Measurement of N-terminal proatrial natriuretic peptide in plasma of cats with and without cardiomyopathy

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Objective—To determine whether plasma N-terminal proatrial natriuretic peptide (Nt-proANP) concentrations in cats with cardiomyopathy (CM) differ from values in healthy cats and evaluate whether plasma Nt-proANP concentrations can be used to discriminate cats with CM and congestive heart failure (CHF) from CM-affected cats without CHF.

Animals—16 cats that had CM without CHF, 16 cats that had CM with CHF, and 11 healthy control cats.

Procedures—All cats underwent a physical examination, assessment of clinicopathologic variables (including plasma thyroxine concentration), thoracic radiography, and echocardiography. On the basis of findings, cats were assigned to 1 of 3 groups (control cats, cats with CM and CHF, and cats with CM without CHF). Venous blood samples were obtained from all 43 cats, and plasma Nt-proANP concentrations were measured by use of a human proANP(1-98) ELISA.

Results—Plasma Nt-proANP concentrations differed significantly among the 3 groups. Median Nt-proANP concentration was 381 fmol/mL (range, 52 to 450 fmol/mL), 763 fmol/mL (range, 167 to 2,386 fmol/mL), and 2,443 fmol/mL (range, 1,189 to 15,462 fmol/mL) in the control group, in cats with CM without CHF, and in cats with CM and CHF, respectively.

Conclusions and Clinical Relevance—Measurement of plasma Nt-proANP concentration could be of benefit in the assessment of cats with naturally occurring CM and might have potential as a screening marker for the disease. Furthermore, measurement of plasma Nt-proANP concentration may be useful for distinguishing cats with CM and CHF from those with CM and no CHF. (Am J Vet Res 2009;70:216–222)
An increase in plasma concentration of Nt-proANP is the result of increased synthesis of ANP in atrial myocytes and potentially in ventricular myocytes; the main trigger for ANP release is an increase in wall stress of the atria.\(^1\)\(^-\)\(^3\)\(^-\)\(^4\) Because ANP is mainly localized in the atrial appendages in cats, it was suggested that the atricles are more sensitive to stretching stress, compared with the atrial bodies.\(^2\)\(^5\)

In humans, correlations between ANP concentration and several cardiac conditions such as chronic heart failure (even in asymptomatic patients) and between ANP concentration and survival have been identified.\(^1\)\(^2\)\(^6\)\(^-\)\(^8\)\(^-\)\(^20\) Furthermore, results of several studies\(^26\)\(^,\)\(^29\)\(^,\)\(^30\) in humans have indicated that the plasma concentration of natriuretic peptides is a good indicator for the efficacy of cardiac interventions. Some studies to investigate the importance of ANP in veterinary medicine have been performed. A correlation between ANP concentration and heart failure has been identified in dogs.\(^31\)\(^-\)\(^34\)

Contradictory findings from studies\(^35\)\(^-\)\(^37\) in cats have been published. In 1 study,\(^35\) no significant difference in plasma ANP concentration between cats with HCM and healthy control cats was detected. However, results of 2 other recent studies\(^36\)\(^,\)\(^37\) indicated that plasma ANP concentrations were significantly increased in cats with CM, compared with values in healthy cats.

The purpose of the study reported here was to determine whether plasma Nt-proANP concentrations in cats with CM differ from values in healthy cats. An additional objective was to determine whether plasma Nt-proANP concentrations could be used to discriminate CM-affected cats without CHF from CM-affected cats with CHF. The hypotheses were that cats with CM have higher plasma Nt-proANP concentrations than healthy cats and that among cats with CM, those with CHF have higher plasma Nt-proANP concentrations than those without CHF.

### Materials and Methods

**Animal selection and group allocation**—Cats that were examined and that had evidence of cardiac disease and those examined for other (noncardiopulmonary) reasons (eg, orthopedic diseases) in the period from October 2005 to November 2006 were included in the study. Exclusion criteria were azotemia (plasma urea concentration $> 65$ mg/dL and plasma creatinine concentration $> 1.8$ mg/dL) and high plasma thyroxine concentration ($> 4$ µg/dL). Informed consent was obtained from the owners.

Various examination procedures were undertaken for each cat; these included physical examination, CBC, serum biochemical analyses, assessment of plasma thyroxine concentration, echocardiography, and thoracic radiography. Electrocardiography was performed prior to and during echocardiography.

Cats were allocated to 1 of 3 groups. The control group (group 1) included cats with no clinical, radiographic, or echocardiographic signs of cardiac or pulmonary disease. Group 2 included cats with CM without signs of CHF. For these cats, there was echocardiographic evidence of myocardial alterations, such as abnormal thickness ($> 6$ mm) of the interventricular septal wall or of the left ventricular posterior wall in diastole, papillary muscle hypertrophy, diastolic dysfunction (determined via evaluation of mitral valve inflow patterns), or large left atrium with apparently normal appearance of the left ventricle. Cats with heart disease and signs of decompensation during clinical, radiographic, or echocardiographic examination were allocated to the third group (ie, cats with CM and CHF).

Signs of decompensation were dyspnea and identification of congested pulmonary vessels, pleural effusion, or pulmonary edema in radiographic views.

**Echocardiography**—Transthoracic echocardiography was performed by use of an ultrasound machine equipped with a broadband high-frequency 7-MHz phased-array transducer.\(^1\) All examinations were digitally stored, and the internal analysis software of the echocardiographic device was used to perform calculations. The cats were examined in right and left lateral recumbency by 1 investigator (FM). M-mode recordings such as end-diastolic dimension of the interventricular septum, left ventricular posterior wall, and fractional shortening were obtained from the right parasternal long and short axes; all measurements were made according to the recommendations of the American Society of Echocardiography.\(^21\)\(^-\)\(^24\) To calculate the LA:Ao ratio (by use of the so-called Swedish method), dimensions of the left atrium and aorta were measured in a 2-dimensional short-axis plane in the right parasternal view at the level of the aortic valves just after their closure. The aorta was measured (edge to edge) from the midpoint of the right coronary cusp to the commissures of the noncoronary and left cusps. By continuing this line from edge to edge, the left atrium was measured.\(^39\)\(^,\)\(^40\) The left atrium was considered large if the LA:Ao ratio was $> 1.5$.\(^40\)

The left ventricular outflow velocity and mitral valve inflow pattern were recorded from the left apical long-axis view during pulsed- and continuous-wave spectral Doppler echocardiography. Also, the presence or absence of systolic anterior motion was recorded.

**Thoracic radiography**—Thoracic radiographs were obtained while the cats were in right lateral and ventral recumbency; images were processed digitally.\(^1\)\(^2\)\(^4\) In these radiographic views, cardiac size, lung patterns, pulmonary vasculature, and pleural effusion were evaluated.

**Analysis of Nt-proANP(1-98)**—A blood sample (1 mL) was collected from a cephalic or femoral vein of each cat into a tube containing EDTA. The sample was kept at room temperature (approx $20\degree$C) and centrifuged within 5 hours; the supernatant was collected, placed in a 1.8-mL cryotube,\(^1\) and stored at $-20\degree$C for as long as 6 months prior to batched Nt-proANP measurement. The laboratory personnel were unaware of the clinical condition of the cat from which each sample was obtained. The analysis was performed by use of a human Nt-proANP(1-98) ELISA.\(^4\) Because feline Nt-proANP(1-98) is highly homologous (94%) with that of humans, a test kit designed for use in humans can be used for the detection of Nt-proANP in cats.\(^9\)\(^,\)\(^41\)

The test kit used was a sandwich enzyme immunoassay designed to determine Nt-proANP(1-98) directly in
biological fluids. The kit incorporated a pair of immunoaffinity-purified polyclonal antibodies derived from sheep. The capture antibody, which was specific for proANP(10-19), was coated onto the microtiter plate. The detection antibody, which was specific for proANP(85-90), was labeled with biotin. If it is present in a sample, Nt-proANP binds to the precoated capture antibody and forms a complex with the detection antibody. For this test kit, cross-reactivity of Nt-proANP(1-98) with proANP(1-30), proANP(31-67), proANP(79-98), α-ANP, proBNP(8-29), proBNP(32-57), proCNP(1-19), proCNP(30-50), and proCNP(31-97) 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. An ELISA reader was used for the measurement. Repeat Nt-proANP(1-98) measurement was performed in every sample, and the mean of these 2 values was calculated.

Statistical analysis—Data obtained from the 3 groups are presented as median and range. For evaluation of data distribution, a Kolmogorov-Smirnov test was performed. According to the results of this test, nonparametric tests were applied for further statistical analyses. A Kruskal-Wallis test was used to compare plasma Nt-proANP concentrations values 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 used. A value of \( P < 0.05 \) was considered significant.

Results

Forty-three cats were included in the study; each was assigned to 1 of the 3 study groups (Table 1). Thirty cats were male, of which 1 was sexually intact. Thirteen cats were female, of which 1 was sexually intact. The cats' ages ranged from 1 to 15 years (median age, 7 years). Breeds included domestic shorthair (n = 32), British Shorthair (5), Maine Coon (2), Persian (2), Norwegian Forest Cat (1), and Abyssinian (1). The cats' weights ranged from 2.3 to 8.8 kg (median weight, 5.0 kg).

Among the 11 cats in the control group (group 1), 1 cat had nasal stridor because of a piece of grass lodged in the nasopharynx, 1 cat had cystitis, 2 cats had a history of trauma, and 1 was evaluated because of lameness. The remaining 6 cats were examined as part of routine checkup procedures.

In group 2, 15 cats had hypertrophy of the left ventricular posterior wall and 1 cat had hypertrophy of the papillary muscles. In 8 of these cats, there was a dynamic obstruction of the left ventricular outflow tract. The LA:Ao ratio in this group ranged from 1.2 to 1.8 (median, 1.4).

In group 3, all 16 cats were initially examined because of diminished appetite and lethargy; 9 cats also had dyspnea. In 14 of the 16 cats, the left atrium was considered markedly large (LA:Ao ratio > 2 [reference limit ≤ 1.50]). In 1 cat, the LA:Ao ratio was slightly high (1.6), and in another cat, the LA:Ao ratio was within reference limits (1.4). The median LA:Ao ratio among cats in this group was 2.3 (range, 1.4 to 2.8).

In group 3, 10 cats had hypertrophy of the left ventricle; in 3 other cats, the thickness of the interventricular septum and left ventricular posterior wall during diastole were within reference limits, but mild left ventricular dilation and a severely large left atrium

<table>
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<th>Variable</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
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<td>Age (y)</td>
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<td>7 (1–13)</td>
<td>7 (1–15)</td>
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<tr>
<td>Weight (kg)</td>
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*For this variable, value is significantly \( P < 0.001 \) different from the values in the other 2 groups. DSH = Domestic shorthair. BSH = British Shorthair. NFC = Norwegian Forest Cat.
were evident. The remaining 3 cats were categorized as having nonclassified CM but with a markedly large left atrium (LA:Ao ratio, 2.2, 2.3, and 2.0, respectively).

Plasma Nt-proANP concentration was greatest in group 3 (ie, in cats with CM and CHF; Figure 1). Significantly lower values were detected in group 2 (ie, in cats with CM but no CHF). The lowest plasma Nt-proANP concentrations were detected in the control group (group 1). Median plasma Nt-proANP concentrations differed significantly among the 3 groups. Comparisons of plasma Nt-proANP concentration between pairs of groups (ie, groups 1 and 2, groups 1 and 3, and groups 2 and 3) also revealed significant differences ($P < 0.001$). There was a moderate correlation ($r = 0.69; P < 0.001$) between plasma Nt-proANP concentration and LA:Ao ratio in the study cats.

Discussion

As an indicator of circulating ANP concentration, assessment of plasma Nt-proANP concentration in humans and dogs is known to be a good marker of cardiac failure, efficacy of cardiac treatments, and prognosis.26–34,42–47

In the present study, median Nt-proANP concentrations in the groups of healthy cats, cats with CM without signs of CHF, and cats with CM and signs of CHF differed significantly. On the basis of these results, measurement of plasma concentration of Nt-proANP may have the potential to distinguish not only between healthy cats and cats with CM but also between cats with CM and CHF and those with CM but no CHF. The findings of the present study are consistent with results of investigations in humans and in dogs.31–34,42–50 Moreover, the plasma Nt-proANP concentrations, age, weight, and sex of the cats of our study were comparable with those of 2 other studies in which a significant increase in serum Nt-proANP or plasma Ct-ANP concentration was detected in cats with CHF as well as in those with nonclinical CM. However, in 1 of those studies,36 median serum Nt-proANP concentration in the healthy control group was higher than the median plasma Nt-proANP concentration for the hearts of our study. This difference may be attributable to the fact that echocardiography was not performed in the control cats of the former study; therefore, the possibility that some cats had CM could not completely be ruled out.

The 3 highest plasma concentrations of Nt-proANP among the healthy control cats of the present study were 450, 445, and 437 fmol/mL; these cats had a history of a lameness of the hind limb, fracture of the symphysis of the mandible, and fracture of the femoral head, respectively. These all were painful conditions, which lead to the secretion of prostaglandins and some degree of inflammation. It has been reported that increases in circulating concentrations of prostaglandins as well as inflammatory cytokines (especially interleukins 1 and 6) can be associated with an increase in plasma ANP concentration. Thus, the relatively high Nt-proANP concentrations in the control cats may be a consequence of a painful or inflammatory condition. Without inclusion of the data from these 3 cats, the median plasma Nt-proANP concentration in the control group would be lower (median value, 346 fmol/mL vs 381 fmol/mL).

Also, in a study involving cats with age and sex distributions similar to those of the cats in the present study, no significant differences in Nt-proANP concentration were observed between healthy cats and those with HCM. Because the atra are the main location for synthesis and secretion of ANP,22–25 a possible explanation for this discrepancy with findings of the present study could be related to the dimensions of the left atrium in the 2 study populations of cats. The left atrium was only mildly large in most of the HCM-affected cats in the previous study, which contrasts with the markedly large left atrium in many of the cats with CM and CHF in our study.

Also in that previous study,33 the plasma Nt-proANP concentration in control cats was higher than the value in the control group of the present study. This difference may be attributable to the fact that aprotinin was added to blood samples and the samples were immediately placed on ice, centrifuged within 15 minutes, and stored at −70°C in the previous study. In our investigation, blood samples were kept at room temperature for as long as 5 hours, aprotinin was not used, and samples were stored at −20°C (according to the directions of the manufacturer of the human proANP[1-98] ELISA and previous investigations). In a study involving blood samples collected from humans, no differences in plasma Nt-proANP concentrations were detected between samples collected with aprotinin and those collected without aprotinin, even after samples were stored at room temperature for 2 to 3 days. Another investigation revealed that Nt-proANP is stable for as long as 6 hours at room temperature in EDTA-anticoagulated whole blood samples and that 89% of
the initial concentration was present after storage for 24 hours.

Another interesting aspect of the present study was the correlation (r = 0.69, P < 0.001) between plasma Nt-proANP concentration and the LA:Ao ratio. A similar correlation has been identified in dogs as well as previously in cats. A major limitation of the present study is that measurement of blood pressure was not performed routinely in each cat. This is in contrast to an investigation in cats in which a blood pressure value > 180 mm Hg was an exclusion criterion. In another study, blood pressure was measured in 31 of 50 cats with CM but the value was > 175 mm Hg in only 2 cats; however, those 2 cats were not excluded from that study. Although researchers determined that mean arterial blood pressure values in cats with polycystic kidney disease were higher than findings in unaffected control cats, plasma ANP concentrations in those cats were not significantly higher than control group values. In another study in dogs, intracardial administration of angiotensin II resulted in an increase in blood pressure but no significant change in plasma ANP concentration. 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 remain to be investigated in future studies. Another limitation of the present study is perhaps the somewhat low number of cats in each group. However, the differences detected were significant and comparable to results of another study in which more cats were included.

Simultaneous measurement of circulating Nt-proBNP concentration, as was used to distinguish the control group from the study group in a previous study, was not performed in the present study. Eventually, measurement of plasma Nt-proBNP concentration may have a greater potential to identify cats with CM because of the finding of novel expression in cardiomyocytes in cats with HCM. Also, other studies in dogs revealed that measurement of plasma BNP concentration was superior to measurement of plasma ANP concentration for detection of occult CM. Nevertheless, a correlation between tissue ANP concentration and the thickness of the left ventricular posterior wall in diastole has been identified, and ventricular ANP gene expression may occur in human, canine, and feline patients with HCM because of disease-specific changes such as hypertrophy, fibrosis, or myocardial fiber disarray. Also, in our study, simultaneous measurement of concentrations of cardiac troponins (especially cardiac troponin I, which might help to verify myocardial damage) was not performed. However, studies of myocardial diseases in cats including assessment of such variables are warranted.

In the present study, plasma Nt-proANP concentrations differed significantly among healthy cats, cats with CM but without signs of CHF, and cats with CM and signs of CHF. Therefore, plasma Nt-proANP concentration may be able not only to indicate the presence of CM but also to distinguish cats with CM and CHF from those with CM and no CHF. However, further studies involving more cats and additional sequential examinations should be performed to confirm these results and to evaluate the potential prognostic value of Nt-proANP concentration measurement in cats with heart failure. Also, a time course experiment may be helpful for evaluation of the efficacy of treatments in cats. Furthermore, simultaneous measurements of Nt-proBNP and cardiac troponin I concentrations might be helpful in supporting the diagnosis of left ventricular stress and myocardial damage, respectively. Additionally, the assay used in our study yielded reliable results under daily routine conditions because no special procedures for blood sampling and storage are necessary. Given that Nt-proANP is stable in blood samples for 2 to 3 days even at room temperature, it appears feasible that samples collected in clinical settings could be shipped to a diagnostic laboratory for assessment.

References

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