Evaluation of the association between plasma concentration of N-terminal proatrial natriuretic peptide and outcome in cats with cardiomyopathy

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Objective—To determine whether plasma N-terminal proatrial natriuretic peptide (NT-proANP) concentration could predict the outcome (survival duration) of cats with cardiomyopathy (CM).

Design—Case-control study.

Animals—51 cats with CM (25 with and 26 without congestive heart failure [CHF]) and 17 healthy cats.

Procedures—Cats were thoroughly examined and assigned to 1 of 3 groups (control, CM with CHF, and CM alone). Plasma NT-proANP concentrations were measured by use of a human proANP(1-98) ELISA. Survival durations were compared between CM groups.

Results—Plasma NT-proANP concentrations differed significantly among the 3 groups, and survival durations differed significantly between the 2 CM groups. Median (range) NT-proANP concentration was 413 fmol/mL (52 to 940 fmol/mL) in the control group, 1,254 fmol/mL (167 to 2,818 fmol/mL) in the CM alone group, and 3,208 fmol/mL (1,189 to 15,462 fmol/mL) in the CM with CHF group. At a cutoff of 517 fmol/mL, NT-proANP concentration had a sensitivity of 90% and specificity of 82% for detecting CM. Multivariate analysis revealed that only the variable left atrium-to-aortic diameter ratio was a significant predictor of survival duration.

Conclusions and Clinical Relevance—Plasma NT-proANP concentration may have potential as a testing marker for distinguishing healthy cats from cats with CM. It may also be useful for distinguishing CM cats with CHF from those without CHF. The value of NT-proANP concentration as a predictor of survival duration was not supported in this study and requires further evaluation. (*J Am Vet Med Assoc* 2010;237:665–672)

Natriuretic peptides are a family of structurally similar but genetically distinct hormones that mainly regulate salt and water homeostasis and control blood pressure. The members of the natriuretic peptide family include types A through D, urodilatin, and synthetic vasonatrin peptide (a chimera of ANP and CNP).[1-7] Atrial natriuretic peptide and BNP are primarily synthesized in the atria of the heart and have an endocrine function.[2,3] The biological actions of ANP and BNP are mediated by a natriuretic peptide receptor type A and include diuresis, natriuresis, vasodilation, and inhibition of both the sympathetic nervous system and the renin-angiotensin-aldosterone axis. They also possess growth-suppressing and antiproliferative properties.[2,8–11]

Atrial natriuretic peptide is synthesized as a 153–amino acid preprohormone, which is stored after enzymatic transformation as proANP(1-126) in atrial myocyte granules.[12] During secretion, proANP is further processed by a serinprotease into an N-terminal fragment(1-98) (NT-proANP) and the biologically active C-terminal ANP(99-126) (α-ANP) in equimolar amounts.[1,2,10] Because NT-proANP is more stable and has an approximately 10-fold longer half-life in plasma, it is considered a reliable marker of the concentration of the active hormone.[11,13] Atrial natriuretic peptide (and BNP) is cleared from the circulation via natriuretic peptide receptor type C as well as via proteolytic cleavage by neutral endopeptidase.[1,6,10] Whereas the main trigger for ANP release is an increase in atrial wall stress, a certain release from the ventricles has also been demonstrated.[6,11,14,15] In cats, ANP is reportedly mainly lo-

Abbreviations

ANP	Atrial natriuretic peptide
AUC	Area under the curve
BNP	B-type natriuretic peptide
CHF	Congestive heart failure
CI	Confidence interval
CNP	C-type natriuretic peptide
HCM	Hypertrophic cardiomyopathy
LA:Ao	Left atrium-to-aortic diameter ratio
NT-proANP	N-terminal proatrial natriuretic peptide
NT-proBNP	N-terminal pro-B–type natriuretic peptide
ProANP	Proatrial natriuretic peptide
ROC	Receiver-operating characteristic
SAM	Systolic anterior motion

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calized in the auricular appendages, suggesting the auricles are particularly sensitive to stretching stress.[16]

Studies[11,17–21] in humans with heart disease have revealed that plasma ANP concentration is considerably elevated in asymptomatic patients and correlates with the severity of CHF, left ventricular mass, and left ventricular systolic and diastolic dysfunction as well as with severity of diastolic dysfunction alone. Natriuretic peptides are also reportedly good indicators of the effectiveness of medical and invasive cardiac treatments.[22–24] Findings[6,11,22,25–28] in humans suggest a prognostic value of natriuretic peptides for predicting further events such as hemodynamic abnormalities, heart failure in asymptomatic patients, and death.

In veterinary medicine, several studies[29–35] have been conducted to investigate the importance of ANP, and correlations between plasma ANP concentration and heart failure have been identified in dogs and cats. However, conflicting findings exist in cats with HCM but no clinical signs of disease. In 1 study,[36] no significant difference in plasma ANP concentration was detected between cats with HCM and healthy control cats. Results of 3 other studies[33–35] suggested plasma ANP concentrations are significantly increased in cats with cardiomyopathy, compared with values in healthy cats. Studies designed to investigate the prognostic value of natriuretic peptides in veterinary medicine are lacking. Only 1 study,[37] which involved 10 dogs with mild to moderate heart disease and 13 with severe heart disease (ie, mitral and tricuspid insufficiency and dilated cardiomyopathy), has been reported in which a significantly greater median survival time was found in dogs with an ANP concentration lower than the cutoff value. However, regression analysis to evaluate whether a correlation existed between ANP concentration and survival time was not performed.[37]

The purpose of the study reported here was to investigate whether plasma NT-proANP concentrations in cats with cardiomyopathy could predict the survival time of these cats and whether the variable could be useful in determining the prognosis. Another aim was to validate the results of a previous study[35] in which fewer subjects were used.

Materials and Methods

Animals—Included in the study were client-owned cats with cardiac disease or cats examined for other (noncardiopulmonary) reasons at the Small Animal Hospital of the University of Veterinary Medicine Hannover from October 2005 through November 2007. Exclusion criteria were detection of azotemia (plasma concentrations of urea > 65 mg/dL and creatinine > 1.8 mg/dL) and high plasma thyroxine concentration (> 4 µg/dL) as well as prior treatment with medications affecting the cardiovascular system (ie, angiotensin-converting-enzyme inhibitors, ion-channel and β-adrenoceptor blockers, and furosemide). For each cat, a physical examination, CBC, plasma biochemical analysis, measurement of plasma thyroxine concentration, thoracic radiography, ECG, and echocardiography were performed. Consent was obtained from all cat owners. The study protocol was approved by an institutional animal care and use committee.

On the basis of clinical findings, cats were assigned to 1 of 3 groups. The control group (group 1) comprised cats with no clinical, radiographic, or echocardiographic signs of cardiac or pulmonary disease. Classification of cardiomyopathy was made in accordance with systems used in other studies.[38–40] Cats with cardiomyopathy without signs of CHF were allocated to group 2 (cardiomyopathy alone). In those cats, echocardiographic findings included increased thickness (> 6 mm) of the interventricular septum or of the left ventricular posterior wall in diastole and presence of papillary muscle hypertrophy, diastolic dysfunction, or an enlarged left atrium with unremarkable appearance of the left ventricle. Group 3 (cardiomyopathy with CHF) included cats with cardiomyopathy and signs of associated decompensation such as dyspnea and radiographic detection of congested pulmonary vessels, pleural effusion, or pulmonary edema.

Echocardiography—Standard transthoracic echocardiography was performed with the aid of an ultrasonographic machine equipped with a broad-band, high-frequency, 7-MHz, phased-array transducer.[a] Simultaneous ECG recordings were obtained in all cats. All findings were stored digitally and calculated with the analysis software of the echocardiographic device. The cats were examined in right and left lateral recumbency by 2 investigators (FM and SH). The LA:Ao was calculated as described elsewhere.[41] The left atrium was considered enlarged when the LA:Ao was > 1.5.[42]

M-mode measurements of the thickness of the interventricular septum in diastole, left ventricular posterior wall in diastole, diastolic and systolic left ventricular internal diameter, and fractional shortening were obtained from the right parasternal long and short axes. All measurements were made in accordance with the recommendations of the American Society of Echocardiography.[43]

Mitral valve inflow pattern and left ventricular outflow velocity were recorded from the left apical long-axis view during Doppler echocardiography (color, pulsed, and continuous wave), and the presence or absence of SAM of the mitral valve was recorded. When SAM was present, the associated mitral regurgitation was also assessed via Doppler echocardiography. In cats with a normal appearance of the left ventricle but enlargement of the left atrium, in addition to assessment of mitral valve inflow patterns, Doppler tissue imaging was performed to evaluate diastolic function. During imaging, the early diastolic velocity of the mitral annulus was measured from the left apical 4-chamber view (reference limits, 10.6 to 14.6 cm/s[44,45]). Cats without evidence of diastolic dysfunction during Doppler tissue imaging (ie, low diastolic wall velocity) were categorized as having unclassified cardiomyopathy.

Thoracic radiography—Thoracic radiographs were obtained with cats positioned in right lateral and ventral recumbency, and images were processed digitally.[b–d] In these radiographic views, cardiac size, lung patterns, pulmonary vasculature, and presence of pleural effusion were evaluated.

Analysis of NT-proANP(1-98)—Blood samples for NT-proANP analysis were obtained from a cephalic or

femoral vein during routine hematologic analyses. The sample (1 mL) was collected in an EDTA-treated tube and centrifuged at 10,000 × *g* for 2 minutes within a maximum of 5 hours afterward. The supernatant plasma was harvested, placed in a 1.8-mL cryotube,[e] and stored at –20°C pending NT-proANP measurement. The NT-proANP(1-98) measurement was repeated for every sample, and the mean of the 2 values was calculated. Laboratory personnel were blinded to the clinical condition of the cats and the radiographic and echocardiographic findings.

Because of the high homology of feline and human NT-proANP(1-98), a test kit designed for use in humans was used for the detection of NT-proANP in cats, as described elsewhere.[12,35,36] The kit consisted of a sandwich enzyme immunoassay designed to measure NT-proANP(1-98)[f] directly in biological fluids. For this test kit, cross-reactivity of NT-proANP(1-98) with other proANP, proBNP, and proCNP fragments was < 1%. The standard range was 0 to 10,000 fmol/mL, with a detection limit of 50 fmol/mL. A standard curve was constructed from the standard values. On the basis of 5 replicates, the intra-assay coefficient of variance was 6.0% at a mean concentration of 427 fmol/mL and the interassay coefficient of variance was 7% at a mean of 436 fmol/mL. All measurements were made with an ELISA reader.[g]

Recheck examinations—Outcome of the cardiomyopathy-affected cats was evaluated by means of recheck examinations or by telephone interviews with the owners or referring veterinarians. The time for the recheck examinations depended on the clinical status of individual cats and ranged from a few days in severely affected cats to several months in clinically stable cats. All recheck examinations in cats available for reexamination included physical and echocardiographic evaluation as well as routine laboratory analyses (CBC and serum biochemical analysis).

Statistical analysis—Distribution of data (NT-proANP concentrations) was evaluated by use of a Kolmogorov-Smirnov test. When data were not normally distributed, nonparametric tests were used for further evaluation. Data obtained from the 3 groups of cats (control, cardiomyopathy with CHF, and cardiomyopathy alone) are reported as median and range.

A Kruskal-Wallis test was used to compare plasma NT-proANP concentrations among the 3 groups. To verify differences or similarities between pairs of groups (groups 1 and 2, groups 1 and 3, and groups 2 and 3), a Mann-Whitney *U* test was performed. With the Kaplan-Meier method, survival time for the 2 groups of cats with cardiomyopathy (groups 2 and 3) was estimated. Survival time was defined as the interval from diagnosis of cardiomyopathy until death, and cats euthanatized because of noncardiac problems were censored. Also, cats still alive at the date of the last follow-up were censored at that time.

A log rank test was used to determine whether a difference in survival time existed between the 2 cardiomyopathy groups. A value of $P < 0.05$ was considered significant. A univariate Cox regression analysis was performed for the variables group, age, sex, LA:Ao, and plasma NT-proANP concentration to determine whether any of these variables were associated with survival duration. For selection of variables for the analysis, a value of $P < 0.1$ was used. When significance was achieved, multivariate Cox regression analysis was performed with each significant variable added by means of a backward stepwise approach to model building. To avoid missing any variable as a significant predictor, an additional likelihood model was constructed with all variables and all possible combinations of them. Results of the multivariate analysis were considered significant at values of $P < 0.05$, with a hazard ratio CI of 95%.

The correlation between the plasma NT-proANP concentration and LA:Ao was examined by application of a 2-tailed Spearman rank correlation test. Receiver-operating characteristic analysis was performed to assess the ability of NT-proANP concentrations to discriminate among the 3 groups. Additional ROC analysis was used to identify the NT-proANP cutoff concentration that would best classify cats correctly with respect to sensitivity and specificity. All statistical analyses were performed by use of statistical software.[h]

Results

Animals—Sixty-eight cats were included in the study. Of these, 17 cats were assigned to group 1 (control) and 26 were assigned to group 2 (cardiomyopathy alone). Twenty-five cats were assigned to group 3 (cardiomyopathy with CHF). Cat age, reproductive status, and body weight did not differ significantly among groups (**Table 1**).

Clinical findings—Group 1 included 17 cats, of which 5 young (1- to 3-year-old) cats had been referred for evaluation of a mild heart murmur, 1 cat had nasal stridor because of a piece of grass lodged in the nasopharynx, 1 had cystitis, 2 had a history of trauma (caught by dog and hit by car, respectively), 1 had behavioral problems (urination outside the litter box), 1 was evaluated for lameness, and another 1 was evaluated for a small scleral mass. The remaining 5 cats were examined as part of routine check-up procedures. In 4 of the 5 cats referred for evaluation of a heart murmur, a grade 1/6 to 2/6 murmur could be auscultated. Three of these 4 cats were < 2 years of age (5, 12, and 16 months old), and no echocardiographic abnormalities could be detected. Because a systolic murmur in young adult cats can be associated with ejection of blood into the great vessels without any evidence of disease, the 3 cats were included in the control group. In one 13-year-old cat examined during a routine check-up examination, a heart rate–dependent, mild, dynamic obstruction of the right ventricular outflow tract was detected without any other echocardiographic, ECG, or systemic abnormalities. This cat was also included because this type of obstruction has been found in healthy cats > 4 years of age and was proposed as a physiologic cause of systolic murmurs in cats attributable to right ventricular systolic narrowing.[46]

Group 2 included 16 cats referred for evaluation of a heart murmur; the other cats had been referred for cardiac evaluation because of polypnea (n = 2 cats; di-

Table 1—Characteristics of healthy client-owned control cats (group 1; n = 17), cats with cardiomyopathy alone (group 2; 26), and cats with cardiomyopathy and CHF (group 3; 25).

Variable	Group 1	Group 2	Group 3
Age (y)	7[a] (0.5–14)	8[a] (1–17)	8[a] (1–15)
Body weight (kg)	4.7[a] (3.8–8.4)	5.2[a] (3.6–7.9)	5.1[a] (2.5–8.8)
Plasma NT-proANP (fmol/mL)	413[a] (52–940)	1,254[b] (167–2,818)	3,208[c] (1,189–15,462)
LA:Ao	1.3[a] (1.2–1.5)	1.4[a] (1.2–1.8)	2.3[b] (1.6–3.2)
Reproductive status			
Sexually intact male	1 (6)	0	2 (8)
Castrated male	10 (59)	21 (81)	16 (64)
Spayed female	6 (35)	5 (19)	7 (28)
Breed			
Domestic shorthair	12 (71)	19 (73)	16 (64)
British shorthair	2 (12)	4 (15)	5 (20)
Persian	0	2 (8)	2 (8)
Abyssinian	1 (6)	0	0
Maine Coon	1 (6)	1 (4)	0
Norwegian Forest Cat	0	0	1 (4)
Chartreux	1 (6)	0	0
Siamese	0	0	1 (4)

Values for continuous variables (age, body weight, plasma NT-proANP concentration, and LA:Ao) are reported as median (range). Values for categorical variables are reported as number (%).
[a–c]Within a row, values with different superscript letters are significantly ($P < 0.001$) different.

agnoses were pain associated with hemorrhagic cystitis [1] and an intrathoracic mass [1]), inappetence (4; diagnosis was a mass of the small bowel [1], mass of the tongue [1], mass of the bile duct and liver [1], and peritonitis attributable to ulcer-ruptured duodenum [1]), and hind limb weakness (2; diagnoses were necrosis of the femoral head [1] and myelopathy [1]) and for assessment of seizure-like signs (2; diagnoses were neurologic disorders). Twenty-two cats had hypertrophy of the left ventricular posterior wall or interventricular septum, and 1 cat had hypertrophy of the papillary muscles with an unremarkable diameter of the left ventricular posterior wall in diastole (6 cm). Because the findings in the cat with hypertrophic papillary muscles are reportedly a preliminary sign of HCM, that cat was enrolled in group 2.[47,48] In 10 group 2 cats, a dynamic obstruction was detected in the left ventricular outflow tract (median peak velocity, 2.85 m/s; range, 1.8 to 6 m/s). Three cats, each with an enlarged left atrium but normal appearance of the left ventricle, were categorized as having unclassified cardiomyopathy because no diastolic dysfunction could be detected during Doppler tissue imaging. The LA:Ao in group 2 ranged from 1.2 to 1.8 (median, 1.4).

Group 3 included 25 cats initially examined because of diminished appetite and lethargy; 15 of these cats also had dyspnea. Nineteen cats had hypertrophy of the left ventricle, 8 of which had dynamic obstruction (median peak velocity, 2.6 m/s; range, 2 to 6 m/s). In 4 cats, unclassified cardiomyopathy was present; in another 2, dilated cardiomyopathy was detected. In 1 cat with dilated cardiomyopathy, a taurine deficiency was diagnosed. Nineteen group 3 cats had a markedly enlarged left atrium (LA:Ao, ≥ 2). In 6 cats with hypertrophy of the left ventricle, the LA:Ao ranged from 1.6 through 1.8. The median LA:Ao in group 3 cats was 2.3 (range, 1.6 to 3.2).

Plasma NT-proANP concentration—The median plasma NT-proANP differed significantly ($P = 0.015$) among the 3 groups (**Figure 1**). Comparison of NT-proANP concentrations between pairs of groups also revealed significant ($P < 0.001$) differences. Group 3 had the greatest NT-proANP concentration (median, 3,208 fmol/mL; range, 1,189 to 15,462 fmol/mL), whereas the other cardiomyopathic cats in group 2 had significantly lower values (median, 1,254 fmol/mL; range, 167 to 2,818 fmol/mL). The group 1 control cats had the lowest concentrations (median, 413 fmol/mL; range, 52 to 940 fmol/mL). For all cats, a moderate correlation ($r = 0.71$; $P = 0.01$) between plasma NT-proANP concentration and LA:Ao was found (**Figure 2**).

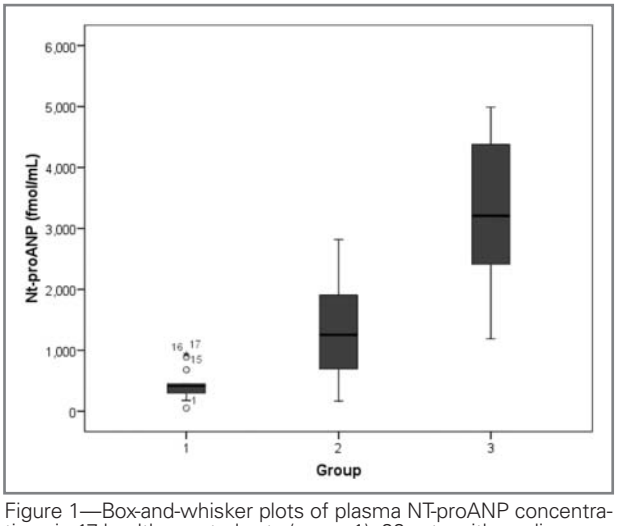


Figure 1—Box-and-whisker plots of plasma NT-proANP concentrations in 17 healthy control cats (group 1), 26 cats with cardiomyopathy but without CHF (group 2), and 25 cats with cardiomyopathy and CHF (group 3). For each box, the horizontal line represents the median value; the lower and upper boundaries of the box represent the 25th and 75th percentiles, respectively; and the whiskers represent the minimum and maximum NT-proANP concentrations. Circles represent outlier values; the outlier value in group 3 was 15,462 fmol/mL. The median NT-proANP concentration differed significantly among groups ($P = 0.015$) and between each pair of groups ($P < 0.001$).

Survival duration—For the analysis of survival duration, 9 cats in group 2 were censored when they were euthanatized for noncardiac reasons (neoplasia [n = 5], urethral obstruction [2], peritonitis [1], and iatrogenic hypoadrenocorticism [1]). An additional 15 cats in group 2 were censored because they did not reach the endpoint (death). Consequently, the median survival duration could not be calculated for group 2 (survival duration ranged from 424 to 808 days). However, when the data were evaluated on the basis of a 78% survival rate, which was met in both groups of cats with cardiomyopathy, the survival duration in group 2 was estimated at approximately 505 days and that in group 3 was 38 days, representing a clinically relevant difference.

In group 3, 3 cats were euthanatized for noncardiac reasons (neoplasia [n = 2] and urethral obstruction [1]) and were censored at that time. An additional 7 cats were censored because they were alive at the study's conclusion. Survival duration was significantly ($P < 0.001$) longer in group 2 than in group 3 (median, 313 days; range, 0 to 747 days; **Figure 3**). Five group 3 cats were euthanatized within 24 hours after study enrollment when treatment was declined by owners, and within the same period, 3 other cats were euthanatized because of arterial thromboembolism. To determine whether those circumstances might have influenced the significance of the difference between survival durations of groups 2 and 3, an additional Kaplan-Meier analysis was performed to exclude data from the 5 group 3 cats. The resulting median survival duration for group 3 was 336 days (range, 3 to 747 days), which still differed significantly ($P < 0.01$) from that of group 2.

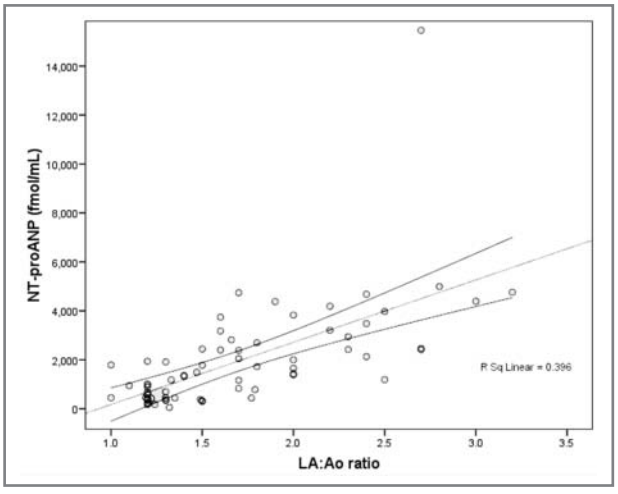


Figure 2—Scatterplot of plasma NT-proANP concentrations and the LA:Ao ratios for 68 cats, showing the linear regression line (middle line; $r = 0.71$; $P = 0.01$) and associated 95% CIs (top and bottom lines).

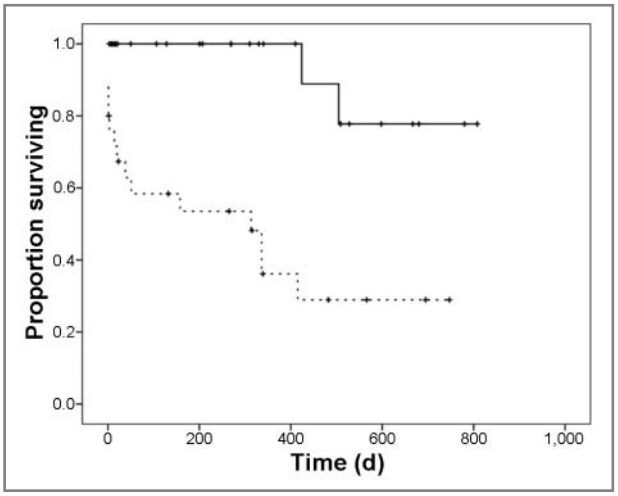
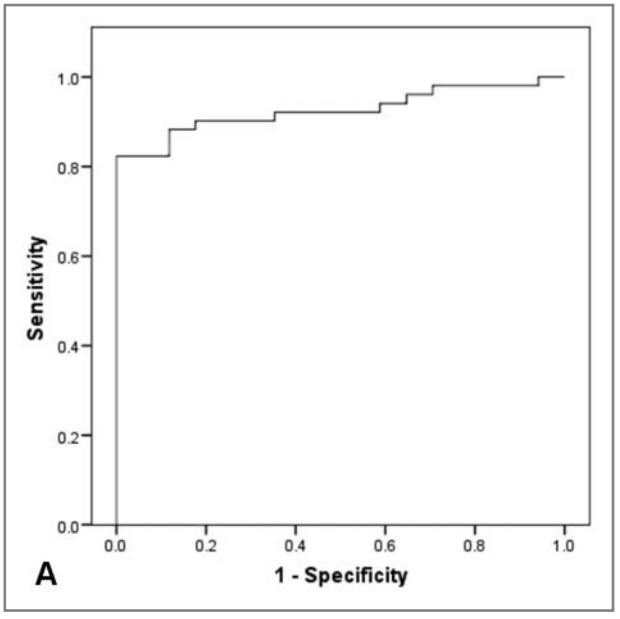


Figure 3—Kaplan-Meier survival curves indicating the survival duration of cardiomyopathic cats with (n = 25) and without (16) CHF. The survival duration of cats with CHF (dotted line; median duration, 313 days; range, 0 to 747 days) was significantly ($P < 0.001$) shorter than that of cats without CHF (solid line; range, 424 to 808 days). Vertical hatch marks indicate cats that were censored from the analysis.



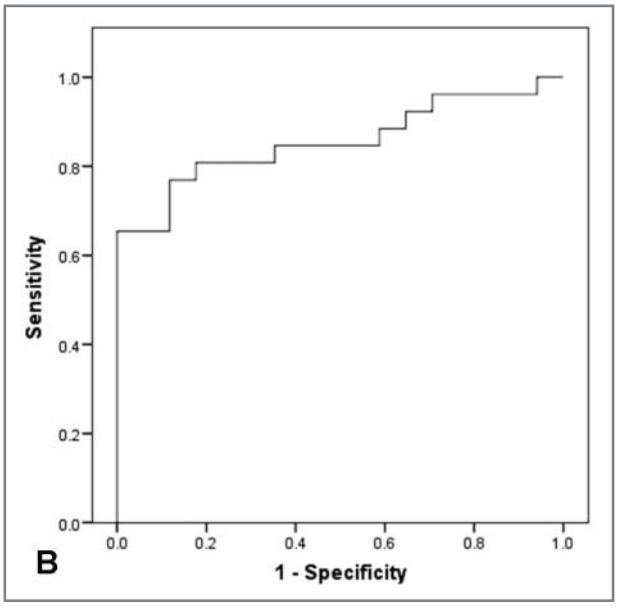


Figure 4—Receiver-operating characteristic curves indicating the utility of plasma NT-proANP concentration to distinguish 17 control cats from 51 cats with cardiomyopathy (A) and to distinguish 26 cardiomyopathic cats without CHF from the 17 control cats (B).

Table 2—Sensitivity and specificity of using plasma NT-proANP concentration at various cutoff values for distinguishing healthy cats from cats with cardiomyopathy and with and without CHF.

Intended distinction	Cutoff value (fmol/mL)	Sensitivity (%)	Specificity (%)
Healthy vs cardiomyopathy*	517	90	82
	965	82	100
Healthy vs cardiomyopathy without CHF*	517	81	82
	965	65	100
Healthy vs cardiomyopathy with CHF†	1,064	100	100
Cardiomyopathy with vs without CHF†	1,374	96	58
	2,089	84	89
	2,882	60	100

*Values greater than the cutoff were used to identify cats with cardiomyopathy. †Values greater than the cutoff were used to identify cardiomyopathic cats with CHF.

Univariate Cox regression analysis revealed that the variables LA:Ao ($P < 0.001$), cat group ($P = 0.005$), and NT-proANP ($P = 0.006$) were significant predictors of survival duration, whereas the variables age ($P = 0.86$) and reproductive status ($P = 0.91$) were not. However, when a multivariate analysis was performed, the only independent predictor of survival duration was LA:Ao ($P < 0.001$). The variables NT-proANP ($P = 0.644$) and cat group ($P = 0.121$) were no longer significant.

Plasma NT-proANP concentration as a diagnostic test—Results of the ROC analysis indicated plasma NT-proANP concentration was successfully able to distinguish between pairs of cat groups. The AUC was greatest for distinguishing cats with CHF from healthy control cats (1.0; 95% CI, 1.0 to 1.0). The AUC for distinguishing control cats from both groups of cardiomyopathic cats was 0.926 (95% CI, 0.865 to 0.987) and the AUC for distinguishing cardiomyopathic cats with CHF from those without CHF was 0.914 (95% CI, 0.838 to 0.989; **Figure 4**).

For distinguishing cardiomyopathic cats from healthy cats, a cutoff value of 517 fmol/mL (with values < 517 fmol/mL indicating healthy cats) resulted in high sensitivity (90%) and good specificity (82%; **Table 2**). For distinguishing healthy cats from cardiomyopathic cats without CHF, the same cutoff value resulted in reduced sensitivity (81%) but equivalent specificity (82%). For distinguishing cardiomyopathic cats with CHF from those without CHF, a cutoff value of 2,089 mol/mL (with values < 2,089 indicating no CHF) resulted in good sensitivity (84%) and specificity (89%).

Discussion

The plasma NT-proANP concentration of cats in the present study differed significantly among healthy control cats, cardiomyopathic cats without CHF, and cardiomyopathic cats with CHF. In addition, survival duration differed significantly between both groups of cardiomyopathic cats. A moderate correlation between the LA:Ao and NT-proANP concentration as well as a significant association between the LA:Ao and survival duration was detected.

The first finding is consistent with results of investigations involving humans and dogs[2,11,17,18,29–32] and also with the results of other investigations in cats.[33–35,i] Therefore, plasma NT-proANP concentration may have the potential to not only distinguish cats with cardiomyopathy from healthy cats but also to differentiate cardiomyopathic cats with CHF from those without CHF. Although another study[36] revealed a significant correlation between plasma ANP concentration and diameter of the left ventricular posterior wall in diastole as well as left atrial size, no significant difference between cats with HCM and the healthy cats could be detected. It is possible that the lower number of cats in that study, compared with those of our study, resulted in reduced power to detect a difference.

A correlation between tissue ANP concentration and thickness of the left ventricular posterior wall in diastole has also been identified in other studies,[49,50,j] and ventricular ANP gene expression may occur in humans, dogs, and cats with HCM because of disease-specific changes such as hypertrophy, fibrosis, or myocardial fiber disarray.

In the present study, cats with cardiomyopathy could be distinguished from the healthy control cats with a sensitivity of 90% and specificity of 82% when the cutoff plasma NT-proANP concentration was 517 fmol/mL. In that situation, 88% of the observations were correctly classified. This might be clinically important in that NT-proANP values higher than that cutoff, particularly when abnormalities such as heart murmurs are present, should be further evaluated for cardiomyopathy via echocardiography. The results of the present study are comparable to those of another,[33] with the exception that the median NT-proANP concentration of the control group and the cutoff value for distinguishing control from diseased cats were higher than those in our study. A possible explanation for this finding is that echocardiography was not performed in the control cats of the other study and therefore some might have had cardiomyopathy. Another small difference between studies is the slightly lower median NT-proANP concentration of the other study's cats versus our cardiomyopathic cats. Given that the atria are the main source of ANP,[2,3] the difference might have been attributable to the lower median (range) LA:Ao (2.21 [2.02 to 2.39]) in that study versus ours. This association was also reflected in the correlation between the plasma NT-proANP concentration and the LA:Ao of the present study. A similar correlation has been identified in dogs[37] and cats.[33]

The survival duration of cardiomyopathic cats without CHF in our study was conspicuously short-

er than the median survival duration of 1,830[51] and 1,129[52] days in cats with subclinical cardiomyopathy from other studies. This finding may simply be attributable to the shorter follow-up period in our study. Further, approximately 80% of cats in this particular group survived much longer than the study period lasted, and several cats had to be censored when they were euthanatized for noncardiac reasons. The median survival duration of our cardiomyopathic cats with CHF was in the range of values for similar cats in the other studies (92[51] and 563[52] days).

Although plasma NT-proANP concentration was a significant predictor of survival duration in univariate analysis, it failed to achieve significance in the multivariate model. One possible explanation is the low number of cats in the present study. Second, many cats, particularly those with cardiomyopathy but without CHF, were censored. It could also be that NT-proANP as an acutely responsive hormone primarily reflects volume overload rather than myocardial dysfunction or that ANP originates from the ventricles to a lesser extent than does BNP.[2,11] Furthermore, in 1 study[16] involving cats with cardiomyopathy, high production of only BNP and not ANP in the ventricular myocardium was detected. On the other hand, in hypertensive rats, hamsters with CHF, and humans with dilated cardiomyopathy, an increase in ANP synthesis by the ventricular myocardium takes place.[11,16] In human medicine, plasma proANP concentration can be used to predict death in patients with heart failure.[21,22,25–28] Whether this is also true for cats with cardiomyopathy requires additional investigation.

In 1 study,[36] the plasma NT-proANP concentration in control cats was higher than the value in the control group of our and another study.[33] This difference may have been attributable to different methods of sample handling, such as the addition of aprotinin to the blood sample. In our investigation, aprotinin was not used, blood samples were kept at room temperature (approx 20°C) for a maximum of 5 hours, and samples were stored at –20°C in accordance with instructions that accompanied the test kit and methods used in other investigations.[53,54]

A limitation of the study reported here was that blood pressure measurement was not performed in all cats; therefore, differentiation of idiopathic HCM from hypertension-related hypertrophy was not always possible. Because the purpose of the study was to evaluate plasma NT-proANP concentrations in different types of cardiomyopathy, we believed differentiation between primary and secondary HCM was not necessary. In another study,[33] blood pressure was measured in approximately 60% of cardiomyopathic cats, but in only 2 cats did a value > 175 mm Hg exist, suggesting hypertension might be a minor cause of HCM. In another study,[55] mean arterial blood pressure values in cats with polycystic kidney disease were higher than those in control cats, but plasma ANP concentration in the diseased cats was not significantly greater. Nevertheless, an additional effect of hypertension on circulating NT-proANP concentration cannot be excluded. Whether plasma ANP concentration is influenced by blood pressure in cats with cardiac disease and whether any such influence is dependent on cardiac disease severity remains to be investigated. Simultaneous measurement of circulating NT-proBNP or cardiac troponin concentrations was not performed in the present study. Parallel measurement of several cardiac biomarkers at the same time, such as NT-proANP, NT-proBNP, and cardiac troponins, or sequential measurement of them or their ratios might have prognostic value and warrants investigation.

Another study limitation was that echocardiography was performed by 2 examiners. Although the examiners were equally experienced and echocardiography was performed in accordance with current recommendations, interobserver variability was not evaluated and, therefore, cannot be excluded as a potential bias.

a. Vivid 7, GE Healthcare Technologies, Solingen, Germany.
b. Philips Optimus, Philips Medizin Systeme GmbH, Hamburg, Germany.
c. AGFA socpix LR 5200, AGFA Gevaert, Leverkusen, Germany.
d. AGFA ADC compact, AGFA Gevaert, Leverkusen, Germany.
e. Nunc CryoTube Vials, Thermo Fisher Scientific, Roskilde, Denmark.
f. proANP (1-98), Biomedica Group, Immundiagnostik AG, Bensheim, Germany.
g. ELISA-Reader DYNATECH MR 5000, Dynatech Deutschland GmbH, Denkendorf, Germany.
h. SPSS, version 15.0, SPSS Inc, Chicago, Ill.
i. Sisson DD, Oyama MA, Solter PF. Plasma levels of ANP, BNP, epinephrine, norepinephrine, serum aldosterone, and plasma renin activity in healthy cats and cats with myocardial disease (abstr). *J Vet Intern Med* 2003;17:483.
j. Biondo AW, Wiedmeyer CE, Sisson DD, et al. Cardiac expression of atrial natriuretic peptide gene in feline cardiomyopathy (abstr). *Vet Pathol* 2001;38:151.

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