Evaluation of N-terminal pro-B-type natriuretic peptide as a diagnostic marker of various stages of cardiomyopathy in Doberman Pinschers

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Objective—To evaluate the diagnostic value of plasma N-terminal pro-B-type natriuretic peptide (NT-proBNP) concentrations in Doberman Pinschers in various stages of dilated cardiomyopathy (DCM).

Animals—328 Doberman Pinschers.

Procedures—Staging of DCM was determined via analysis of results of physical examinations, 24-hour ambulatory ECG (Holter) recordings, and echocardiographic evaluations. Plasma samples for NT-proBNP assays were obtained at each examination. Concentrations of NT-proBNP were measured in 337 samples obtained from 196 healthy Doberman Pinschers (control dogs) and in 195 samples obtained from 132 Doberman Pinschers in various stages of DCM. These included dogs that had ventricular premature contractions (VPCs; 79 samples), echocardiographic changes (23 samples), or both (51 samples); 16 samples were from dogs with overt DCM, and 26 were from dogs that were considered normal during initial examination but developed DCM within 1.5 years after this assessment. Receiver operating characteristic curves were analyzed to determine sensitivity and specificity of NT-proBNP concentrations for detection of DCM.

Results—NT-proBNP concentrations in dogs that had or developed DCM were significantly higher than those of control dogs. Sensitivity and specificity of NT-proBNP concentrations (cut-off value, >400 pmol/L) to detect all stages of DCM were 81.1% and 75.0%, respectively; sensitivity was 90.0% and specificity was 75.0% to predict echocardiographic changes. Specificity to detect echocardiographic changes was 90.4% at a cutoff value of 550 pmol/L.

Conclusions and Clinical Relevance—Plasma concentrations of NT-proBNP were increased in dogs with DCM and in apparently healthy dogs that developed DCM within 1.5 years after samples were obtained, compared with concentrations in control dogs. (Am J Vet Res 2011;72:642–649)
enlargement, initially during systole and later during diastole.\textsuperscript{6,8-10} Doberman Pinschers in stages of DCM in which only VPCs, only echocardiographic changes, or both are detectable while clinical signs of heart disease remain absent are considered to be in an occult phase of the disease.\textsuperscript{1,6,12,16-18} In an earlier study,\textsuperscript{7} sudden death, caused by ventricular tachycardia or ventricular fibrillation, was reported in 14 of 54 (26%) dogs with occult DCM. The overt stage of DCM is characterized by the presence of clinical signs of heart failure.\textsuperscript{1,12,16,18-19} For diagnostic methods within 1.5 years, compared with dogs that did not have DCM and did not develop DCM detectable by use of conventional diagnostic methods including dogs that would be increased in Doberman Pinschers in all stages of DCM (including dogs in the first stage that would develop DCM detectable by use of conventional diagnostic methods within 1.5 years), compared with dogs that did not have DCM and did not develop DCM by the end of the evaluation period.

Materials and Methods

Animals—Three hundred twenty-eight (172 female and 156 male) client-owned Doberman Pinschers were prospectively selected from a larger group of dogs enrolled in an ongoing longitudinal cohort study at our hospital. Dogs were selected by use of the study database from January 1, 2004, to December 1, 2009. Enrollment was restricted to purebred Doberman Pinschers. Dogs were excluded from the study or included and assigned to groups on the basis of the results of clinical examinations, analysis of Holter monitor recordings, and echocardiographic evaluations. Written owner consent was obtained for each dog before enrollment into the study. Dogs enrolled in the study were examined at least once each year. The study protocol was in compliance with the institutional animal care and use committee and with German law regarding laboratory animals and animal care.

Examinations—Each examination included a physical evaluation, analysis of 24-hour Holter monitor recordings, and echocardiographic examination. A blood sample was also collected for plasma NT-proBNP assay at the time of each examination.

Holter monitor recordings were fitted to the dogs after all other examinations were complete. Recordings were obtained in the home environment and analyzed by use of 1 of 2 commercially available software programs.\textsuperscript{4,5} Manual adjustments of the number of VPCs detected were performed, and accuracy of arrhythmia detection by the software was verified. Results were determined to be normal for dogs that had < 50 VPCs recorded/24 h with no couplets, triplets, or salvos detected and abnormal for dogs that had > 100 VPCs/24 h (including couplets, triplets, or salvos). Results were considered equivocal for dogs that had 50 to 100 VPCs/24 h.

Echocardiographic examination of all dogs was performed in right and left lateral recumbency without sedation. A right parasternal long-axis view was used to obtain M-mode LVIDd and LVIDs measurements; these were assessed as normal (LVIDd ≤ 47 mm and LVIDs ≤ 38 mm),\textsuperscript{12} equivocal (LVIDd between 47 and 49 mm, LVIDs between 38 and 40 mm, or both), or abnormal and indicative of DCM (LVIDd ≥ 49 mm,\textsuperscript{2} LVIDs ≥ 40 mm, or both\textsuperscript{12}). All valves were examined by use of color Doppler imaging. Velocities across the aortic and pulmonary valves were measured via continuous-wave Doppler analysis; a value of < 2.2 m/s was required for inclusion in the study.

Blood (5 mL) was collected from a jugular vein into frozen plasma tubes that contained EDTA and was centrifuged at a rotation speed of 2,000 × g in a cooled (4°C) centrifuge for 10 minutes to separate the plasma. Plasma was stored at −70°C until batch analysis was performed in our laboratory. Concentrations of plasma NT-proBNP were determined via a commercially available NT-proBNP assay that was previously validated for use in canine plasma samples.\textsuperscript{26} The quantitative colorimetric end-point assay included the use of immunoaffinity-purified sheep antibody against canine NT-proBNP in a sandwich (capture antibody, antigen, and detection antibody) technique. The detection antibody was conjugated to horseradish peroxidase, and NT-proBNP was detected colorimetrically at a wavelength of 450 nm. Samples were run in duplicate, and the mean of these 2 samples was used for data analysis.
Exclusion criteria—Dogs with evidence of systemic disease, concomitant congenital heart disease, or primary mitral valve disease (determined via echocardiography) were not selected for study enrollment. Dogs that had equivocal results of Holter monitor recording analysis or echocardiographic M-mode measurements that fell between the criteria for normal and abnormal results were also excluded from the study.

Disease staging and group assignment—Dogs were initially assigned to 1 of 5 groups on the basis of results of physical examination, analysis of Holter monitor recordings, and echocardiography. These examinations were used to determine disease stages for group assignment. Group assignment was performed by 1 investigator (GW) before NT-proBNP results were entered into the data sheet. Dogs assigned to the control group had no clinical signs of heart disease, normal results for analysis of Holter monitor recordings, and normal M-mode echocardiographic measurements.

Dogs with no clinical signs of heart disease that were determined to have occult DCM at the time of initial examination were assigned to 1 of 3 groups (VPC, echo, or VPC-and-echo). Dogs that had > 100 VPCs/24-h detected in Holter monitor recordings and normal echocardiographic M-mode measurements were assigned to the VPC group. Those with normal results for analysis of Holter monitor recordings and abnormal M-mode echocardiographic measurements indicative of DCM were assigned to the echo group. Dogs with > 100 VPCs/24-h detected in Holter monitor recordings and abnormal M-mode echocardiographic measurements indicative of DCM were assigned to the VPC-and-echo group. Dogs that had overt DCM were assigned to the clinical group. These dogs had clinical signs of heart disease (including syncope, exercise intolerance, and coughing or dyspnea due to congestive heart failure) as well as ECG abnormalities (> 100 VPCs/24-h), M-mode echocardiographic measurements indicative of DCM, or both.

Dogs that had no clinical signs and were considered normal according to the described ECG and echocardiographic criteria during the initial examination but in which DCM was diagnosed during a follow-up examination within 1.5 years were retrospectively removed from the control group and assigned to a separate group (last-normal) without knowledge of their NT-proBNP status. Dogs were not assigned to the last-normal group if the previous examination had been performed > 1.5 years before the diagnosis of DCM was determined, and previously obtained data from such dogs were not included in the study.

Statistical analysis—A linear mixed model was used to evaluate associations of sex, weight, age, and M-mode measurements (LVIDd and LVIDs) with logarithmized NT-proBNP values in the control group. To compare NT-proBNP in dogs of various ages, the control group was divided into the following age groups: ≤ 3 years, 3.1 to 5.0 years, 5.1 to 8.0 years, and > 8.0 years. Logarithmized values of NT-proBNP measurements were compared among all groups by use of 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 controls the overall type I error rate. Differences in the logarithms of NT-proBNP values between the groups of dogs with DCM and the control group were assessed by use of a linear mixed model and the procedure for simultaneous tests. The best model in terms of Akaike’s information criterion was determined via backward selection. The linear mixed model accounted for age differences between the control group and the VPC, VPC-and-echo, and clinical groups, which comprised dogs that were significantly older than those in the remaining groups. The model also addressed the effect of repeated NT-proBNP measurements in the same dog at different time points. Residuals of the linear mixed models were inspected visually, and ROC curves were used to assess the use of plasma NT-proBNP concentrations to discriminate between dogs with and without DCM in the following comparisons: control group versus all dogs with DCM (including those that developed DCM within 1.5 years of sample collection), control group versus dogs with VPCs only, and control group versus dogs that had echocardiographic abnormalities with or without VPCs. Sensitivity, specificity, and AUC were investigated. Values of \( P < 0.05 \) were accepted as significant. Commercially available software programs were used for analysis.

Results

A total of 532 examinations, which included analysis of Holter monitor recordings, echocardiographic examination, and sample collection for NT-proBNP measurement, were performed for the 328 Doberman Pinschers that met the criteria for study enrollment. Dogs that had multiple examinations at different time points had blood samples collected for plasma NT-proBNP measurement at each examination. The control group consisted of 196 healthy Doberman Pinschers (mean weight, 34.39 kg; mean age, 4.43 years); 337 blood samples were obtained from dogs in this group. The VPC group consisted of 42 dogs with occult DCM (mean weight, 35.52 kg; mean age, 6.79 years) from which 79 blood samples were obtained. The echo group included 16 dogs with occult DCM (mean weight, 36.03 kg; mean age, 6.11 years) from which 23 blood samples were obtained. The VPC-and-echo group comprised 36 dogs with occult DCM (mean weight, 36.78 kg; mean age, 8.00 years) from which 31 blood samples were obtained.

### Table 1—Plasma NT-proBNP concentrations in healthy control Doberman Pinschers (n = 337 samples from 196 dogs) categorized according to age group.

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>No. of samples</th>
<th>Median (pmol/L)</th>
<th>Range (pmol/L)</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 3.0</td>
<td>128</td>
<td>295</td>
<td>23–1,325</td>
<td>318 ± 168</td>
</tr>
<tr>
<td>3.1–5.0</td>
<td>102</td>
<td>290</td>
<td>46–883</td>
<td>310 ± 156</td>
</tr>
<tr>
<td>5.1–8.0</td>
<td>59</td>
<td>304</td>
<td>22–737</td>
<td>340 ± 150</td>
</tr>
<tr>
<td>&gt; 8</td>
<td>48</td>
<td>395</td>
<td>112–1,118</td>
<td>431 ± 231*</td>
</tr>
</tbody>
</table>

Samples were obtained at each examination; some dogs had > 1 examination and thus > 1 sample collected during the study period. Values of \( P < 0.05 \) were considered significant.

*Significantly \( (P < 0.001) \) different from all other age groups.
were obtained. The clinical group included 12 dogs with overt DCM (mean weight, 36.66 kg; mean age, 7.60 years) from which 16 blood samples were obtained. The last-normal group comprised 26 dogs with occult DCM (mean weight, 35.86 kg; mean age, 5.42 years) from which 26 blood samples were obtained.

Age was the only variable that had a significant influence on plasma NT-proBNP concentrations in healthy Doberman Pinschers of the control group (Table 1). Among dogs in the control group, plasma concentrations of NT-proBNP were significantly (P < 0.001) increased in dogs > 8 years of age, compared with younger dogs. Sex, weight, and systolic and diastolic M-mode measurements were not significantly associated with NT-proBNP values.

Among the 6 groups, there were significantly more male dogs in the VPC-and-echo group, compared with the remaining groups. Dogs in the VPC, VPC-and-echo, and clinical groups were significantly older than were dogs in the control, last-normal, and echo groups. Dogs with echocardiographic abnormalities indicative of DCM (echo, VPC-and-echo, and clinical groups) had higher LVIdD and LVIdDs M-mode measurements than did dogs in the remaining groups. Weight was not different among the 6 groups.

Only group, age, and LVIdDs remained significantly (P < 0.001) associated with plasma NT-proBNP concentrations in the linear mixed model. Mean plasma concentrations of NT-proBNP were significantly (P < 0.001) higher in all groups of dogs that had or developed DCM, compared with concentrations for the control group. There was no significant difference in plasma concentrations of NT-proBNP among the 5 groups of dogs in various stages of DCM, with the exception of the clinical group, in which NT-proBNP values were significantly higher than all other groups (Figure 1; Table 2).

Sensitivity and specificity of the use of plasma NT-proBNP concentrations to detect all stages of DCM with a cutoff value of > 400 pmol/L were 81.1% and 75.0%, respectively; the AUC of the ROC curve was 0.84 (Figure 2). The ROC curve analysis revealed > 550 pmol of NT-proBNP/L as the best cutoff value for prediction of echocardiographic abnormalities indicative of DCM; sensitivity was 78.6%, specificity was 90.4%, and the AUC was 0.92. At a cutoff value of > 400 pmol/L, sensitivity to predict echocardiographic abnormalities was 90.0%, specificity decreased to 75.0%, and the AUC was 0.92. Analysis for predic-

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Table 1—Plasma NT-proBNP concentrations in 328 Doberman Pinschers.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of dogs</th>
<th>No. of samples</th>
<th>Median</th>
<th>Range</th>
<th>Mean ± SD</th>
<th>Percentile 5</th>
<th>Percentile 95</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>196</td>
<td>337</td>
<td>303</td>
<td>22–1,325</td>
<td>334 ± 188*</td>
<td>117</td>
<td>670</td>
</tr>
<tr>
<td>Last-normal</td>
<td>26</td>
<td>26</td>
<td>583</td>
<td>222–1,138</td>
<td>593 ± 179</td>
<td>239</td>
<td>1,022</td>
</tr>
<tr>
<td>VPC</td>
<td>42</td>
<td>79</td>
<td>579</td>
<td>153–3,000</td>
<td>661 ± 575</td>
<td>192</td>
<td>2,382</td>
</tr>
<tr>
<td>Echo</td>
<td>16</td>
<td>33</td>
<td>768</td>
<td>406–3,000</td>
<td>1,059 ± 767</td>
<td>409</td>
<td>2,998</td>
</tr>
<tr>
<td>VPC-and-echo</td>
<td>36</td>
<td>51</td>
<td>883</td>
<td>165–3,000</td>
<td>1,019 ± 720</td>
<td>254</td>
<td>2,984</td>
</tr>
<tr>
<td>Clinical</td>
<td>12</td>
<td>16</td>
<td>2,960</td>
<td>386–3,000</td>
<td>2,379 ± 904*</td>
<td>386</td>
<td>3,000</td>
</tr>
</tbody>
</table>

The control group comprised healthy dogs without evidence of DCM. Dogs that were considered normal according to diagnostic criteria at sample collection but in which DCM was diagnosed during a follow-up examination within 1.5 years were included in the last-normal group. Dogs with occult DCM were assigned to the following groups on the basis of diagnostic findings: VPC (> 100 VPCs/24 h detected in Holter monitor recordings), echo (M-mode echocardiographic measurements indicative of DCM), or VPC-and-echo (> 100 VPCs/24 h with M-mode echocardiographic measurements indicative of DCM). Dogs with overt DCM were assigned to the clinical group.

*Significantly (P < 0.001) lower than values for all other groups. †Significantly (P < 0.001) higher than values for all other groups.

See Table 1 for remainder of key.
Figure 2—Receiver operating characteristic curves displaying sensitivity and specificity of the use of plasma NT-proBNP concentrations to detect DCM in 328 Doberman Pinschers (left) and dot plots of plasma NT-proBNP concentrations in individual dogs (right). In panel A, ability of the test to distinguish between healthy control dogs (n = 337 samples from 196 dogs) and dogs that had or that developed DCM within the study period (195 samples from 132 dogs) is displayed (left); the 45° line represents the line of no discrimination. The diagnostic cutoff value of 400 pmol/L is shown as a horizontal line on the dot plot (right); dots above the line in the control group indicate false-positive test results, and dots below the line in the DCM group represent false-negative test results. In panel B, ability of the test to distinguish between healthy control dogs and dogs in the study that had M-mode echocardiographic abnormalities detected (VPC group), as well as dogs that had >100 VPCs/24 h with no echocardiographic changes (VPC-and-echo group), is displayed (left); the horizontal line on the dot plot (right) indicates the diagnostic cutoff value of 550 pmol/L.

Discussion

N-terminal pro-B-type natriuretic peptide is a valuable biomarker that can be used to distinguish cardiac causes of dyspnea from noncardiac causes, and high circulating concentrations of the peptide have been reported in humans and dogs with DCM, myxomatous mitral valve degeneration, hypertrophic cardiomyopathy, and various congenital heart diseases. To the authors’ knowledge, the study reported here is the first to evaluate this biomarker in dogs in various stages of DCM. Analysis of our results revealed that Doberman Pinschers in all stages of DCM, including dogs in the last-normal group (in which the results of conventional diagnostic tests were considered normal at the time of sample collection), had significantly increased plasma concentrations of NT-proBNP, compared with those of control dogs.

Screening for occult disease stages is one of the most promising areas of blood sample–based biomarker research. Because echocardiographic and 24-hour Holter monitor examinations are considered the current gold standard for diagnosis of DCM in Doberman Pinschers, a positive test result for a biomarker such as NT-proBNP would be considered a false-positive result in a dog that was classified as healthy by these methods at the same time point; however, it may be the case that the dog is in an early stage of DCM that is not yet detectable via echocardiography or analysis of Holter monitor recordings. Implementation of a longitudinal study design that included follow-up examinations allowed us to circumvent this problem and to retrospectively identify a group of dogs (last-normal) that were determined to be normal according to gold-standard diagnostic methods at the time of sample collection, but developed DCM within 1.5 years after this evaluation. Plasma NT-proBNP concentrations were significantly increased in this group, compared with concentrations in the control group. Therefore, the disease was detected by measurement of the biomarker earlier than it was detected by means of echocardiography or analysis of Holter monitor recordings in this group.

It appears that NT-proBNP concentrations increase in myocytes because of cellular changes that are present in the first stage of DCM in Doberman Pinschers, before the heart has morphological alterations detectable by conventional diagnostic tests. Increased ventricular stretch and atrial diastolic wall stretch augment synthesis and release of BNP and NT-proBNP from cardiomyocytes and are the principal stimuli controlling BNP production. Studies have revealed that diseases (such as DCM or myxomatous mitral valve degeneration) or experimental conditions that cause cardiac volume or pressure overload result in increased circulating concentrations of NT-proBNP in dogs.

In another study, investigators evaluating plasma BNP concentrations in Boxers with arrhythmogenic right ventricular cardiomyopathy did not find increased BNP values in dogs with VPCs. However, to the authors’ knowledge, no previous studies in veterinary medicine have evaluated associations between arrhythmias and NT-proBNP values. Results of the present study provide new information because plasma concentrations of NT-proBNP were increased, relative to control values, in dogs that had >100 VPCs/24 h with no echocardiographic abnormalities detected (VPC group), as well as in dogs with echocardiographic evidence of volume overload, systolic dysfunction, or both (echo and VPC-and-echo groups). Whereas the information that VPCs are associated with augmented circulating concentrations of NT-proBNP in dogs is new, investigators of several studies in humans have reported high circulating concentrations of BNP and NT-proBNP in patients with ventricular arrhythmias; values are particularly high in patients with an increased risk of sudden cardiac death. Additionally, measurement of NT-proBNP appears to be a valuable test in humans to differentiate
between cardiac and noncardiac causes of syncope. A possible explanation for increased plasma NT-proBNP concentrations in Doberman Pinschers in the VPC group may be that the hearts of some affected dogs might have been gradually enlarging, causing myocardial stretch and thus enhanced NT-proBNP synthesis and release, while echocardiographic measurements remained within the reference range. Further long-term studies would be necessary to test this hypothesis. Nevertheless, ROC curve analysis revealed that NT-proBNP measurement cannot replace analysis of Holter monitor recordings as a diagnostic tool because sensitivity of the assay was considered fairly low (81.1%) when dogs in the VPC group were included in the analysis, indicating that the diagnosis of DCM would be missed in a number of dogs with > 100 VPCs/24 h if Holter monitor recordings were not analyzed.

Sensitivity of the NT-proBNP assay to predict echocardiographic abnormalities indicative of DCM was 90.0% at a cutoff value of 400 pmol/L, and increasing the cutoff value to > 550 pmol/L increased specificity from 75% to 90.4%. Interestingly, the multivariate analysis with stepwise backward regression analysis showed that LVIDd, but not LVIDs, remained significantly associated with plasma NT-proBNP concentrations, in addition to group (indicative of disease stage) and age. Although sensitivity and specificity with the described cutoff values were high, NT-proBNP measurement should be considered an additional diagnostic test, rather than a replacement for the established conventional diagnostic tests (echocardiography and examination of Holter monitor recordings). Ideally, a perfect screening test reaches 100% sensitivity and has a high specificity. Although sensitivity was 90.0% at a cutoff of > 400 pmol of NT-proBNP/L to predict echocardiographic changes, some diagnoses would be missed if echocardiography was not performed. However, plasma NT-proBNP concentrations indicated early DCM in some dogs in which the results of echocardiography were considered normal, and those dogs developed DCM at a later time point. Thus the tests complement each other. However, results of the present study suggest that NT-proBNP measurement may be a valuable screening test if echocardiography is not available or if owners are not yet convinced to have further examinations performed. Assays for NT-proBNP measurement are less expensive than echocardiography and are widely available. If plasma concentrations of NT-proBNP are > 550 pmol/L (in the absence of other cardiac diseases or renal failure), echocardiographic abnormalities in Doberman Pinschers are likely to be detected, and this may help to convince owners that further diagnostic tests are warranted. If plasma concentrations of NT-proBNP are < 400 pmol/L, it is less likely that the dog has echocardiographically detectable DCM.

A recent study in dogs revealed weekly variations in plasma or plasma concentrations of NT-proBNP, which may result in measurements of the biomarker above the cutoff values determined in the present study. Veterinarians should be aware that plasma concentrations of NT-proBNP above the cutoff values described in the present study in a Doberman Pinscher do not necessarily mean that the dog has or will develop DCM; however, detection of these values in the last-normal group, which did not have DCM detectable by means of echocardiography and analysis of Holter monitor recordings, leads to the conclusion that measurement of plasma NT-proBNP concentrations is a useful additional screening test for DCM in Doberman Pinschers. Plasma concentrations of NT-proBNP > 400 pmol/L could be an indicator of DCM, and dogs with such values should be examined further via conventional diagnostic methods. Yearly screening for cardiomyopathy in Doberman Pinschers has been recommended; dogs with high circulating concentrations of NT-proBNP should be re-examined more frequently.

Several variables (sex, weight, age, and echocardiographic M-mode measurements [LVIdd and LVIds]) were investigated for potential associations with plasma concentrations of NT-proBNP among Doberman Pinschers in the healthy control group of the present study. Only age had a significant influence on NT-proBNP values in these dogs; when dogs were grouped by age, significantly increased plasma NT-proBNP concentrations were detected in healthy Doberman Pinschers > 8 years old, compared with younger age groups. This association remained significant in the multivariate analysis that included dogs in various stages of DCM. In humans, plasma NT-proBNP concentrations also increased with age because of changes in metabolic clearance of the peptide. In contrast to results of the study reported here, earlier veterinary studies did not report associations between age and circulating concentrations of BNP and NT-proBNP. The difference in this finding of the present study, compared with results of previous veterinary studies, may be explained by the fact that the present study included more dogs, especially in the older age group.

It is important to realize that the cutoff values for Doberman Pinschers established in the present study are only valid for the NT-proBNP assay that was used and for samples collected according to the described method. At least 1 other test for NT-proBNP evaluation is commercially available for use in dogs, for which sample collection and test characteristics are likely to be different from those described in this report. Cutoff values may also be different for such tests. Further studies are needed to determine the comparability of NT-proBNP assays available through various manufacturers.

A potential limitation of the study reported here was the inclusion of young dogs that might develop DCM at a later time. However, to test for associations with age, it was necessary to include young dogs; additionally, some dogs develop DCM at a young age, and age-matched controls were required. Furthermore, age was taken into account by including it in the statistical model as a covariate. Another potential limitation was that the control group may have included dogs that had some myocardial damage at a cellular level at the time the NT-proBNP analysis was performed, which was not detectable by means of echocardiography or analysis of Holter monitor recordings. The NT-proBNP test results of these dogs were counted as false-positive results, and these might have increased the mean plasma NT-proBNP concentration of the control group and might have re-
suited in decreased specificity of plasma NT-proBNP concentrations to detect DCM. Further longitudinal follow-up studies are needed to determine whether such dogs develop DCM at a later time.

References

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8. MedCalc statistical software, version 11.1, Mariakerke, Belgium.
10. Cardiopet proBNP test, IDEXX Laboratories, Westbrook, Me.

d. Cardiopet proBNP test, IDEXX Laboratories, Westbrook, Me.


