Cardiac Troponins as Indicators of Acute Myocardial Damage in Dogs

Iwan A. Burgener, Alan Kovacevic, G. Neal Mauldin, and Christophe W. Lombard

Cardiac troponin I (cTnI) and T (cTnT) have a high sequence homology across phyla and are sensitive and specific markers of myocardial damage. The purpose of this study was to evaluate the Cardiac Reader, a human point-of-care system for the determination of cTnT and myoglobin, and the Abbott AxSYM System for the determination of cTnI and creatine kinase isoenzyme MB (CK-MB) in healthy dogs and in dogs at risk for acute myocardial damage because of gastric dilatation-volvulus (GDV) and blunt chest trauma (BCT). GDV results in significant decreases in cardiac output, mean arterial pressure, and coronary blood flow, causing subendocardial necrosis and ventricular arrhythmias. Blunt thoracic injuries, resulting from road traffic accidents or falls, may cause myocardial injury, commonly referred to as myocardial contusion or traumatic myocarditis. Histopathologic findings include subendocardial, subepicardial, and intramyocardial hemorrhage, as well as acute degeneration or necrosis of muscle fibers.

Current techniques for assessing the severity of myocardial damage include physical examination, ECG, radiographs, and echocardiography. Although ECG is often used, comparisons on the basis of postmortem diagnosis of acute myocardial infarction in canine and human patients have indicated that its diagnostic sensitivity is only 30–53%. Currently, the tests of choice for the diagnosis of myocardial infarction in humans are the measurements of serum concentrations of cardiac troponin I (cTnI) and troponin T (cTnT). Compared with lactate dehydrogenase and creatine kinase isoenzymes, these proteins are more specific for cardiac damage and are more persistent in blood in humans and common laboratory animals, as well as in dogs. Troponins are structurally bound proteins of the contractile apparatus, which regulate the calcium-mediated interaction between actin and myosin in skeletal and heart muscle. cTnI and cTnT are encoded by genes disparate from those encoding the skeletal isoforms. Their unique amino acid sequences allow the production of monoclonal antibodies and immunoassays for use in clinical laboratories.

The similarity of the canine heart to the human heart has resulted in the use of dogs as an experimental model for induced myocardial infarction, in which cTnT and cTnI have been found to be sensitive and specific biomarkers of cardiac injury. A limited number of clinical studies on cardiac troponins in dogs have been published in recent years. Results of these studies indicate that cardiac troponins may be helpful in the identification of myocardial contusion, myocarditis, and congestive heart failure, doxorubicin-induced cardiotoxicity, and in distinguishing between idiopathic pericardial effusion and pericardial effusion caused by hemangiosarcoma.

The purpose of the present study was to evaluate the Cardiac Reader, a human point-of-care system for the determination of cTnT and myoglobin, and the Abbott AxSYM System for the determination of cTnI and creatine kinase isoenzyme MB (CK-MB) in healthy dogs and in dogs at risk for acute myocardial damage because of GDV and BCT.

Materials and Methods

**Study Design**

Fifty-six healthy dogs were used to establish reference ranges. These were dogs presented at regular intervals to the Small Animal Internal Medicine, Vetsuisse Faculty of the University of Bern, Switzerland; e-mail: iwan.burgener@kkh.unibc.ch.
Clinic of the University of Bern for routine preventative health care or blood donations, and were judged to be free of heart disease on the basis of history, a thorough physical examination, normal routine blood work, and unremarkable follow-up. The follow-up period of all but 2 of these dogs was at least 1 year, including a recheck with a normal clinical examination and routine blood work, but no echocardiography was performed. Two dogs were lost to follow-up after 3 and 6 months because of owner relocation.

All dogs presenting with GDV and BCT during the period from April 2000–October 2001 were enrolled in the study. The dogs were examined, and blood samples were drawn at presentation and at 24 and 48 hours after the onset of clinical signs. After physical examination and diagnosis, an indwelling catheter was placed in both cephalic veins of each dog with GDV. Balanced electrolyte solutions and colloids were infused as deemed necessary by the primary clinician. Gastric decompression was then achieved by a combination of orogastric intubation and needle gastrocentesis, followed by celiotomy with repositioning of the stomach and gastropexy. BCT was defined as thoracic injury resulting from collisions (car/train) or falls from a height of at least 3 m. The clinical suspicion was confirmed by thoracic radiographs revealing rib fractures, evidence of lung contusions, or both. The BCT dogs were treated with various combinations of intravenous fluid therapy, nasogastric tube feeding, analgesia, antibiotics as regarded necessary by the attending clinicians. The present study was approved by the Animal Care and Use Committee of the University of Bern and conducted following the ethical principles outlined by the Society of Swiss Veterinarians.

Data Collection and Analysis

Blood samples were drawn at presentation and at 24 and 48 hours after the onset of clinical signs. All initial blood samples were drawn before any treatments were initiated. Three hundred microliters of whole blood were collected into lithium heparin tubes for cTnT and myoglobin determinations, which were performed within 30 minutes of sampling with an electrochemiluminiscent immunoassay. Two mL of whole blood were drawn into plain tubes and centrifuged, and the serum was separated and stored at 4°C for assays of cTnI and CK-MB with a microparticle enzyme immunoassay performed within 48 hours. Finally, 1 mL of blood was collected into sodium fluoride/EDTA tubes and centrifuged, and the plasma was separated and stored at −20°C for lactic acid measurements performed within 48 hours.

The Cardiac Reader is a point-of-care system for the quantitative determination of cTnI and myoglobin in heparinized whole blood samples. Two mL of whole blood were drawn into plain tubes and centrifuged, and the serum was separated and stored at 4°C for assays of cTnI and CK-MB with a microparticle enzyme immunoassay performed within 48 hours. Finally, 1 mL of blood was collected into sodium fluoride/EDTA tubes and centrifuged, and the plasma was separated and stored at −20°C for lactic acid measurements performed within 48 hours.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Not Detectable</th>
<th>Range of Values Obtained</th>
<th>Median (2.5–97.5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cTnI (μg/L)</td>
<td>35 of 56</td>
<td>0.1–1.1</td>
<td>0 (0–0.7)</td>
</tr>
<tr>
<td>cTnT (ng/mL)</td>
<td>55 of 56</td>
<td>0.2</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td>Myoglobin (ng/mL)</td>
<td>42 of 56</td>
<td>31–78</td>
<td>0 (0–74)</td>
</tr>
<tr>
<td>CK-MB (μg/L)</td>
<td>42 of 56</td>
<td>0.2–5.3</td>
<td>0.8 (0.3–2.9)</td>
</tr>
<tr>
<td>Lactic acid (mmol/L)</td>
<td>0</td>
<td>0.59–4.36</td>
<td>2.00 (0.73–3.27)</td>
</tr>
</tbody>
</table>

cTnI, cardiac troponin I; cTnT, cardiac troponin T; CK-MB, creatine kinase isoenzyme MB. For cTnI, cTnT, and myoglobin: 0 refers to values below detection limits.

Both assays had a very good repeatability for canine blood samples as tested with 2 healthy and 8 diseased dogs (<10% difference among 3 measurements of the same sample in all 10 dogs).

Statistical Analysis

Data were entered into a commercially available personal computer statistical package. Reference ranges were calculated as 2.5–97.5 percentiles. All results were tested for normality by a Kolmogorov-Smirnov test. Nonparametric analysis was used as appropriate. The Kruskal-Wallis 1-way ANOVA on ranks was used to test for statistical differences between groups, and multiple comparisons were conducted with Dunn’s test. Within groups, the Friedman statistic was used to compare the values at presentation and after 24 and 48 hours, and post-hoc comparisons were conducted with Dunn’s test. The level of significance was set at $P < .05$.

Results

Control Group and Reference Ranges

Of the 56 healthy control dogs, 22 different breeds and 10 mixed breed dogs were represented (33 females and 23 males). Ages ranged from 0.8–10.8 years (median 3.9 years) and body weights from 5–63 kg (median 33 kg). In healthy dogs, cTnI was below the detection limit (<0.1 μg/L) in 35 of 56 dogs, and cTnT was not measurable (<0.05 ng/mL) in all but 1 dog (Table 1). Myoglobin was detected in 14 of 56 control dogs, whereas CK-MB and lactic acid were detected in all healthy dogs (Table 1).

Patient Population

Twenty-eight dogs with GDV were enrolled in the study. The median age was 7.85 years (range 9 months–11 years), and male dogs were overrepresented (18 males, 10 females). A total of 6 dogs were euthanized because of gastric necrosis (3 dogs), concomitant systemic histiocytosis (1), pre-existing dilated cardiomyopathy (1), and financial reasons and poor surgical recovery (1). The owners of 3 dogs (2 with gastric necrosis, 1 with histiocytosis) consented to a complete postmortem examination. Subendocardial and endocardial hemorrhage and multifocal myocardial necrosis were found in all 3 dogs. The other 22 dogs were...
discharged from hospital 2–5 days after presentation. Only one of the 28 dogs suffering from GDV was treated with an antiarrhythmic drug (Mexitil®) because of persistent ventricular tachycardia, multif orm ventricular premature complexes, and consequential circulatory problems. The dog was loaded with a slow IV bolus of 2.5 mg/kg followed by a constant rate infusion with 30 μg/kg/min for 6 hours and 5 μg/kg/min for an additional 8 hours, and responded well to therapy.

Eight dogs with BCT were enrolled in the study. The median age was 6.4 years (range 1–11.8), and the sexes were equally represented. Five dogs were hit by a car, 1 by a train, and 2 fell from a height of more than 3 m. All dogs suffering from BCT survived and were discharged from hospital 3–8 days after presentation.

Because of the study design and the nature of the cases (all emergencies, mostly arriving outside regular hours), it was not possible and was not deemed appropriate to perform an echocardiography initially to exclude pre-existing cardiac problems. Chest radiographs were performed in all dogs with BCT and in 10 of 28 dogs with GDV with no obvious signs of heart problems detected by board-certified radiologists. All dogs with GDV had an ECG written before anaesthesia, and obvious signs of pre-existing heart problems were only noted in a dog already diagnosed with a dilated cardiomyopathy (atrial fibrillation). In most of the dogs with GDV (21 of 28), a 48-hour Holter recording was performed postoperatively and indicated no obvious signs of pre-existing heart problems. To exclude metabolic problems possibly damaging cardiomyocytes, complete blood work was done in all dogs within the first 2 to 14 hours and as deemed appropriate by the treating clinicians thereafter.

Comparison at Initial Presentation

The cTnI concentrations in GDV and BCT dogs were significantly higher than those in control dogs (P < .001), but there was no significant difference between concentrations in dogs with GDV and BCT (Fig 1). For cTnT, the only significant difference at initial presentation was between the BCT group and the control group, with higher values in dogs suffering from BCT (P = .033). Dogs with GDV and BCT had significantly higher myoglobin (P < .001; Fig 2), CK-MB (P < .001), and lactic acid (P < .001) concentrations compared with values for the control group, but no significant differences were noted between concentrations in GDV and BCT cases.

Dogs with GDV over 48 Hours

Throughout the test period, high cTnI or cTnT values were found at least once in 26 of 28 (range 0.3–369 μg/L) and 16 of 28 dogs (0–1.7 ng/mL), respectively. Myoglobin was high in all but 2 cases (range 47–700 ng/mL), and CK-MB was high in all dogs (3.1–79.7 μg/L). The lactic acid concentration was increased in 18 of 28 dogs at presentation (range 0.87–13.4 mmol/L) and returned to normal ranges within 48 hours. All 6 dogs euthanized during the study had increased cTnI (range 1.8–369 μg/L), and all but the dog with dilated cardiomyopathy had increased cTnT concentrations (range 0–1.5 ng/mL).

The cTnI and cTnT concentrations were significantly higher after 24 and 48 hours compared with values at presentation (P < .001), but there was no significant difference between the 2 time points (Figs 3, 4). No significant changes were found in myoglobin concentrations over time. The CK-MB concentrations decreased over 48 hours (P < .001), but there was no difference between the initial and the 24-hour values. Lactic acid concentrations were significantly lower after 24 and 48 hours than at presentation (P < .001; Fig 5).

The dog treated with antiarrhythmic drugs had some of the highest cardiac troponin concentrations (cTnI 292 μg/L; cTnT 1.7 ng/mL), peaking after 24 hours and decreasing thereafter parallel to clinical improvement.

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**Fig 1.** cTnI concentrations at initial examination in control, GDV, and BCT dogs. Significant differences between control and GDV (a) and between control and BCT (b) (both P < .001), but not between GDV and BCT.

**Fig 2.** Myoglobin concentration at initial examination in control, GDV, and BCT dogs. Significant differences between control and GDV (a) and between control and BCT (b) (both P < .001), but not between GDV and BCT.
Throughout the test period, high cTnI or cTnT values were found at least once in 6 of 8 (range 0–82.4 μg/L) and 3 of 8 dogs (range 0–0.29 ng/mL), respectively. Myoglobin and CK-MB were high in all cases (ranges 118–700 ng/mL and 2.2–27.4 μg/L, respectively). The lactic acid concentrations at presentation were increased in 3 of 8 dogs (range 1.17–5.79 mmol/L).

In dogs with BCT, no significant changes were found in cTnI, cTnT, and CK-MB concentrations over time. Myoglobin and lactic acid concentrations decreased significantly over 48 hours (P < .004, Fig 6; and P < .001, Fig 5: Lactate in GOV; respectively).

Discussion

Several test systems for cTnI and cTnT have been developed in human medicine. To the authors’ knowledge, canine reference values for the 2 test systems used in this study have not been previously established. The cTnT test systems previously evaluated in dogs (ELECSYS 1010 Troponin STAT18 and ELECSYS Troponin T21) are both second-generation assays for the detection of human cTnT and use the same 2 monoclonal murine antibodies as does the Cardiac Reader used in the present study.10 The Cardiac Reader has been calibrated with both second and third generations of this assay.24,25 Compared with the former 2 cumbersome and expensive laboratory machines, the Cardiac Reader is an affordable, rapid point-of-care system, which is suited for use in an intensive care unit or an emergency laboratory. In the 2 previous studies,18,21 cTnT was below detection limits in healthy dogs (n = 40 and 15, respectively), which was corroborated by the present study, in which cTnT was undetectable in all but one of 56 controls. The single control animal in which cTnT was detected had no obvious signs of heart disease, a negative cTnT on a recheck, and has been free of clinical signs of heart disease for a follow-up period of 2 years. Given this follow-up period and subsequent negative cTnT value, a false-positive reading from the Cardiac Reader cannot be ruled out.

In contrast to the available test systems for cTnT, which are all produced by Boehringer Mannheim, a unit of Roche Diagnostics, the commercially available systems for cTnI are produced by a variety of different companies by means of different antibodies. To the authors’ knowledge, reference ranges for canine cTnI have been established by using 3 disparate human test systems: OPUS Troponin I (Behring Diagnostics, Westwood, MA),18 Stratus CS stat fluorometric analyzer (Dade Behring, Newark, DE),29 and Access AccuTnI assay (Beckman Coulter, Fullerton, CA).23 The cTnI values obtained in healthy dogs fluctuated between 0–1.37 ng/mL,18 0–0.07 ng/mL,29 0.01–0.15 ng/mL,23 and 0–0.7 μg/L (=ng/mL) in the present study. This disparity of reference values illustrates the inherent problem of a lack of standardization among different assay systems, which is already known from studies in human medicine.30,31 Nevertheless, these differences do not probably have any clinical impact compared with the positive values that are as high as 369 μg/L and 82.4 μg/L in dogs with GDV and BCT.

One of the major limitations of the present study is the lack of a gold standard for the antemortem diagnosis of myocardial cell damage. In dogs with suspected cardiac contusion, only 30% displayed relevant ECG abnormalities,18 whereas dogs with babesiosis developed a variety of ECG abnormalities that were not associated with histological changes, disease severity, outcome, or plasma cardiac troponin concentrations.19 The only exception to this was the presence of ventricular premature complexes, which are easily missed by short-term ECG. Holter ECG over 24–48 hours might be more helpful for this purpose, but it is not suitable for dogs with BCT. Despite the lack of a gold standard, subendocardial and endocardial hemorrhages as well as multifocal myocardial necrosis were found in all 3 dogs that underwent postmortem examination after euthanasia, along with increased cTnI and cTnT in all of them. Furthermore, all dogs with GDV and 48-hour Holter

![Fig 3. cTnI concentration over time in GDV dogs. Significant differences between 0 and 24 hours (a) and between 0 and 48 hours (b) (both P < .001), but not between 24 and 48 hours.](image-url)

![Fig 4. cTnT concentration over time in GDV dogs. Significant differences between 0 and 24 hours (a) and between 0 and 48 hours (b) (both P < .001), but not between 24 and 48 hours.](image-url)
monitoring (21) showed signs of myocardial damage (arrhythmias, ventricular premature complexes, tachycardia).

Assuming that all dogs with GDV and BCT really had myocardial damage because of their problems, and that all control dogs had no cardiac problems, sensitivity and specificity of the test systems used could be calculated. With the upper end of the reference range as cut-off values (0.7 μg/L for cTnI, not detectable for cTnT), both test systems reach a very high specificity of 96% (Abbott Axsym system for cTnI) and 98% (Cardiac Reader for cTnT). If the overall sensitivity is calculated (dogs with GDV and BCT together), the cTnI assay reaches 89% (32 of 36 cases positive), but the cTnT assay reaches only 53% (19 of 36). These values get slightly better when taking only the dogs with GDV into account (93% and 57%, respectively). The overall accuracy of the 2 test systems used would be very good for cTnI and the Abbott Axsym system (93%; 86 of 92 dogs accurately tested) and good for cTnT and the Cardiac Reader (80%; 74 of 92 accurate). And again, these values would also be higher when excluding the dogs with BCT from the calculation (95% and 85%, respectively).

Increased cardiac troponins suggestive of myocardial damage occurred in 26 of 28 dogs with GDV and 6 of 8 dogs with BCT. At initial presentation, the cTnI concentrations were high in both groups, whereas cTnT concentrations were significantly increased only in BCT cases. The time elapsed between the initial insult and presentation may be partially responsible for this disparity: the majority of the GDV patients were presented within 1.5 hours after the first clinical signs occurred, whereas the BCT dogs were mostly referral cases and were presented 3–4 hours after the incident because of first emergency treatments by the referring veterinarian. Another possible explanation for this difference can be found in an experimental study carried out by Ricchiuti and coworkers,6 in which cTnI increased earlier than did cTnT after coronary artery occlusion. In another canine model of myocardial infarction,13 the cTnT concentration did not increase in the first 90 minutes of ischemia but correlated highly with infarction size within 3 hours of reperfusion. Given the hypovolemic state of most dogs suffering from GDV, this might be another possible explanation for the lack of increases in cTnT values in GDV cases at presentation.

The highly significant increase in cTnI and cTnT in dogs suffering of GDV after 24 and 48 hours indicates myocardial damage secondary to ischemia and reperfusion injury. Increased cTnT or cTnI values were found in 93% and 57% of dogs, respectively, and were as high as 527 times the upper limit of the reference range. Only 2 dogs were found to have no increases in serum cTnI. In both of these cases, blood cTnT was not detected, myoglobin concentrations were low (all 6 measurements <190 ng/mL), only partially gastric rotation was found at surgery, and both were discharged after 2 days without any complications. The myocardial damage as a sequela of GDV is further supported by the significantly high CK-MB in all GDV dogs and increased myoglobin in 93% of these dogs in the absence of obvious skeletal muscle damage. Myoglobin and CK-MB are considered short-lived and nonspecific compared with cardiac troponins, with a half-life of approximately 9 minutes (myoglobin)32 and 2 hours (CK-MB)33 in dogs. The skeletal muscle damage due to surgery is a possible explanation for the absence of a decrease in myoglobin concentrations and a delayed decrease in CK-MB in GDV dogs.34

The mortality rate in GDV dogs of 21% (6 of 28) corroborates findings in previous studies, in which mortality rates ranged from 15–24%.4,3,36 All 6 dogs euthanized had increased cTnI concentrations, and all but the single dog with pre-existing dilated cardiomyopathy had increased cTnT concentrations, consistent with acute myocardial damage. A deficiency of the structural protein cTnT in the myocardium of dogs with idiopathic dilated cardiomyopathy37 could explain the undetected cTnT concentrations in this dog. Compared with the nonsurvivors, in whom all but one had

![Fig 5. Lactate concentration over time in GDV dogs. Significant differences between 0 and 24 hours (a) and between 0 and 48 hours (b) (both P < .001), but not between 24 and 48 hours.](image)

![Fig 6. Myoglobin concentration over time in BCT dogs. Significant differences between 0 and 48 hours (a) (P = .004), but not between 0 and 24 hours or between 24 and 48 hours.](image)
increased cTnT, only 11 of 22 survivors had high cTnT concentrations. Due to the small number of dogs in this study, further studies will be necessary to investigate any possible prognostic value of increased cTnT in GDV cases.

The highest cTnI and cTnT values measured in dogs with BCT were 4.5–6 times lower than the highest values measured in dogs with GDV, suggesting a less severe degree of myocardial damage. The values found for cTnI and cTnT in the present study were comparable to the cases described by Schober et al. Both their and our test systems found similar ranges for cTnT in BCT dogs (0–0.29 ng/mL), and a similar number of dogs with increased measurements (25–27%). All 8 dogs with BCT in the present study had obvious skeletal muscle trauma and increased blood myoglobin, which decreased significantly over 48 hours. No significant differences were found in cTnI, cTnT, and CK-MB concentrations over time, although the lowest cTn concentrations were observed at 48 hours, and CK-MB and cTn values tended to decrease over time. These values must, however, be interpreted cautiously because of the low number of cases involved in the present study.

Hyperlactatemia has been associated with various canine diseases and preoperative plasma lactate concentration was found to be a good predictor of gastric necrosis and outcome in dogs with GDV. Fifty-eight percent of the dogs with lactic acid concentrations higher than 6 mmol/L died in that study compared to only 1 of 9 dogs in the present study population. In all GDV dogs, lactic acid concentration returned to reference ranges after 24 hours. This was the case even for the 3 dogs in which postmortem examination revealed gastric necrosis, which is considered a major cause of hyperlactatemia. Therefore, the lactic acid concentration was not a reliable prognostic indicator in our hands. Rigorous IV fluid administration and gastric decompression resolved hypovolemia and hypoxia and invariably decreased the plasma lactic acid concentrations.

In conclusion, the results of the present study suggest a high incidence of cardiac damage in dogs suffering from GDV and BCT. In animals suffering from GDV, cardiac troponins significantly increase within the first 24 to 48 hours in contrast to the animals with BCT. Serum cTnI appears to be more sensitive in detecting myocardial cell injury than cTnT, but cTnT may prove to have value as a negative prognostic indicator in GDV. The Abbott AxSYM system and the Cardiac Reader appeared suitable for the measurement of canine cardiac troponins, with the Cardiac Reader as a small point-of-care system particularly suitable for use in emergency settings.

Footnotes


Acknowledgments

The work was done at the Small Animal Hospital of the University of Bern, Switzerland, and was supported by the Department of Clinical Veterinary Medicine of the University of Bern. The authors would like to thank F. Hannes and M. Burri from Roche Diagnostics (Switzerland) Ltd for their support, Drs. F. Gaschen and J. Howard for their critical reading of the manuscript, and the clinicians of the Small Animal Hospital of the University of Bern for help in recruiting the cases.

References


