Journal of Feline Medicine and Surgery (2010) **12**, 942–947
doi:10.1016/j.jfms.2010.08.004

ELSEVIER

jfms

NT-proBNP measurement fails to reliably identify subclinical hypertrophic cardiomyopathy in Maine Coon cats

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The purpose of this study was to evaluate the value of measuring plasma NT-proBNP concentration as a screening tool in cats with varying severity of subclinical hypertrophic cardiomyopathy (HCM). Plasma NT-proBNP concentration was measured in 35 cats that had previously been classified as normal, equivocal, moderate HCM or severe HCM via echocardiography. No cat had ever been in congestive heart failure. Cats with severe HCM had a significantly higher NT-proBNP concentration compared to the other groups ($P < 0.0003$), however, the sensitivity of NT-proBNP for diagnosing cats with severe disease was only 44% (cutoff $\leq$ 100 pmol/l) to 55% (cutoff $\leq$ 40 pmol/l). There was no significant difference in NT-proBNP concentration between normal, equivocal and moderate categories (sensitivity for detecting moderate HCM was 0%). Based on the results of this study, NT-proBNP concentration is not considered adequate as a screening test for detecting mild to moderate HCM in Maine Coon cats and it appears that it may miss many cats with severe HCM.

Date accepted: 2 August 2010

The natriuretic peptides (NPs) are a group of substances whose main function is the regulation of fluid homeostasis.[1] The utility of measuring NP concentrations in human medicine has been intensively studied and the NPs have become increasingly regarded as sensitive, specific and reliable in the recognition and management of patients with heart disease and heart failure.[1–3] Measurement of B-type natriuretic peptide (BNP) in plasma appears to provide greater diagnostic accuracy when compared with atrial natriuretic peptide (ANP).[1] This has led to extensive clinical investigation of this hormone.[1–3] BNP is found wherever myocardial tissue is present but the greatest quantity is in the ventricles.[1] BNP is rapidly produced by cardiomyocytes after stimuli such as myocardial stretch, ischemia and hypoxia; however, other stimuli such as endothelin-1, angiotensin II, interleukin 1β and adrenergic agonists also increase its production.[1]

BNP is initially expressed as the pre-prohormone (pre-proBNP).[1] This is rapidly converted to the prohormone (proBNP).[1] ProBNP is then cleaved and released from the myocytes as active BNP and the inactive N-terminal of proBNP (NT-proBNP).[1] NT-proBNP is less labile and has a longer plasma half-life than active BNP.[4] Its plasma concentration reflects that of active BNP, thus, it has been used as a more stable marker of BNP activity.[4] BNP and its cleavage equivalent NT-proBNP have been shown to be powerful biomarkers for the diagnosis and prognosis of cardiovascular disease in human medicine.[2,3] The recent availability of a commercial assay for NT-proBNP in plasma of cats has sparked widespread interest in its use as a biomarker for feline cardiac disease. Studies have shown that NT-proBNP concentration may be useful in helping to discriminate cardiac from non-cardiac causes of dyspnea in cats in the emergency setting,[5,6] and in detecting cats with subclinical cardiac disease.[4,7]

Feline hypertrophic cardiomyopathy (HCM) is a common primary myocardial disease characterized by thickening of the left ventricular (LV) myocardium.[4] It can be idiopathic or due to mutations in cardiac myosin binding protein C in Maine Coon and Ragdoll cats and is common in these breeds.[8,9] Disease can range from mild to severe and affected cats may show no clinical signs, including the absence of a heart murmur, making detection of affected cats by physical examination difficult to impossible.[4] The sensitivity of thoracic radiographs for diagnosing HCM is limited

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1098-612X/10/120942+06 $36.00/0

due to the concentric nature of the LV hypertrophy and the specificity of radiography is considered poor.[4] Echocardiography by an experienced individual is currently considered the gold standard for diagnosing HCM. The diagnosis can be made unequivocally when the entire LV wall or a region of it measures 6 mm or more in diastole with the exclusion of secondary causes of LV hypertrophy.[4] Echocardiography however, is an expensive and somewhat time consuming process. As a result, serum or plasma biomarkers are being explored as a less expensive, readily available screening tool.[4,5,7,10–13] In humans with HCM an elevated NT-proBNP concentration has been positively associated with NYHA class of heart failure, left atrial size, severity of diastolic dysfunction, LV outflow tract gradient and severity of LV hypertrophy.[14–16] The roles of ANP, NT-proBNP, cardiac troponin I and plasma endothelin (ET-1) reactivity have been explored in various feline cardiac diseases, including HCM.[4,7,10–13] These studies have shown variable results with plasma ET-1, cTNI and NT-proBNP showing statistically significant elevations when compared to normal cats but with considerable overlap (ie, poor sensitivity and specificity).[7,12,13] Plasma ANP concentration was not statistically significantly elevated in diseased cats compared to normal control cats in one study,[10] but was shown to be significantly elevated in another.[7]

The aim of this study was to determine the accuracy of measuring plasma NT-proBNP concentration for identifying cats with subclinical HCM. Each cat in this study had previously had an NT-proBNP determination done by another laboratory (CardioCare NT-proBNP, Veterinary Diagnostics Institute, Irvine, CA, USA) and those results have been published.[4] When the original laboratory was bought by the current laboratory, the collection technique and possibly other aspects of the test were changed. Consequently, the current study was undertaken to determine if the changes resulted in alterations in the accuracy of the test.

Materials and methods

The study included adult Maine Coon and Maine crossbred cats from a feline HCM research colony at the University of California, Davis. Animals were cared for according to the guidelines in the National Institute of Health Guide for the Care and Use of Laboratory Animals. All cats had previously been genotyped as heterozygous or negative for the A31P myosin binding protein C (MYBPC) mutation.[8] Cats had full physical examination and echocardiography performed in the 2 months prior to blood collection. All cats previously had serum creatinine and T_4 concentrations measured to rule out secondary causes of LV hypertrophy (ie, hyperthyroidism and renal disease as a cause of systemic hypertension). Systemic blood pressure measurements were not recorded due to the fractious nature and need for sedation in many cats in this colony. Instead, it was chosen to rule out the most common causes of hypertension in cats via measurement of serum T_4 and creatinine. All cats were euthyroid as defined by a serum T_4 concentration <4 μg/dl (50 nmol/l) and no subject had evidence of renal failure as defined by a serum creatinine concentration of <2.2 mg/dl (190 nmol/l).

Echocardiography

All cats were screened for HCM by one investigator (MDK) via echocardiography (Philips iE33 echocardiograph machine, Philips Medical Systems, NA, Bothell, WA, USA). Echocardiography was performed within 2 months of blood collection for NT-proBNP concentration. Cats were sedated with 0.1 mg/kg acepromazine SC. Maximum LV diastolic wall thickness was measured from two to three cross-sectional 2D views. The greatest thickness measured at any site in the LV wall was considered to represent maximal LV wall thickness. Cats were classified as normal if the maximal wall thickness was <6 mm. Ventricular hypertrophy was classified as moderate or severe based on maximal LV wall thickness of 6–7 mm and >7 mm, respectively. An equivocal classification was given when the maximal wall thickness was <6 mm but the papillary muscles were subjectively assessed to be at least moderately enlarged.

Measurement of plasma NT-proBNP concentration

Plasma NT-proBNP concentration was measured using a commercially available assay (Cardiopet proBNP, Idexx Laboratories, Westbrook, ME, USA). Blood samples were collected by venepuncture into standard glass EDTA tubes and centrifuged within 30 min of collection. The supernatant was then placed into transport tubes provided by the laboratory, frozen at -80°C and shipped overnight on dry ice in one batch within 4 weeks of collection. Plasma NT-proBNP was measured using a commercially available horse-radish peroxidase, colorimetric end-point assay for the quantitative determination of feline NT-proBNP (Cardiopet proBNP, Idexx Laboratories, Westbrook, ME, USA).

Statistical analysis

Cats were categorized according to presence and severity of disease: group 1 – normal, group 2 – equivocal, group 3 – moderate HCM, group 4 – severe HCM. Differences in NT-proBNP concentration between groups were examined using the Kruskal–Wallis one-way analysis of variance test. $P < 0.05$ was considered significant. Pair wise post-hoc comparisons using the Mann–Whitney test with Bonferroni-adjusted P value of 0.0167 were used to compare which groups were significantly different between group 1 vs 4, group 2 vs 4 and group 3 vs 4. Sensitivity and specificity were calculated using the cutoff values currently used by the commercial laboratory running the assay (<100 nmol/l

suggests that the likelihood of significant cardiac disease is low).

Results

Thirty-five cats were included in the study (15 intact females and 20 intact males). The median age was 8 years (range 7−15 years). Echocardiographically, eight cats were classified as normal (four heterozygous for the A31P MYBPC mutation, four negative), 11 as equivocal (four heterozygous, seven negative), seven as moderately affected (three heterozygous, four negative) and nine as severely affected (four heterozygous, five negative). No cat changed disease category in the time period between this study and the previous study on the same population of cats.[4] All echocardiographically normal cats (group 1) had an NT-proBNP concentration of 0 pmol/l. Most cats in the equivocal category (group 2) had an NT-proBNP concentration of 0 pmol/l except for one cat with a value of 33 pmol/l. The majority of cats in the moderate category (group 3) had an NT-proBNP concentration of 0 pmol/l except two cats with values of 38 pmol/l and 48 pmol/l. Cats in the severe category had NT-proBNP concentrations ranging from 0 to 482 pmol/l (median 45 pmol/l) (Fig 1, Table 1).

Cats with severe HCM had a significantly greater NT-proBNP concentration when compared to all other groups of cats ($P < 0.0003$). Group 1 was significantly different from group 4 ($P = 0.009$) and group 2 was significantly different from group 4 ($P = 0.008$) while group 3 was not found to be significantly different from group 4 ($P = 0.09$). If an upper limit of 100 pmol/l was used as the cutoff for normal NT-proBNP concentration (which is the current laboratory recommended cutoff), the sensitivity for detecting cats with severe HCM in this population was only 44% (95% confidence interval [CI] of 15−77%), with 0% sensitivity for detecting cats with moderate and equivocal disease. The sensitivity for detecting cats with any category of disease was only 15% (95% CI of 5−35%). All cats without HCM or those with equivocal/moderate disease had an NT-proBNP concentration of <100 pmol/l, resulting in a specificity for detecting cats without severe disease (normal, equivocal, or moderate disease) of 100% (95% CI of 84−100%). If an NT-proBNP concentration <100 pmol/l was used to identify only unaffected cats, the specificity remained at 100% as all cats in this group had an NT-proBNP concentration of 0 pmol/l. There was no significant difference in NT-proBNP concentration between normal, equivocal and moderate HCM groups. Even if the upper limit of normal was reduced to 40 pmol/l, the sensitivity for detecting cats with severe disease was only 55% (95% CI of 23−85%). The sensitivity for detecting any affected cat remained low at 22% (95% CI of 9−43%). With an NT-proBNP concentration of <40 pmol/l the specificity for accurately identifying unaffected cats remained at 100% and the specificity for detecting cats without severe disease remained high at 96%.

There was no significant relationship between cats that were heterozygous for the A31P MYBC mutation and severity of disease. There was also no significant relationship between cats with the A31P MYBC mutation and NT-proBNP concentration.

Table 1. NT-proBNP concentrations (pmol/l) in 35 cats. Group 1: normal echocardiogram ($n = 8$), group 2: equivocal disease ($n = 11$), group 3: moderate disease ($n = 7$), group 4: severe disease ($n = 9$) (see text for details).

Group 1	Group 2	Group 3	Group 4
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	19
0	0	0	45
0	0	38	282
0	0	48	345
0	0		434
	0		482
	0		
	33		

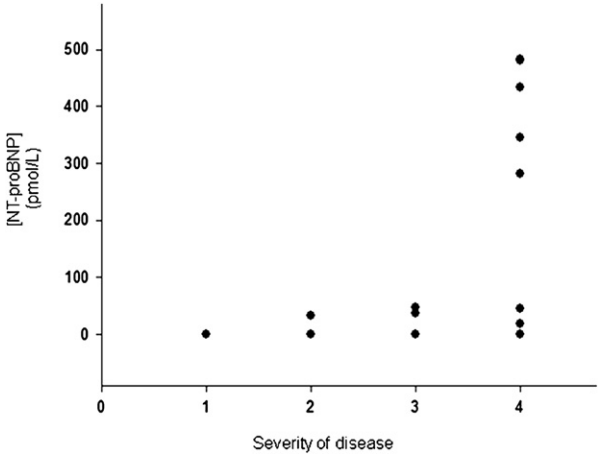

Fig 1. Vertical scatter plot showing NT-proBNP concentrations in 35 cats. The *x*-axis represents the category of disease. An NT-proBNP of 0 is represented by multiple cats in each category (see Table 1). There was a significant difference in NT-proBNP concentrations when comparing group 4 cats to all other groups ($P < 0.0003$). There was no significant difference in NT-proBNP concentration between group 1, 2 and 3 cats. There was a significant difference when comparing group 1 to group 4 and group 2 to group 4 ($P = 0.009$ and 0.008, respectively). There was not a significant difference when comparing group 3 to group 4 ($P = 0.09$).

Discussion

The aim of this study was to determine if the currently available commercial test for feline NT-proBNP

concentration resulted in similar findings to that of the previous test and to determine if the current test is useful for screening for HCM in cats. The results of this study show that NT-proBNP may be useful for identifying cats with severe disease but even then the sensitivity was low at 44% (95% CI of 15–77%) using the currently supplied reference range. This value was appreciably less than the one generated in the previous study using these same cats in which the sensitivity was found to be 90% for cats with severe disease (cutoff 44 pmol/l).[4] The current test resulted in close to 50% of severely affected cats remaining undetected in this population, even if the upper cutoff was reduced to 40 pmol/l. The test was completely insensitive for detecting cats with moderate HCM (LV maximal wall thickness 6–7 mm) and cats with equivocal disease (LV maximal wall thickness < 6 mm, moderately to severely enlarged papillary muscles) giving a 0% sensitivity for both of these categories singly and in combination. Therefore, if NT-proBNP was to be used as a method of screening cats for HCM, a positive result would represent a <50% chance of accurately identifying cats with severe disease and many cats with disease (equivocal, moderate and severe) would be falsely identified as normal unless echocardiography was performed.

Increased concentrations of NPs have been documented in humans, cats and dogs with heart disease and congestive heart failure. An increased circulating concentration of NT-proBNP has been identified in human patients with HCM and its concentration has been shown to correlate positively with the severity of hypertrophy, presence of LV diastolic dysfunction, New York Heart Association heart failure class, and subaortic pressure gradient >30 mm Hg.[14–16] In cats, one study found that both NT-proANP and NT-proBNP concentrations were significantly elevated in various forms of subclinical heart disease (including HCM) when compared to normal cats.[7] Those with heart disease and congestive heart failure also had significantly elevated concentrations of both NT-proANP and NT-proBNP when compared with normal cats and those with heart disease but without congestive heart failure. The authors suggested on the basis of this that both serum NT-proANP and serum NT-proBNP concentrations can be used to distinguish cats with heart disease (including HCM) from healthy controls and that these biomarkers could be potentially useful for screening breeding animals for heart disease.[7] The authors did state that further investigation was required and they did not specify if cats without heart failure had mild, moderate or severe disease. In contrast, another study did not show any statistically significant difference between NT-proANP concentrations when comparing cats with HCM to normal controls.[10]

The current study and the previously discussed study on the same population of cats[4] found that NT-proBNP concentrations were only useful in identifying subclinical cats with severe HCM. In that study the same group of cats was evaluated but a different diagnostic laboratory and testing protocol were used. That protocol performed better giving a sensitivity for detecting cats with severe disease of 90% using an upper cutoff of 44 pmol/l. Although higher NT-proBNP values were obtained in the current study, more cats in the previous study had values above the reference range, resulting in the greater sensitivity obtained in that study (Fig 2).[4] That testing protocol was also insensitive for detecting cats with

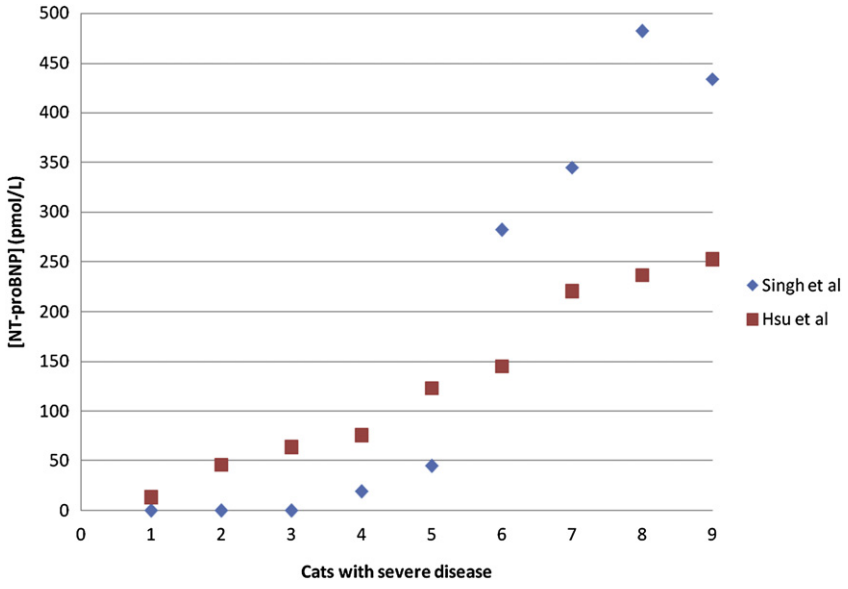

Fig 2. Comparison of NT-proBNP concentrations in nine cats with severe disease in the current study and the previously done study on the same population of cats.[4]

equivocal (mild) and moderate disease.[4] With the current protocol, if NT-proBNP was to be used as a screening test for identification of subclinical HCM in cats, a positive result would be strongly correlated with the presence of severe disease (100% positive predictive value was obtained in this study), however, the test failed to identify 50% of severely affected cats and all mild-moderately affected cats, making NT-proBNP a poor screening test for HCM in cats.

Some limitations do exist in this study. Only Maine Coon cats and primarily their crosses with and without the A31P mutation were examined. It is possible that this cohort of research colony cats is different from the general population of cats with HCM even though they are identical echocardiographically to other cats with HCM. Echocardiography and blood work varied with regard to the time of blood collection for evaluation of NT-proBNP concentration. It is possible, although highly unlikely, that the HCM classification was different at the time of blood collection or that cats may have been affected by diseases causing LV hypertrophy such has hypertension or hyperthyroidism. Systemic blood pressure measurements were not recorded due to the fractious nature and need for sedation in many cats in this colony, making the results of this technique highly likely to be difficult to obtain or interpret. Instead, it was chosen to rule out the most common causes of hypertension in cats via measurement of serum T_4 and creatinine. This group of research cats has been well characterized and followed for years. An echocardiogram was done within 2 months of NT-proBNP measurement on each cat and blood work (creatinine, T_4) was done within 6 months. In addition all cats were clinically normal, making recent changes in disease severity or presence of systemic disease unlikely.

It is possible that the different results obtained in this study compared to the previous study on the same population of cats[4] are merely due to chance and day-to-day variability. In addition, the small study population in both studies results in very wide confidence intervals around the obtained sensitivities and specificities, increasing the chance of variable results. The HCM classification scheme used in this study was arbitrary. However, with the current methods available to us for diagnosing HCM, the distinction between mild disease and normal cats is probably impossible and, therefore, the equivocal rather than a mild category was used. A maximal septal or LV free wall thickness of >7 mm was chosen for severe disease as this is the commonly accepted cutoff for this category among veterinary cardiologists.[17]

Conclusion

The measurement of plasma NT-proBNP concentration was insensitive for detecting anything less than severe HCM in cats as judged by this NT-proBNP assay in a cohort of Maine coon and Maine coon cross-research cats. While an elevated concentration gave a good chance of there being severe disease, cats with equivocal and moderate disease were not identified via NT-proBNP concentrations. In addition, 56% of cats with severe disease in this group would also have been identified as normal based on an NT-proBNP concentration <100 pmol/l. Based on the results of this study, NT-proBNP measurement is not considered an accurate screening tool for the diagnosis of HCM in cats.

Acknowledgments

Thank you to Idexx Laboratories for organizing shipping and running of the NT-proBNP assay. Idexx Laboratories were not involved in the study design or data review. We would also like to thank Dr Philip Kass for help with statistical analysis of the data.

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