

Cardiac Troponin I in Doberman Pinschers with Cardiomyopathy

G. Wess, J. Simak, M. Mahling, and K. Hartmann

Background: Cardiac troponin I (cTnI) is useful for detection of cardiac myocyte damage, but its efficacy in detecting various stages of dilated cardiomyopathy (DCM) in Doberman Pinschers is unclear.

Objectives: To evaluate the diagnostic value of cTnI in various stages of DCM in Dobermans.

Animals: Six hundred and fifty-three cTnI measurements of 336 Doberman Pinschers.

Methods: Using a longitudinal study design, staging of the disease was based upon 24-hour-ambulatory-ECG (Holter) and echocardiography. A total of 447 cTnI measurements were performed in 264 healthy Dobermans, and 206 cTnI measurements in 75 Dobermans with cardiomyopathy. Eighty-eight cTnI samples were from dogs with > 100 ventricular premature contractions (VPCs)/24 hour, but without echocardiographic changes (“VPC group”). Additional 19 samples originated from dogs with only echocardiographic changes (“ECHO group”), and 56 samples from dogs with both VPCs and echocardiographic changes (“VPC plus ECHO group”). Twenty samples were from dogs with clinical signs (“clinical group”). The group “incipient” included 23 dogs, that were considered to be normal according to Holter and echocardiography at the time of the exam, but that developed DCM within 1.5 years.

Results: cTnI values of dogs in all disease groups, including the “incipient” (0.30 ± 0.20) and “VPC group” (0.36 ± 0.34), were significantly ($P = .04$, $P < .001$) higher than the control group (0.07 ± 0.16). A cut-off value of >0.22 ng/mL had a sensitivity of 79.5% and a specificity of 84.4% to detect all forms of cardiomyopathy.

Conclusions and Clinical Importance: cTnI measurement is a valuable diagnostic test that can detect cardiomyopathy in dogs that are otherwise clinically normal.

Key words: Arrhythmia; Biomarker; Dilated cardiomyopathy; Dogs; Occult; Ventricular premature contraction.

Measurement of serum cardiac troponin I (cTnI) is a highly specific and sensitive test for myocardial cellular damage in humans and animals. The primary value of cTnI as a biomarker in humans is to detect myocardial infarcts.¹ In humans, this protein is detectable in the blood 3–12 hour after cardiac injury, peaks at 1–2 days, and dissipates by 5–10 days.² Persistent increases in cTnI are associated with chronic or ongoing disease processes.² High concentrations of cTnI in animals occur in association with a number of diseases.^{3–17}

Cardiomyopathy in Doberman Pinschers is a common, inherited, slowly progressive primary myocardial disease.^{18–22} The natural progression of dilated cardiomyopathy (DCM) can be described by 3 distinct stages/phases.^{20,22–25} Stage I is characterized by a morphologically and electrically normal heart and no evidence of clinical signs of heart disease. The occult stage of the disease, stage II, is characterized by evidence of morphologic or electrical derangement in the absence of clinical signs of heart disease. The morphologic abnormality consists of left ventricular (LV) enlargement in systole and later in diastole. Ventricular premature contractions (VPCs) are the cardinal finding in the occult phase of DCM in Doberman Pinschers.^{a,18,19,22–31} Sudden death caused by ventricular tachycardia-fibrillation

Abbreviations:

AUC	area under the curve
cTnI	cardiac troponin I
DCM	dilated cardiomyopathy
Holter	24-hour-ambulatory-ECG
LV	left ventricle
LVIDd	left ventricular internal end-diastolic dimension
LVIDs	left ventricular internal end-systolic dimension
ROC	receiver operator curves
VPC	ventricular premature contraction

occurs during the occult phase in at least 25–30% of affected dogs.^{18,26,30} These abnormalities, morphologic or electrical, can coexist or may be of predominantly one form at any time during this occult stage.^{22,26,30,32,33} Stage III is characterized by the presence of clinical signs of heart failure and is also called the overt stage of DCM.^{22,26,30,32,33} For diagnostic, prognostic, therapeutic, and breeding purposes, it is essential to detect the occult phase of the disease. Diagnosis primarily depends on evidence of ventricular arrhythmias, detected by 24-hour-ambulatory-ECG (Holter) and echocardiographic evaluation of LV dimensions and function.

Although these criteria represent the current criterion-referenced standard, they have several drawbacks, including limited availability because of the need for experienced echocardiographers, the need for specialized equipment, such as ultrasound machines or Holter monitors, high interobserver variability particularly in measurement of ventricular function, and financial cost, which may be substantial for owners with large number of dogs.¹⁰ Blood-based testing to detect cardiomyopathy is attractive because it is minimally invasive, ease of sample collection, potential for widespread availability, quantitative nature and theoretic cost efficiency. Cardiac

From the Clinic of Small Animal Medicine (Wess, Simak, Hartmann), and the Statistical Consulting Unit, LMU University, Munich, Germany (Mahling). Preliminary data of this study have been presented as an abstract at the 18th ECVIM Meeting 2008 in Ghent, Belgium.

Corresponding author: Gerhard Wess, DVM, Dipl ACVIM (Cardiology), Dipl ECVIM-CA (Cardiology and Internal Medicine), Clinic of Small Animal Medicine, LMU University, Veterinaerstr. 13, 80539 Munich, Germany; e-mail: gwess@lmu.de.

Submitted November 23, 2009; Revised December 27, 2009; Accepted February 26, 2010.

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10.1111/j.1939-1676.2010.0516.x

biomarkers such as cTnI or natriuretic peptides are all elaborated by the affected heart, presumably along a continuum associated with disease severity. Therefore, they might be found in dogs with early stages of nonclinical disease.

The hypothesis of this study was that Doberman Pinschers with DCM would have increased serum cTnI concentrations, even in early stages of the cardiomyopathy, in which arrhythmias are detected by Holter examination. Therefore, the objective of this prospective study was (1) to evaluate several influence factors on cTnI using a large cohort of healthy control dogs and (2) to evaluate serum cTnI concentrations in Doberman Pinschers in various stages of cardiomyopathy.

Material and Methods

Animals

The study population was 336 (186 female and 150 male) client-owned Doberman Pinschers that were prospectively selected according to the inclusion and exclusion criteria from a longitudinal cohort study starting in 2004. A total of 653 examinations from these 336 Doberman Pinschers met the selection criteria and were included in the study. Enrollment was restricted to purebred Doberman Pinschers without evidence of systemic disease. Written owner consent to participate in the study was obtained. The dogs were examined at least once a year. Each exam included a clinical examination, a Holter examination, echocardiography, and cTnI measurement.

Examinations

All dogs were examined without sedation in right and left lateral position. M-mode measurements were performed using the right parasternal long-axis view. All valves were examined with color-Doppler. Velocities over the aortic and pulmonary valves were measured with continuous wave Doppler examinations and had to be below 2.2 m/s. Twenty-four hour Holter recordings were performed at each exam and analyzed by 1 of 2 commercial available software programs.^{b,c} Manual adjustments and accuracy verification of the arrhythmias recognized by the software were performed. Plasma cTnI concentration was determined by a human assay^d validated for use in canine blood samples.³⁴ The detection limit was 0.2 ng/mL; if a value was below the detection limit, the value was entered into the data sheet as 0.02 ng/mL for statistical analysis, as this is the reported mean value of healthy dogs.³⁵ Additionally, to test if the significances between groups would be the same as by using 0.02 ng/mL, values below the detection limit were entered as 0.2 into the data sheet.

Disease Staging

Dogs were grouped according to the results of the Holter and echocardiographic examinations into 6 different groups:

- (1) Control group: Dogs in this group had no clinical signs and < 50 VPCs/24 h and normal echocardiographic measurements. M-mode values that were considered to be normal were as follows: left ventricular internal end-diastolic dimension (LVIDd) ≤ 47 mm and left ventricular internal end-systolic dimension (LVIDs) ≤ 38 mm.^{19,26}
- (2) Incipient group: Dogs were assigned to this group retrospectively. At the time of the examination dogs had no clinical signs and were considered normal according to the Holter and echocardiographic criteria as described above. However, at the

next exam within 1.5 years these dogs were abnormal according to the Holter and/or echocardiographic criteria as above.

- (3) VPC group: Dogs in this group had no clinical signs and > 100 VPCs/24 h on Holter examination and normal echocardiographic M-mode measurements as described above. The dogs were in the occult phase of the disease.
- (4) ECHO group: Dogs in this group did not show clinical signs, and were also in the occult phase of the disease. They had < 50 VPCs/24 h on Holter examination, but abnormal echocardiographic M-mode measurements. Values that were considered abnormal and indicative of DCM were as follows: LVIDd ≥ 49 mm^{31,36} and/or LVIDs ≥ 40 mm.^{19,21}
- (5) VPC and ECHO groups: Dogs in this group had no clinical signs and > 100 VPCs/24 h on Holter examination and abnormal echocardiographic M-mode measurements as described above. They were also in the occult phase of the disease.
- (6) Clinical group: In these dogs, clinical overt DCM was present. Dogs showed clinical signs (syncope, exercise intolerance, coughing, or dyspnea due to congestive heart failure) and were electrocardiographically and/or echocardiographically abnormal.

Exclusion Criteria

Dogs with evidence of systemic disease, concomitant congenital heart disease, or evidence of primary mitral valve disease (based on echocardiography) were not selected from the data-base. Dogs that had between 50 and 100 VPCs/24 h were considered "equivocal" and were not included in the study. Dogs with echocardiographic measurements that fell between the criteria for normal and abnormal were also not included in the study. Dogs were not included into the "incipient" group, if the last exam was dated back > 1.5 years, before they were diagnosed with cardiomyopathy.

Statistical Analysis

A linear mixed model was used to evaluate the effect of sex, weight, age, and M-mode measurements (LVIDd and LVIDs) on cTnI values in the control group. Dogs in the control group were assigned to different age groups: age group 1 (0–<2 years), age group 2 (2–<4 years), age group 3 (4–<6 years), age group 4 (6–<8 years), and age group 5 (>8 years). cTnI measurements were compared among the different groups using a linear mixed model and a procedure for simultaneous tests and confidence intervals for general linear hypotheses in parametric models, which adjusts for multiplicity and thus controls the overall type I error rate.³⁷ Differences in cTnI values between the disease groups and the control group were assessed using a linear mixed model and the procedure for simultaneous tests. The best model in terms of AIC was determined using backward selection. Additionally, the linear mixed model was used to test differences in cTnI values between the disease groups and the control group. The linear mixed model takes into account age differences between the control group and the disease groups 3, 5, and 6, which were significantly ($P < .001$) older than the control group and groups 2 and 4. Moreover, the model addresses the effect of repeated cTnI measurements in the same subject at different examinations. Residuals of the linear mixed models were inspected visually. Receiver operator curves (ROC) were used to illustrate sensitivity and specificity of cTnI to detect cardiomyopathy for various scenarios: (1) control versus all stages of cardiomyopathy, (2) control versus dogs with VPCs only, and (3) control versus dogs with echocardiographic changes with or without VPCs. The association between numbers of VPCs/24-hour and cTnI was tested by a linear regression analysis. Significance was defined as $P < .05$. Commercially available software programs were used for analysis.^{e,f,38}

Results

Six hundred and fifty-three examinations of 336 (186 female and 150 male) Doberman Pinschers fulfilled the inclusion and exclusion criteria. Each examination included Holter exam, echocardiographic exam, and cTnI measurement. The control group consisted of 264 healthy Dobermans Pinschers (mean weight, 35.0 kg; mean age, 4.35 years). cTnI was measured at 447 different examinations (time points) in this group. The group “incipient” consisted of 23 samples from dogs that were considered to be normal at the time of the examination, but that developed the disease within 1.5 years (mean weight, 35.8 kg; mean age, 5.59 years). The VPC group consisted of 88 cTnI samples obtained at different time points (at least 3 months apart) from 39 dogs (mean weight, 35.5; mean age, 7.34 years) in the occult phase of DCM with >100 VPCs/24h, but without echocardiographic changes. The ECHO group included 19 samples from 9 dogs (mean weight 35.0 kg, mean age 5.94 years) with only echocardiographic changes. Fifty-six samples were from 22 dogs that had both VPCs and echocardiographic changes (mean weight, 36.9 kg; mean age, 7.98 years). Twenty samples were obtained from 13 dogs with clinical signs (mean weight, 35.0 kg; mean age 7.58 years).

Age was the only variable that had a significant ($P < .001$) association on cTnI in healthy Doberman Pinschers (Table 1). Systolic and diastolic M-mode measurements, weight, and sex were not associated with cTnI values in the control group. The analysis between the different age groups showed significantly ($P < .001$) higher cTnI values in the oldest age group (>8 years) compared with the other groups.

Comparing the baseline characteristics between the control and the various disease groups, there were significantly ($P = .06$) more male dogs in the VPC plus ECHO group ($n = 37$, 66.6%) compared with the remaining groups ($n = 241$, 41.7%). Dogs in the VPC group, the VPC plus ECHO group, and the clinical group were significantly ($P < .001$) older than the control (4.35 ± 2.50 years), the incipient (5.59 ± 2.04 years), and the ECHO group (5.93 ± 2.99 years). Weight was not different among groups. The effects of all these covariates on cTnI values were accounted for in the linear mixed models. Only age and disease stage remained

Table 1. Serum cardiac troponin I concentrations (ng/mL) in different age groups from healthy control Doberman Pinschers ($n = 453$ examinations).

Age (Years)	N	cTnI Mean \pm SD (Range)
1-<2	75	0.06 \pm 0.18 (0.02-1.18)
2-<4	165	0.03 \pm 0.05 (0.02-0.48)
4-<6	101	0.07 \pm 0.15 (0.02-0.98)
6-<8	51	0.12 \pm 0.21 (0.02-0.94)
>8	55	0.22* \pm 0.19 (0.02-0.78)
Total	447	0.08 \pm 0.15 (0.02-1.18)

*Significantly different compared with all other age groups.

Table 2. Cardiac troponin-I concentrations (ng/mL) in various disease stages.

	N	cTnI Mean	95% CI
Healthy	447	0.07* \pm 0.16	0.05-0.08
Incipient	23	0.30 \pm 0.20	0.22-0.39
Only VPC	88	0.36 \pm 0.34	0.29-0.43
Only ECHO	19	0.33 \pm 0.23	0.22-0.44
Occult VPC plus ECHO	56	0.45 \pm 0.22	0.39-0.51
Clinical	20	1.04* \pm 0.48	0.82-1.27
Total	653	0.18 \pm 0.30	0.16-0.21

*Significantly lower compared with all other groups.

*Significantly higher compared with all other groups.

significant ($P = .001$) influence factors on cTnI concentrations in the linear mixed model.

cTnI was significantly higher in all disease groups compared with the control group using the linear mixed model ($P < .001$), which adjusted the effect of repeated measurements in some animals (Table 2). There was no significant difference of cTnI concentrations between the disease groups, with the exception of the group 5 with clinical signs, in which cTnI values were significantly higher than all other groups (Table 2 and Fig 1). The same significant differences between the control group and the disease groups were reached using 0.2 ng/mL as data entry for values below the detection limit; only mean values of all groups were higher than using 0.02 ng/mL as data entry for values below the detection limit. As the mean value (0.07 ng/mL, Table 2) for the control group using 0.02 ng/mL as data entry was in accordance with other studies (and even slightly higher) all further statistical tests were made using 0.02 ng/mL.^{3,35}

Sensitivity and specificity were illustrated by ROC curves for different scenarios. Using a cut-off value of >0.22 ng/mL, sensitivity was 78.9% and specificity 87.3% to detect all disease stages (Fig 2). The area under the curve (AUC) of the ROC plot was 0.85. To analyze the ability to predict arrhythmias or echocardiographic changes, sensitivity and specificity were calculated for a combination of different disease stages. The AUC was better to predict echocardiographic changes (AUC = 0.89), with a sensitivity of 86.6 and a specificity of 87.3%, compared with the ability to predict VPCs (AUC = 0.79) with a sensitivity of 70.5% and a specificity of 87.3%. Although cTnI was able to predict VPCs, no significant linear association ($R^2 = 0.008$, $P = .29$) between number of VPCs/24 hour and cTnI levels was found (Fig 3).

Discussion

The results of this study are unique in several aspects. First of all, if a new test is evaluated against the current gold standard (in the case of cardiomyopathy in Doberman Pinscher echocardiography and Holter examinations), it can only be as good as the gold standard, but it cannot perform better than the current test. If the new test would reveal a positive result in a patient that is classified by the current gold standard as healthy, it would be

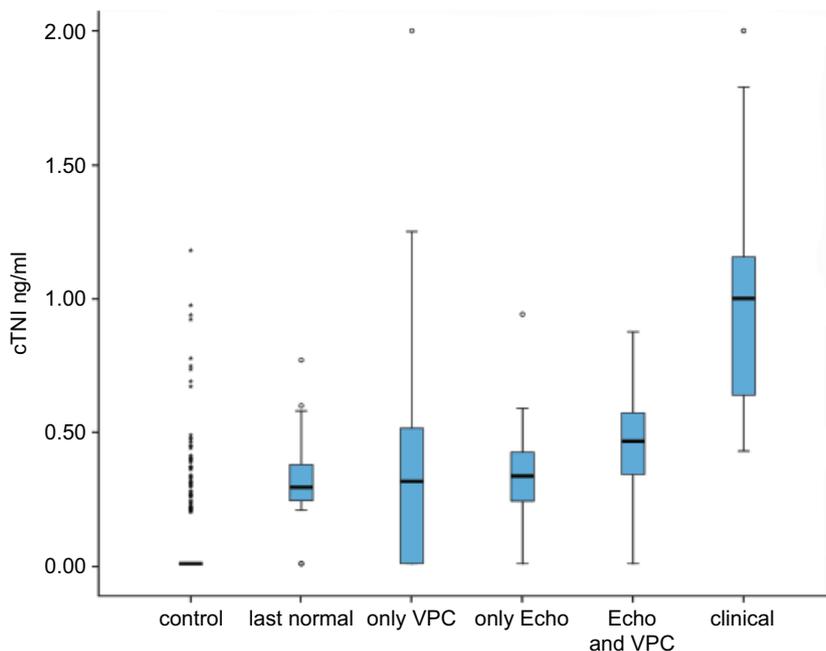


Fig 1. Distribution of 653 cardiac troponin-I of 336 Doberman Pinschers with different forms of cardiomyopathy and a control group. For each plot, the box represents the inter quartile range, the horizontal line in the middle of the box represents the median, and the whiskers denote the range. Outlier values between 1.5 and 3 times the IQR are indicated by a circle. Cases with values > 3 box lengths from the upper or lower edge of the box are shown with an asterisk.

considered a false positive result, even if the patient has the disease (and the gold standard would be unable to diagnose it at this time point). To circumvent this problem, this study introduced the disease group named “incipient,” a group consisting of animals that were classified as healthy by the current gold standards, but a diagnosis of cardiomyopathy based upon echocardiography, Holter, or both was made within 1.5 years after the last examination in this study. The last sample before the

dogs were classified as abnormal by the gold standards was retrospectively reclassified as “incipient.” cTnI was elevated significantly in this group compared with the control group—and thus, the biomarker detected the disease earlier than the current gold standards. In accordance with these findings, cTnI concentrations are increased in humans with mild cardiac injury, indicating that measurements of cTnI concentration can be useful in the detection of early disease.³⁹

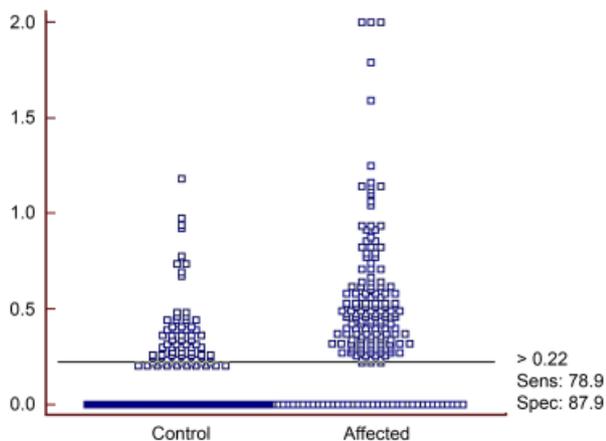


Fig 2. Dot diagram displaying individual cardiac troponin I concentrations of Doberman Pinschers in the control group (n = 447) and Doberman Pinschers with cardiomyopathy (groups 2–6, n = 206). The cut-off value of 0.22 ng/mL is shown as a line, demonstrating false positive (dots above the line in the control group) and false negative results (dots below the line in the cardiomyopathy group).

Another unique feature of this study is that it evaluated cTnI in different stages of cardiomyopathy in Doberman Pinschers. Cardiomyopathy in Dobermans is a common, inherited, slowly progressive primary myocardial disease.^{18–22} VPCs are a common finding in Doberman Pinschers with cardiomyopathy and can lead to sudden cardiac death in 25–30% of the cases in the occult phase of the disease.^{18,21,26,30,40} Because of the high frequency of electrical abnormalities in this breed, Holter examination has been suggested to be the best test to detect early changes.^{26,30,40} Usually, affected dogs will develop typical echocardiographic changes later in the disease progression.^{18,21,26,30,40} Increased troponin concentrations occur in dogs and humans with DCM, but the value of cTnI in the diagnosis of various forms of DCM in Doberman Pinschers has not been reported.^{10,41,42} cTnI is a marker of myocyte injury and is increased in various cardiac and noncardiac conditions.^{3,6,8,9,11,14,15,43,44} Therefore the results of this study, that the group with the most advanced disease stage had the highest cTnI levels, were not surprising. However, even the group with arrhythmias as the only finding had significantly increased cTnI levels. A small

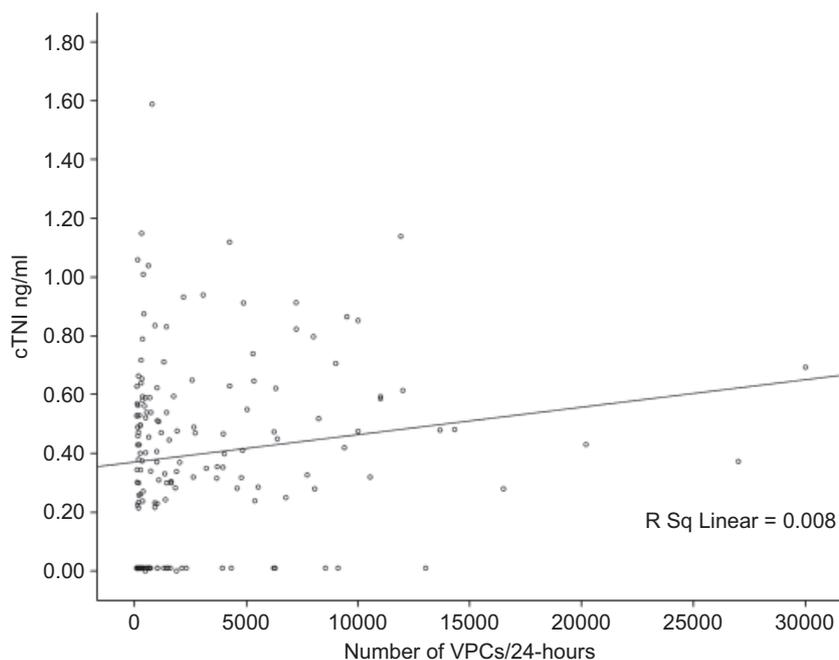


Fig 3. Graph of serum cardiac troponin I concentration versus number of VPCs/24 hour for Doberman Pinschers with >100 VPCs/24 h. No significant correlation ($R^2 = 0.008$; $P = .290$) was identified between serum cTnI concentration and the number of VPCs.

study evaluating cTnI in Boxer dogs with arrhythmogenic right ventricular cardiomyopathy found increased cTnI levels in dogs with VPCs but without echocardiographic changes, similar to this study.¹⁴ This study also found a significant positive correlation ($r = 0.78$; $P < .001$) between serum cTnI concentration and the amount of VPCs.¹⁴ In contrary, the present study found no correlation ($R^2 = 0.008$, $P = .290$; Fig 3) between the amount of VPCs and cTnI levels; therefore it seems that it is not the actual amount of VPCs that is responsible for increased cTnI concentrations, and it should be remembered that it has not yet been demonstrated that the number of VPC correlate with severity of disease or risk of unexpected death. An attempt to correlate the grade of arrhythmias (ie, couplets, triplets, ventricular tachycardia, R-on-T phenomenon) was not undertaken in this study, but in humans cTnI levels increased with the severity of arrhythmia detected.⁴⁵ If higher numbers of VPCs do not lead to higher cTnI levels, as shown in the present study, this could mean, that higher numbers of VPCs do not reflect more myocyte damage, and that the number of VPCs alone might not increase the risk to die from sudden death. Rather, the severity grade of the arrhythmia might be related to myocyte damage and thus cTnI levels in Doberman Pinschers. This needs to be investigated in future studies.

Several potential physiologic influence factors on cTnI were tested using the control group. Gender and weight did not influence cTnI levels. Only age had a significant influence factor on healthy control Doberman Pinschers in this study. Significantly higher cTnI values were found in healthy Doberman Pinschers older than 8 years, compared with younger age groups. Results of a previ-

ous report that included 176 healthy dogs revealed a modestly but significantly positive correlation with cTnI concentration and age ($r = 0.488$; $P = .001$).³ In humans, higher cTnI values have been reported for healthy elderly people as well.⁴⁶ Although in this study, Doberman Pinschers in the group with VPCs, in the group with VPCs and echocardiographic changes, and the group with clinical cardiomyopathy were older than control Doberman Pinschers, the increased serum cTnI concentrations in the affected groups are not simply the result of age, because age was taken into account by including it into the statistical model as a covariate. An explanation for higher cTnI values in older dogs might be age-related changes of the myocardium, leading to mild myocyte damage. Another explanation might be that some dogs with increased cTnI levels in the group of the control animals might actually have early cardiomyopathy, which Holter and echocardiography did not detect.

ROC curve analysis revealed that cTnI measurement cannot replace echocardiography or Holter analysis, as the sensitivity is too low. Despite the fact that cTnI was able to predict VPCs, the sensitivity of 70.5% is not high enough to replace a Holter examination. The sensitivity of cTnI to detect echocardiographic changes was 86.6%, but still too low to replace echocardiography. The AUC of the ROC plot was best for the ability of cTnI to predict echocardiographic changes. This might be explained by the fact that dogs with echocardiographic changes are in an advanced disease state and that these dogs have more myocyte damages. The sensitivity of 86.6% using a cut-off value of >0.22 ng/mL to detect echocardiographic changes was similar to the sensitivity in a previous study evaluating occult cardiomyopathy, but the specificity of

87.3% calculated in the present study was higher, which might be explained by the higher number of animals used in this study, using only 1 breed and stricter diagnostic criteria (ie, no Holter analysis was performed in the mentioned study).¹⁰ Specificity of cTnI on the other side was fairly high, and the ability to detect the “incipient” stage that the currently used methods (echocardiography and Holter) were unable to detect might lead to the conclusion that cTnI is a useful additional screening test for cardiomyopathy in Doberman Pinschers. Any elevation of cTnI > 0.22 ng/mL could be an indicator of cardiomyopathy, and dogs with such values should be investigated using echocardiography and Holter examination. Yearly screening for cardiomyopathy in Doberman Pinschers has been recommended.^{26,30,40} Dogs with increased cTnI levels should probably be re-examined more frequently.

A limitation of this study is that the control group might include dogs that already have myocardial damage at a cellular base, which neither Holter nor echocardiography can detect yet. These dogs might have correctly increased cTnI levels, but because they are judged as “healthy” according to the current gold standard, the cTnI levels of these dogs will be counted as false positive results and elevate the mean cTnI level of the control group. These cases would decrease the specificity of cTnI to detect cardiomyopathy. Further longitudinal follow-up studies on this issue are necessary and under way, but not yet finished. Another potential limitation is the inclusion of young dogs that might develop the disease at a later time. However, to test an influence of age it was necessary to include also young dogs. Additionally, early DCM will develop at a young age and, therefore, an age-matched control group has to be used. Furthermore, age was taken into account by including it into the statistical model as a covariate.

At the time the study was initiated, none of the new ultrasensitive assays for cTnI detection was available. Therefore, a test with comparatively high detection limit was used. For statistical reasons, values below the detection limit were entered as 0.02 ng/mL into the data sheet, despite the fact that the test is not able to detect these low levels. This value was used because other studies using higher sensitive assays reported mean values of 0.02 ng/mL in healthy dogs.³⁵ To test if the statistical differences between the groups would be the same, additionally a value of 0.2 ng/mL was entered for values below the detection limit. The same significant differences between the control group and the disease groups were reached using 0.2 ng/mL as data entry for values below the detection limit; only mean values of all groups were higher. However, as it is known from previous studies that the mean cTnI values are much lower than 0.2 ng/mL, the obtained mean values for the disease/control group would be inappropriately high. As the mean value (0.07 ng/mL, Table 2) for the control group using 0.02 ng/mL as data entry was in accordance with other studies (and even slightly higher), all further statistical tests were made using 0.02 ng/mL.^{3,35} Ultrasensitive tests may detect even earlier changes compared with this study and may lead

to lower cut-off values. This needs to be assessed in future studies.

Footnotes

- ^aO’Sullivan ML, O’Grady MR, Minors SL, et al. Occult dilated cardiomyopathy in the Doberman Pinscher, a retrospective study of prognosis in 163 cases. *J Vet Intern Med* 2005;19:406 (abstract)
- ^bCusto tera, Arcon Systems GmbH, Starnberg, Germany
- ^cAmedtech ECGpro Holter software, EP 810 Digital Recorder, Medizintechnik Aue GmbH, Aue, Germany
- ^dImmulate 2000 troponin-I, Siemens Medical Solutions Diagnostics, Deerfield, IL
- ^eSPSS for Windows, Version 13.0, SPSS Inc, Chicago, IL
- ^fMedCalc Statistical software, Version 11.1, Mariakerke, Belgium
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