Title

Investigation into the use of plasma NT-proBNP concentration to screen for feline hypertrophic cardiomyopathy.

Authors

Hsu, Adonia
Kittleson, Mark D
Paling, Anna

Publication Date

2009-05-22

Peer reviewed

Journal of Veterinary Cardiology (2009) 11, S63—S70

ELSEVIER

Journal of Veterinary Cardiology
ESVC

www.elsevier.com/locate/jvc

Investigation into the use of plasma NT-proBNP concentration to screen for feline hypertrophic cardiomyopathy

Adonia Hsu VMD, Mark D. Kittleson DVM, PhD*, Anna Paling DVM

Cardiology Service, William R. Pritchard Veterinary Medical Teaching Hospital, School of Veterinary Medicine, One Shields Avenue, University of California Davis, Davis, CA 95616, USA

Received 8 October 2008; received in revised form 27 January 2009; accepted 2 February 2009

KEYWORDS
Cat;
Hypertrophic cardiomyopathy;
BNP;
NT-proBNP;
Natriuretic peptide

Abstract *Objective:* To evaluate the utility of feline NT-proBNP plasma concentration [NT-proBNP] as a screening tool for cats with subclinical hypertrophic cardiomyopathy (HCM).
Animals, materials and methods: Forty adult Maine Coon or Maine Coon crossbred cats from the feline HCM research colony at the University of California, Davis were studied. All cats had previously been genotyped as heterozygous or negative for the A31P myosin binding protein C (MYBPC) mutation. Echocardiograms were performed to assess the severity of HCM in each cat. Blood samples were collected for evaluation of [NT-proBNP].
Results: In these cats with severe HCM, [NT-proBNP] was significantly elevated ($P < 0.0001$) when compared to all other groups of cats and an [NT-proBNP] > 44 pmol/L accurately predicted the presence of severe HCM. However, [NT-proBNP] was not increased in cats with moderate or equivocal HCM when compared to normal cats. Cats heterozygous for the MYBPC mutation had a significantly elevated [NT-proBNP] when compared to cats without the A31P mutation ($P = 0.028$).
Conclusions: Measurement of [NT-proBNP] has a high sensitivity and specificity as a means of detecting severe HCM in cats, but it is not sensitive for the identification of moderate HCM as judged by the evaluation of Maine Coon and Maine Coon cross cats in our colony. Consequently, we conclude that this test cannot be used to screen cats for the presence of mild to moderate HCM.

Introduction

Feline hypertrophic cardiomyopathy (HCM) is a common, primary myocardial disease characterized

* Corresponding author.
E-mail address: mdkittleson@ucdavis.edu (M.D. Kittleson DVM).

1760-2734/$ - see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jvc.2009.02.005

by thickening of the left ventricular myocardium. It is either idiopathic or due to mutations in cardiac myosin binding protein C in Maine Coon and Ragdoll cats.[1,2] HCM ranges in severity from mild to severe. Cats with mild to moderate disease generally show no clinical signs (i.e., they are subclinical) while those with severe disease range from having no clinical signs to experiencing congestive heart failure, aortic thromboembolism, and sudden death.

The diagnosis of feline HCM is made using echocardiography, although it is a diagnosis of exclusion. An unequivocal diagnosis of HCM can be made when the entire LV wall or some region is 6 mm or more thick in the absence of hyperthyroidism, systemic hypertension and severe dehydration.[3] The thickening is almost always accompanied by moderate to severe papillary muscle enlargement in cats. Commonly there is end-systolic cavity obliteration. Systolic anterior motion of the mitral valve may or may not be present. The left atrium may or may not be enlarged. Other echocardiographic techniques, including assessment of transmitral flow via pulsed wave Doppler and Doppler tissue imaging, may be useful for identifying the diastolic dysfunction often present with severe HCM.[4]

Because feline HCM is common in certain breeds of cats, screening for this disease is a frequent request of breeders who want to attempt to reduce the incidence of HCM in their line(s). Screening for HCM is fraught with difficulties. The sensitivity and specificity of auscultation in detecting cardiac disease in cats is compromised by the absence of auscultatory abnormalities in many cats with HCM, especially those with mild to moderate disease, and the occasional presence of a physiologic murmur, most commonly due to dynamic right ventricular outflow tract obstruction, in cats.[5,6] The sensitivity of thoracic radiography for diagnosing even severe HCM in cats is limited due to the concentric nature of the left ventricular hypertrophy coupled with the more cranial location of the left atrium in cats when compared to dogs.[3] In addition, the specificity of thoracic radiography for HCM is very poor. As such, screening for HCM has historically required an echocardiographic examination by an experienced individual, which is often an expensive and time consuming process.

Consequently, there is an obvious desire for a simpler, more readily available, and less expensive tool to screen cats for HCM. Serum or plasma biomarkers are widely utilized in human medicine to assess cardiac disease. Recently, a plasma assay for the feline N-terminal of the prohormone of brain natriuretic peptide (NT-proBNP) has become available. This has sparked profound interest in exploring the usefulness of this biomarker in feline cardiac disease. BNP is a peptide that is synthesized in the cardiac atria and ventricles. The putative reason for increased expression of BNP is myocardial stretch. BNP is initially expressed as a prohormone (proBNP).[7] ProBNP is cleaved and released from myocytes as active BNP and the inactive N-terminal or NT-proBNP. NT-proBNP is less labile with a longer plasma half life compared to active BNP. Although NT-proBNP is not the active polypeptide product, its plasma concentration reflects the plasma concentration of active BNP ([BNP]).[8,9] Therefore, it has been utilized as a more stable marker of BNP activity.

In humans with HCM, an elevated plasma NT-proBNP concentration ([NT-proBNP]) has been associated with various features of the disease.[10,11] Specifically, a positive correlation has been found between [NT-proBNP] and several variables including NYHA class of heart failure, left atrial size, severity of diastolic dysfunction, left ventricular outflow tract gradient and severity of left ventricular hypertrophy.[12–14] Examination of the role of cardiac biomarkers in feline cardiac disease as predictors of disease severity has been limited. The roles of N-terminal and C-terminal atrial natriuretic peptide (ANP), NT-proBNP, cardiac troponin I, and plasma endothelin reactivity have been explored in various feline cardiac diseases, including HCM.[15–20] Cats with subclinical HCM were included in some of these studies, however subject numbers and the description regarding severity of HCM have been limited.

The present study was designed to examine plasma NT-proBNP concentration in cats with moderate to severe HCM in a colony of cats with HCM where the disease has been carefully characterized over years in each cat. The specific aim of the study was to determine the feasibility of using plasma NT-proBNP concentration to identify cats with moderate or severe disease.

Animals, materials and methods

Animals

The study included adult Maine Coon and Main Coon crossbred cats from the feline HCM research colony at the University of California, Davis. Animals were cared for according to the guidelines in the NIH 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.[1] Clinical evaluation included physical examination, echocardiography, and measurement of serum T4, creatinine, and urea nitrogen concentrations. All subjects were euthyroid as defined by a serum T4 concentration < 4 μg/dL. No subject had renal failure (serum creatinine concentration < 2.2 mg/dL.

Echocardiography

All cats were screened for HCM over a 2 week period using an echocardiograph machine.[a] The diagnosis of HCM was based on the 2-D echocardiographic measurement of a thickened left ventricular wall with left ventricular papillary muscle hypertrophy in the absence of any other systemic disease capable of producing the magnitude of hypertrophy observed and the absence of fixed aortic stenosis. Noninvasive systemic arterial blood pressure was not measured because of the inaccuracy of this technique, especially in a group of cats where some are quite fractious. Instead, the two most common causes of systemic hypertension (renal failure and hyperthyroidism) were documented to not be present.

Cats were sedated with 0.1 mg/kg SQ acepromazine. All echocardiograms were performed by one investigator (MDK). Maximum left ventricular wall thickness was measured from 2–3 cross-sectional views and a subjective assessment of left atrial size was made from a right parasternal two-dimensional cross-sectional view. The greatest thickness measured at any site in the LV wall was considered to represent maximal LV wall thickness. Cats were designated normal if the maximal LV wall thickness was <6 mm. Ventricular hypertrophy was classified as moderate or severe based on a maximal LV wall thickness of 6–7 mm and >7 mm, respectively. An equivocal designation was given when the maximal wall thickness was <6 mm and the papillary muscles were subjectively assessed to be moderately enlarged. One cat with a 6.5 mm maximal wall thickness and very severe papillary muscle hypertrophy was categorized as having severe HCM.

Measurement of plasma [NT-proBNP]

Plasma [NT-proBNP] was measured using the Feline CardioCare NT-proBNP assay.[b] Blood samples were collected by venipuncture and placed in standard glass tubes containing EDTA. Samples, within 1 h of withdrawal, were centrifuged and the supernate collected and placed into a standard transport tube provided by the company and immediately placed in a standard freezer until overnight shipment to the company on ice. Samples were then stored at the company until batch analysis that was performed once weekly. Most samples were not obtained at the time of echocardiographic evaluation and were drawn within a time period spanning 6 months prior to 3 months after the echocardiographic examination.

Statistical analysis

Groups (normal, equivocal, moderate HCM, severe HCM) were compared using the Kruskal–Wallis one-way analysis of variance test. Differences in [NT-proBNP] between cats with the A31P mutation and those without were looked for using the Wilcoxon rank sum test. $P < 0.05$ was considered significant.

Results

Forty cats were studied (19 intact females and 21 intact males). The median age for the group was 6.5 years (range 5–16 years). Echocardiographically nine cats were classified as normal, 12 as equivocal, nine as having moderate HCM, and 10 as having severe HCM (Fig. 1). Three of the cats with severe HCM had mild to moderate left atrial enlargement while no other cat did. Of the 40 cats, 19 were heterozygous for the A31P mutation (5/9 normal cats, 4/12 equivocal cats, 5/9 cats with moderate HCM, and 5/10 cats with severe HCM).

Cats with severe HCM had a significantly greater [NT-proBNP] compared with all other cats ($P < 0.0001$). The median (range) [NT-proBNP] in the severe, moderate, equivocal, and normal groups was 134 pmol/L (12–252 pmol/L), 22 pmol/L (5–77 pmol/L), 19 pmol/L (5–53 pmol/L), and 21 pmol/L (10–79 pmol/L), respectively (Fig. 2). Comparing the [NT-proBNP] of the severe group to each of the other individual groups yielded significance in each instance: moderate vs. severe ($P = 0.0025$), equivocal vs. severe ($P = 0.0003$), and normal vs. severe ($P = 0.007$). The normal, equivocal and moderate HCM groups were not significantly different from each other.

A cut-off value of 44 pmol/L was used to distinguish between a normal and an elevated [NT-proBNP] based on previous studies and visual

[a] Hewlett Packard Sonos 5500 echocardiograph machine, Philips Medical Systems, NA, Bothell, WA, USA.
[b] Veterinary Diagnostics Institute, 9272 Jeronimo Road #118, Irvine, CA 92618.

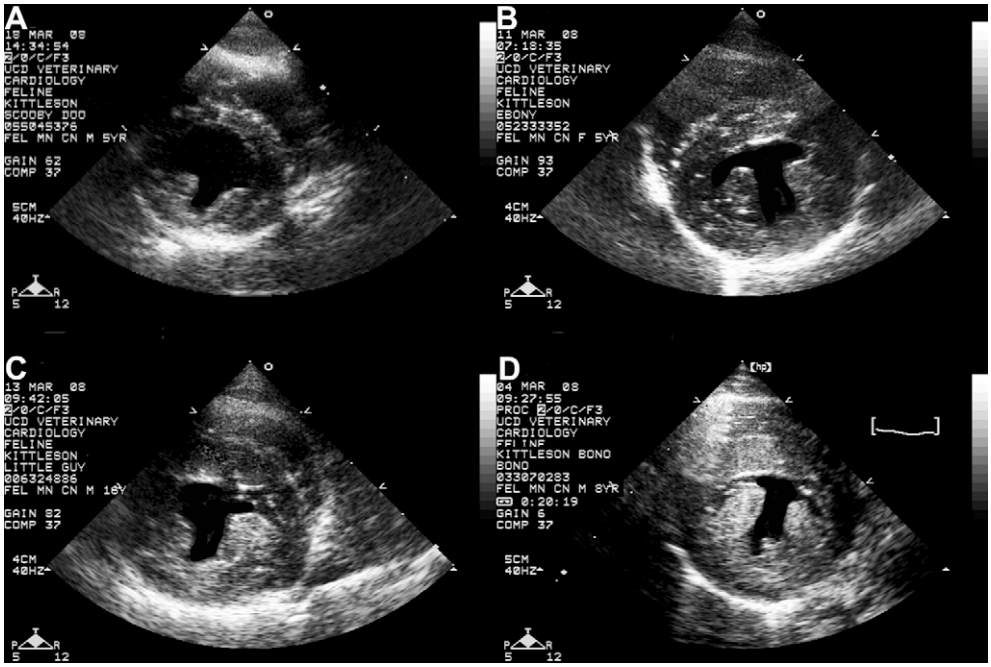

Figure 1 Right parasternal cross-sectional views of the left ventricle from a normal cat (A), a cat equivocal for HCM (B), a cat with moderate HCM (C), and a cat with severe HCM (D). Note that A and D are at a depth of 0—5 cm while B and C are at 0—4 cm.

assessment of the data.[c] An elevated [NT-proBNP] was reasonably sensitive (90%) and specific (83%) for identifying severe HCM. When used to try to distinguish cats with moderate HCM from normal cats and cats with an equivocal echocardiogram, [NT-proBNP] was only 20% sensitive but 86% specific. When cats with moderate and severe HCM were combined, the [NT-proBNP] value was only 58% sensitive and 86% specific for detecting HCM. No other cut-off value improved sensitivity and specificity.

Cats that had the A31P MYBPC mutation had a significantly elevated [NT-proBNP] when compared to those without the mutation. When looking at all cats in the population with the mutation, the median (range) was 33 pmol/L (5—252 pmol/L) vs. 12 (5—145 pmol/L) in negative cats ($P = 0.04$). The [NT-proBNP] in cats with the mutation with severe HCM was also elevated (220 [123—252] pmol/L) when compared to [NT-proBNP] in cats without the mutation and severe HCM (63 [12—145] pmol/L, ($P = 0.016$)).

Discussion

The goal of this study was to determine if measuring feline [NT-proBNP] in cats is a reasonable screening test for detecting HCM in cats. The current method of screening is echocardiographic quantitative assessment of LV wall thickness and the qualitative assessment of ancillary echocardiographic measures including papillary muscle size, left atrial size and the presence or absence of systolic anterior motion (SAM) of the mitral valve. Echocardiographic screening of domestic cats for HCM is expensive, is ambiguous when mild disease is present, and suffers from inter- and intra-observer variability. Consequently, the identification of a more accurate, reliable, less costly, and unambiguous means of screening for feline HCM would be advantageous. The ideal test would need to be very sensitive (i.e., there would be few false negative findings) at detecting mild to severe HCM while still being reasonably specific (i.e., there would be few false positive findings). Additionally, it would help an echocardiographic examiner decide if a cat with an equivocal exam either had or did not have HCM. Based on our findings, it appears that measuring the plasma concentration of NT-proBNP is not that ideal test.

The results of this study show that [NT-proBNP] is only accurate for identifying cats with severe HCM. Although the test was highly sensitive and specific for cats classified with severe disease (maximal wall thickness > 7 mm), it was extremely insensitive for detecting cats with moderate HCM (i.e., those with a maximal wall thickness between 6 and 7 mm (20% sensitivity)). As expected, when

[c] Fox PR, personal communication.

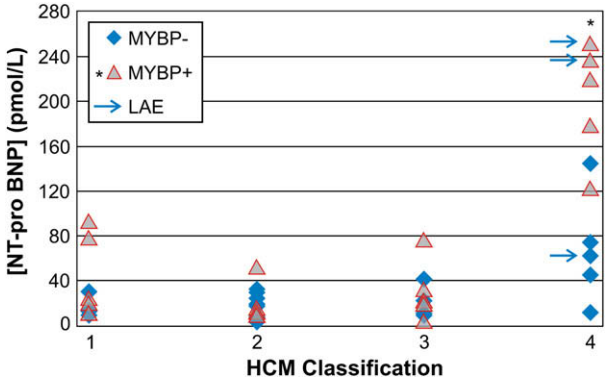

Figure 2 A scatter plot displaying plasma NT-proBNP concentration [NT-proBNP] in 40 cats, including nine normal cats (five MYBPC+, four MYBPC−), 12 cats equivocal for HCM (four MYBPC+, eight MYBPC−), nine cats with moderate HCM (five MYBPC+, four MYBPC−), and 10 cats with severe HCM (five MYBPC+, five MYBPC−). Those cats classified with severe HCM had a significantly greater [NT-proBNP] compared with all other groups ($P < 0.0001$)*. The normal, equivocal and moderate HCM groups were not significantly different from each other. Cats that had the A31P MYBPC mutation had a significantly elevated [NT-proBNP] when compared to those without the mutation ($P = 0.04$)*. Three cats had evidence of left atrial enlargement (LAE) on echocardiographic examination as indicated by the arrows on the figure. HCM classifications: 1, normal; 2, equivocal; 3, moderate; 4, severe.

cats with moderate and severe HCM were combined (i.e., those with a maximal wall thickness > 6 mm), sensitivity was also poor (58% sensitivity). Therefore, if [NT-proBNP] were to be used as a screening test, although a positive result would confer a good chance of severe disease being present, many cats with moderate HCM would be falsely identified as normal unless there was further evaluation performed, defeating the purpose of the screening tool. Similarly there was no apparent benefit in measuring [NT-proBNP] in cats with an equivocal echocardiographic exam since the [NT-proBNP] in this group was indistinguishable from the group of normal cats and from the cats with moderate HCM.

A recently published study also looked at [NT-proBNP] as a means of distinguishing cats with cardiac disease from normal cats.[16] The investigators from this study concluded that ''measurement of BNP concentrations may prove clinically useful as an initial screening test for cats with suspected cardiac disease.'' The study included 36 cats with HCM along with 14 cats with other forms of cardiac disease. Although the study did not describe the severity of HCM (i.e., maximal wall thickness and/or papillary muscle hypertrophy), 23 of the cats with HCM were in heart failure at the time of evaluation and all of the cats with HCM including those not in heart failure had an enlarged left atrium (LA/Ao ratio > 1.5). Consequently, it would appear that all of the cats with HCM in this study, including those not in heart failure, would have fit into the severe category of our study. We therefore conclude that the results of that study are concordant with the results of our own study with regard to cats with severe HCM and [NT-proBNP]. However, in contrast, we conclude that measurement of [NT-proBNP] is not a valid screening test for feline HCM.

Another study has used immunohistochemistry to show that there is increased BNP content in both the atria and ventricles of cats with HCM.[17] The severity of HCM was also not characterized here. However, all cats had a thicker than normal left atrial wall suggesting increased left atrial pressure. Therefore, it is likely that these cats also had severe HCM.

Conventional wisdom suggests that diastolic myocardial wall stretch is responsible for increased BNP synthesis and secretion. Diastolic wall stretch (strain) is brought about by placing stress on the wall. Diastolic wall stress is determined by diastolic intraventricular pressure, chamber radius and wall thickness (the law of Laplace). Most of the cats with severe HCM in our study did not have left atrial enlargement, which suggests that left atrial pressure and in turn left ventricular diastolic pressure was not increased. Therefore, in the present study, diastolic intraventricular pressure was presumed to be normal, chamber radius was normal to decreased and wall thickness was increased in most of the cats with severe HCM. This would indicate that diastolic wall stress was decreased and therefore diastolic myocardial stretch was decreased. Yet [NT-proBNP] was increased in our cats with severe HCM, as has been shown in humans with HCM that are not in heart failure.[10,13] In addition, although a significant correlation between BNP and E/E_a, a measure of left atrial pressure, has been demonstrated in both humans and cats, the relationship is weak ($r < 0.5$). Both of these suggest that there is a mechanism in addition to myocardial stretch (strain) that can stimulate increased BNP synthesis and release in HCM.

It is worth noting that cats with the A31P MYBPC mutation had a significantly increased [NT-proBNP] when compared to those without the mutation despite the fact that the number of cats with the mutation was reasonably evenly distributed between the four groups (5/9 normal cats; 4/12 cats with equivocal findings; 5/9 with moderate

HCM; 5/10 with severe HCM). This suggests that the presence of the A31P mutation itself may have a modulatory effect on BNP synthesis and secretion.

The usefulness of measuring [NT-proBNP] to screen for diseases such as arrhythmogenic right ventricular cardiomyopathy, chronic myxomatous AV valve disease, and dilated cardiomyopathy in dogs has been explored previously in the veterinary literature.[21–24] In general, the findings from those studies are similar to the results of the current investigation. In one study the measurement of [BNP] was not useful for detecting arrhythmogenic right ventricular cardiomyopathy in Boxer dogs. In another, [NT-proBNP] was increased in Cavalier King Charles spaniels with severe mitral regurgitation but was not in those with mild to moderate disease. Another study examined the ability of measuring [BNP] in Golden Retrievers with muscular dystrophy to identify early cardiomyopathy. The test was useful in dogs over 12 months of age with more advanced disease but not in younger dogs. Lastly, the ability of [NT-proBNP] measurement to identify Doberman Pinschers with dilated cardiomyopathy was examined. Disease severity was not presented in that investigation; however it appeared that the test was able to detect early (occult) disease, although there were many false positive results. Recently [NT-proBNP] measurement was evaluated in a small group of Beagle dogs with experimentally induced severe aortic stenosis in which it was found that [NT-proBNP] was increased.[25]

The HCM categorization scheme used in this study was arbitrary but we believe logical. With the current methods of diagnosing HCM, the distinction between mild HCM and normal is probably impossible and therefore, the equivocal designation was used and a mild category was not used. Within the population of cats in this study, there was left atrial enlargement in three cats. Severe disease is usually required to cause an increase in left ventricular diastolic pressure high enough to cause left atrial enlargement. Consequently, those cats and all cats that were quantitatively (i.e., maximal wall thickness) and anatomically indistinguishable from the cats with left atrial enlargement were categorized in the severe group. Cats with an LV wall thickness that was increased but was not as thick as those cats in the severe group were termed moderate. Not all cats with HCM in this research colony have the A31P mutation. Consequently, it is most likely that at least one more mutation exists that is responsible for HCM in this colony and in Maine Coon cats.

Limitations

Only Maine Coon and Maine Coon cross cats with and without the A31P mutation from a research colony were used in this study. It is possible that this cohort of cats is somehow different from other cats with HCM. However, phenotypically these cats appear to be the same as other cats with HCM seen in veterinary clinics.

The presence and severity of a dynamic left ventricular outflow tract obstruction, a common manifestation of HCM in human patients, has been positively correlated with BNP concentration.[12,13] Although some of the cats in this study with moderate or severe HCM did have systolic anterior motion of the mitral valve, the cats were sedated at the time of study and so the incidence and severity of SAM was almost assuredly reduced from what it would have been had the cats been unsedated. Consequently, this variable was not examined in relation to [NT-proBNP].

Blood obtained for the [NT-proBNP] measurement was performed at a different time than the echocardiographic examination. Therefore, it cannot be excluded that some cats had a higher value due to increased dynamic left ventricular outflow tract obstruction during blood collection.

Cats were sedated for the echocardiographic examination but often were not sedated for phlebotomy. Although there is evidence that BNP can decrease anxiety there is no evidence that sedation with an anxiolytic such as a phenothiazine tranquilizer alters [NT-proBNP].[26]

Since echocardiographic examination to time of blood collection varied between subjects, it is possible that HCM classification was different at the time of blood collection than at the time of echo examination. However, this group of research cats has been well characterized echocardiographically on a yearly basis and followed for years. Cats that had samples collected before the echocardiographic examination remained within the same severity group as the previous echocardiogram (1 year prior) and all cats in the study were over 5 years of age so it is highly unlikely that changes in disease severity accounted for any error.

Systemic blood pressure was not measured and so systemic hypertension cannot be definitively ruled out as the cause of LV wall thickening in each cat. However, if we had measured systemic blood pressure we would almost assuredly have identified some cats with a blood pressure elevation that was not actually due to systemic hypertension but rather due to so-called ''white coat'' hypertension (i.e., excitement/stress).

Because of the high rate of false positive diagnoses of systemic hypertension in this species and because of the inherent inaccuracy of the technique overall in cats, we decided not to attempt noninvasive blood pressure measurement in this cohort but instead ruled out the most common causes of true systemic hypertension in cats in our study groups.

Conclusion

Measurement of [NT-proBNP] has a high sensitivity and specificity as a means of detecting severe HCM in cats, but it is not sensitive for the identification of moderate HCM as judged by the evaluation of Maine Coon and Maine Coon cross cats in our colony. Consequently, we conclude that this test cannot be used to screen cats for the presence of mild to moderate HCM.

Conflict of Interest

The measurement of [NT-proBNP] was done free-of-charge by the Veterinary Diagnostics Institute. However, they had no access to the compiled data.

Acknowledgements

This study was funded by the American College of Veterinary Internal Medicine Cardiology Group and by the Veterinary Diagnostics Institute.

References

1. Meurs KM, Sanchez X, David RM, Bowles NE, Towbin JA, Reiser PJ, Kittleson JA, Munro MJ, Dryburgh K, Macdonald KA, Kittleson MD. A cardiac myosin binding protein C mutation in the Maine Coon cat with familial hypertrophic cardiomyopathy. Hum Mol Genet 2005;14: 3587—3593.
2. Meurs KM, Norgard MM, Ederer MM, Hendrix KP, Kittleson MD. A substitution mutation in the myosin binding protein C gene in ragdoll hypertrophic cardiomyopathy. Genomics 2007;90:261—264.
3. Kittleson MD, Kienle RD. Small animal cardiovascular medicine. St. Louis, MO: Mosby; 1998.
4. Gavaghan BJ, Kittleson MD, Fisher KJ, Kass PH, Gavaghan MA. Quantification of left ventricular diastolic wall motion by Doppler tissue imaging in healthy cats and cats with cardiomyopathy. Am J Vet Res 1999;60:1478—1486.
5. Bonagura JD. Feline echocardiography. J Fel Med Surg 2000; 2:147—151.
6. Rishniw M, Thomas WP. Dynamic right ventricular outflow obstruction: a new cause of systolic murmurs in cats. J Vet Intern Med 2002;16:547—552.
7. Goetze JP. Biochemistry of pro-B-type natriuretic peptide-derived peptides: the endocrine heart revisited. Clin Chem 2004;50:1503—1510.
8. Sisson DD. Neuroendocrine evaluation of cardiac disease. Vet Clin North Am Small Anim Pract 2004;34:1105—1126.
9. de Lemos JA, McGuire DK, Drazner MH. B-type natriuretic peptide in cardiovascular disease. Lancet 2003;362: 316—322.
10. Kaski JP, Tome-Esteban MT, Mead-Regan SJ, Pantazis A, Marek J, Deanfield JE, McKenna WJ, Elliott PM. B-Type natriuretic peptide predicts disease severity in children with hypertrophic cardiomyopathy. Heart 2008;94: 1307—1311.
11. Maron BJ, Tholakanahalli VN, Zenovich AG, Casey SA, Duprez D, Aeppli DM, Cohn JN. Usefulness of B-type natriuretic peptide assay in the assessment of symptomatic state in hypertrophic cardiomyopathy. Circulation 2004;109: 984—989.
12. Efthimiadis GK, Hitoglou-Makedou A, Giannakoulas G, Mitakidou A, Karamitsos T, Karvounis H, Mochlas S, Styliadis I, Stefanidis H, Parcharidis G, Louridas G. Clinical significance of N-terminal-probrain natriuretic peptide in hypertrophic cardiomyopathy. Heart Vessels 2007;22: 322—327.
13. Arteaga E, Araujo AQ, Buck P, Ianni BM, Rabello R, Mady C. Plasma amino-terminal pro-B-type natriuretic peptide quantification in hypertrophic cardiomyopathy. Am Heart J 2005;150:1228—1232.
14. Kim SW, Park SW, Lim SH, Kwon SU, Choi YJ, Park MK, Lee SC, Lee SH, Park JE, Jeon ES. Amount of left ventricular hypertrophy determines the plasma N-terminal pro-brain natriuretic peptide level in patients with hypertrophic cardiomyopathy and normal left ventricular ejection fraction. Clin Cardiol 2006;29: 155—160.
15. Hori Y, Yamano S, Iwanaga K, Kano T, Tanabe M, Uechi M, Kanai K, Nakao R, Hoshi F, Higuchi S. Evaluation of plasma C-terminal atrial natriuretic peptide in healthy cats and cats with heart disease. J Vet Intern Med 2008; 22:135—139.
16. Connolly DJ, Magalhaes RJ, Syme HM, Boswood A, Fuentes VL, Chu L, Metcalf M. Circulating natriuretic peptides in cats with heart disease. J Vet Intern Med 2008; 22:96—105.
17. Biondo AW, Ehrhart EJ, Sisson DD, Bulmer BJ, De Morais HS, Solter PF. Immunohistochemistry of atrial and brain natriuretic peptides in control cats and cats with hypertrophic cardiomyopathy. Vet Pathol 2003;40: 501—506.
18. MacLean HN, Abbott JA, Ward DL, Huckle WR, Sisson DD, Pyle RL. N-terminal atrial natriuretic peptide immunoreactivity in plasma of cats with hypertrophic cardiomyopathy. J Vet Intern Med 2006;20:284—289.
19. Prosek R, Sisson DD, Oyama MA, Biondo AW, Solter PE. Measurements of plasma endothelin immunoreactivity in healthy cats and cats with cardiomyopathy. J Vet Intern Med 2004;18:501—504.
20. Herndon WE, Kittleson MD, Sanderson K, Drobatz KJ, Clifford CA, Gelzer A, Summerfield NJ, Linde A, Sleeper MM. Cardiac troponin I in feline hypertrophic cardiomyopathy. J Vet Intern Med 2002;16:558—564.
21. Baumwart RD, Meurs KM. Assessment of plasma brain natriuretic peptide concentration in Boxers with arrhythmogenic right ventricular cardiomyopathy. Am J Vet Res 2005;66:2086—2089.
22. Tarnow I, Olsen LH, Kvart C, Hoglund K, Moesgaard SG, Kamstrup TS, Pedersen HD, Haggstrom J. Predictive value of

natriuretic peptides in dogs with mitral valve disease. Vet J 2008 (on-line Aug 1, 2008).
23. Chetboul V, Tessier-Vetzel D, Escriou C, Tissier R, Carlos C, Boussouf M, Pouchelon JL, Blot S, Derumeaux G. Diagnostic potential of natriuretic peptides in the occult phase of golden retriever muscular dystrophy cardiomyopathy. J Vet Intern Med 2004;18:845—850.
24. Oyama MA, Sisson DD, Solter PF. Prospective screening for occult cardiomyopathy in dogs by measurement of plasma atrial natriuretic peptide, B-type natriuretic peptide, and cardiac troponin-I concentrations. Am J Vet Res 2007;68: 42—47.
25. Hori Y, Tsubaki M, Katou A, Ono Y, Yonezawa T, Li X, Higuchi SI. Evaluation of NT-Pro BNP and CT-ANP as markers of concentric hypertrophy in dogs with a model of compensated aortic stenosis. J Vet Intern Med 2008;22:1118—1123.
26. Gardi J, Bíró E, Vecsernyés M, Julesz J, Nyári T, Tóth G, Telegdy G. The effects of brain and C-type natriuretic peptides on corticotropin-releasing factor in brain of rats. Life Sci 1997;60:2111—2117.