

Plasma Big Endothelin-1, Atrial Natriuretic Peptide, Aldosterone, and Norepinephrine Concentrations in Normal Doberman Pinschers and Doberman Pinschers with Dilated Cardiomyopathy

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Background: Dilated cardiomyopathy (DCM) results in progressive myocardial and circulatory dysfunction causing activation of a number of neurohormonal systems, including the endothelin (ET) system, which is only beginning to be described in clinical veterinary medicine. Measurement of these circulating neurohormones possesses potential utility in the diagnosis, staging, and assessment of prognosis in cardiac disease.

Hypothesis: We hypothesized that plasma big ET-1, norepinephrine (NE), aldosterone, and atrial natriuretic peptide (ANP) concentrations in normal Dobermans would differ from those in Dobermans with DCM, and that concentrations of these hormones would be associated with time to congestive heart failure (CHF) or death.

Animals: Thirty client-owned Dobermans (10 each of normal, occult DCM, and overt DCM) were included in the study.

Methods: Dogs underwent an echocardiogram, ECG, and blood sample collection. Neurohormones were measured by high-pressure liquid chromatography (NE) or commercial assays.

Results: Dogs with occult DCM had significantly higher ANP concentrations compared with normal dogs (least squares means [95% confidence interval, CI]: occult female 53.7 pg/mL [40.2-71.7] versus normal female 31.6 pg/mL [24.8-40.3], $P = .026$; occult male 86.1 pg/mL [64.7-115] versus normal male 12.1 pg/mL [5.1-28.7], $P = .011$). Dogs with overt DCM had significantly higher concentrations of all neurohormones compared with the normal group. Furthermore, increasing big ET-1 (risk ratio [RR] 2.7, CI 1.3-8.6, $P = .01$) and NE concentrations (RR 3.9, CI 1.1-18.1, $P = .03$) over 1 month were associated with a shorter survival time.

Conclusions and Clinical Importance: High ANP concentrations can identify dogs with advanced occult DCM. Increasing big ET-1 or NE concentrations over time can be useful predictors of poor prognosis.

Key words: Biomarker; Canine; Congestive heart failure; Neurohormone.

The evaluation of circulating biomarkers in cardiac disease, in the form of neurohormones or myocardial enzymes or proteins, has become a popular topic of research and discussion among the veterinary cardiology community. This information not only furthers understanding of the pathophysiology of cardiac diseases but also has the potential to revolutionize the way we diagnose and monitor disease and assess prognosis. In dilated cardiomyopathy (DCM), specifically, clinical challenges include diagnosis of equivocal cases, prediction of the time to congestive heart failure (CHF) in subclinical or occult cases, objective assessment of therapeutic response, and prediction of survival time. Measurement of circulating neurohormones activated in the setting of DCM has the potential to contribute favorably to these issues. Studies in symptomatic and asymptomatic human DCM patients have found increases in markers of the sympathetic nervous system

(SNS) (ie, norepinephrine, arginine vasopressin),^{1,2} the renin-angiotensin-aldosterone system (RAAS) (ie, renin, angiotensin, aldosterone, hyponatremia),³ the natriuretic peptides (ie, atrial and brain natriuretic peptides),⁴⁻⁶ and the endothelin (ET) system (ie, ET-1, big ET-1),⁷⁻¹¹ and this data has proven useful to assess prognosis and therapeutic response, serving as a rationale for investigation of these same neurohormones in dogs with DCM.

In dogs with CHF secondary to DCM, high norepinephrine (NE),¹² aldosterone,^{13,14} atrial natriuretic peptide (ANP),^{14,15} and, more recently, brain natriuretic peptide (BNP)^{16,a} and ET-1¹⁷ have been described. Of these, only BNP has been reported to be significantly increased in the occult stage of DCM, identifying it in particular as a potential diagnostic aid. The prognostic utility of these neurohormones, however, is unknown in dogs with DCM and not routinely put to clinical use.

Endothelin-1, a 21-amino-acid peptide, is the predominant isoform of the ET system in the vasculature. It is a potent vasoconstrictor, with additional actions including positive inotropy and chronotropy, stimulation of the RAAS and SNS, and mitogenic properties.¹⁸ For clinical purposes, measurement of its precursor molecule, big ET-1, is favored because ET-1 has a very short half-life, whereas big ET-1 circulates longer, and the predominant fraction of ET immunoreactivity in heart failure patients is big ET-1 as opposed to ET-1.^{18,19} Big ET-1 is potentially a stronger indicator of prognosis in human heart failure than other clinical data including other neurohormones.⁷⁻¹¹ To the authors' knowledge, quantification of big ET-1 in dogs with various stages of naturally occurring DCM has not been reported.

The purpose of this study was to characterize select plasma neurohormone concentrations (big ET-1, NE,

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aldosterone, ANP) in Doberman Pinschers with sub-clinical (occult) and clinical (overt) DCM in comparison with normal Doberman Pinschers, and to investigate the presence of any associations between these hormone concentrations and prognosis.

Materials and Methods

The study protocol was approved by the Animal Care Committee of the University of Guelph, and informed consent was obtained from all owners.

Dogs

All dogs were client-owned Doberman Pinschers ≥ 20 months of age, examined between March 2001 and May 2002 at the Small Animal Teaching Hospital of the Ontario Veterinary College, University of Guelph. Each dog underwent a physical examination, 9-lead ECG, and echocardiography. Echocardiography was performed on dogs manually restrained in lateral recumbency with an echocardiographic system equipped with either a 2–4 or 3–5 MHz transducer.^b All 2-dimensional images and M-mode measurements were performed according to standard recommendations.^{20,21} The average of 3 measurements, with reacquisition of the image for each, was used. Dogs were included in the normal group (NL) if they were free of clinical signs of cardiac disease and if all of the following criteria were met: left ventricular internal dimension in diastole (LVIDd) < 42.7 mm for males or < 40.9 mm for females; left ventricular internal dimension in systole (LVIDs) < 34.7 mm for males or < 33.1 mm for females; and no ventricular premature beats during the ECG and echocardiogram.^{c,d} Dogs were included in the occult DCM group (OccDCM) if they were free of clinical signs of cardiac disease and if the following criteria for LV enlargement were met: LVIDd > 49 mm or LVIDs > 42 mm.^e Dogs were included in the overt DCM group (OvDCM) if the following criteria were met: LV enlargement as described above for the occult DCM group; fractional shortening (FS) $\leq 16\%$; respiratory clinical signs (any of cough, wheeze, dyspnea, orthopnea); and radiographic evidence of pulmonary edema. Exclusion criteria included evidence of mitral valve disease (marked mitral regurgitation, abnormal mitral valve morphology, and exuberant septal motion) or any cardiac disease other than DCM; the presence of atrial fibrillation (due to a concurrent echocardiography study); or evidence of significant noncardiac disease based on CBC and biochemistry profile. The latter included serum sodium concentration measured by ion-specific potentiometry,^f which was used in the subsequent analysis of included dogs.

Blood Sampling and Neurohormone Assays

Blood samples were collected for measurements of neurohormones following echocardiography in all dogs. For the OvDCM group only, a second sample was collected at a 1-month recheck visit. Dogs were placed in a quiet room with their owner for a 15–20 minute rest period. Jugular venipuncture was then performed with the dog in lateral recumbency on the floor. Blood was collected in 4×7 mL K_3 ethylenediamine tetra-acetic acid (EDTA) tubes^g prechilled on ice, 2 of which also contained 700 μ L aprotinin (for preservation of ANP and big ET-1).^h The samples were immediately centrifuged at 4°C for 8 minutes. The plasma was transferred to 4×3 mL cryovialsⁱ and immediately stored at -70°C .

All neurohormones were measured by an endocrinology laboratory at the Western Hospital in Toronto, Ontario. Plasma samples were transported to the lab by the investigator (MLO) in a dry shipper containing liquid nitrogen, then again stored at -70°C until the assays were performed, at which time the samples

Table 1. Validation of neurohormonal assays for canine plasma.

	Precision	Recovery	Linearity	
			Range	Sensitivity
NE	4.1%	70%	0.1–25 nM	0.1 nM
Aldosterone	6.7%	80%	2–200 pg/mL	4 pg/mL
ANP	10.4%	75%	40–2500 pg/mL	5 pg/mL
Big ET-1	4.9%	70%	2–100 pg/mL	0.3 pg/mL

NE, norepinephrine; ANP, atrial natriuretic peptide; ET, endothelin.

were thawed in ice water. Extraction procedures specific for each neurohormone were performed to remove any potential interfering proteins, and neurohormones were measured either by high-pressure liquid chromatography or commercial kits.²² Briefly, plasma catecholamines were separated by high-pressure liquid chromatography^j with a reverse-phase analytical column.^k Catecholamine detection was done with an electrochemical detector,^l and data analysis was performed by a computer-based software package.^m Plasma aldosterone was measured with a commercial radioimmunoassay kitⁿ and gamma counter.^o according to manufacturer's recommendations for canine samples. ANP was measured by a competitive enzyme-linked immunosorbent assay (ELISA), demonstrating 100% cross-reactivity with human and canine ANP^p because human and canine ANP share the identical 28-amino acid sequence.²³ Measurement of plasma big ET-1 was performed with a human commercial solid-phase sandwich ELISA, specific for big ET-1 ($\leq 0.1\%$ cross-reactivity with big ET-2, big ET-3, ET-1, ET-2, and ET-3).^q The sequence of antigen peptides used for antibody production for this kit is identical between humans and canines according to the manufacturer (written communication with Shinya Abe of Immuno-Biological Laboratories Co, Gunma, Japan, December 2002).

Validation of the assays with canine plasma was performed before running experimental samples and included tests of linearity, sensitivity (detection limit), recovery, and precision. Linearity was tested by canine plasma spiked with the test standard (a known concentration of hormone) then serially diluted to yield 8 concentrations. Standard curves were plotted, and the range of concentrations over which the curves were linear was determined. Sensitivity (detection limit) was determined as the concentration that produced a signal 2 to 3 standard deviations higher than the zero standard. Recovery was tested by canine plasma spiked with 5 different known concentrations of the hormones. Precision was assessed by running the same sample 4 times in the same run (intra-assay variability), and expressed as coefficient of variability (CV). The results of validation tests are presented in Table 1. The assays were determined to be linear over clinically relevant ranges of values, and detection limits were thought to be adequate based on relevant human and canine literature. CV for catecholamines, aldosterone, and big ET-1 was determined to be satisfactory at $< 10\%$, while CV for ANP was slightly greater at just over 10% but was still deemed acceptable. Recovery for all neurohormones was lower than expected but satisfactory. While the extraction procedures were optimized for the individual assays, the use of these purification techniques may account for the recovery findings.

Follow-up

For the OccDCM group, time to CHF or sudden death was recorded as the number of days from enrollment (day of data collection) to the onset of respiratory signs and need for diuretics, or to sudden death. Dogs experiencing noncardiac death before one of the above endpoints or those still occult at the end of the study

Table 2. Characteristics of dogs.

	Normal	Occult DCM	Overt DCM
Sex	3 M, 7 F	4 M, 6 F	9 M, 1 F
Age (years)	5.2 ± 2.2	8.3 ± 2.5 ^a	7.9 ± 1.6 ^a
Body weight (kg)	36.1 ± 4.4	37.4 ± 4.8	38.1 ± 6.2
LVIDd (mm)	38.4 ± 2.3	55.6 ± 4.4 ^b	60.4 ± 7.1 ^{b,c}
LVIDs (mm)	29.2 ± 3.7	48.9 ± 4.3 ^b	55.5 ± 6.2 ^{b,d}
FS (%)	24.0 ± 7.2	11.9 ± 3.6 ^b	7.7 ± 4.7 ^{b,d}

DCM, dilated cardiomyopathy; M, male; F, female; LVIDd, left ventricular internal dimension in diastole; LVIDs, left ventricular internal dimension in systole; FS, fractional shortening. Data are presented as mean ± standard deviation.

^a $P < .05$ versus normal group.

^b $P < .001$ versus normal group.

^c $P < .001$ versus occult DCM group.

^d $P < .05$ versus occult DCM group.

follow-up period (March 2003) were right censored. For the OvDCM group, survival time was calculated as the number of days from enrollment (initial data collection) to CHF death, sudden death, or euthanasia because of CHF. Dogs experiencing non-cardiac death before one of the above endpoints were right censored.

Statistical Analysis

Statistical analyses were performed by computer-based statistical software.[†] Tests of normality (Shapiro-Wilk and Kolmogorov-Smirnov, significance level $P > .1$) were applied and verified by examining residual plots. Log transformations were performed if residuals were not normally distributed. Differences among groups in baseline characteristics were assessed by analysis of variance (ANOVA) and the Least Squares Difference (LSD) test for multiple comparisons. Differences among groups in neurohormone concentrations were evaluated by analysis of covariance (ANOVA

of values adjusted for regression on an independent variable),²⁴ with the following potential covariates included: sex, age, body weight (BW), and heart rate (HR). Interactions between covariates, between covariates and the group effect, and quadratic terms for age and BW were also included. Least squares means (LS means), which incorporate and thus account for the effects of significant covariates (essentially adjusting the means to account for differences among groups in significant covariates), were calculated for each variable based on the respective model.²⁴ The LS means for variables with continuous covariates are those calculated using the average values of the continuous covariates (age, BW, HR). Separate LS means were reported for males and females if sex was a significant covariate. Tests of multiple comparisons were then applied (LSD test for comparison of 3 groups and Tukey's test for comparison of 6 groups when sex was a significant covariate). The correlation between neurohormone concentrations and echocardiographic parameters was evaluated by Pearson's correlation. Bivariate Cox proportional hazards regression analysis was used to determine if any of the baseline neurohormone concentrations or change in neurohormone concentrations over 1 month (for the OvDCM group) was significantly associated with time to endpoint in the OccDCM or OvDCM groups. A proportional hazard ratio (risk ratio, RR) with 95% confidence interval (CI) was calculated for each variable. Significance was defined as $P < .05$.

Results

A total of 30 Doberman Pinschers were included, with 10 dogs in each of the 3 groups. Sex, age, BW, LV dimensions, and FS for each of the 3 groups are summarized in Table 2. Mean age was significantly lower in the NL group compared with the OccDCM and OvDCM groups. BW was not significantly different among groups. As expected, LV dimensions and FS were significantly different among the 3 groups.

NE was significantly higher only in OvDCM compared with NL and OccDCM (Table 3). Regardless of group, NE was significantly higher in females than in

Table 3. Neurohormone concentrations in normal Doberman Pinschers and Doberman Pinschers with occult and overt dilated cardiomyopathy.

	Normal	Occult DCM	Overt DCM	Significant Covariates
NE (nM)				
M	0.7 (0.4–1.2)	0.8 (0.5–1.3)	1.5 (1.1–2.0) ^{a,b}	S
F	1.0 (0.7–1.4)	1.1 (0.8–1.6)	5.8 (2.4–14.2) ^{a,b}	
Aldosterone (pg/mL)	20.9 (13.1–33.2)	20.4 (12.8–32.4)	55.4 (34.8–88.1) ^{c,d}	
Sodium (mM)	150 (148–151)	149 (148–151)	148 (146–150)	
ANP (pg/mL)				
M	12.1 (5.1–28.7)	86.1 (64.7–115) ^c	176 (144–215) ^{a,b}	S, A, ^e A × A, Gr × S, Gr × A, A × S
F	31.6 (24.8–40.3)	53.7 (40.2–71.7) ^c	121 (57.1–255) ^c	
Big ET-1 (pg/mL)				
M	6.5 (4.6–9.1)	9.3 (6.9–12.5)	12.3 (10.2–14.9) ^c	Gr × S, BW ^f
F	4.5 (3.6–5.6)	5.6 (4.4–7.1)	20.2 (11.6–35.4) ^{a,b}	

DCM, dilated cardiomyopathy; NE, norepinephrine; M, male; S, sex; F, female; ANP, atrial natriuretic peptide; A, age; Gr, group; ET-1, endothelin-1; BW, body weight. Data are presented as least squares mean (95% confidence interval).

^a $P < .001$ versus normal group.

^b $P < .001$ versus occult DCM group.

^c $P < .05$ versus normal group.

^d $P < .05$ versus occult DCM group.

^e Positive (direct) relationship between variable and covariate.

^f Negative (inverse) relationship between variable and covariate.

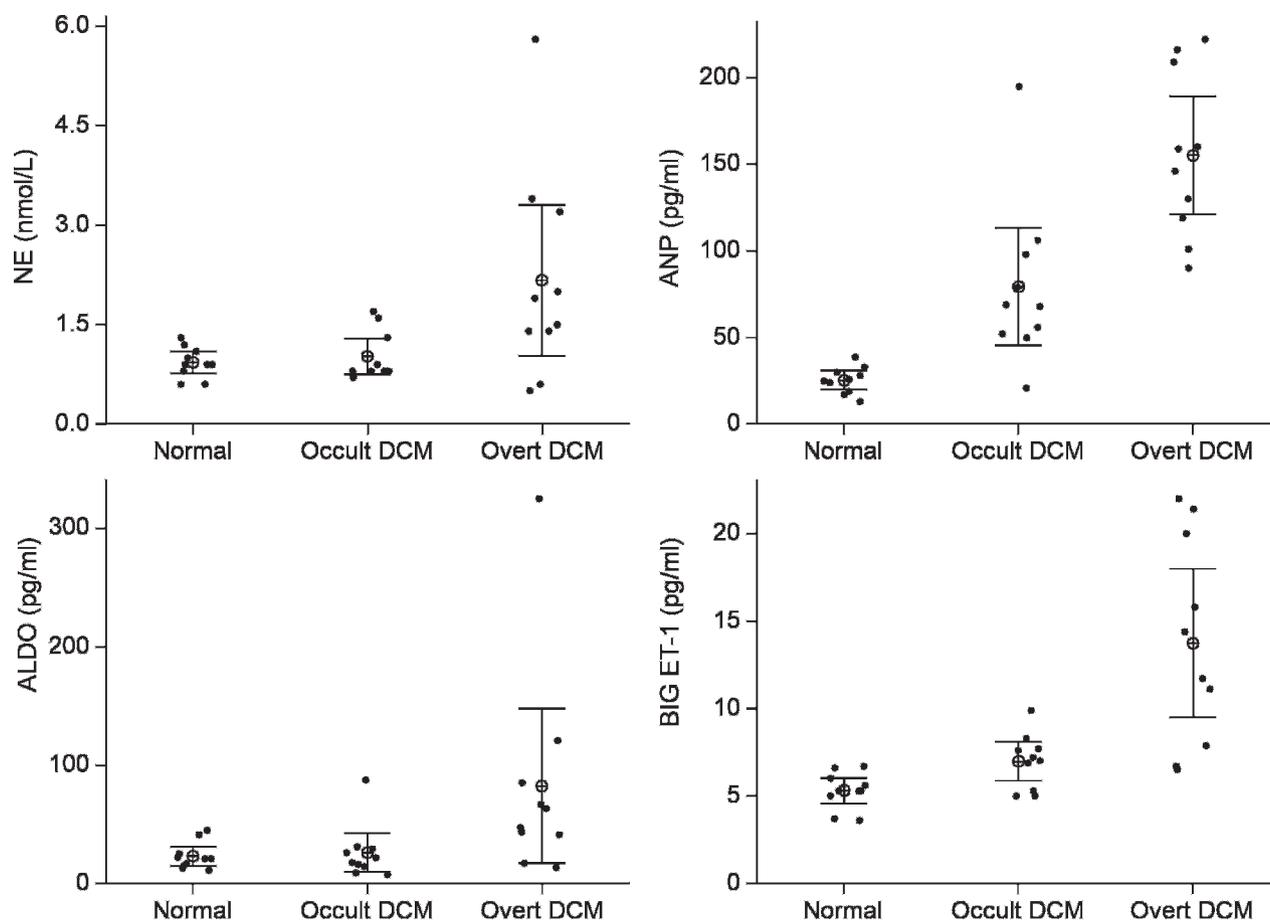


Fig 1. Plasma concentrations of norepinephrine (NE), atrial natriuretic peptide (ANP), aldosterone (ALDO), and big endothelin-1 (ET-1) in 10 normal Dobermans, 10 Dobermans with occult DCM, and 10 Dobermans with overt DCM (DCM and CHF). Individual dogs are represented by the filled circles. The geometric mean for each group is represented by the cross in an open circle, and the interval bars represent the 95% confidence intervals for the mean.

males. Aldosterone was similarly significantly higher in OvDCM than NL and OccDCM. With the removal of 1 potential outlier in the OvDCM group (Fig 1, highest value), the results for aldosterone were unchanged. Serum sodium was not significantly different among groups. ANP concentrations were significantly increased in both OvDCM and OccDCM compared with NL in both sexes. In addition to sex, age (positive relationship) and the quadratic term for age (negative quadratic) were also significant covariates for ANP (Table 3). Big ET-1 was significantly increased in OvDCM compared with NL for both sexes. In addition to sex, BW (negative relationship) was also a significant covariate for big ET-1 (Table 3).

Within the group of 30 dogs, there were substantial and significant positive correlations between ANP and LVIDD ($r = 0.73$, $P < .001$; Fig 2) and LVIDs ($r = 0.71$, $P < .001$; Fig 3), and modest but significant positive correlations between big ET-1 and LVIDD ($r = 0.48$, $P = .007$; Fig 4) and LVIDs ($r = 0.47$, $P = .009$; Fig 5). Weak but significant negative correlations were found between aldosterone and left ventricular free wall (LVFW) thickness in diastole ($r = -0.39$, $P = .033$) and systole ($r = -0.37$, $P = .046$). Modest but significant negative correlations were found between

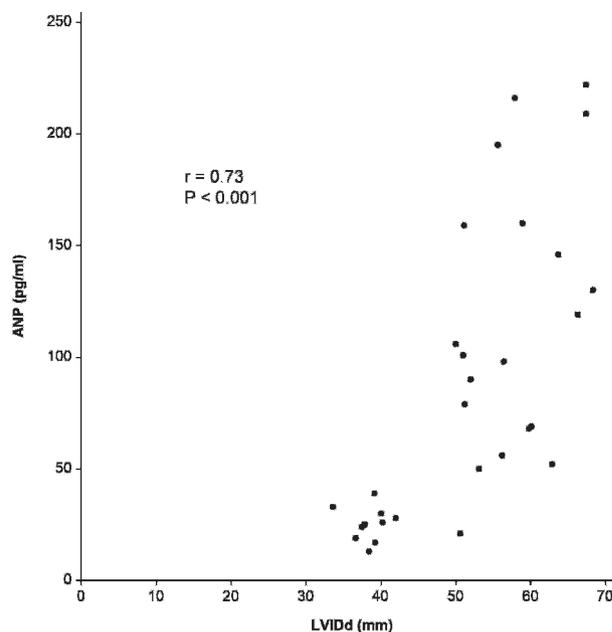


Fig 2. Correlation between plasma concentration of atrial natriuretic peptide (ANP) and left ventricular internal dimension in diastole (LVIDD) in all 30 Dobermans. There was a significant positive correlation between ANP and LVIDD.

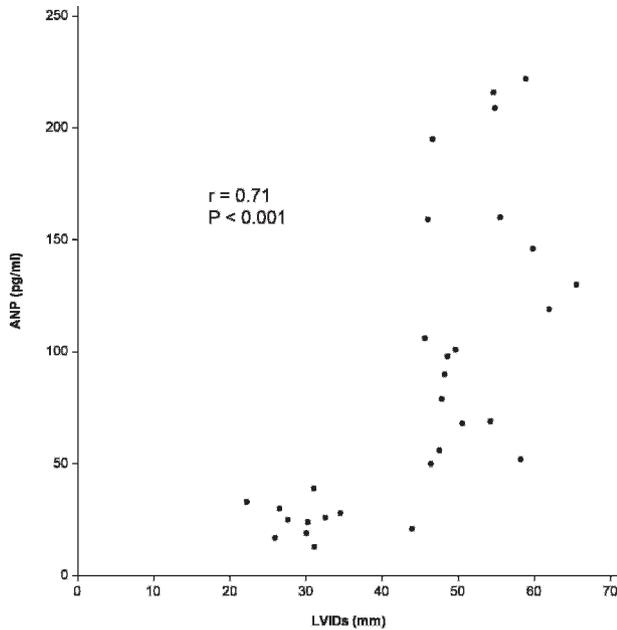


Fig 3. Correlation between plasma concentration of atrial natriuretic peptide (ANP) and left ventricular internal dimension in systole (LVIDs) in all 30 Dobermans. There was a significant positive correlation between ANP and LVIDs.

the amplitude of LVFW motion and aldosterone ($r = -0.44$, $P = .019$), ANP ($r = -0.45$, $P = .017$), and big ET-1 ($r = -0.44$, $P = .018$).

In the OccDCM group, 7 dogs reached a cardiac endpoint: 6 CHF and 1 sudden death. Of the remaining 3 dogs, 2 experienced noncardiac death (1 died secondary to hemorrhage from a pulmonary mass; 1 was euthanized for an intrathoracic mass), and 1

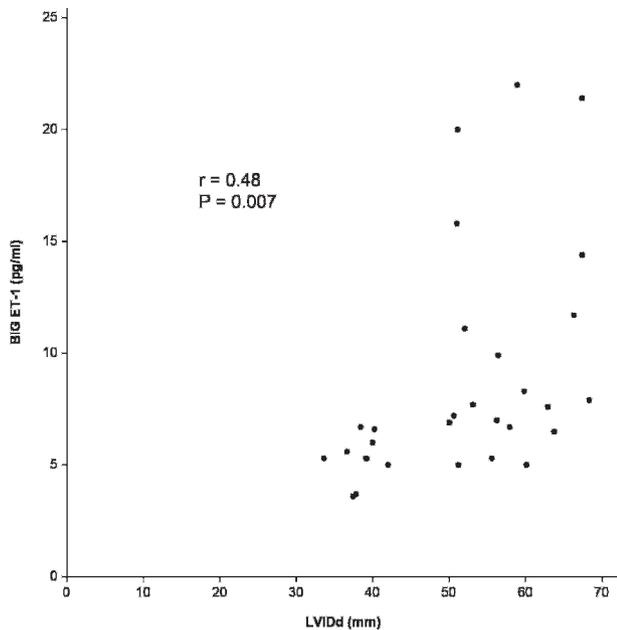


Fig 4. Correlation between plasma concentration of big endothelin-1 (ET-1) and left ventricular internal dimension in diastole (LVIDd) in all 30 Dobermans. There was a significant positive correlation between big ET-1 and LVIDd.

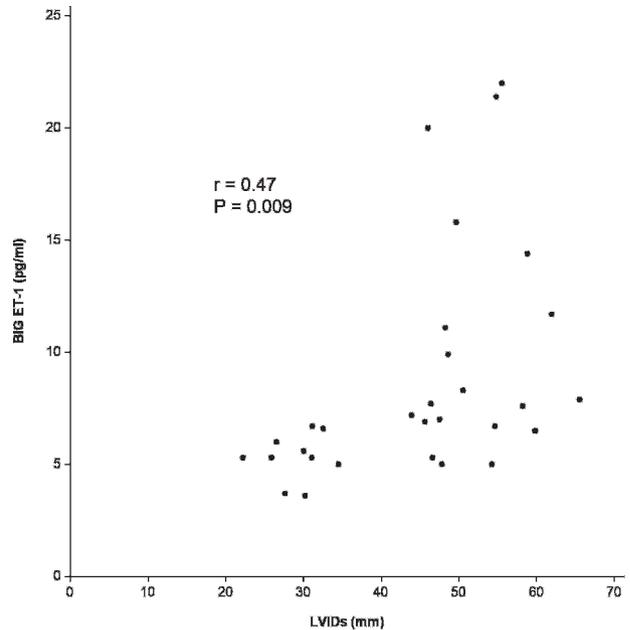


Fig 5. Correlation between plasma concentration of big endothelin-1 (ET-1) and left ventricular internal dimension in systole (LVIDs) in all 30 Dobermans. There was a significant positive correlation between big ET-1 and LVIDs.

remained occult at the time of analysis. For those that experienced CHF or sudden death, the median time to endpoint was 143 days (range 47–357 days). None of the neurohormone concentrations were significantly associated with time to CHF or sudden death.

In the OvDCM group, 9 dogs died because of heart disease (5 were euthanized because of refractory CHF and 4 died of sudden death). The remaining 1 dog was euthanized for appendicular osteosarcoma with stable CHF at the time. The median time to cardiac death was 62 days (range 13–214 days). Variables significantly associated with survival time included absolute and percentage change in NE over 1 month (RR 2.0, CI 1.1–4.4, $P = .03$ and RR 3.9, CI 1.1–18.1, $P = .03$, respectively), and absolute and percentage change in big ET-1 over 1 month (RR 1.2, CI 1.0–1.4, $P = .007$ and RR 2.7, CI 1.3–8.6, $P = .01$, respectively).

Discussion

Activation of neurohormonal systems in heart failure, while beneficial in the short term to maintain cardiac output and organ perfusion, is detrimental in the long term and contributes to progressive myocardial dysfunction and clinical deterioration in CHF patients.²⁵ In the present study, increased NE was present in the OvDCM group only. At least 1 other canine study investigating SNS stimulation in naturally occurring canine DCM has similarly documented high NE in dogs with DCM and CHF, and lack of increased NE in dogs with occult DCM.¹² In humans, plasma NE increases in proportion to the degree of LV systolic dysfunction even in asymptomatic disease, suggesting that the increase is not simply a consequence of worsening congestion.² Similarly, in dogs with rapid pacing-induced heart

failure, there is a rapid and significant rise in NE during the 28-day asymptomatic phase after the initiation of pacing, and a further significant rise at the onset of CHF.²⁶ Increasing NE is correlated to hemodynamic changes including increasing pulmonary capillary wedge pressure, mean pulmonary artery pressure, right atrial pressure, and decreasing cardiac output.²⁶ The lack of increased SNS activity in the OccDCM group is somewhat surprising considering that their end-systolic dimensions and FS were significantly abnormal.

The association between high NE concentrations and poor prognosis in human DCM patients is well documented, and some suggest NE should be routinely measured for this purpose.^{1,27,28} For the OvDCM group, an increase in NE over a 1-month period was associated with a shorter survival time (2 times the risk of death for every unit increase in NE). This may have reflected worsening hemodynamic status and consequent increase in SNS activation, direct detrimental effects of catecholamines (including vasoconstriction, tachycardia, myocardial necrosis, apoptosis, calcium overload, and arrhythmogenesis), or a combination of the two. Nevertheless, rising NE concentrations may be regarded as reflective of poor outcome and indicative of ineffective management.

Circulating aldosterone concentration was significantly high in the OvDCM group only, which is in agreement with other canine studies.^{13,14} While the presence of hyponatremia identifies human heart failure patients with the most marked activation of RAAS and poor prognosis,³ there were no significant differences among groups in serum sodium concentration in these Doberman Pinschers, similar to another study of canine DCM.¹⁴ The lack of high circulating aldosterone in the OccDCM group does not preclude activation of local RAAS, which play an important role in myocardial remodeling.^{29,30} The weak correlation between increasing aldosterone concentrations and decreasing LVFW thickness and amplitude of motion may in part reflect this remodeling effect. Data in humans support that aldosterone is produced in the left ventricle of patients with systolic dysfunction in response to increased wall tension or stretch, and the concentration in the heart greatly exceeds that in the circulation, such that circulating concentrations do not reflect the magnitude of local activity.³⁰ The fact that all OccDCM dogs were on angiotensin converting enzyme (ACE) inhibitors could also have contributed to these findings.

Unlike the other neurohormones in this study, plasma ANP concentrations were significantly high in both the OccDCM and OvDCM groups and were substantially correlated with LV dimensions. Studies in humans with DCM and dogs with pacing-induced heart failure have demonstrated significant increases in ANP both in the presence and absence of clinical signs.^{2,4,26,31} Furthermore, correlations between ANP concentrations and echocardiographic measures of systolic and diastolic function have been shown in human patients with DCM.³² These findings suggest a potential role for ANP concentrations in the prediction of time to onset of CHF. Although we did find significant correlations

between ANP concentrations and LV dimensions (Figs 2, 3), this study failed to demonstrate a role for ANP in predicting prognosis with these small numbers. In contrast to these findings, other studies of naturally occurring canine DCM have found a lack of increases in ANP in the occult phase of disease.¹⁴⁻¹⁶ Presumably the dogs in the present study were at a more advanced stage of the occult phase with a greater degree of left atrium enlargement and stretch because of greater systolic or diastolic dysfunction or both compared with these other studies. This idea is supported by the relatively short time to CHF or sudden death (median 143 days) in this study. ANP is unlikely to be useful in the diagnosis of early occult DCM considering its stimuli for release. Rather, BNP, which is secreted mainly from the ventricles in response to volume or pressure overload, is likely more sensitive and specific for identification of subclinical LV dysfunction.^{16,a} However, it may be worth investigating whether serial ANP concentrations have utility in prediction of time to onset of CHF once occult DCM is diagnosed, because it may be anticipated that concentrations rise as diastolic dysfunction and atrial dilation (the precursors to CHF) progress.

Big ET-1, the prohormone to the potent vasoconstrictor ET-1, has a longer half-life and circulates in higher concentration than ET-1, and it represents the dominant fraction of ET immunoreactivity in heart failure, rendering its measurement advantageous to that of ET-1.^{18,19} Increases in big ET-1 have been demonstrated in human DCM patients with severe or chronic heart failure; however, normal concentrations are typically found in asymptomatic or mildly symptomatic patients.^{7-9,19} In the Dobermans in this study, only the OvDCM group had significantly high big ET-1. Quantitation of ET-1 has been reported in a group of dogs with DCM or mitral valve disease with or without CHF, and similarly only those with CHF demonstrated high ET-1 concentrations.¹⁷ While big ET-1 is expected to circulate longer and in higher amounts than ET-1, measurement of big ET-1 did not appear to offer any advantage in terms of distinguishing dogs with advanced occult disease in this study. We were able to confirm activation of the ET system in canine DCM and CHF, but we also found increasing big ET-1 concentrations over a 1-month period to be associated with shorter survival time. Like NE concentrations, this finding may reflect worsening hemodynamic status, as potentially indicated by the significant correlations between big ET-1 concentrations and LV dimensions (Figs 4, 5), or detrimental effects of ET itself. A number of human clinical studies have shown that the concentration of plasma big ET-1 is a strong, independent predictor of prognosis in patients with DCM and severe heart failure, even superior to other clinical, neurohormonal, hemodynamic, and exercise testing data.⁷⁻⁹ Furthermore, studies have shown the value of sequential measurement of big ET-1 concentration in therapeutic monitoring, with the change in concentration predicting the magnitude of therapeutic response and future outcome.^{10,11,33,34} Unfortunately, a significant limitation to the clinical application of neurohormone measurement in veterinary

medicine is the lack of commercially available assays for rapid and easy determination of most neurohormones. It is hoped that this will change as more data become available regarding the utility of this information.

Several limitations of this study are worth noting. A small number of dogs were included, and it is well recognized that small sample sizes are by nature underpowered to detect anything but substantive differences among groups. With respect to survival analysis in particular, we were limited to investigating the effect of single parameters alone (bivariate analysis). This must be regarded, therefore, as a preliminary look at potential prognostic utility of these hormones. Follow-up studies in larger numbers of dogs are required. The medications received by and the diets fed to the dogs were not standardized, and this factor was not accounted for in the analyses. All OccDCM and OvDCM dogs were receiving ACE inhibitors, and all OvDCM dogs were receiving furosemide; however, other medications were variably used in the 2 groups, including beta-blockers, sotalol, spironolactone, and positive inotropes. With respect to the neuroendocrine data, plasma samples were stored for 1 to 6 months before analysis. The slow decay of catecholamines in stored, frozen canine plasma has been described previously.³⁵ Some researchers have suggested that ANP is stable for up to 6 months when stored at -80°C and treated with aprotinin,³⁶ while others have found that plasma ANP degraded by 50% after 1 month storage at -80°C , even with the addition of aprotinin.³⁷ Therefore, the potential for significant decay of ANP in frozen plasma must be considered when comparing these results with previous or future studies. Similar to the proposed advantages of measuring big ET-1 over ET-1, it may have been preferable to measure the N-terminal fragment of ANP, which is released on an equimolar basis with the active hormone but has slower plasma clearance, higher circulating concentration, and prolonged in vitro stability.¹⁴ The effect of storage at -70°C on the concentrations of aldosterone and big ET-1 is uncertain, and this issue was not addressed in the methodology.

In conclusion, this study provides some insight into the potential sequence of activation of various neuroendocrine systems at various stages of DCM. As ANP was the only neurohormone increased in the OccDCM group, it may be interesting to investigate the utility of serial measurements of ANP in predicting time to onset of CHF in this group. Big ET-1 and NE were significantly increased in the OvDCM group, and increasing concentrations over a 1-month period were associated with reduced survival. This finding may be clinically useful in the event that an easily performed commercial canine assay becomes available for big ET-1 or NE.

Footnotes

^a Oyama MA, Sisson DD, Solter PF. Prospective screening for occult canine dilated cardiomyopathy using B-type and atrial

natriuretic peptide and cardiac troponin-I assay. *J Vet Intern Med* 2005;18:453-454 (abstract)

^b HDI 5000CV, ATL Instruments, Bothell, WA

^c O'Grady MR, Horne R. Echocardiographic findings in 51 normal Doberman Pinschers. *J Vet Intern Med* 1995;9:202 (abstract)

^d O'Grady MR, Horne R. Outcome of 103 asymptomatic Doberman Pinschers: Incidence of dilated cardiomyopathy in a longitudinal study. *J Vet Intern Med* 1995;9:199 (abstract)

^e O'Grady MR, Horne R, Gordon SG. Does angiotensin converting enzyme inhibitor therapy delay the onset of congestive heart failure or sudden death in Doberman Pinschers with occult dilated cardiomyopathy? *J Vet Intern Med* 1997;11:138 (abstract)

^f Ion Selective Electrode (ISE) system, Hitachi 911, Roche Diagnostics, Laval, QC, Canada

^g Vacutainer system, Becton-Dickinson, Franklin Lakes, NJ

^h Product No. A6279, Sigma Chemicals Co, St Louis, MO

ⁱ Fisher Scientific Ltd, Nepean, ON, Canada

^j Waters 2695 HPLC system, Waters Canada, Mississauga, ON, Canada

^k Waters Spherisorb, 3 μm ODS2, 4.6 \times 100 mm, Waters PSS832112, Waters Canada, Mississauga, ON, Canada

^l Coulochem II model 5200A, ESA Inc, Bedford, MA

^m Millenium 2010 chromatography manager, Waters Canada, Mississauga, ON, Canada

ⁿ ImmuChem Double Antibody Aldosterone ¹²⁵I RIA Kit, ICN Pharmaceuticals Inc, Costa Mesa, CA

^o 1270 Rackgamma II, LKB Wallac, Fisher Scientific Ltd, Nepean, ON, Canada

^p α -ANP 1-28 (human, canine) EIA kit (Product EIAH8798), Peninsula Laboratories, San Carlos, CA

^q Big Endothelin-1 EIA kit (Product No. 17168), Immuno-Biological Laboratories Co, Ltd, Gunma, Japan

^r Statistical Analysis System (SAS) for Windows, Version 8.0, SAS Institute Inc, Cary, NC

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