Cardiac Troponins I and T in Dogs with Pericardial Effusion

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Cardiac troponin I (cTnI) and cardiac troponin T (cTnT) are sensitive and specific markers for myocardial ischemia and necrosis. Dogs with pericardial effusion frequently have myocardial ischemia and necrosis, and these changes are more severe in dogs with hemangiosarcoma (HSA). We investigated the utility of using serum cTnI and cTnT concentrations to identify the idiopathic pericardial effusion from that associated with HSA. Blood samples for measurement of cTnI and cTnT concentrations were collected before pericardiocentesis in 37 dogs with pericardial effusion. Eighteen dogs had a mass consistent with HSA, 6 dogs had idiopathic pericardial effusion, 1 dog had mesothelioma, and 1 dog had a heart base tumor. No final diagnosis was achieved for 11 dogs. Dogs with pericardial effusion had significantly higher serum concentrations of cTnI (P < .001) but not cTnT (P = .16) than did dogs with idiopathic pericardial effusion (0.05 ng/dL; range: 0.03–0.09 ng/dL) (P < .001). There was no difference in the concentration of cTnT between dogs with HSA and those with idiopathic pericardial effusion (P = .08). Measurement of cTnI may be useful in helping to distinguish between idiopathic pericardial effusion and pericardial effusion caused by HSA.

Key words: Biomarkers; Canine; Hemangiosarcoma; Mesothelioma; Neoplasia.

Pericardial effusion in dogs most commonly results from hemangiosarcoma (HSA) but can be associated with other types of neoplasia or can be idiopathic.1,4 Approximately 60–80% of dogs with pericardial tamponade evaluated by echocardiography have a cavitated cardiac lesion consistent with HSA.5 In some dogs, no mass may be detected at the initial echocardiographic examination, but a mass may be identified later by echocardiography or at postmortem examination. Dogs with cardiac HSA have a grave prognosis, with survival times of days to weeks, whereas those with other types of pericardial effusion, even from other neoplastic causes, have a better prognosis.3,6

Pericardial fluid analysis is seldom diagnostic because HSA cells do not usually exfoliate. In addition, pericardial effusion of both malignant and benign origin frequently contains reactive mesothelial cells that are difficult to differentiate from neoplastic cells.7 Thus, in cases in which no mass is visualized with echocardiography, a final diagnosis may not be available unless thoracotomy or thoracoscopy is performed. The survival benefit of pericardiectomy has been clearly established in dogs with idiopathic pericardial effusion.1,3 In contrast, HSA has a poor prognosis even with pericardiectomy.1,3

Recently, cardiac-sensitive biomarkers, the troponins, have become available for the diagnosis of cardiac ischemia and subsequent myocardial necrosis in humans.8,9 The troponins are 3 distinct proteins (I, C, and T) that are expressed in cardiac and skeletal muscle and are encoded by different genes. Troponin C molecules found in both cardiac and skeletal muscle are structurally identical in both muscle types, thus limiting the diagnostic utility of troponin C for cardiac disease. The cardiac forms of troponin I (cTnI) and troponin T (cTnT) are present in high concentrations only within cardiac myocytes. The release of cardiac troponins into the blood stream occurs only during cardiac ischemia and necrosis.8,9 Measurement of cardiac troponins has been widely used to diagnose acute myocardial infarction in humans, and high concentrations have been associated with numerous other disease processes, including sepsis, cardiac contusion, and advanced congestive heart failure.8 Theoretically, anything that causes myocardial ischemia and necrosis will result in increased serum troponin concentrations. Veterinary experience with the measurement of cardiac troponins is more limited. Sleeper et al10 established a normal range for cTnI in dogs and cats. In other studies, increased circulating troponin concentrations have been associated with congestive heart failure, doxorubicin therapy, babesiosis, cardiac contusion, and gastric dilatation-volvulus.11-13

We explored the use of measurement of cTnI or cTnT concentrations to aid in the diagnosis of cardiac neoplasia. Malignant cardiac tumors are associated with myocardial necrosis; therefore, dogs with neoplastic pericardial effusion should have higher circulating concentrations of cardiac troponin than would dogs with idiopathic pericardial effusion.14

The goals of this study were to determine whether serum cTnI and cTnT concentrations are increased in dogs with pericardial effusion and whether dogs with HSA have higher concentrations of cTnI or cTnT than do dogs with idiopathic pericardial effusion.

Materials and Methods

Dogs presenting to the emergency or cardiology service at Tufts University School of Veterinary Medicine were prospectively recruited into the study. The presence of pericardial effusion was confirmed by 2-dimensional echocardiography. Dogs that had been previously identified as having heart disease requiring medical therapy, that had received blood products within 24 hours before sampling, or that were <1 year of age were not eligible. Additionally, dogs that had undergone pericardiocentesis before study enrollment also were excluded, because the possibility of inadvertent myocardial trauma during pericardiocentesis could not be excluded.

Prior to pericardiocentesis, 6 mL of blood was collected and evenly divided between serum separator and lithium heparin tubes. Samples
were centrifuged, and the serum or plasma was removed and frozen at \(-70^\circ\mathrm{C}\) until analysis.

On the basis of echocardiography reports and subsequent follow-up examinations dogs were diagnosed as having HSA, heart base tumor, mesothelioma, or idiopathic pericardial effusion. The HSA group consisted of dogs in which a cavitary mass was attached to or invading either the right atrium or right auricular appendage was identified on initial or subsequent echocardiographic examination or in which there was a histopathologic diagnosis of HSA. The heart base tumor group consisted of dogs in which a mass attached to the aorta and judged to be consistent with a heart base tumor was visualized using 2-dimensional echocardiography or in which there was a histopathologic diagnosis of a heart base tumor. The mesothelioma group consisted of dogs with a histopathologic diagnosis of mesothelioma. The idiopathic pericardial effusion group consisted of those dogs with a histologic diagnosis of idiopathic pericarditis or dogs that survived \(>1\) year after the initial diagnosis of pericardial effusion without a specific cause being identified. The normal control group consisted of dogs that were determined to be normal based upon results of physical examination, CBC, biochemical profile, and echocardiographic examination.  

**cTnI and cTnT Analysis**  
Plasma cTnI concentrations were determined using a fluorometric enzyme immunoassay for cTnI. This assay involves the use of 2 mouse monoclonal antibodies that recognize 2 different epitopes on the human cTnI molecule. The minimal detectable concentration of cTnI is 0.03 ng/mL. Serum samples were analyzed for cTnT concentration with an electrochemiluminescence immunoassay. The assay involves the use of 2 mouse monoclonal antibodies against different epitopes of human cTnT. The minimal detectable concentration of cTnT in serum is 0.01 ng/mL. Although validation of this specific assay has not been performed in dogs, this assay has been used in several previous studies.  

**Statistical Analysis**  
All statistical analyses were performed using commercially available software. Normally distributed data are reported as a mean and standard deviation. Data that were not normally distributed are reported as a median and range. Where possible, data that were not normally distributed were transformed before analysis. Comparisons using normally distributed data were performed with a Student \(t\)-test or an analysis of variance (ANOVA). Data that were not normally distributed were analyzed using a Kruskal-Wallis 1-way ANOVA. Significance of correlation was determined using a Pearson’s correlation analysis. A \(P\) value of <.05 was considered significant.

**Results**  
Blood samples were collected from 37 dogs with pericardial effusion and 5 normal control dogs. The mean age of the 5 normal dogs was 8.0 ± 2.6 years. The median concentration of cTnI in normal dogs was 0.02 ng/mL (range: 0.00–0.03 ng/mL). None of the dogs had any detectable circulating cTnT (<0.01 ng/mL).

The median serum concentrations of cTnI and cTnT for the 37 dogs with pericardial effusion were 0.64 ng/mL (range: 0.03–47.18 ng/mL) and 0.00 ng/mL (range: 0.00–6.40 ng/mL), respectively. Dogs with pericardial effusion had significantly higher serum cTnI concentrations than did control dogs (\(P < .001\)). There was no difference in cTnT concentration between normal dogs and dogs with pericardial effusion (\(P = .16\)).

A definitive clinical or histopathologic diagnosis was not reached in 11 of the dogs with pericardial effusion. As a result, they were excluded from intergroup analyses. The remaining 26 dogs had a final diagnosis of HSA (\(n = 18\)), idiopathic pericardial effusion (\(n = 6\)), heart base tumor (\(n = 1\)), and mesothelioma (\(n = 1\)).

Dogs with HSA had a mean age of 9.6 ± 2.0 years. The breeds with HSA were Golden Retriever or Golden Retriever mix (\(n = 9\)), German Shepherd Dog (\(n = 3\)), mixed breed (\(n = 2\)), Greyhound (\(n = 1\)), Labrador Retriever (\(n = 1\)), Cocker Spaniel (\(n = 1\)), and Rottweiler (\(n = 1\)). There were 11 castrated males and 7 spayed females. Fifteen dogs had a mass consistent with HSA that was identified on initial echocardiographic examination. One additional dog had a mass consistent with HSA identified on follow-up echocardiographic examination. HSA was identified during postmortem examination in 2 dogs. The 3 dogs in which a mass was not identified during the initial echocardiographic examination had high cTnI levels (0.2, 2.7, and 5.0 ng/dL). Dimensions of the mass were measured from the echocardiogram or during postmortem examination in 7 dogs. Masses ranged in diameter from 2.3 to 5.5 cm. The median concentration of cTnI was 2.77 ng/dL (range: 0.09–47.18 ng/dL), and the median concentration of cTnT was 0.00 ng/dL (range: 0.00–6.40 ng/dL).

Dogs with idiopathic pericardial effusion had a mean age of 9.7 ± 2.7 years. Breeds diagnosed with idiopathic pericardial effusion were Golden Retriever (\(n = 2\)), Labrador Retriever (\(n = 1\)), Australian Cattle Dog (\(n = 1\)), English Bulldog (\(n = 1\)), and Doberman Pinscher (\(n = 1\)). There were 4 castrated males and 2 spayed females. The median concentration of cTnI was 0.05 ng/dL (range: 0.03–0.09 ng/dL), and the median concentration of cTnT was 0.00 ng/dL (range: 0.00–0.010 ng/dL). All dogs with idiopathic pericardial effusion had a minimum of 2 years of follow-up. A pericardial biopsy consistent with idiopathic pericardial effusion was available for 4 of the 6 dogs. The remaining 2 dogs had no cardiac mass identified after more than 2 years of follow-up.

The single dog with a heart base tumor was an 11-year-old spayed female Boston Terrier with a cTnI concentration of 9.36 ng/dL and a cTnT concentration of 5.4 ng/dL. The dog with a mesothelioma was a 9-year-old castrated male Labrador Retriever mix with a cTnI concentration of 0.06 ng/dL and cTnT concentration of <0.01 ng/dL.

Dogs with HSA had significantly higher concentrations of cTnI than did dogs with idiopathic pericardial effusion \(P < .001\); Fig 1). There was no difference in the concentration of cTnT between the two groups (\(P = .08\)).

There was no correlation between the size of the mass and the concentration of cTnI (\(P = .3, r = -.55\)) or cTnT (\(P = .4, r = .47\)).

**Discussion**  
There was a significant difference in cTnI concentration when dogs with idiopathic pericardial effusion were compared with dogs with pericardial effusion resulting from HSA. In the present study, dogs with pericardial effusion had significantly higher concentrations of cTnI but not cTnT than did the normal control dogs. This finding is consistent with reports of gastric dilatation-volvulus and cardiac contusion, in which more dogs had increases in cTnI than in cTnT. The exact reason for this difference is unknown, but it may result from a structurally closer binding of cTnT to the tropomyosin chain.
All 37 dogs with pericardial effusion had significantly higher concentrations of cTnI compared with the normal control dogs, which probably reflects the fact that coronary perfusion is decreased in the presence of pericardial tamponade. Mild elevations in cTnI and cTnT concentrations can be seen in cases of ischemic injury that does not result in necrosis. These concentration increases are thought to be associated with release from the relatively small cytosolic pool rather than the much larger portion of cTnI and cTnT bound to the tropomyosin filament, which is only released upon death of the cardiac myocyte.

There was no correlation between the size of the mass in dogs with HSA and the circulating concentrations of cTnI and cTnT. Thus, even those dogs with smaller masses not visualized by echocardiography may have significant increases in cTnI concentration. The 3 dogs in which a mass was not identified on initial echocardiographic examination but was later identified on follow-up examination or during postmortem examination all had cTnI concentrations of $>$0.1 ng/mL. This cutoff would have correctly identified 17 of the 18 dogs with HSA. If these results are confirmed by future studies, cTnI concentrations may be useful for discrimination between cases of pericardial effusion caused by HSA and those from idiopathic causes.

Limitations of the present study include variation in the normal range and the lower detection limit for the currently available commercial cTnI analyzers. However, these limitations do not impair the ability of the clinician to compare results between analyzer types; normal ranges must be established for each individual analyzer. This study included only those cases where a definitive clinical or histologic diagnosis was reached. A prospective study is needed to investigate the utility of cTnI evaluation for the discrimination between idiopathic pericardial effusion and that resulting from HSA. Further prospective studies in dogs are needed to better define the role of cTnI when no mass can be visualized utilizing echocardiography and in dogs with heart base tumor or mesothelioma.

### Footnotes

- Stratus CS stat fluorometric analyzer, Dade Behring Inc, Newark, DE
- Elecsys Troponin T STAT immunoassay, Elecsys 2010 analyzer, Boehringer Mannheim, Mannheim, Germany
- Systat 9.01 software, SPSS, Chicago, IL

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### References