Circulating Natriuretic Peptides in Cats with Heart Disease

D.J. Connolly, R.J. Soares Magalhaes, H.M. Syme, A. Boswood, V. Luis Fuentes, L. Chu, and M. Metcalf

Background: Circulating natriuretic peptide concentrations are increased in cats with myocardial dysfunction.

Hypothesis: Serum N-terminal fragment of proatrial natriuretic peptide (NT-proANP) and NT-probrain natriuretic peptide (proBNP) concentrations may predict the presence of heart disease (HD) and congestive heart failure (CHF). A positive relationship is also predicted among natriuretic peptide (NP) concentrations, a noninvasive estimate of left ventricular filling pressure \(E/E_a\), and an echocardiographic measure of left atrial (LA) size \(\text{LA/Ao diameter} \) \(E/E_a\).

Methods: Serum NP concentrations were measured in 28 healthy control and 50 study cats using sandwich enzyme immunoassays. The study group comprised cats, with HD but no CHF (HD \(E/E_a=n=17\)) and cats with CHF (HD + CHF, \(n=33\)). The relationship among NP concentrations, LA size, and \(E/E_a\) was examined. The ability of NP to distinguish control from study cats, and HD – CHF from HD + CHF cats, was explored using receiver operator curve analysis.

Results: NP concentrations were significantly lower in control than in study cats \((P=.0001)\). The NT-proBNP concentrations were positively correlated with \(\text{LA/Ao ratio} \ (p=0.34; \ P=.02)\) and with \(E/E_a\) ratio \((p=0.68; \ P<.05)\). An NT-proBNP concentration of 49 fmol/mL gave a sensitivity and specificity of 100 and 89.3%, respectively, for correctly distinguishing 96.2% of control from study cats. Pairwise comparisons of the areas under the curve identified a statistically significant difference \((P=.011)\) between NT-proANP and NT-proBNP to distinguish control from study cats, NT-proANP and NT-proBNP concentrations were significantly higher in HD + CHF cats than in HD – CHF cats \((P=.0023 \text{ and } .0001, \text{ respectively})\).

Conclusions: Serum concentrations of NT-proANP and particularly NT-proBNP were different in healthy control cats, asymptomatic cats with HD, and cats with CHF, suggesting that measurement of NP concentrations may prove clinically useful as an initial screening test for cats with suspected cardiac disease.

Key words: Diastolic dysfunction; Doppler tissue imaging; Feline cardiomyopathy; Left atrial pressure.

Natriuretic peptides (NP) are a group of hormones synthesized by cardiomyocytes, and include atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP). They are released into the circulation as a result of myocardial stretch of the atria and ventricles, respectively, and are responsible for the regulation of body fluid homeostasis and blood pressure.1 In human patients, they are increasingly being used as markers for the diagnosis and prognosis of cardiac disease.2–5 They also have a potential role in treatment of heart disease.6

The primary stimulus for ANP release is increased atrial transmural pressure; however, its synthesis is also upregulated in hypertrophied cardiac myocytes.7,8 ANP is encoded as a 126-amino acid precursor, which on release is cleaved to the physiologically active 28-amino acid carboxy-terminal fragment and a 98-amino acid amino-terminal fragment (NT-proANP), both of which circulate in plasma. Usually, circulating NT-proANP is measured because it is far more stable at room temperature than ANP, making it more suitable as a diagnostic test.9

BNP undergoes similar posttranslational modification. In healthy humans, cats, and dogs, circulating BNP probably originates from storage granules in the atria, allowing a rapid increase in plasma concentrations in response to sudden atrial wall stretch. Sustained increases in circulating BNP, as seen in patients with chronic heart failure, are facilitated by increased protein synthesis with the major site of BNP production switching from the atria to the ventricles.10 BNP concentrations therefore are principally regulated by ventricular wall stress and pressure load.11

Increased circulating concentrations of these hormones have been identified in human patients with hypertrophic cardiomyopathy (HCM),2,12 and NT-proBNP has been shown to correlate positively with the severity of hypertrophy.2 HCM is the most prevalent cardiac disease in the adult cat and, similar to other feline cardiomyopathies, causes diastolic dysfunction, which frequently results in left atrial (LA) enlargement and congestive heart failure (CHF).13,14 Increased concentrations of NPs might therefore be expected in affected cats. Plasma NT-proANP immunoreactivity has been previously compared in cats with and without HCM. No significant difference was found between the 2 study groups, but the majority of cats in the HCM group were asymptomatic. The study identified a positive correlation between NT-ANP and echocardiographic parameters of LA size and left ventricular wall thickness.15 Plasma BNP concentrations in cats with myocardial disease and CHF were found to be 10 times greater than those of control animals, and increased expression of myocardial BNP was identified in the atria and ventricles of cats with HCM.3, a

In studies of humans and dogs, a positive correlation between the presence and severity of CHF and NP concentrations has been recognized.16–21 Increased BNP concentrations in cats with HCM and CHF have also been reported.a Circulating NP concentrations have been shown to be positively correlated with disease severity and pulmonary capillary wedge pressure in canine and human patients17,21–24 and in experimental studies of dogs.25 In veterinary cardiology, LA size is sometimes used as an
indicator of disease severity. More sophisticated noninvasive estimates of left ventricular filling pressure using Doppler echocardiography have also been described. Studies in humans have shown a correlation between various echocardiographic measures, including the ratio \((E/E_a)\) of early mitral inflow velocity \((E)\) to the longitudinal velocity of the mitral valve annulus during early diastole \((E_a)\) with invasive measures of mean LA pressure. \(E_a\) behaves as an index of left ventricular relaxation that is less preload dependent than traditional echo-Doppler variables, and, when included in the ratio with \(E\), can compensate for the numerous influences on ventricular diastolic function, explaining why this ratio has a much stronger correlation with left ventricular filling pressure than \(E\) alone. A recent report using dogs with experimentally induced acute mitral valve insufficiency also confirmed a high correlation between the \(E/E_a\) ratio and mean LA pressure. The \(E/E_a\) ratio has also been shown to correlate well with left heart filling pressures in human HCM patients. Similar studies have not been reported for cats with HCM, despite the fact that mitral inflow and Doppler tissue imaging (DTI) waveforms are frequently measured in cats.

The aims of this study were to:

1. Investigate the ability of serum NT-proANP and NT-proBNP concentrations to distinguish control cats from study cats, and to distinguish cats with HD but without CHF (HD − CHF) from those with CHF (HD + CHF).
2. Investigate the relationship among serum NT-proANP and NT-proBNP concentrations, \(E/E_a\), and LA/aortic diameter (Ao).

The hypothesis of the study was that serum NT-proANP and NT-proBNP concentrations could be used to distinguish HD − CHF from HD + CHF cats, and to distinguish both groups from healthy control cats.

Materials and Methods

Animals and Diagnostic Tests

Healthy control cats consisted of animals seen at 2 private practices. Most cats were being screened as part of an annual health check before vaccination or elective surgery such as neutering or dental procedures. Five of the 28 control cats were being treated for conditions unlikely to affect ANP or BNP concentrations: ear mite infestation (2), tail trauma (1), impacted anal glands (1), and skin laceration (1). All cats received thorough physical examinations by veterinarians with postgraduate qualifications in cardiology or internal medicine (Royal College of Veterinary Surgeons Certificate in Veterinary Cardiology or Diploma of the European College of Veterinary Internal Medicine). Cats were excluded if they had concurrent renal disease, thyroid disease, neoplasia, hematologic disturbances, palpable goiter, or heart disease. Inclusion was determined by the absence of clinical signs (eg, absence of murmur, gallop rhythm, tachypnea, and arrhythmia), previous history, CBC, and biochemistry analysis, and in older cats, serum total T4 concentration.

Study cats were referred to the Royal Veterinary College cardiology service for further assessment of cardiac dysfunction. Thirty-five of 50 cats had received medication before referral. Medications included 1 or more of the following: furosemide, spironolactone, angiotensin converting enzyme inhibitor, atenolol, aspirin, and diltiazem. All affected cats received thorough physical examinations, echocardiography (two-dimensional [2D], M-mode, and Doppler) with simultaneous electrocardiogram (ECG) recording. One or more of the following tests were also performed: ECG, serum biochemistry, CBC, thoracic radiography, systolic blood pressure analysis using a Doppler flow detector, serum cardiac troponin I concentration, or serum total T4 concentration. Cats were classified as having CHF on the basis of clinical signs and thoracic radiographic evidence consistent with pulmonary edema or pleural effusion, or ultrasonographic evidence of pleural fluid, in the presence of structural heart disease.

Standard echocardiographic studies were performed using a 7S phased array probe (3.0–6.7 MHz) with harmonic imaging. The frame rate generally used was 87 frames/s, although it varied from 43 to 97 frames/s. An ECG was recorded simultaneously in all cats. For each variable, 3 measurements were averaged from 3 consecutive cardiac cycles. The LA to Ao ratio (LA/Ao) was obtained using 2D echocardiography from the right parasternal short-axis heart base view. Measurements were made at the first diastolic frame just after aortic valve closure. LA/Ao was measured using the method described by Hansson et al (Fig 1). A ratio of > 1.5 was considered consistent with LA enlargement.

M-mode measurements of thickness of the interventricular septum in diastole, left ventricular internal diameter in diastole and systole, and left ventricular freewall in diastole were made at the level of the chordae tendineae in the short-axis view. Where asymmetrical hypertrophy was identified, the maximum thickness of the hypertrophied wall in diastole was measured from the 2D right parasternal long- and short-axis views. Papillary muscle size and wall motion (hypokinesia and asymmetry) were judged subjectively, and all images were reviewed by the principal author. The right parasternal long-axis view was used to identify systolic anterior motion of the mitral valve.

Doppler echocardiography (color, pulsed wave, and continuous wave) was used to characterize flow disturbances, including dynamic right ventricular outflow tract obstruction and dynamic left ventricular outflow tract obstruction with associated mitral insufficiency. The specific diagnosis for the type of myocardial disease or congenital disease was made with regard to previous publications.
The BNP assay uses immunoaffinity-purified sheep anti-NT-proBNP antibody diluted with synthetic human NT-proANP and the recovery of the peptide measured.

**Assay Validation**

Serum samples from cats with known NP concentrations were pooled to provide samples with low, medium, and high NP concentrations. These samples were then used to calculate intra- and interassay coefficients of variation (CV) and to determine the precision and reproducibility of the assay. Serum samples were diluted by the addition of sample diluent and the resulting measured NP concentration compared with the predicted concentration to evaluate dilutional parallelism. The analytical sensitivity (minimum detection limit) was determined by reading the + 3 standard deviation (SD) response from 10 replicate measurements of the zero standard. In addition, feline NT-proANP serum samples were spiked with synthetic human NT-proANP and the recovery of the peptide measured.

**Statistical Analysis**

The results were analyzed using a commercially available statistical software package. The age distribution of the 3 groups was compared by the Kruskal–Wallis equality-of-populations rank test. A $\chi^2$ test was used to assess the difference of proportions of sex in the 3 groups.

The concentrations of NT-proANP and NT-proBNP were not normally distributed across groups, and the Kruskal–Wallis equality-of-populations rank test was used. The strength of the relationship between NT-proANP and NT-proBNP and their correlations with $E/E_0$ and LA/Ao ratio were assessed by Spearman’s rank correlation coefficient ($r$). Receiver operator curves (ROC) were derived for each of the natriuretic hormone concentrations and the areas under the curve were calculated for each. ROC analysis was performed for all possible pair combinations to assess the capacity of pro-ANP and pro-BNP concentrations to discriminate cats in each of 3 clinical outcomes (healthy, HD – CHF, HD + CHF). These ROC analyses enabled estimation of the concentration of hormone cut-off that would best classify cats correctly. Cut-off concentrations for both NT-proANP and NT-proBNP were estimated based on the highest percentage of correctly classified observations. Other reported results were the AUC and the sensitivity and specificity of correct classification. Pairwise comparisons of the AUCs were made by testing the equality of 2 ROC areas obtained. A logistic regression model was used to assess the effect of the concentrations of urea and creatinine on the concentrations of natriuretic hormones. A maximum logistic regression model was specified that included disease status as a binary outcome variable and hormone, urea, and creatinine concentrations as explanatory variables. The effect of retaining or dropping variables from the model was assessed using Akaike’s Information Criteria (AIC) scores. The AIC scores are statistical criteria that enable logistic regression model comparisons; the smaller the AIC score, the better the model.

**Results**

**Animals**

There were 78 cats of 11 breeds; they included 56 domestic short hair, 8 domestic long hair, 3 Persian, and 3 Siamese cats. The groups comprised healthy control cats ($n = 28$), HD – CHF cats ($n = 17$), and HD + CHF cats ($n = 33$). The mean age ± SD of all cats in the study was NT-proBNP (60–80) conjugated to HRP. The ANP assay uses polyclonal sheep anti-human NT-proANP antibody. The sandwich comprises anti-NT-proANP (10–19) precoated to the wells of the plate and anti–NT-proBNP (85–90) conjugated to HRP. Samples were run as duplicates with the mean value used for the study.
7.2 ± 4.5 years (range, 1–19 years). The mean age for the control group was 7.0 ± 5.0 years (range, 6 months – 19 years), for HD – CHF cats 6.3 ± 3.6 years (range, 1–14 years), and for HD + CHF cats 7.6 ± 4.6 years (range, 1–15 years). There were no significant differences in age among the 3 groups (P > 0.05).

There were 54 male cats, with a higher proportion of male cats in the study group (40) than in the control group (14) (P > 0.05). The mean body weight of normal cats was similar in all 3 groups (P > 0.05) at 4.3 kg (95% CI: 3.97–4.6) for control cats, 4.4 kg (95% CI: 3.82–4.94) for HD – CHF cats, and 4.6 kg (95% CI: 4.25–4.98) for HD + CHF cats.

The diagnoses in the study group included HCM, hypertrophic obstructive cardiomyopathy, or both (n = 36); restrictive cardiomyopathy (RCM, n = 10); dilated cardiomyopathy (n = 1); mitral dysplasia (n = 1); double-chambered right ventricle (n = 1); and idiopathic third-degree atrio-ventricular block (n = 1). Of the HD – CHF cats, 4 had RCM and 13 had HCM. In the HD + CHF group, 6 cats had RCM and 23 had HCM.

Cats were classified according to the International Renal Interest Society classification of chronic renal insufficiency. Of the 33 HD + CHF cats, 6 (18.2%) were classified as stage II (mild azotemia) with serum creatinine concentration <2.8 mg/dL (reference range, 1.21–2.18) and 5 (15.2%) were classified as stage III (moderate azotemia) with serum creatinine concentration between 2.8 and 5.0 mg/dL.39

Serum total T4 concentration was measured in 5 of the control cats, and was within the reference range for the laboratories used by the 2 different practices. Serum total T4 concentration was measured in 15 cats in the study group, with results from 8.3 to 61 nmol/L (reference range, 19–65 nmol/L). Five of the cats had a T4 concentration below the normal reference range; 3 of these cats had CHF. Systolic blood pressure was measured in 31 of the study cats (range, 85–190 mmHg) and was increased (systolic arterial pressure >175 mmHg) in 2 of the cats. Blood pressure was not measured in any of the control cats.

**Diagnostic Tests**

The limit of detection of the NT-proANP assay was 145 fmol/mL. The intra-assay CVs (n = 6) were 8.1, 7.5, and 4.9% and the interassay CVs (n = 6) were 13.6, 8.7, and 20.7% for samples with low (274 fmol/mL), medium (791 fmol/mL), and high (2,584 fmol/mL) NT-proANP concentrations, respectively. Spiking feline plasma samples (n = 6) with 1,095 fmol/mL human NT-proANP yielded an average recovery of 100% (range, 92–107%).

The limit of detection of the NT-proBNP assay was 7.0 fmol/mL. The intra-assay CVs (n = 20) were 13.1, 10.1, and 7.0%, and the interassay CVs (n = 3) were 15.3, 12.2, and 7.7% for samples with low (112.2 fmol/mL), medium (276.0 fmol/mL), and high (854.1 fmol/mL) NT-proBNP concentrations, respectively. The NT-proBNP dilution parallelism indicated a mean recovery of 148%. Spiking of feline serum with human NT-proBNP was not performed.

**Table 1.** The mean and the 95% CI of serum NT-proANP and serum NT-proBNP concentrations obtained for cats in the control group, for cats with HD without heart failure (HD – CHF cats), and cats with heart failure (HD + CHF cats).

<table>
<thead>
<tr>
<th>Group of cats</th>
<th>NT-proANP (fmol/mL)</th>
<th>NT-proBNP (fmol/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>682 (530.2–833.8)</td>
<td>33.6 (11.2–56.1)</td>
</tr>
<tr>
<td>HD – CHF</td>
<td>1176.4 (809.96–1542.85)</td>
<td>184.1 (111.03–257.08)</td>
</tr>
<tr>
<td>HD + CHF</td>
<td>1865.03 (1499.3–2230.7)</td>
<td>524.7 (437.2–612.3)</td>
</tr>
</tbody>
</table>

95% CI, 95% confidence interval; NT-proANP, N-terminal fragment of proatrial natriuretic peptide; NT-proBNP, NT-probrain natriuretic peptide; HD, heart disease; CHF, congestive heart failure.

**Serum NT-proANP and NT-proBNP Concentrations**

The mean and ranges of NT-proANP and NT-proBNP concentrations in the 3 groups are shown in Table 1 and Figure 3a,b. We found a positive linear relationship
between the NT-proANP and NT-proBNP concentrations for all cats in the study ($r = 0.73; P < 0.05$) (Fig 4).

The NT-proANP and NT-proBNP concentrations were both significantly lower in control cats than in study cats ($P < 0.0001$). HD + CHF cats had significantly higher concentrations of both NT-proANP and NT-proBNP than HD − CHF cats ($P = .0023$ and .0001, respectively).

The results of the ROC analysis for the ability of NT-proBNP and NT-proANP to distinguish between all combinations of the 2 groups are summarized in Table 2.

The AUC of the ROC was greatest when a serum NT-proBNP concentration was used for distinguishing control cats from HD + CHF cats (0.99; 95%CI: 0.97–1.0) (Fig 5a). The second greatest AUC was seen when NT-proBNP concentration was used to distinguish control cats from study cats (0.97; 95%CI: 0.94–1.0) (Fig 5b).

When pairwise comparisons of the AUCs were made, we found a statistically significant difference ($P = .011$) between using NT-proANP and NT-proBNP to distinguish control cats from study cats (0.97; 95%CI: 0.94–1.0) (Fig 5b).

When pairwise comparisons of the AUCs were made, we found a statistically significant difference ($P = .011$) between using NT-proANP and NT-proBNP to distinguish control cats from study cats (0.97; 95%CI: 0.94–1.0) (Fig 5b).

Table 2. AUC, SD, and 95% CIs of the ROC analysis for NT-proANP and NT-proBNP, estimated for pairwise group comparisons of cats belonging to the control group, cats with HD without heart failure (HD − CHF), and cats with heart failure (HD + CHF).

<table>
<thead>
<tr>
<th>Groups</th>
<th>NP</th>
<th>Observations</th>
<th>AUC</th>
<th>SD</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control versus (HD − CHF and HD + CHF)</td>
<td>NT-proANP</td>
<td>77</td>
<td>0.8783</td>
<td>0.369</td>
<td>0.796–0.961</td>
</tr>
<tr>
<td></td>
<td>NT-proBNP</td>
<td>78</td>
<td>0.9764</td>
<td>0.155</td>
<td>0.942–1.00</td>
</tr>
<tr>
<td>Control versus HD − CHF</td>
<td>NT-proANP</td>
<td>45</td>
<td>0.7647</td>
<td>0.502</td>
<td>0.618–0.912</td>
</tr>
<tr>
<td></td>
<td>NT-proBNP</td>
<td>45</td>
<td>0.9475</td>
<td>0.236</td>
<td>0.878–1.00</td>
</tr>
<tr>
<td>Control versus HD + CHF</td>
<td>NT-proANP</td>
<td>60</td>
<td>0.94</td>
<td>0.263</td>
<td>0.87–1.00</td>
</tr>
<tr>
<td></td>
<td>NT-proBNP</td>
<td>61</td>
<td>0.99</td>
<td>0.071</td>
<td>0.974–1.00</td>
</tr>
<tr>
<td>HD − CHF versus HD + CHF</td>
<td>NT-proANP</td>
<td>49</td>
<td>0.77</td>
<td>0.537</td>
<td>0.62–0.92</td>
</tr>
<tr>
<td></td>
<td>NT-proBNP</td>
<td>50</td>
<td>0.895</td>
<td>0.320</td>
<td>0.81–0.98</td>
</tr>
</tbody>
</table>

AUC, areas under the curve; SD, standard deviation; 95% CI, 95% confidence interval; ROC, receiver-operator curve; NT-proANP, N-terminal fragment of proatrial natriuretic peptide; NT-proBNP, NT-probrain natriuretic peptide; HD, heart disease; CHF, congestive heart failure; NP, natriuretic peptide.
classify 96.2% of the observations with a sensitivity and specificity of 100 and 89.3%, respectively. Using a cut-off of 10 fmol/mL, the sensitivity was 100% but the specificity decreased to 14.3%; with a cut-off of 468 fmol/mL, the specificity increased to 100% but the sensitivity decreased to 11.8%.

A statistically significant positive correlation was seen between the LA/Ao and the $E/E_a$ ratio obtained for all study cats ($r = 0.57; P < 0.05$) (Fig 6). The LA/Ao ratio was significantly higher ($P < 0.05$) in HD + CHF cats at 2.21 (range, 2.02–2.39) compared with 1.70 (range, 1.53–1.87) in HD – CHF cats. The $E/E_a$ ratio was also higher in HD + CHF cats at 24.12 (range, 19.9–28.34) and at 12.1 (range, 10.2–13.9) than in HD – CHF cats ($P < 0.05$).

Both the NT-proANP and NT-proBNP concentrations were significantly positively correlated with the LA/Ao and $E/E_a$ ratios (Fig 7a,b). The correlation between NT-proBNP concentration and LA/Ao and $E/E_a$ ratios was greater than that for NT-proANP concentra-

### Table 3. Sensitivity and specificity of classification for the NT-proANP and NT-proBNP cut-off values with the highest percentage of correct classification estimated for pairwise group comparisons of cats belonging to the control group, cats with HD without heart failure (HD – CHF), and cats with heart failure (HD + CHF).

<table>
<thead>
<tr>
<th>Groups</th>
<th>NP</th>
<th>Cut-off value (fmol/mL)</th>
<th>Correctly classified (%)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control versus (HD – CHF and</td>
<td>NP</td>
<td>NT-proANP</td>
<td>960</td>
<td>83.12</td>
<td>83.67</td>
</tr>
<tr>
<td>HD + CHF)</td>
<td></td>
<td>NT-proBNP</td>
<td>49</td>
<td>96.15</td>
<td>100.0</td>
</tr>
<tr>
<td>Control versus HD – CHF</td>
<td>NP</td>
<td>NT-proANP</td>
<td>828</td>
<td>67.86</td>
<td>76.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NT-proBNP</td>
<td>49</td>
<td>93.33</td>
<td>100.0</td>
</tr>
<tr>
<td>Control versus HD + CHF</td>
<td>NP</td>
<td>NT-proANP</td>
<td>919</td>
<td>88.33</td>
<td>96.88</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NT-proBNP</td>
<td>148</td>
<td>96.43</td>
<td>96.97</td>
</tr>
<tr>
<td>HD – CHF versus HD + CHF</td>
<td>NP</td>
<td>NT-proANP</td>
<td>1250</td>
<td>73.47</td>
<td>75.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NT-proBNP</td>
<td>220</td>
<td>86.00</td>
<td>93.94</td>
</tr>
</tbody>
</table>

NT-proANP, N-terminal fragment of proatrial natriuretic peptide; NT-proBNP, NT-probrain natriuretic peptide; HD, heart disease; CHF, congestive heart failure; NP, natriuretic peptide.

![Fig 6](image6.png)

**Fig 6.** Scatter plot of 36 paired measurements of left atrial (LA)/aortic diameter (Ao) ratio and the Doppler-derived $E/E_a$ ratio in study cats, showing the linear regression line, the regression equation, and the proportion of explained variation ($R^2$) ($r = 0.57$).

![Fig 7](image7.png)

**Fig 7.** (a) Scatter plot of 35 paired measurements in study cats of N-terminal fragment of proatrial natriuretic peptide (NT-proANP) concentration and the $E/E_a$ ratio, showing the regression line and 95% CIs of the line, the regression equation, and the proportion of explained variation ($R^2$) ($r = 0.59$). (b) Scatter plot of 36 paired measurements in study cats of N-terminal probrain natriuretic peptide (NT-proBNP) concentration and the $E/E_a$ ratio, showing the regression line, regression equation, and the proportion of explained variation ($R^2$) ($r = 0.68$).
...tions. The highest correlation was seen between NT-proBNP and the $E/E_a$ ratio ($\rho = 0.68; P < .05$) (Fig 7b).

The concentrations of urea and creatinine were found to act as potential confounding factors on the circulating concentrations of both hormones as expressed by a reduction of the AIC score from 23.5 to 8.9 after inclusion of both variables in the logistic regression model.

Discussion

The results of this study indicate that both serum NT-proANP and serum NT-proBNP concentrations can be used to distinguish cats with HD from healthy controls. The increase in serum NT-proANP and NT-proBNP concentrations with HD shown in Fig 3a,b suggests that both tests may be clinically useful. These findings are consistent with previously published studies in dogs and humans.\textsuperscript{17,20,21} The pairwise comparisons of the AUCs identified a statistically significant difference ($P = .011$) between NT-proANP and NT-proBNP to distinguish control cats from study cats (Fig 5b) in favor of NT-proBNP. Although the other pairwise AUC comparisons were not statistically significant, this may be a consequence of the small group numbers. If a larger number of cats had been available, a statistical difference in the AUC between NT-proANP and NT-proBNP for the other ROC curves may have been observed.

One possible reason as to why NT-pro-BNP appears to perform better is that the BNP assay uses antibody raised against feline-specific NT-ProBNP peptide, where-as the ANP assay uses antibody raised against the human peptide. Although the ANP sequence is highly conserved across mammalian species, amino acid differences are present between feline and human NT-proANP, and it is possible that this difference may reduce the sensitivity and specificity of the test.\textsuperscript{10} An alternative explanation may be that myocardial disease in cats frequently results in marked diastolic dysfunction and substantial remodeling of the left ventricle, especially in cats with HCM. Indeed, previous studies have shown that this remodeling is accompanied by sufficient myocardial cell damage to cause an associated increase in serum cardiac troponin I concentrations.\textsuperscript{31,42} This chronic left ventricular remodeling is likely to induce increased BNP protein synthesis in the ventricles as a result of increased left ventricular end-diastolic pressure, which would be detectable by measuring NT-proBNP in the serum of affected cats. In fact, immunohistochemistry of ANP and BNP in the feline myocardium has confirmed upregulation of BNP but not ANP in cats with HCM.\textsuperscript{3}

With regard to distinguishing cats with CHF from controls, both assays performed well. The most likely explanation for the improved performance of ANP in this particular analysis is that cats in CHF invariably have enlarged left atria, and the associated left atrial wall stress acts as a stimulus for ANP release. This stimulus will be decreased or absent in cats with HD but not signs of CHF, which would explain why NT-proANP performed less well than NT-proBNP in distinguishing HD – CHF cats from controls. Furthermore, the ability of serum NT-proBNP to outperform NT-proANP with respect to this cohort may be related to its increased synthesis as a result of increased left ventricular diastolic pressure. This would support the use of NT-proBNP as a potential initial screening test to identify HD in breeding animals, but additional trials are required before definitive recommendations can be made. A cut-off concentration of 49 fmol/mL for serum NT-proBNP correctly classified 96% of control and study cats with a sensitivity of 100% and a specificity of 89.3%. These results suggest that using this concentration in a screening test rigorously identifies diseased animals, and also identifies some false positives. Consequently, it would be prudent to follow up positive cats with a more specific test such as echocardiography to confirm their disease status. Interestingly, it has been shown in dogs that BNP can detect occult myocardial disease before the onset of obvious hemodynamic disturbances. This was demonstrated in a colony of Golden retrievers with occult muscular dystrophy cardiomyopathy in which increased circulating BNP concentrations were identified in dogs with occult disease than in controls.\textsuperscript{43} In the present study, serum NT-proBNP was used to distinguish cats with cardiac disease from healthy controls rather than from cats with other conditions such as primary respiratory disease. It is possible that if the test were used to distinguish cats with HD from healthy cats as well as those with other noncardiac diseases, the accuracy may not be as high as reported here.

The significant increase in serum NT-proANP and NT-proBNP in study cats over controls in this study is similar to the finding of a previous study using both an enzyme-linked immunoassay and a radioimmunoassay for ANP and a radioimmunoassay for BNP, but ROC analysis was not reported.\textsuperscript{3} In a recent report, plasma NT-proANP immunoreactivity was compared between cats with and without HCM. No significant difference was identified between the 2 study groups, but unlike the present study, the majority of the HCM group were asymptomatic.\textsuperscript{15}

The $E/E_a$ ratio has been used as an estimate of left ventricular filling pressure in studies of dogs and humans,\textsuperscript{26–29} including human patients with HCM.\textsuperscript{31} A significant positive correlation was identified between the $E/E_a$ and LA/Ao ratios in the study group (Fig 6). This indicates that cats with increased left ventricular end diastolic pressure estimated by $E/E_a$ also have enlarged LA, and suggests that both ratios may be useful indicators of left ventricular diastolic dysfunction and disease severity in the cat. However, these results do not allow the $E/E_a$ ratio to be properly validated in cats with HCM because no direct pressure measurements were recorded. Furthermore, validation also requires the inclusion of these measurements from the control group, which was not done in this study.

The NT fragments of ANP and BNP are at least in part excreted by the kidneys; circulating concentrations of these peptides therefore may be influenced by renal function. One study determined that dogs with chronic renal failure have a 2-fold higher plasma carboxy-terminal ANP concentration than healthy dogs.\textsuperscript{16} Human patients with renal disease were found to have increased...
BNP concentrations, and in humans, glomerular filtration rate has been shown to be negatively correlated with both ANP and BNP. The effect of renal function on NP concentrations in the cat has not been reported and was not evaluated in the present study. Therefore, the observed higher NP concentrations in HD + CHF cats than in HD – CHF cats may be explained (at least in part) by impairment of renal function. This impairment may be treatment-induced in some cases. At present, it is not possible to separate the effect of renal function on serum NT-proANP and NT-proBNP concentrations from the effect of worsening HD, nor is it possible to quantify the effect of renal impairment on NP concentrations. Nevertheless, the results indicate that NT-proBNP, and to a lesser extent NT-proANP, can clearly distinguish HD – CHF cats (all but 1 of which had serum urea and creatinine concentrations within the normal range) from controls. It therefore is likely that progression of HD in cats with CHF is the main stimulus for NP secretion.

Study Limitations

The samples were stored at –20 °C for a variable period no longer than 2 weeks, and at –80 °C for a variable period up to 6 months. Unpublished studies performed by 1 of the authors (L.C.) indicate that both peptides appear stable at –20 °C for > 6 months. With regard to the storage of NT-proBNP, these finding are similar to a previous publication; hence, it is unlikely that the storage method used in this study would have had a major effect on the serum concentrations measured.

The dilution parallelism for the NT-proBNP assay indicates a greater than expected recovery. Repeat analysis gave the same results, and may suggest that in addition to the test peptide, other related peptides are being detected. However, the results from the ROC analysis strongly imply that the test remains clinically useful with a high degree of accuracy, despite the suboptimal dilution parallelism analysis.

A single NT-proANP/NT-proBNP measurement was made for each cat during the study, and no attempt was made to assess the effect of daily variation in concentrations, stage of disease, or severity of myocardial remodeling. Furthermore, the influence of medication on peptide concentrations was not analyzed.

The echocardiographic studies were performed by 3 different but equally experienced clinicians working in the referral clinic. This may have introduced some additional study variation, although all images and measurements were reviewed by the principal author.

The $E_E$ and $E_A$ Doppler measurements were taken from different echocardiographic time frames. Breathing and intrinsic cardiac forces result in translational movements of the heart within the thorax, and such movement can lead to some variation in the amplitude of the DTI waveforms.

The control group of cats did not undergo an echocardiographic examination, and although no abnormalities were detected on thorough physical and careful cardiac examinations, HD cannot be completely ruled out. In the same way, hypertensive HD cannot be ruled out, because blood pressure was not measured in any of the control cats.

$E_E$ and LA/Ao measurements were not performed in the control group, and it is likely that if we had included these measurements, the correlation coefficient would be different. Interpretation of this analysis therefore is valid only for the diseased cats (HD – CHF and HD + CHF groups).

Two study cats had a systolic blood pressure > 175 mmHg. Both resented having the procedure performed and unfortunately were lost to follow-up, and repeat measurements were not obtained. Neither cat underwent a retinal examination. Furthermore, blood pressure was measured in only 31/50 of the study cats and it is possible that some of these cats had hypertensive cardiomyopathy rather than HCM.

In conclusion, despite these limitations, we found that measurement of serum NT-proANP and NT-proBNP concentrations allowed cats with HD to be clearly distinguished from normal controls. Furthermore, both NPs clearly differentiated HD – CHF from HD + CHF cats. In addition, measurement of serum NT-proBNP also allowed HD – CHF cats to be differentiated from healthy controls. Serum NT-proBNP may prove useful as an initial screening test for HCM in cats.

Footnotes

b Ultrasonic Doppler flow detector model 811-B, Parks Medical Electronics, Aloha, OR
c Vivid 7, GE Vingmed Ultrasound A/S, Horten, Norway
d Echo Pac 7 for Vivid 7, GE Vingmed Ultrasound A/S
eproANP(1-98) Guildhay Ltd, Biomedica Guildford, Surrey, UK
f Feline Cardioscreen NT-proBNP Guildhay Ltd, Biomedica Guildford

References


