Evaluation of serum cardiac troponin I concentration in Boxers with arrhythmogenic right ventricular cardiomyopathy

Ryan D. Baumwart, DVM; João Orvalho, DVM; Kathryn M. Meurs, DVM, PhD

Objective—To evaluate serum cardiac troponin I (cTnI) concentrations in Boxers with arrhythmogenic right ventricular cardiomyopathy (ARVC), unaffected (control) Boxers, and control non-Boxers.

Animals—10 Boxers with a clinical diagnosis of ARVC defined by ≥1,000 ventricular premature complexes (VPCs)/24 h on an ambulatory ECG, 10 control Boxers assessed as normal by the presence of <5 VPCs/24 h, and 10 control non-Boxers.

 Procedures—Serum was extracted from a blood sample from each dog. Analysis of serum cTnI concentrations was performed.

Results—Mean ± SD serum cTnI concentration was 0.142 ± 0.05 ng/mL for Boxers with ARVC, 0.079 ± 0.03 ng/mL for control Boxers, and 0.023 ± 0.01 ng/mL for control non-Boxers. A significant difference in serum cTnI concentrations was observed among the 3 groups. In the combined Boxer population (ie, Boxers with ARVC and control Boxers), a significant correlation was found between serum cTnI concentration and number of VPCs/24 h (r = 0.78) and between serum cTnI concentration and grade of ventricular arrhythmia (r = 0.77).

Conclusions and Clinical Relevance—Compared with clinically normal dogs, Boxers with ARVC had a significant increase in serum cTnI concentration. For Boxers, correlations were found between serum cTnI concentration and number of VPCs/24 h and between concentration and the grade of arrhythmia. Because of the overlap in serum cTnI concentrations in control Boxers and Boxers with ARVC, future studies should evaluate the correlation of serum cTnI concentration with severity of disease in terms of degree of myocardial fibrofatty changes. (Am J Vet Res 2007;68:524–528)

Abbreviations

<table>
<thead>
<tr>
<th>AECG</th>
<th>Ambulatory ECG</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARVC</td>
<td>Arrhythmogenic right ventricular cardiomyopathy</td>
</tr>
<tr>
<td>cTnI</td>
<td>Cardiac troponin I</td>
</tr>
<tr>
<td>VPC</td>
<td>Ventricular premature complex</td>
</tr>
<tr>
<td>LVId</td>
<td>Left ventricular internal diastolic diameter</td>
</tr>
<tr>
<td>LVIds</td>
<td>Left ventricular internal systolic diameter</td>
</tr>
<tr>
<td>FS</td>
<td>Fractional shortening</td>
</tr>
</tbody>
</table>

Arrhythmogenic right ventricular cardiomyopathy is recognized in Boxers and is characterized clinically by ventricular tachyarrhythmias and histopathologically by fibrofatty replacement of the right and sometimes left and interventricular myocardium.1-14 Affected dogs may have syncope or signs associated with congestive heart failure. However, many dogs die of sudden cardiac death without ever having any clinical signs. Screening for the occult form of the disease may be performed with a 24-hour AECG, but this test is not readily available to all clinicians. Considerable interest exists in the early diagnosis of ARVC through alternative noninvasive techniques.

Biochemical markers such as brain natriuretic peptide, atrial natriuretic peptide, endothelin, and C-reactive protein are used to provide a noninvasive means for detecting the presence of cardiac disease in dogs.5-10 Previously, we reported the evaluation of plasma brain natriuretic peptide concentration in Boxers with ARVC. Increases in plasma brain natriuretic peptide concentration were not associated with the presence of the disease.11

Cardiac troponins are increasingly used as a biomarker by clinical cardiologists. Cardiac troponin is a regulatory protein of the thin filament and consists of 3 subunits as follows: troponin C, troponin I, and troponin T.12 Hematologic assays for cTnI and cardiac troponin T are used to specifically identify cardiac muscle injury in humans.13,14 An increase in serum cardiac troponin concentration is an important marker for the diagnosis of acute myocardial infarction in humans and in dogs with experimentally induced disease.13,14-17 Additionally, an increase in serum cTnI concentration is observed with myocardial injury associated with myocarditis in mice and humans.18,19 Small increases in serum cardiac troponin concentrations have also been reported for humans with congestive heart failure.20-23

Received August 28, 2006.
Accepted October 30, 2006.
From the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43220. Dr. Orvalho’s present address is Veterinary Medical Teaching Hospital, School of Veterinary Medicine, University of California, Davis, CA 95616. Dr. Meurs’ present address is the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, WA 99164. Supported by grants from the American Boxer Charitable Foundation and the American Kennel Club Canine Health Foundation. Address correspondence to Dr. Meurs.
Cardiac troponin I is structurally bound within the myocyte and is released into circulation after cell disruption and necrosis.\textsuperscript{13,24-33} Reference range values of cTnl concentrations in healthy dogs have been published.\textsuperscript{24-33} In dogs, cardiac troponin concentrations increase with mitral valve disease, dilated cardiomyopathy, subaortic stenosis, pericardial effusion, babesiosis, adriamycin administration, suspected cardiac contusions, and gastric dilatation and volvulus.\textsuperscript{26-33}

We hypothesized that Boxers with ARVC, a disease characterized by myocardial atrophy with fibrofatty replacement, would have increased serum cTnl concentrations, compared with clinically normal dogs. The objective of the study reported here was to evaluate serum cTnl concentrations in Boxers with ARVC, unaffected control Boxers, and unaffected control non-Boxers to determine whether a significant increase in serum cTnl concentrations could be identified in Boxers with ARVC.

Materials and Methods

**Animals**—This study was conducted in accordance with the guidelines of the Animal Care and Use Committee of the Ohio State University College of Veterinary Medicine. Written consent authorizing study participation was obtained from each client.

Three groups of dogs were selected for evaluation. The first 2 Boxer groups (Boxers with ARVC and control Boxers) were dogs selected from an ongoing study of ARVC in Boxers in which annual echocardiography and 24-hour AECG were performed. The first group consisted of Boxers with a clinical diagnosis of ARVC (n = 10) defined by the presence of \( \geq 1,000 \) VPCs/24 h on a 24-hour AECG and echocardiographic variables within reference range limits.\textsuperscript{24} The second group consisted of control Boxers (n = 10) assessed as clinically normal by the presence of \(< 5 \) VPCs/24 h on the AECG monitor and echocardiographic variables within reference limits. The third group consisted of control non-Boxers (n = 10) screened by physical and echocardiographic examination and selected as negative controls. The control non-Boxer group included the following breeds: Australian Shepherd (n = 1), Labrador Retriever (2), Collie (1), and mixed (6).

**Procedures**—An AECG was obtained by use of a 3-channel monitoring recorder. The monitor was placed, and the dog was sent home to allow monitoring of the electrical activity of the dog in its normal environment. All tapes were obtained by use of an AECG analysis system with prospective user interaction.\textsuperscript{3} Any tape that did not have \( \geq 20 \) hours of readable data was excluded. The total number of VPCs were tabulated, and the complexity of the ventricular arrhythmia was graded according to the following scheme: grade 0 = no VPCs; grade 1 = only single monomorphic VPCs; grade 2 = single VPCs (in a bigeminal or trigeminal pattern) or multiform complexes; grade 3 = couplets or triplets; grade 4 = runs of ventricular tachycardia (4 beats or longer) or an R-on-T phenomenon. Lead II was used for analysis.

Boxers currently on antiarrhythmic medication were not excluded from the study. Four Boxers with ARVC were being treated with sotalol as an antiarrhythmic at a mean dosage of \( 2.44 \pm 0.29 \) mg/kg, PO, twice daily. No other antiarrhythmic was prescribed for any Boxers with ARVC. None of the Boxers with ARVC were syncopal, although this was not used as inclusion or exclusion criterion.

All dogs were assessed by echocardiography to identify and exclude dogs that may have underlying cardiac disease that would alter results of the study. The echocardiographic examination was conducted with standard clinical techniques and without sedation. Echocardiographic recordings were made with a simultaneous ECG, and all raw data were captured digitally to maintain optimal fidelity for off-line measurement.\textsuperscript{3} M-mode measurements of the LVIDd and LVIDs were obtained. Dogs with abnormal left ventricular FS (\(< 25\%\)), increased left ventricular chamber size for their body size, or considerable valvular regurgitation were excluded from the study.\textsuperscript{34}

In all dogs, 2 to 3 mL of blood was collected from the jugular vein and placed in serum collection tubes. Samples were then centrifuged, and serum was extracted and stored at \(-80^\circ\)C for batch analysis. Sample storage varied from 1 week to 11 months. Samples were thawed only once, at the time of analysis. Analysis of serum cTnl concentration was performed by use of an immunoassay system\textsuperscript{35} with a detection level of 0.01 ng/mL.

**Statistical analysis**—All calculations, statistical analyses, and graphing were done by use of commercial software.\textsuperscript{36} Serum cTnl concentrations that were reported as \(< 0.01 \) ng/mL were given a value of 0.01

<table>
<thead>
<tr>
<th>Groups (10 dogs/group)</th>
<th>Mean (y)</th>
<th>Range</th>
<th>Weight (kg)</th>
<th>Mean</th>
<th>Range</th>
<th>MI</th>
<th>MC</th>
<th>FI</th>
<th>FS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boxers with ARVC</td>
<td>7.3</td>
<td>2.0–10.0</td>
<td>33.8</td>
<td>26.0–36.0</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Control Boxers</td>
<td>4.0</td>
<td>1.0–6.5</td>
<td>26.5</td>
<td>21.4–34.7</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Control non-Boxers</td>
<td>5.6</td>
<td>3.1–7.2</td>
<td>32.3</td>
<td>20.5–38.7</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

MI = Sexually intact male. MC = Castrated male. FI = Sexually intact female. FS = Spayed female.
ng/mL for statistical analysis. Descriptive statistics were obtained for serum cTnl concentration, age, weight, and echocardiographic variables for each group as well as total VPCs/24 h and grade of arrhythmia for the 2 Boxer groups. A 1-way ANOVA was performed to detect significant differences in age, weight, echocardiographic variables (FS, LVIDd, and LVIDs), and cTnl concentrations among groups. When significant differences were found, a Tukey post hoc test was performed. A Mann-Whitney rank sum test was performed to detect significance differences in number of VPCs and grade of ventricular arrhythmia in Boxer groups. A Spearman correlation analysis was performed to determine whether the number of VPCs/24 h on AECG was correlated with the serum cTnl concentration for the combined Boxer population (ie, Boxers with ARVC and control Boxers). A Spearman correlation was also used to determine whether the grade of the arrhythmia on AECG was correlated with the serum cTnl concentration for the combined Boxer population. A value of $P < 0.05$ was considered significant for all tests.

**Results**

A significant ($P = 0.01$) difference was found for age between Boxers with ARVC and control Boxers. No significant difference was found between control non-Boxers and Boxers with ARVC or between control non-Boxers and control Boxers (Table 1).

Median number of VPCs obtained from the AECG for Boxers with ARVC was 2,375 VPCs/24 h (range, 1,157 to 24,057 VPCs/24 h) and for control Boxers was 2 VPCs/24 h (range, 0 to 5 VPCs/24 h). A significant ($P < 0.001$) difference was found between the 2 Boxer groups for number of VPCs. Median grade of arrhythmia obtained from the AECG for Boxers with ARVC was 3 (range, 2 to 4) and for control Boxers was 1 (range, 1 to 2). A significant ($P < 0.001$) difference was found between the 2 Boxer groups for grade of ventricular arrhythmia. Mean $\pm$ SD FS was 34.1 $\pm$ 3.3% for Boxers with ARVC, 34.7 $\pm$ 1.8% for control Boxers, and 28.7 $\pm$ 2.6% for control non-Boxers. Although all values were within reference range limits, a significant ($P = 0.017$) difference in FS was found for control non-Boxers, compared with Boxers with ARVC and control Boxers. Mean left ventricular diastolic dimension indexed to body surface area was 4.26 $\pm$ 0.54 cm/m² for Boxers with ARVC, 4.36 $\pm$ 0.54 cm/m² for control Boxers, and 4.19 $\pm$ 0.64 cm/m² for control non-Boxers. Mean left ventricular systolic dimension indexed to body surface area was 2.84 $\pm$ 0.38 cm/m² for Boxers with ARVC, 2.77 $\pm$ 0.44 cm/m² for control Boxers, and 2.98 $\pm$ 0.44 cm/m² for control non-Boxers. A significant difference was not observed among groups for LVIDd/m² and LVIDs/m².

Mean serum cTnl concentration was 0.142 $\pm$ 0.05 ng/mL for Boxers with ARVC, 0.079 $\pm$ 0.03 ng/mL for control Boxers, and 0.023 $\pm$ 0.01 ng/mL for control non-Boxers. A significant ($P < 0.001$) difference was
observed for serum cTnI concentration among groups (Figure 1). A significant ($P < 0.001; r = 0.78$) correlation was found between the serum cTnI concentration and number of VPCs/24 h for the combined Boxer population (ie, Boxers with ARVC and control Boxers; Figure 2). A significant ($P < 0.001; r = 0.77$) correlation was also identified between the serum cTnI concentration and the grade of the arrhythmia for the combined Boxer population (Figure 3).

**Discussion**

In our study, Boxers with a clinical diagnosis of ARVC had significantly greater serum concentrations of cTnI, compared with the control groups (Boxers without ARVC and control non-Boxers). Interestingly, the mean concentration in Boxers with ARVC (0.142 ng/mL) is similar to that previously described for a large group of dogs (Doberman Pinschers, Great Danes, Boxers, Rottweilers, Labrador Retrievers, and mixed-breed dogs) with cardiomyopathy (median concentration of 0.14 ng/mL).\(^{27}\) The high serum cTnI concentration could be associated with reported histopathologic findings of myocardial atrophy, fibrofatty replacement, and focal areas of myocytolysis described with ARVC and the resultant release of cTnI into circulation after cell disruption and necrosis.\(^{3,4}\) However, a limitation of our study is that we were not able to assess the degree of myocardial damage in Boxers with ARVC and correlate it with serum cTnI concentrations. Arrhythmogenic right ventricular cardiomyopathy is a progressive disease that predominantly affects the right ventricle. Most myocardial changes can only be observed histologically until the disease is quite advanced and more global ventricular dilation or dysfunction may be observed. The most accurate assessment of the myocardial changes would have required histologic examination of postmortem tissue specimens or endomyocardial biopsy specimens, which was beyond the scope of our study. However, it is interesting that a significant correlation between serum cTnI concentration and number of VPCs/24 h as well as between serum cTnI concentration and grade of ventricular arrhythmia for the Boxer groups was identified. This suggests that cTnI may be an indicator of stage or severity of disease, but the chronologic relationship between the fibrofatty infiltrates and the development of the arrhythmias is unclear. The clinical utility of serum cTnI concentrations in Boxers to evaluate the progression of the disease deserves further study.

Affected Boxers in our study were selected on the basis of the presence of at least 1,000 VPCs/24 h. This was an arbitrary cut-off point used to select Boxers with convincing evidence of this arrhythmic disease. It is not actually known what represents an acceptable or normal number of VPCs in Boxers. However, in 1 study, clinically normal control non-Boxers had a median of 2 VPCs in a 24-hour period.\(^{35}\)

To our knowledge, this is the first study to specifically evaluate serum cTnI concentrations in Boxers with ARVC. A previous study\(^{22}\) on cTnI included 4 Boxers with sustained or nonsustained right ventricular tachycardia; however, these dogs were evaluated with a larger group of dogs with cardiomyopathies.

A significant difference was identified in age between Boxers with ARVC and control Boxers. Results of a previous report\(^{27}\) that included 176 healthy dogs revealed a modest but significant positive correlation with cTnI concentration and age ($r = 0.488; P = 0.001$). Although Boxers with ARVC in our study were older than control Boxers, it is unlikely that the increased serum cTnI concentrations in Boxers with ARVC are simply the result of age because a significant difference in serum cTnI concentration was also observed between Boxers with ARVC and control non-Boxers, yet a significant difference in age was not found between these 2 groups.

Results of our study indicate that serum cTnI concentrations were highest in Boxers with ARVC, compared with other groups. However, serum cTnI concentrations in control Boxers were also higher than that of control non-Boxers. It is possible that Boxers as a breed may have higher reference range values for serum cTnI concentrations, but this would seem to be a surprising breed difference. Additionally, 2 previous studies performed to evaluate serum cTnI concentrations in clinically normal dogs that included Boxers did not indicate any clear variations for this breed.\(^{24,25}\) Therefore, it may be more likely that the group of control Boxers selected in our study may have included at least a few dogs that...
were not truly clinically normal but represented affected Boxers not readily identifiable with current methods of diagnosis. In 1 study\(^6\) of humans, cTn concentrations were increased even with a small amount of cardiac injury, indicating that measurements of cTn concentration may be useful in the detection of early disease.

We detected a significant increase in serum cTn concentrations in Boxers with ARVC, compared with other groups. This observation is of interest; however, serum cTn concentrations in an individual dog should be interpreted with caution given the overlap in serum concentrations between the apparently unaffected (control) Boxers and Boxers with ARVC and the relatively small number of dogs evaluated in our study. Future studies that include evaluation of myocardial fibrofatty changes either noninvasively (via magnetic resonance imaging) or invasively (via histologic evaluation of endomyocardial biopsy specimens) may allow for development of a more definitive correlation between serum cTn concentrations and stage of ARVC in Boxers.

### References