI, and cTnT in healthy newborn foals and to compare these variables in surviving and nonsurviving septic neonates.

Materials and Methods

Fifty two clinically normal foals between 12 and 48 hours of age were determined to be nonseptic and free of cardiac disease based on

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Table 1. Distribution of cTnI, cTnT, and CK-MB in normal and septicemic neonatal foals.

	Controls (n = 52)			Septicemic (n = 38)		
	cTnI (ng/mL)	cTnT (ng/mL)	CKMB (ng/mL)	cTnI (ng/mL)	cTnT (ng/mL)	CKMB (ng/mL)
Minimum	0.01	0.009	0.40	0.01	0.009	0.50
25th percentile	0.08	0.009	1.3	0.05	0.009	2.0
Median	0.14	0.009	2.3	0.12	0.009	4.4
75th percentile	0.25	0.009	4.0	0.22	0.05	7.8
95th percentile	0.49	0.03	7.4	1.10	0.16	24
Maximum	0.51	0.041	9.3	1.3	0.20[a]	26[a]

[a] The mean ranking was higher for septicemic foals than for control foals for cTnT ($P = .004$) and CKMB ($P = .005$).

physical examination, CBC data, and serum IgG concentrations that were greater than 800 g/dL.[a] The foals were examined and sampled by jugular venipuncture either at the time of admission to the veterinary teaching hospital at the University of Wisconsin (n = 14) as healthy foals accompanying sick mares or at the time of new foal check on farms in southern Wisconsin (n = 16) and central Kentucky (n = 22). There were 28 males and 24 females. Breed distribution was 27 Thoroughbreds, 8 Arabians, 7 Quarter Horses, 4 Warmbloods, 2 Paints, 1 Andalusians, 1 National Showhorse, 1 Norwegian Fjord, and 1 grade horse.

The septic foals enrolled in the study came from admissions to the large-animal hospital at the University of Wisconsin over the 2002 and 2003 foaling seasons. Thirty-eight foals were determined to be septic by virtue of a sepsis score of greater than 11. The sepsis score was calculated using clinical and clinicopathologic data obtained at admission to the hospital using the system developed by Brewer and Koterba.[18] These authors identified a sensitivity of 93% and a specificity of 86% for their scoring system when compared with the gold standards of positive blood culture or culture-positive sites of infection. In a recent multiyear prospective study involving 75 neonatal admissions to our own neonatal intensive-care unit, this scoring system has demonstrated a sensitivity of 76% and a specificity of 58% when compared only with the gold standard of blood culture (Peek, personal communication). Nineteen of the 38 foals categorized as septic on the basis of sepsis score had positive blood cultures obtained at admission, while the other 19 were blood-culture negative. Foals in the septic group ranged from 6 hours to 7 days of age, with a median age of 48 hours at the time of admission and blood sampling for cardiac-enzyme evaluation.

For both foal populations, serum samples were centrifuged within 2–4 hours of collection, the samples aliquoted and stored at −20°C, and later batched for analysis. Cardiac troponin I was measured by the ACCESS® Immunoassay,[b] a second-generation, two-site chemiluminescent assay that uses 2 mouse monoclonal antibodies directed against human cTnI. The lower limit of detection for this assay is 0.01 ng/mL. Cardiac troponin T was measured using the Elecsys 2010® Immunoassay,[c] a third-generation, two-site electrochemiluminescent assay that uses 2 mouse monoclonal antibodies directed against human cTnT. The lower limit of detection for this assay is 0.009 ng/mL. CKMB mass measurements were performed using the Elecsys 2010® assay,[c] with a lower limit of detection of 0.1 ng/mL.

Statistical Analyses

The distributions of cTnI, cTnT, and CKMB within the normal and septic foal populations were described as the minimum and maximum values, alongside the 25th, 50th, 75th, and 95th percentiles. Comparisons between values obtained in normal and septic foal populations for each biochemical marker measured were made using 2-sided Wilcoxon's rank-sum tests. Specifically, cTnI, cTnT, and CKMB were compared between all septic foals and the normal foal population as well as between surviving and nonsurviving septic foals. Significance was set at $P < .05$. Comparisons were also made for each of the variables within the septic group between those foals that were blood-culture positive (n = 19) and those that were blood-culture negative (n = 19) using the same statistical methodology. The potential for correlation between cTnI or CKMB and the numerical value of the sepsis score was investigated using the Spearman's rho rank correlation coefficient, with significance set at $P < .05$. Survival rates were compared between bacteremic and nonbacteremic septic groups using the chi-squared statistic, with significance again set at $P < .05$.

Results

Of the 38 septic foals enrolled in the study, 19 were blood-culture positive and 19 were blood-culture negative. Thirteen of the foals with positive blood cultures had single gram-negative isolates, 3 had single gram -positive isolates, and 3 had more than one bacterial species obtained from admission samples. For all septic foals, the overall survival rate was 23/38 (61%). Although the survival rate in blood-culture–positive foals was 10/19 (52%) compared with 13/19 (68%) in the blood-culture–negative group, there was not a statistically significant difference in survival between these two groups ($P > .05$).

Significantly increased values were observed for cTnT ($P = .004$) and CKMB ($P = .005$) in septic compared with healthy neonatal foals (Table 1). There were no differences in nonsurviving septic foals compared with the surviving group. No significant differences ($P \geq .08$) in cTnI, cTnT, and CKMB were noted between bacteremic and nonbacteremic foals within the septic-foal population. No statistically significant correlation was observed between the value of the sepsis score and CKMB or cTnI levels.

Discussion

There are several commercially available assays for the detection of cTnI and cTnT in the serum or plasma of human patients. Previous case reports documenting cTnI values in the equine literature have varied in the type of commercial assay used.[10–13] For this study, we employed immunoassays that were specifically developed to detect human cTnI and cTnT using mouse monoclonal antibodies directed against the human subunit proteins. This same methodology was used in the study by Phillips et al[10] documenting cTnI values in pastured and race-training thoroughbreds, but different from the enzyme-linked immunosorbent assays (ELISAs) used in the case reports by Schwarzwald et al,[11] Cornelisse et al,[12] and Frye et al.[13] Although the mouse immunoassays are designed for human

use, there is good reason to anticipate cross reactivity based on the highly conserved nature of the cardiac troponins across mammalian species.[14] Furthermore, a similar mouse cTnI immunoassay designed for human diagnostic use had a demonstrated ability to detect cTnI in equine cardiac tissue while not cross reacting with equine skeletal troponin I.[19] Both the ELISAs and the chemiluminescent immunoassays used in our study have importantly reduced previous problems in cross reactivity with skeletal muscle troponin and therefore demonstrate greater specificity for myocardial injury than previously used enzymatic markers such as CKMB and lactic dehydrogenase.[20,21] Although tissue-specific markers for myocardial necrosis are primarily used in the diagnosis of acute myocardial infarction in human patients,[1,2] of considerable relevance to our study is the fact that elevations in cardiac troponins occur in human patients with nonischemic heart disease, including adults and children with sepsis and septic shock.[22,23] Elevations in these markers have been reported to correlate with increased mortality in pediatric patients with sepsis.[24,25] We demonstrated that septic foals have significantly higher cTnT and CKMB levels compared with healthy neonatal foals. This suggests that acute cardiac injury is a component of the multiple-organ dysfunction syndrome observed in foals suffering from sepsis. It is worth noting that our definition of septic was based on the scoring system initially developed by Brewer and Koterba,[18] which has recently been questioned as to its accuracy.[26] When measured against the more stringent gold standard of proven bacteremia, this scoring system has diminished sensitivity (76 versus 93%) and specificity (58 versus 86%) in our hands compared with Brewer and Koterba.[18] However, defining septic patients as being only those that are demonstrably bacteremic fails to correctly identify many and hence our sensitivity and specificity data should be taken as only approximate values for comparative purposes. It is possible, therefore, that we incorrectly identified some foals as septic; however, our own data has demonstrated that the higher the sepsis score the poorer the chances of survival in critically ill foals admitted to our hospital,[27] such that we at least used a defining criterion for sepsis that correctly identifies the most critically ill individuals. It was not possible to adequately investigate with any statistical power whether the type of bacterial species isolated (gram-positive versus gram-negative) made an important impact on the magnitude of cardiac biomarker elevation due to the comparatively low numbers of foals (3 of 19) that had single gram-positive isolates from blood cultures obtained at admission. Thiru et al[24] demonstrated that cardiac biomarkers increased in human pediatric patients with sepsis due to streptococcal infection and that this correlated with survival, although none of the foals in this study were blood-culture positive with *Streptococcus* spp; further study in foals is merited to investigate whether gram-negative infections, which are the most common form of sepsis in foals, carry a greater or lesser prognosis.

Consistent and detailed pathologic investigation of nonsurviving septic foals was not available for foals in the current study. Detailed gross and histologic postmortem information was available from 5 of the 15 nonsurviving septic foals; in 2 of the 5, there were no gross or histologic lesions noted, while the other 3 demonstrated cardiac lesions that included fibrinous myocarditis, pericardial effusion with both myocardial and endocardial necrosis, and endocardial necrosis, respectively. The cTnI values for each of these 5 foals were above the median but less than the 75th percentile value for cTnI in the whole septic foal population. For CKMB, the admission values for these 5 nonsurvivors were between the median and 75th percentile for 4 individuals and between the 75th and 95th percentiles for 1 foal (foal with endocardial necrosis) when compared with the entire septic foal population. In 4 of the 5 nonsurvivors for which detailed pathology was available, cTnT was undetectable, and in 1 nonsurvivor (with pericardial effusion, myocardial and endocardial necrosis), it was 0.049, the third highest value in the study. The small number of foals for whom specific cardiac postmortem information is available preclude a useful statistical analysis, but the fact that each of the 3 foals that had demonstrable cardiac lesions at postmortem had elevated cTnI and CKMB values compared with the rest of the septic foal population suggest that this is an area that merits further study. Due to the lack of comprehensive and confirmatory postmortem data, it is important to consider noncardiac sources of cTnI, cTnT, and CKMB in our patient population. In humans, cTnT can be expressed in diseased and healthy skeletal muscle, but even in diseased muscle states, cTnT is not detected by the diagnostic assays used in this study,[28] but whether this holds true for horses is not known. Similarly, CKMB can be expressed in human skeletal muscle[28] and hence is not 100% specific for cardiac muscle injury, consequently, one could speculate that skeletal muscle damage, common in recumbent, dehydrated, and septic neonates might potentially result in false elevations detectable in serum. Cardiac troponin I has also been documented to increase in association with renal failure[29] and several other noncardiac diseases in humans,[30] although it is still considered the gold standard and a highly specific marker of acute myocardial injury.[2]

Although cTnT values were significantly elevated in the septic foals, it is worth noting that this variable was below the lower limit of detection (0.009 ng/mL) for most foals in the study (43/52 healthy foals and 23/38 septic foals). cTnI, on the other hand, was only below the lower limit of detection (0.01 ng/mL) in 1 foal in each group, and none of the samples tested in the study was below the lower limit of detection for CKMB. It is interesting to us to speculate on the influence of sample timing with respect to all 3 of the biomarkers examined, because the release of cTnI and cTnT into the serum of human patients occurs within 3 hours of myocardial ischemia and necrosis and peaks approximately 24–72 hours after onset.[31] With many of our septic foals being admitted during the first 24–48 hours of life, it is possible that we missed peak increased circulating levels of both troponins by limiting ourselves to a single-time-point sample at admission to the hospital. It is also worth pointing out that our septic foal population and the control, healthy foal population did differ slightly in their age at sampling. The median age at sampling for the septic foal group was 48 hours of age, such that half of the foals in this population would have been between 48 hours and 7 days of age. By comparison, the control foal group was younger, all being less than 48 hours of age. Further study is indicated to establish the kinetics of troponin and CKMB

release in both adult horses and neonatal foals. However, it is unlikely that serial sampling will be feasible for equine patients outside of a very select group of referral cases and we were looking for prognostic information from a pragmatic and convenient sampling point. Further studies are indicated to determine whether strategic sequential sampling during therapy will provide greater diagnostic and prognostic information. Similarly, further studies attempting to correlate echocardiographic assessment of cardiac function (ejection fraction, fractional shortening, systolic left ventricular function) with biochemical markers of cardiac injury are needed to further investigate cardiac performance in septic foals and to establish the specificity of these markers for acute cardiac insult.

Footnotes

[a] Snap® IgG Test, Idexx Laboratories, Westbrook, ME
[b] Beckman Instruments, Fullerton, CA
[c] Roche Diagnostics, Indianapolis, IN

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