

Correlation between activation of the sympathetic nervous system estimated by plasma concentrations of norepinephrine and Doppler echocardiographic variables in dogs with acquired heart disease

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Objective—To evaluate correlations between plasma concentrations of norepinephrine and Doppler echocardiographic variables for dogs with degenerative mitral valve disease (DMVD) or dilatative cardiomyopathy (DCM) to better understand the time course and magnitude of sympathetic activation in dogs with heart failure (HF).

Animals—15 healthy dogs, 15 dogs with DMVD, and 15 dogs with DCM.

Procedures—Dogs were positioned in lateral recumbency with minimal restraint for at least 20 minutes. Plasma samples were obtained and assayed by use of high-performance liquid chromatography. Concentrations were correlated with HF classification and with the main Doppler echocardiographic variables for each group.

Results—Mean \pm SD norepinephrine concentration was significantly higher in dogs with DMVD (494.4 ± 204.8 pg/mL) or DCM (655.7 ± 652.5 pg/mL) than in healthy dogs (205.8 ± 78.9 pg/mL), but concentrations did not differ significantly between the 2 groups with HF. Correlations were not detected between norepinephrine and heart rate or any M-mode echocardiographic variables evaluated, except for fractional shortening (FS) in DCM dogs. In that group, norepinephrine was inversely correlated with FS values. In DMVD dogs, no significant correlation was found between norepinephrine and the left atrium-to-aortic root ratio or mitral regurgitation.

Conclusions and Clinical Relevance—A proportional inverse correlation exists between norepinephrine and FS values in dogs with DCM. However, norepinephrine concentration was not correlated with the evaluated echocardiographic variables in dogs with DMVD. Sympathetic antagonists should be evaluated as a treatment option because of the increased plasma concentrations of norepinephrine detected in dogs with HF. (*Am J Vet Res* 2006;67:1163–1168)

Some neuroendocrine mechanisms are triggered in humans and other animals during the development of HF that contribute to the progression of the disease.¹⁻⁷ Among the main mechanisms are stimulation of the SNS, activation of the renin-angiotensin system, and release of

ABBREVIATIONS

HF	Heart failure
SNS	Sympathetic nervous system
DMVD	Degenerative mitral valve disease
DCM	Dilatative cardiomyopathy
EGTA	Ethyleneglycol-bis-(β -aminoethylether)-N, N, N', N'-tetraacetic acid
LA:Ao	Left atrium-to-aortic root ratio
LVIDs	Systolic left ventricular internal dimension
LVIDd	Diastolic left ventricular internal dimension
LVIDs-Ao	Aortic root index of LVIDs
LVIDd-Ao	Aortic root index of LVIDd
FS	Fractional shortening

vasopressin.^{2,4,8,9} Activation of the SNS is one of the first mechanisms triggered in HF, and plasma norepinephrine concentrations increase in patients with HF.^{2,4,6,8,10-22} Some authors have reported¹⁹⁻²⁴ that this increase in circulating norepinephrine concentration induces stimulation of tachycardia and arrhythmias, is toxic to cardiomyocytes, and also causes malfunction and necrosis of cardiomyocytes. Many studies^{16,17,24-26} in humans have correlated norepinephrine concentrations of cardiac patients with prognosis, fatalities, and severity of clinical signs, which indicates that assessment of norepinephrine concentrations is a reliable index for evaluating sympathetic tonus.

Although other neuroendocrine markers, such as atrial natriuretic peptide and brain natriuretic peptide, have been examined in dogs with naturally acquired HF,¹¹ only a few investigators^{2,27} have determined norepinephrine concentrations in these dogs, and neither of those studies was aimed at correlating norepinephrine concentrations with Doppler echocardiographic variables in dogs with naturally acquired HF. The shortage of information on dogs with naturally acquired HF may be related to the difficulty in assessing norepinephrine concentrations in client-owned dogs because such animals are often nervous or excited during physical examination and stress can interfere with accurate evaluation of sympathetic tonus.

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In an initial study² in which results for dogs with naturally acquired HF (DMVD or DCM) were compared, plasma norepinephrine concentrations were higher in dogs with DCM, which typically have a poorer prognosis than dogs with DMVD. The authors of that study did not examine correlations between echocardiographic variables and sympathetic tonus of dogs with DMVD. However, those authors did mention that no correlation existed between norepinephrine concentrations and measures of left ventricular function in dogs with DCM, which differs from data reported¹³ for humans. In another study,²⁷ norepinephrine concentrations in dogs with naturally acquired or surgically induced mitral regurgitation were significantly greater than norepinephrine concentrations in healthy dogs; however, the correlation between sympathetic tonus and Doppler echocardiographic variables was not evaluated.

The objective of the study reported here was to correlate sympathetic tonus, as estimated by plasma norepinephrine concentrations, with Doppler echocardiographic variables in dogs with DCM or DMVD. Our intent was to provide a better understanding of the time course and magnitude of sympathetic activation in dogs with HF.

Materials and Methods

Animals—The study comprised 3 groups of client-owned dogs. The control group consisted of 15 healthy dogs (8 females and 7 males). Mean \pm SD age of the healthy dogs was 3.8 ± 3.15 years. On the basis of body weight, dogs were classified as small breed (range, 3 to 7 kg), medium breed (range, 8 to 15 kg), and large breed (range, 16 to 40 kg). For the control group, there were 6 dogs classified as small breed, 5 dogs classified as medium breed, and 4 dogs classified as large breed. Dogs of the control group were healthy and results for echocardiographic, electrocardiographic, and radiographic evaluations were considered normal.

For the HF groups, we selected 30 client-owned dogs with heart disease. The DMVD group comprised 15 dogs (6 females and 9 males; 10 small breed, 4 medium breed, and 1 large breed) with DMVD. Mean \pm SD age of the dogs with DMVD was 11.5 ± 2.44 years. The DCM group comprised 15 dogs (5 females and 10 males; 3 medium breed and 12 large breed) with DCM. Mean age of the dogs with DCM was 8.4 ± 3.09 years. A definitive diagnosis of DCM was obtained during echocardiographic examination by use of an ultrasound system^a with a 5-MHz microconvex transducer. Dogs with DCM had ventricular dilatation and reduced contractile function. Heart disease in dogs with DMVD or DCM was classified as grade Ia, Ib, II, IIIa, or IIIb by use of the International Small Animal Cardiac Health Council HF scoring system.^{28,29}

Informed written consent was obtained from each owner. The study was approved by the Ethics Committee of the Veterinary and Animal Science School of the University of São Paulo.

Procedure—All dogs were evaluated, which consisted of a thorough physical examination; ECG; measurement of arterial blood pressure; thoracic radiography; a CBC; serum biochemical analysis; and 2-dimensional, M-mode, and spectral-pulsed Doppler echocardiography. No dogs in the study had abnormal results for laboratory tests of renal and hepatic function. In addition, results of the CBC were within the respective reference ranges, and none of the dogs were receiving medications.

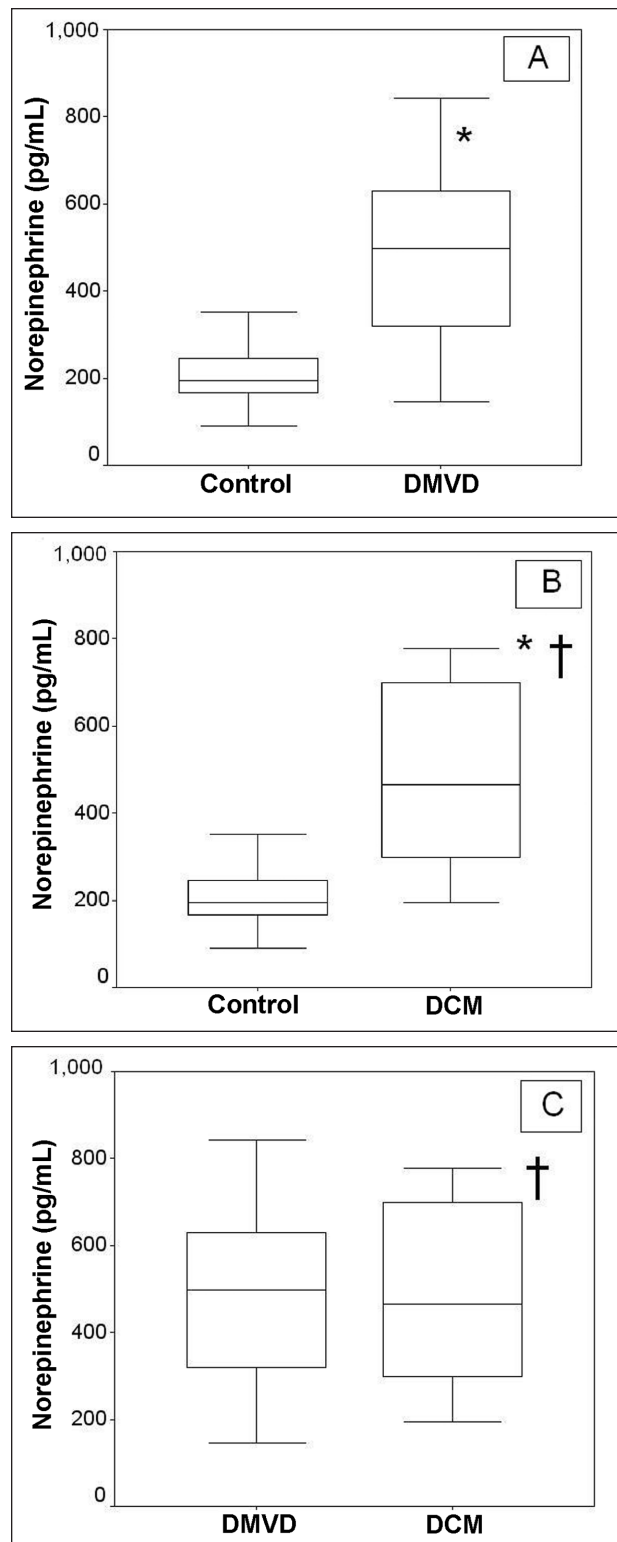


Figure 1—Box-and-whiskers plots comparing plasma concentrations of norepinephrine between 15 healthy (control) dogs and 15 dogs with DMVD (A), between the control dogs and 15 dogs with DCM (B), and between the dogs with DMVD and the dogs with DCM (C). Boxes represent the interquartile range (25th to 75th percentiles). Whiskers extending from boxes capture approximately 95% of the data. The horizontal line within each box represents the median values. *Values differed significantly ($P < 0.001$; Mann-Whitney test) from values for the control dogs. †Two norepinephrine concentrations for this group were outliers ($> 1,000$ pg/mL).

Blood samples for measurement of plasma concentrations of norepinephrine were collected early in the morning. An appropriately sized heparinized catheter^b was inserted into a saphenous vein of each dog. Then, dogs were positioned in lateral recumbency on a table with minimal restraint for 20 minutes.^{26,30} The first milliliter of blood collected via the catheter was discarded. The subsequent 3 to 5 mL of blood was collected and immediately transferred into ice-chilled tubes containing a mixture of EGTA-glutathione (20 µL of anticoagulant/mL of blood).

Within 1 hour after blood samples were collected, plasma was separated by use of cold centrifugation, harvested, and immediately frozen at -70°C. Plasma samples were assayed for norepinephrine content by use of high-performance liquid chromatography^{c,e} with electrochemical detection.³¹⁻³⁴ A calibration curve was used to calculate the amount of norepinephrine contained in each sample.

Several M-mode echocardiographic variables were tested to determine whether they were correlated with norepinephrine concentrations in each group of dogs. Those variables consisted of LA:Ao, LVIDs, LVIDd, LVIDs-Ao, LVIDd-Ao, and FS. Values for FS were calculated by use of the following equation: $FS = (LVIDd - LVIDs)/LVIDd \times 100$. Severity of mitral regurgitation of dogs with DMVD was estimated by use of spectral-pulsed Doppler ultrasonography with careful placement of the pulsed-wave Doppler gate at various depths within the left atrium to provide information on the width and depth of the regurgitant jet in the left atrium. An aliased signal was generated at points where the gate revealed a regurgitant jet; the signal was generated on the basis of the percentage of the left atrium occupied by the regurgitant jet (mild, < 20%; moderate, ≥ 20 to ≤ 50%; and severe, > 50%).³⁵⁻³⁸

Statistical analysis—Data were expressed as mean ± SD. The Kolmogorov-Smirnov normality test was used to test for normal distribution of data for the quantitative variables. On the basis of those results, the nonparametric Mann-Whitney and Kruskal-Wallis tests were selected for analysis of variables. The Spearman rank test was used to measure the degree of association (or dependence) between quantitative variables, and logarithmic regression was calculated when applicable. Values were considered significant at values of $P < 0.05$.

Results

Chromatographic analysis revealed that plasma concentrations of norepinephrine were significantly ($P < 0.001$) higher for dogs with DMVD (mean ± SD, 494.4 ± 204.8 pg/mL; median, 498.0 pg/mL) and dogs with DCM (mean, 655.7 ± 652.5 pg/mL; median, 464.0 pg/mL), compared with concentrations for the control group (mean, 205.8 ± 78.9 pg/mL; median, 194.0 pg/mL; **Figure 1**). Plasma concentrations of norepineph-

rine were compared between dogs with DMVD and DCM, and no significant ($P = 0.950$) difference was found, despite the higher mean norepinephrine concentration for dogs with DCM.

Mean ± SD heart rate measured by use of ECG in dogs with DMVD was 136.7 ± 27.53 beats/min, which did not differ significantly from the mean heart rate for control dogs (137.3 ± 30.8 beats/min; **Table 1**). Mean heart rate for dogs with DCM (198.0 ± 30.0 beats/min) was significantly ($P < 0.001$) higher, compared with the mean heart rate for the control dogs.

Values for the M-mode echocardiographic variables LVIDs-Ao, LVIDd-Ao, and FS for dogs with DMVD and DCM were compared with the corresponding values for the control dogs (**Table 1**). Dogs with DMVD had significantly higher values for FS ($P = 0.016$) and a lower LA:Ao ($P < 0.001$), compared with values for control dogs. For dogs with DCM, values were significantly higher for the echocardiographic variables LVIDs, LVIDd ($P < 0.001$), LVIDs-Ao ($P < 0.001$), and LVIDd-Ao ($P = 0.018$), compared with values for the control group. In contrast, the mean value for FS in dogs with DCM was significantly ($P < 0.001$) lower, compared with the mean value for the control dogs.

The M-mode echocardiographic variables (LVIDs, LVIDd, LVIDs-Ao, LVIDd-Ao, LA:Ao, and FS) and heart rate were tested by use of the Spearman rank test to detect correlations with norepinephrine concentrations in each group. None of the variables were correlated with norepinephrine concentrations, except FS values, which were correlated with norepinephrine concentrations for dogs with DCM. In this group, a significant negative correlation ($r = -0.545$; $P = 0.036$) was detected between plasma concentrations of norepinephrine and FS values (**Figure 2**).

Severity of mitral regurgitation in dogs with DMVD was estimated by use of spectral-pulsed Doppler ultrasonography, which revealed mild to moderate regurgitation for only 3 dogs and severe regurgitation for the other 12 dogs. Mean ± SD norepinephrine concentrations for the 3 dogs with mild to moderate regurgitation (441.0 ± 182.3 pg/mL) did not differ significantly ($P = 0.47$), compared with the mean norepinephrine concentration for the 12 dogs with severe regurgitation (507.8 ± 215.4 pg/mL).

To analyze the relationship between norepinephrine concentrations and functional classifications of

Table 1—Mean ± SD plasma concentration of norepinephrine, heart rate, and values for main echocardiographic variables assessed in 15 healthy (control) dogs, 15 dogs with DMVD, and 15 dogs with DCM.

Variable	Control	DMVD	DCM
Norepinephrine (pg/mL)	205.8 ± 78.9	494.4 ± 204.8*	655.7 ± 652.5*
Heart rate (beats/min)	137.3 ± 30.8	136.7 ± 27.5	198.0 ± 30.0*
FS (%)	36.75 ± 4.63	41.15 ± 3.92†	15.41 ± 4.01*
LVIDs-Ao	1.16 ± 0.15	1.28 ± 0.25	2.41 ± 0.75*
LVIDd-Ao	1.84 ± 0.14	2.20 ± 0.25	2.91 ± 0.94‡
LA:Ao	1.08 ± 0.05	1.71 ± 0.63*	2.18 ± 0.46*

* , † , ‡ Within a row, value differs significantly ($*P < 0.001$; $†P = 0.016$; $‡P = 0.018$; Mann-Whitney test) from the value for the control group.
Heart rate was measured by use of ECG at the time the plasma sample was obtained for measurement of the norepinephrine concentration.

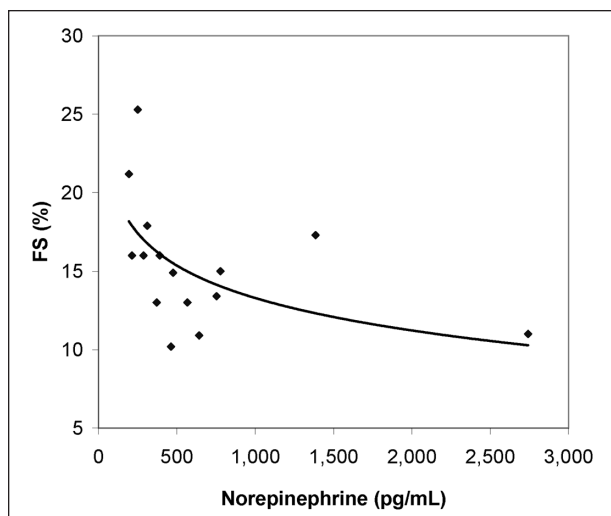


Figure 2—Graph of plasma concentrations of norepinephrine and FS in dogs with DCM. The values were significantly and negatively correlated ($r = -0.545$; $P = 0.036$), as determined by use of the Spearman rank method. The equation for the line was as follows: $y = (-2.988 \times \ln(x)) + 33.937$, where $\ln(x)$ is the natural logarithm of x .

Table 2—Mean \pm SD plasma concentrations of norepinephrine in dogs, based on functional classification for HF.

Functional classification	DMVD (No. of dogs)	DCM (No. of dogs)	Norepinephrine (pg/mL)
Healthy*	0	0	205.8 \pm 78.9
Ia	2	0	474.0 \pm 239.0
Ib	11	2	454.1 \pm 218.9
II	1	4	385.8 \pm 190.8
IIIa	1	4	605.0 \pm 176.5
IIIb	0	5	1,089.4 \pm 1,015.3

*Represents results for the 15 healthy dogs that comprised the control group.

dogs with DMVD or DCM, dogs were allocated to the functional classifications as follows: Ia, 2 dogs; Ib, 13 dogs; II, 5 dogs; IIIa, 5 dogs; and IIIb, 5 dogs. Mean plasma concentrations of norepinephrine for dogs in classes IIIa and IIIb were higher but did not differ significantly ($P = 0.383$), compared with concentrations for dogs in the other classifications (Table 2).

Discussion

Difficulty in assessing plasma concentrations of norepinephrine during physical examination of client-owned dogs as a result of dogs being nervous or excited could be a reason for the shortage of studies on dogs with naturally acquired HF. In the study reported here, this limitation was overcome by inserting a catheter in a saphenous vein of each dog and then having each dog lie on a table with minimal restraint for at least 20 minutes before collection of blood samples via the catheter. This assured animal stress would be only a minor interference in our study.

Differences in ages observed for the 3 groups resulted because of the necessity to obtain normal patterns for echocardiographic variables for the control dogs (youngest dogs) and because of the high survival rate for dogs with DMVD (oldest dogs), compared with survival rate for the dogs with DCM. Because of the

prevalence of DMVD in small breeds and DCM in large breeds, a control group composed of a mixture of breed sizes was chosen. These limitations were also observed in another study.² In that study, the DMVD group consisted of dogs with a mean age of 12.2 years and body weight of approximately 7.9 kg, whereas their DCM-group dogs had a mean age of 8.4 years and body weight of 37.4 kg. These characteristics are similar to those of the groups of dogs in the study reported here.

Our evidence that plasma concentrations of norepinephrine are increased in dogs with heart disease caused by DMVD or DCM corroborates results in other studies^{2,27} for dogs with naturally acquired and surgically induced heart disease. On the other hand, we failed to find a difference ($P = 0.950$) between the plasma concentration of norepinephrine for dogs with DMVD and the concentration for dogs with DCM. This is in contrast to results reported in a study² in which investigators detected significantly higher mean \pm SD norepinephrine concentrations for dogs with DCM, compared with concentrations for dogs with DMVD (950.7 \pm 117.5 pg/mL vs 524.5 \pm 117.5 pg/mL, respectively). It is possible that the higher variability of norepinephrine concentrations for dogs with DCM, compared with that for dogs with DMVD, accounted for the lack of a significant difference during our study. This variability could be explained by the fact that dogs with DCM in the study reported here were within a broader range of functional classifications (classes Ib, II, IIIa, and IIIb), whereas most dogs with DMVD were in functional classifications Ia and Ib. To overcome this heterogeneity between these groups and perhaps confirm the difference in norepinephrine concentrations between dogs with DCM and DMVD, we suggest that a higher number of dogs in each functional classification should be evaluated in future studies.

We detected a significant increase in plasma concentrations of norepinephrine in dogs with naturally acquired mitral valve disease but without clinical signs of the condition. In the same manner, this early systemic sympathetic activation was detected in dogs with experimentally induced mitral valve regurgitation but without clinical signs of the condition (functional classification Ia)³⁹ and in asymptomatic humans with mitral valve regurgitation.⁴⁰

Regarding treatment in humans, β -blockers have become an established treatment for patients with HF. A broad program of clinical studies^{41,42} conducted since 1992 has tested the effect of the β -adrenoceptor blocker carvedilol and found it has the potential to enhance survival in human patients with HF, chiefly those with DCM. Furthermore, many studies^{16,17,24-26} in humans have related the increase in norepinephrine concentrations with fatalities and the fact that severity of clinical signs is an important indicator of prognosis. Analysis of these results suggests that the use of β -adrenoceptor blockers to prevent sympathetic action on cardiomyocytes would be especially helpful in reducing progression of heart disease in dogs, although studies relating fatalities and prognosis on the basis of plasma concentrations of norepinephrine in dogs with acquired HF must be conducted to evaluate whether this species has the same relationship as

humans with regard to norepinephrine concentrations and prognosis.

Analysis of data obtained for echocardiographic variables revealed, as expected, significant differences between control dogs and dogs with DMVD (FS and LA:Ao). Similarly, there were significant differences between control dogs and dogs with DCM (all variables evaluated).

Within the dogs with DMVD was a subgroup of dogs with mild to moderate HF. For this subgroup, echocardiographic variables were not correlated with norepinephrine concentrations. In this subgroup, the increase observed for FS could be explained by the Franklin-Starling mechanism that leads to an increase in the force of contraction on the basis of the increase in preload volume during mitral valve regurgitation.⁴³ Nevertheless, the chronic volume overload and cardiac hypertrophy cause a slow but progressive decrease in myocardial contractility, even in clinically compensated dogs.⁴³

The proposed mechanism of systolic dysfunction characteristic of primary mitral valve regurgitation is related to increases in SNS tone, which causes a reduction of the absolute number of cardiomyocytes as well as the number of contractile elements within each cardiomyocyte.^{43,44} This is the reason that some authors have reported^{39,44} the hypothesis of possible beneficial effects of carvedilol treatment in dogs with DMVD, which includes dogs with DMVD that do not have clinical signs of the condition. Studies to evaluate the effect of β -adrenoceptor blockers during the various phases of DMVD treatment should be conducted to confirm this hypothesis.

For the dogs with DCM, we found a significant negative correlation ($r = -0.545$; $P = 0.036$) between FS and plasma concentrations of norepinephrine. In agreement with our results, investigators in another study¹³ also obtained a significant negative correlation ($r = -0.54$; $P < 0.05$) between norepinephrine concentrations and left ventricular function in human patients. Therefore, the higher the norepinephrine concentration, the smaller the FS value. Similarly, some authors have reported^{13,26f} that the increase in norepinephrine concentrations is directly proportional to left ventricular dysfunction in human patients with severe HF. In contrast, an initial study² conducted in dogs with naturally acquired DCM did not find any correlation. It must be mentioned that data obtained in that study in dogs² was limited to dogs that were receiving medications. Thus, those authors indicated that the results may differ from results obtained without therapeutic intervention, as was seen in the study reported here.

To our knowledge, the study reported here is the first to confirm a negative correlation between norepinephrine concentration and FS in dogs with naturally acquired DCM. This fact may be helpful in the selection and monitoring of dogs with DCM treated by the use of β -adrenoceptor blockers. In addition, this correlation may be of use in prognostic evaluations.

Analysis of the data for the study reported here did not reveal significant differences between norepinephrine concentrations among functional classifications. This is in contrast to results of another study² in which the dogs had an increase in norepinephrine concentra-

tion that was in accordance with a worsening heart condition. This discrepancy could be explained by the small number of dogs per functional classification in our study.

Increases in sympathetic activity during HF lead us to the adoption of β -adrenoceptor blockers treatments and to question when use of β -adrenoceptor blockers should be initiated and how they should be monitored. In the future, measurement of various neurohormones involved in the progression of HF will be helpful in the development of treatments by use of antagonists specific for each animal, initiated at specific time points, and monitored with regard to administration of other drugs.

On the basis of our analysis of the findings of the study reported here, we conclude that a proportional inverse correlation exists between norepinephrine concentrations and FS values in dogs with DCM and that norepinephrine concentrations could not be correlated with the evaluated echocardiographic variables in dogs with DMVD. Considering the increase in norepinephrine concentrations observed in these 2 groups of dogs with HF, it is important to establish the effects of therapeutic interventions with sympathetic antagonists in dogs affected by DMVD or DCM.

- a. Hitachi EUB 515A ultrasound system, Hitachi Medical Systems America Inc, Twinsburg, Ohio.
- b. Angiocath, Becton Dickinson, Juiz de Fora, Brazil.
- c. Waters 464 electrochemical detector, Waters Corp, Milford, Mass.
- d. 515 high-performance liquid chromatography pump, Waters Corp, Milford, Mass.
- e. 717 autosampler, Waters Corp, Milford, Mass.
- f. Nastari L. *Relação entre norepinefrina intramiocárdica e função ventricular esquerda em pacientes portadores de cardiopatia da doença de Chagas*. PhD thesis, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil, 2002.

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