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Cardiac Biomarkers in Cats

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Abbreviations

NTproBNP, N-terminal pro-B type natriuretic peptide; cTnI, cardiac troponin I; cTnT, cardiac troponin T; HCM, hypertrophic cardiomyopathy; LV, left ventricular

Introduction

For over a decade, the measurement of cardiac biomarkers has been reported in cats with a variety of cardiac and systemic diseases, and measurement of N-terminal pro-B type natriuretic peptide (NTproBNP) and cardiac troponin I (cTnI) has now become commonplace in general and referral practice. The diagnosis of cardiac disease in cats poses particular challenges to many practitioners, including a high prevalence of sub-clinical (or "occult") cardiomyopathy, the inconsistent implications of a heart murmur in asymptomatic cats of different ages, and a tendency for the first clinical signs of heart failure to be sudden onset and severe. These assays offer a straightforward and accessible test, which often feature in the diagnostic investigation of feline cardiac disease by vets in general and referral practice alike. However, our understanding of the clinical utility of these laboratory tests is ever evolving.

This review aims to update the reader on the published veterinary literature regarding cardiac biomarkers in cats. The physiology of the natriuretic peptides and cardiac troponins is reviewed in detail elsewhere,[1, 2] and the focus of this review will be on the role of cardiac biomarkers in clinical decision-making.

Review Methods

Published literature was searched using Medline, Web of Science and Google Scholar electronic databases.[a,b,c] Terms used in various combinations are listed in Table 1. The reference lists of retrieved articles were also manually searched and relevant citations retrieved. Conference proceedings were not included. Information recorded from each publication included citation details; biomarkers measured; the assay used; the number of animals, and the hypothesis, findings, conclusions and limitations of each study. This was then cross-checked by a second party for errors.

A summary of the studies reviewed in this manuscript is tabulated (Tables 2-5) for quick reference. This review initially considers biomarkers measured in reference laboratories. Some of the newer, point-of-care tests are considered later in this manuscript.

Distinguishing cardiac from non-cardiac causes of respiratory distress

Cats with respiratory distress are often unstable and tolerate poorly any handling or diagnostic interventions. Although useful to identify cats with cardiogenic respiratory distress, echocardiography may not be available. Measurement of a cardiac biomarker may be useful if a blood sample can be obtained with minimal restraint, and this may be safer than thoracic radiography. Eight studies were identified that compared cardiac biomarker concentrations in cats with cardiac and non-cardiac causes of respiratory distress: 4 studies investigated cTnI[3-6] and 4 investigated natriuretic peptides (of which, all 4 featured NTproBNP and 1 featured NTproANP)[7-10] (Table 2).

Of the cTnI studies, 3/4 reported a higher median cTnI concentration in the cardiac vs. the non-cardiac group and one failed to detect a statistically significant difference. The study where no difference was detected[3] also had the smallest sample size, so this finding may reflect that the statistical comparison was under-powered. All studies where a difference was identified reported a considerable overlap in cTnI values between cardiac and non-cardiac groups, suggesting that a single cut-off value to identify cardiogenic dyspnea in cats is unlikely to be clinically useful.

In contrast, NTproBNP has shown greater accuracy in distinguishing cats with cardiac dyspnea from those with non-cardiac causes: all 4 studies reviewed reported higher median NTproBNP concentration in cats with cardiogenic respiratory distress.[7-10] The one study investigating NTproANP reported that the overall accuracy of this test was lower than NTproBNP, but despite this, the test was still useful to detect cardiogenic dyspnea.[8] Published NTproBNP cut-off values for identifying cats with acute congestive heart failure range from 214-277 pmol/L, with a sensitivity generally over 85% and a

specificity of 84-88%. However, because these studies were published over a 5 year time frame and used 3 different commercially available assays, the cut-off values and sensitivity/specificity data should not be directly compared. Also, recent changes in commercial assay methodology may mean that established cut-off values are replaced by new publications using contemporary assays. Despite this, the evidence convincingly supports the use of NTproBNP to differentiate between cardiac and non-cardiac causes of respiratory distress in cats. A significant practical limitation of the published literature is the current lack of studies investigating the utility of a patient-side NTproBNP test, which has only recently become available.

Pleural fluid and urinary NTproBNP measurement

In humans, NTproBNP concentration can be accurately measured in pleural fluid and urine.[11, 12] The same has been shown in 40 cats presenting to an emergency department with pleural effusions.[10] In both urine and pleural effusion samples, NTproBNP was detectable with adequate performance. NTproBNP concentration was significantly higher in pleural fluid than in plasma with a strong correlation between measurements, suggesting that measurement of NTproBNP in pleural fluid obtained during therapeutic thoracocentesis is an adequate and reliable substitute for blood sampling, thereby reducing handling of these dyspneic patients. Urine NTproBNP to creatinine ratio was higher in the cats with cardiogenic pleural effusion than those with noncardiac causes. Despite this, further analysis failed to determine useful cut-off values to distinguish between cardiac and noncardiac patients using urinary NTproBNP, possibly related to variable handling or processing time for urine samples in the study. Further studies with improved standardisation of urine sample collection from cats with suspected cardiac disease are needed.

Identification of cats with occult cardiomyopathy

Occult heart disease is common in cats, and currently most cats are screened by a veterinary cardiologist using echocardiography. However, some cat owners may be reluctant to travel or pay for a cardiologist to perform echocardiography, highlighting a possible role for cardiac biomarkers to pre-screen for cats most likely to benefit from echocardiography. Eleven published studies compared a control group of healthy cats to a group of cats with echocardiographic evidence of heart disease, but no clinical signs of congestive heart failure[3, 13-21] (Table 3). Of these, only the 2 largest studies were specifically designed to test the ability of cardiac biomarkers to identify occult cardiomyopathy in a screened population of cats. One investigated the use of a quantitative NTproBNP assay,[16] whilst the second evaluated a patient-side SNAP colorimetric assay.[21] In all, the 10 published studies describe a total of 393 healthy cats, 350 cats with HCM, 38 with RCM or UCM, 5 with DCM and 1 with ARVC. Broadly, all but one of these studies reported that cardiac biomarkers were significantly higher in cats with echocardiographic evidence of cardiomyopathy than in cats without cardiac disease.

In three studies, NTproANP was reported to be significantly higher in cats with echocardiographic evidence of heart disease than healthy controls in 3 studies.[18-20] In contrast, one small study[17] did not detect a significant difference between cats with and without heart disease but reported a weak positive correlation between NTproANP and left atrial size.

NTproBNP has been reported to be significantly higher in cats with heart disease than healthy cats in all 5 studies reporting this comparison. Three studies report an optimal NTproBNP cut-off value for detecting occult cardiomyopathy: 2 studies identified 100pmol/L (71-92% sensitivity, 94-100% specificity),[15, 16] the third identified 49pmol/L (sensitivity 100%, specificity 89%).[19] However, in those studies that tested the ability of NTproBNP to distinguish between different grades of severity of cardiomyopathy,[14-16, 21] this biomarker was less accurate at identifying mild grades of disease. This suggests that echocardiography remains preferable to screen for mild/early stage disease. In one study, Maine Coons positive for the MYBPC3:A31P mutation had significantly higher NTproBNP than mutation-negative cats.[14]

Both studies comparing cTnI in cats with heart disease (no heart failure) to healthy controls reported that circulating concentrations of this biomarker were significantly higher in cats with heart disease,[3, 13] as did a recent validation study for a high sensitivity cTnI assay.[22] However, with the larger samples sizes reported and the similarities in results between the published studies, NTproBNP currently seems the better cardiac biomarker to use when screening for occult cardiomyopathy.

Effects of systemic disease

In humans, circulating cardiac biomarker concentrations are known to be influenced by age, sex, renal and thyroid function, body condition (especially obesity) and the presence of anemia.[23] Although the effects of body weight, age and sex have not been well studied in cats, the effects of some non-cardiac diseases have been investigated. Six published studies evaluated the effects of non-cardiac, non-respiratory disease on circulating cardiac biomarkers (Table 4). Three investigated hyperthyroid cats,[24-26] 2 investigated chronic kidney disease (CKD, plus/minus hypertension),[27,28] and 1 reported preliminary data in anaemic cats.[29] All 3 studies regarding the effect of hyperthyroidism provided strong evidence that NTproANP (n=61),[25] NTproBNP (n=84)[25, 26] and cTnI (n=46)[24, 26] are increased in a hyperthyroid state and return to the same level as non-hyperthyroid controls after restoration of a euthyroid state by radioactive iodine therapy. Only one of these studies[26] compared hyperthyroid cats to separate control groups of normal cats and a separate group of cats with cardiomyopathy. In this study, both NTproBNP and cTnI were higher in cats with cardiomyopathy than hyperthyroidism, but significant overlap was present between groups, suggesting that neither biomarker can effectively differentiate between cats with hyperthyroidism and primary cardiomyopathy. The consistent finding that cTnI is increased in hyperthyroid cats suggests that not only are cardiac filling pressures affected by changes in systemic vascular resistance and circulating volume induced by the endocrinopathy, but that thyrotoxicosis affects the myocardium on a cellular level to cause myocardial cell damage (either

by a direct effect of thyroid hormones or an indirect effect, for example via sympathetic nervous system activation).

In cats with azotemic CKD, cTnI is commonly higher than the reference interval for healthy cats. However, no correlation between the severity of azotemia and the degree of cTnI elevation was present, suggesting that the azotemic state may be more important than the degree of renal functional impairment when comparing individual cats to reference intervals.[27] However, the results of this study merit some additional research, because no control group was used and none of the 14 cats investigated had echocardiography performed.

Natriuretic peptides have also been evaluated in cats with CKD. One study reported values of NTproANP and NTproBNP in cats with CKD (with and without hypertension) and compared them to a control group of non-hypertensive cats without evidence of CKD.[28] NTproBNP was significantly higher in cats with severe azotemia (>440umol/L or 5mg/dL) than those with less severe CKD and healthy controls but, again, no correlation with creatinine concentration was detected. Also, NTproBNP was higher in hypertensive CKD than non-hypertensive cats with CKD, and circulating concentrations reduced after successful treatment of hypertension. Similarly to NTproBNP, NTproANP was higher in severely azotemic cats and those with hypertension. However, unlike NTproBNP, NTproANP did correlate with serum creatinine concentration and did not normalise with treatment of systemic hypertension. The reasons for these differences are unclear, but this and the findings of several other studies suggest that NTproBNP is more sensitive to dynamic changes than NTproANP. As with the cTnI study above,[27] this study of natriuretic peptides in cats with CKD did not include echocardiographic examination of any of the subjects. Also, the control group was not age-matched with the hypertensive and non-hypertensive CKD groups. These weaknesses mean that further, prospective, standardised studies in cats with CKD ± hypertension are warranted, where echocardiography is performed to account for the presence of occult cardiac disease and where a control group is age- and sex-matched to the cats with CKD.

One recent study investigating the effect of anemia on cTnI in cats showed that anemic cats had significantly higher circulating cTnI than a control population of unwell, non-anemic cats.[29] However, this study was subject to similar methodological flaws, in that echocardiography was not performed on all cats and the authors did not report the frequency with which auscultated abnormalities (such as a gallop sound, arrhythmia or murmur) were present in the anaemic group. Cats with auscultated abnormalities were, however, excluded from the control group of non-anaemic cats.

Point of care tests

Much interest has been expressed in developing patient-side cardiac biomarker assays, primarily for use in cats with respiratory distress, in the hope that this may help practitioners more confidently diagnose and treat acute congestive heart failure (CHF). Another potential application for these in-clinic tests would be to help primary clinicians stratify cats with heart murmurs, according to which are most likely to have occult cardiomyopathy and therefore require echocardiography. Two studies evaluating patient-side cardiac biomarker tests in cats with cardiac disease have been published.[6, 21]

The performance of the first patient-side NTproBNP ELISA (IDEXX Ltd) was tested in a recent study of 146 cats.[21] The ability of this colorimetric test to identify cats with moderate to severe heart disease amongst a population of cats referred for cardiac investigation suggested a good performance in identifying moderate and severe grades of occult heart disease. The assay had a positive cut-off NTproBNP concentration of between 108-122pmol/L, similar to the cut-off of 100pmol/L for detection of moderate/severe occult cardiomyopathy previously reported.[16] The negative predictive value (NPV) of the patient-side test was 94%; i.e. in a cat with a negative test result, there was a 94% probability that this patient truly did not have moderate to severe heart disease. Although the positive predictive value (PPV) was only 64%, this also aids identification of a population of asymptomatic cats where echocardiography is indicated to screen for occult disease. These figures suggest that the patient-side

183 test has significantly greater utility to “rule-out” more advanced heart disease than it does to confirm
184 the presence of disease. The population described in this study had a high prevalence (24%) of
185 moderate-severe heart disease. In a different population of lower prevalence, such as the background
186 general practice population, the PPV would be even lower. Importantly however, the NPV of the test
187 would increase. For example, if screening a population of shelter cats for hypertrophic
188 cardiomyopathy (HCM), which has an estimated overall prevalence of 15%,[30] the NTproBNP point of
189 care test would have a PPV of 49% and an NPV of 97%, presuming the same test sensitivity and
190 specificity. This would make it a useful test for identifying cats with none or mild disease, for which
191 further diagnostic interventions such as echocardiography are unlikely to add significant additional
192 information to the practitioner. A notable exception to this would be when screening cats used for
193 breeding, where echocardiography would still be required to detect mildly affected cats. Also, the
194 confounding influence of hyperthyroidism to increase circulating NTproBNP concentration should be
195 considered in older cats where point-of-care testing is performed.[25, 26]

196 To date, the ability of this point of care NTproBNP test to distinguish between cardiac and noncardiac
197 causes of respiratory distress has not been evaluated. Despite this, it is reasonable to assume that a
198 negative colorimetric test in a dyspneic cat is likely to reflect a noncardiac cause of clinical signs. In
199 contrast, patient-side cTnI analysis has been investigated in 37 cats to identify cats with a cardiac cause
200 of acute respiratory distress.[6] In this study, cTnI was significantly higher in cats with cardiac disease
201 than those with a respiratory cause of dyspnea. Cats with a circulating cTnI concentration below
202 0.24ng/mL all had a noncardiac cause of clinical signs, whereas above 0.66ng/mL all cats had
203 cardiogenic dyspnea. In this population, none of the cats had sepsis such as pyothorax as a cause of
204 dyspnea. Sepsis is known to increase circulating cTnI concentrations in humans and dogs[31-33] and the
205 same may be true in cats. If so, the findings of this study may not be applicable to a wider population
206 of cats which included patients with pyothorax.

207

Prognostic utility of cardiac biomarkers

In humans, NTproBNP, cTnI and cTnT have been used to stratify patients with HCM according to prognosis.[34-38] Three studies have been published investigating the association of cardiac biomarker concentration with outcome in cats with heart disease; one investigating NTproANP,[39] one investigating both cTnI and cTnT,[40] and one investigating NTproBNP and cTnI (Table 5).[41]

NTproANP was the first cardiac biomarker evaluated for an association with survival time in cats with cardiomyopathy, in a study evaluating 68 cats with varying degrees of cardiomyopathy severity.[39] Although significant at the univariable level, NTproANP did not remain significant in multivariable analysis when included alongside echocardiographic measures of left atrial size, suggesting that once left atrial size was known, no additional prognostic information was gained by the measurement of NTproANP. Similarly, higher NTproBNP was associated with reduced survival time in cats with HCM at the univariable level, but did not remain additionally useful once clinical signs or left atrial size were accounted for in another study of 41 cats.[41]

Cardiac troponins may be more useful than natriuretic peptides in providing prognostic information in cats. The circulating concentration of cTnI and cTnT at diagnosis were significantly higher in cats that suffered cardiac death than survivors in one recent study investigating 36 cats with HCM.[40] cTnT was a better prognostic marker in this study, with cTnI showing no additional prognostic utility. Results also suggested that serial biomarker monitoring may be clinically useful, because cTnT measurement repeated before the end of the follow-up period in this cohort of cats provided independent prognostic information, even when accounting for cTnT concentration at the time of diagnosis. A second study, reporting 41 cats with HCM, identified this same association between increased cTnI and a greater risk of cardiac death.[41] Cats with a circulating concentration of cTnI >0.7ng/ml at the time of diagnosis had a shorter time to cardiac death, independent of both clinical signs of congestive heart failure and echocardiographic measures of left atrial size or function. In this study, cats with regional LV hypokinesis detected on echocardiography had significantly higher cTnI than cats without

regional hypokinesis, possibly reflecting an association between increased cTnI and regional myocardial ischemia or infarction. It is worth noting that these two studies[40, 41] used different cTnI assays, so despite the similar patient demographics and the publication of these two articles within months of once another, reported cTnI values are not comparable.

Currently, no published studies have thoroughly evaluated how cardiac biomarkers may change over time in cats with cardiomyopathy. One recently presented research abstract[42] suggested that a >70% change in quantitative NTproBNP measurement was required to indicate a genuine change, instead of day-to-day biological or assay variability. In our own clinics, it is advised that an increased NTproBNP test (or positive patient-side SNAP test) be followed up with echocardiography rather than re-testing, so our personal experience with this is limited. In dogs, the rate of change in cardiac biomarker concentration appears important in long-term prognosis, but this has not been reported in cats and it is not understood how this may help to predict outcome in feline patients.

Limitations

Most of the studies reviewed here had a prospective design and investigative protocol was standardized where possible. However, treatment protocols were not standardized and differences in decision making between different veterinarians and different owners will no doubt have affected survival time in studies evaluating prognosis.

No consensus exists amongst veterinary cardiologists as to how best grade severity of cardiomyopathy in cats, other than to say most cats that have experienced clinical signs of congestive heart failure have severe disease. In the screening studies reviewed here, where cats with cardiomyopathy were classified into groups of equivocal, mild, moderate and severe by some studies, this lack of consensus means that different authors classified cats in different ways. For example, severe cardiomyopathy in some groups took into account left atrial size but in others only accounted for degree of wall

thickening. As a result, cats classified as “severe” by some studies may have been classified as “moderate” by others. This lack of consensus means that the reader cannot directly compare the findings of different screening studies utilising cardiac biomarkers. In addition, a lack of consensus and an inconsistency in the echocardiographic measurement technique between veterinary cardiologists means that even the current ‘gold-standard’ of diagnosing mild (or equivocal) HCM may not be reliably comparable between publications, and this may account for some of the differences in optimal NTproBNP cut-off values reported in different studies.

Misclassification of patients into even broad groups is possible, such as presence or absence of cardiac disease, or suffering a cardiac versus noncardiac death. An example of this was identified by Humm and colleagues,[10] who reported a suspected misclassification of one cat in their study, despite all patients having been assessed by a veterinary cardiologist using echocardiography. This may have led to falsely low specificity of pleural fluid NTproBNP to detect cardiogenic pleural effusion in their article. Other studies did not perform echocardiography on all cats included, so the presence of cardiac disease in many patients was not assessed, and some studies did not exclude patients with systemic hypertension or hyperthyroidism from their cardiac disease group. Although a heterogeneous population of cats is more likely to be reflective of the wider general practice population, misclassification of cases and the presence of concurrent systemic disease affecting the cardiovascular system are likely to reduce the discriminative ability of the cardiac biomarker tests reported in certain studies. This is especially true in studies with a small sample size or low event rate, such as is the case in many veterinary studies.

Finally, two aspects of laboratory technique are important to consider. The use of different assays in different studies limits the utility of published cut-off values to clinicians comparing their measured biomarker concentration to those reported in the literature. This is especially important to note for cTnI, where 5 different assays were used across the 13 papers reviewed here. Another limitation is the difference between NTproBNP concentration measured by the “second generation” IDEXX Feline

Cardiopet NTproBNP assay and the previous assay used by the same laboratory (although the manufacturer states that values are likely to be comparable). It is likely that studies published before 2014 did not use the more sensitive second generation NTproBNP assay from IDEXX, so cut-off values may not apply to practitioners testing cats at the time of writing, even though they are using the same reference laboratory.

Summary

The published literature on cardiac biomarkers in cats is widespread, and recent studies have widened our understanding of the emerging roles for cardiac biomarkers in cats with heart disease, especially in relation to the development of the convenient patient-side assay and the use of these biomarkers for long-term prognosis.

NTproBNP appears to most reliably differentiate between cardiac and noncardiac causes of respiratory distress in cats. Measurement of NTproBNP is reliable from pleural fluid obtained by thoracocentesis, without the need for blood sampling restraint in an unstable patient. The patient-side test will increase the convenience of NTproBNP testing in cats in general practice and this test has great potential to assist in the emergency room. However, the usefulness of NTproBNP in prognostication is limited where left atrial dilation and a history of congestive heart failure can be reliably confirmed. In contrast, measurement of cTnI can be used not only as a patient-side test to differentiate cardiac from noncardiac causes of respiratory distress (albeit less accurately than NTproBNP), but can also be used as part of prognostication together with heart failure status and echocardiographic measurements.

On the basis of current published data, future studies evaluating urinary NTproBNP:creatinine ratio as a diagnostic test are warranted. The value of the patient-side NTproBNP ELISA in distinguishing between cardiac and respiratory causes of respiratory distress has not yet been published, so its use

306 in the acute patient would currently be based upon limited data. There is notable overlap between
307 the NTproBNP concentrations reported in cats with respiratory disease and the established cut-off
308 value used for the diagnosis of occult cardiomyopathy by the patient-side test, so the potential for
309 false-positive results when using the point-of-care test in acute patients should be considered, as
310 supported by data recently presented as a research abstract.

311 The current evidence base for how systemic non-cardiac diseases affect the circulating concentrations
312 of cardiac biomarkers is weak for cats with CKD and systemic hypertension, and further studies are
313 warranted in these patients. However, hyperthyroid cats are relatively well studied and the evidence
314 from which to draw conclusions is more reliable.

315 In the authors' opinions, practitioners should interpret absolute cut-off values from the published
316 literature cautiously, due to differences in the commercial troponin assays used by different studies,
317 and differences in NTproBNP assay sensitivity at reference laboratories. However, available results
318 suggest that cardiac biomarkers will continue to have clinical utility for veterinary cardiologists and
319 are likely to have an increasingly important role in both primary and referral veterinary practice.

320

321 **Conflicts of Interest**

322 In the last 3 years, the authors have each received discounted biomarker analysis from IDEXX
323 laboratories in the UK for the purposes of research in cats. We wish to confirm that there are no
324 conflicts of interest associated with this publication and there has been no financial support for this
325 work from any third parties or funding bodies.

Table 1: Search terms used in combination to review electronic online databases for recent published literature on feline cardiac biomarkers

Search term	Feline
	Cat
	Natriuretic
	Peptide
	Cardiac
	Troponin
	NTproBNP
	NT-proBNP
	NTproANP
	NT-proANP
	cTnI
	cTnT
	Cardiomyopathy
	Biomarker

330 Table 2: Summary of the studies reporting the use of cardiac biomarkers to differentiate between

331 cardiac and noncardiac causes of respiratory distress in cats

332 *cTnI, cardiac troponin I; NTproANP, N-terminal pro atrial natriuretic peptide; NTproBNP, N-terminal pro B-type natriuretic peptide; AUC,*

333 *area under the curve, pertaining to receiver operating curve statistical analysis of diagnostic test utility; P, prospective study design; M,*

334 *multicenter recruitment; C, control group appropriate; B, blinding specified in manuscript; E, echocardiography on all cats*

Biomarkers	**Number of cats**	**Conclusions**	**Assay**	**Citation**	**Evidence category**
cTnI	16 cats with HCM, 18 healthy controls	cTnI can help distinguish between HCM and non-HCM groups, but less able to identify cats with CHF	Immulyte (Diagnostic Products Co.)	Connolly[3]	P;C;E
	43 cats with dyspnea (31 cardiac)	Able to identify cardiac causes of dyspnea: AUC 0.84. Overlap between groups	Stratus (Dade Behring)	Herndon[4]	P;M;C;E
	53 cats with dyspnea (23 cardiac)	Significant difference between cardiac and noncardiac: AUC 0.84. Overlap between groups	Immulyte (Siemens Medical Diagnostics)	Connolly[5]	P;M;C
	39 dyspneic cats (25 cardiac) 37 healthy controls	A patient-side cTnI assay can be used to differentiate cats with cardiac causes of dyspnea from those with noncardiac disease and normal controls	i-Stat 1 analyser (Heska Corp.)	Wells[6]	P;M;C;E
NTproANP NTproBNP	85 dyspneic cats (44 cardiac)	Both NTproANP and NTproBNP were able to discriminate between cardiac and noncardiac patients, but NTproBNP better performance (cut-off 220pmol/L, AUC 0.96)	proANP 1-98 (Guildhay Ltd), Cardioscreen NTproBNP (Guildhay Ltd)	Connolly[8]	P;C;B

NTproBNP	162 dyspneic cats (101 cardiac)	Reliable discrimination between cardiac and respiratory causes of dyspnea: cut-off 207pmol/L, AUC 0.98	CardioPet proBNP (IDEXX Ltd)	Fox[7]	P;M;C;E
	21 cats with pleural effusion (11 cardiac disease)	NTproBNP successfully discriminated between cardiogenic and noncardiac causes of pleural effusion: cut-off 258pmol/L, AUC 1.0	Cardiopet proBNP (IDEXX Ltd)	Hassdente-ufel[9]	P;C;B;E
	40 cats with pleural effusion (22 cardiac)	Plasma NTproBNP reliably identified cats with cardiogenic pleural effusion: cut-off 214pmol/L, AUC 0.91	Vetsign Feline Cardiopet NTproBNP (IDEXX Ltd)	Humm[10]	P;C;E

335

336

337 Table 3: Summary of the studies reporting the use of cardiac biomarkers to detect occult heart

338 disease in cats

339 *cTnI, cardiac troponin I; NTproANP, N-terminal pro atrial natriuretic peptide; NTproBNP, N-terminal pro B-type natriuretic peptide; AUC,*

340 *area under the curve, pertaining to receiver operating curve statistical analysis of diagnostic test utility; P, prospective study design; M,*

341 *multicenter recruitment; C, control group appropriate; B, blinding specified in manuscript; E, echocardiography on all cats*

Test	**Number of cats**	**Conclusions**	**Assay**	**Citation**	**Study design**
cTnI	16 cats with HCM, 18 healthy controls	cTnI can help distinguish between HCM and non-HCM groups, but less able to identify cats with CHF	Immulyte (Diagnostic Products Corp.)	Connolly[3]	P;C;E
	53 cats: 20 HCM and 33 healthy controls.	cTnI could discriminate between HCM and control cats, and was also correlated with LV wall thickness	Status CS troponin I (Dade Behring Inc.)	Herndon[13]	P;M;B;C
	73 cats: 53 cardiac and 20 healthy controls	cTnI was higher in cats with heart disease than control cats (assay validation study)	ADVIA Centaur CP TnI-ultra (Siemens)	Langhorn[22]	P;C;E
NTproANP	36 cats: 17 HCM and 19 healthy controls	No significant difference between HCM and control cats, but a mild positive correlation with LVFW thickness and LA size was detected	proANP 1-98 Biomedica, (American Laboratory Products Co.)	Maclean[17]	P;B;C;E
	Study 1 - 5 cats Study 2 - 22 cats: 14 cardiomyopathy and 8 controls	Positively correlated with LA pressure. Significant difference between cardiac and control groups	Shinonoria-ANP radioimmunoassay (Shionogi Co.)	Hori[18]	P;C;E
	43 cats: 16 heart disease/CHF, 16	Positively correlated with LA size. Discriminated between all 3 groups	proANP 1-98 (Biomedica	Zimmering[20]	P;B;C;E

	heart disease/NO-CHF, 11 controls		Group, Immundiagnostik AG)		
NTproANP NTproBNP	78 cats: 33 heart disease/CHF, 17 heart disease/NO-CHF, 28 controls	Both biomarkers distinguished between all 3 groups, and were correlated with each other. NTproBNP was more accurate at detecting cardiac disease: cut-off 49pmol/L, AUC 0.98. Both markers positively correlated with LA size and E:E' ratio.	proANP 1-98 (Guildhay Ltd) Feline Cardioscreen proBNP (Guildhay Ltd)	Connolly[19]	P;B;C;E
NTproBNP	41 cats: 9 normal, 12 equivocal HCM, 19 moderate/severe HCM. Maine Coon or Maine Coon-cross only	Higher NTproBNP in severe group, no significant difference between all other groups: not an effective screening test in this population. Severe HCM cut-off 44pmol/L, AUC not reported. MYBPC3:A31P mutation positive cats had higher NTproBNP	Feline Cardiocare NTproBNP (Veterinary Diagnostics Institute)	Hsu[14]	P;C;E
	201 cats: 99 normal, 9 equivocal HCM, 15 mild HCM, 17 moderate HCM, 61 severe HCM	No difference in NTproBNP between equivocal and healthy cats. Severe HCM had significantly higher NTproBNP than other groups. Cut-off for mild HCM detection 100pmol/L, AUC 0.96	Feline Cardioscreen proBNP (Guildhay Ltd)	Wess[15]	P;C;B;E
	227 cats: 114 normal, 87 HCM, 22 UCM, 3 UCM, 1 DCM	NTproBNP effectively discriminated between normal cats and those with occult cardiomyopathy. Cut-off	Cardiopet proBNP (IDEXX Ltd)	Fox[16]	P;M;C;B;E

		99pmol/L, AUC 0.92. Correlation of NTproBNP with LV wall thickness and LA size.			
	146 cats: 43 normal, 16 equivocal, 50 mild heart disease, 37 moderate/severe	NTproBNP SNAP test can be used to help exclude moderate to severe occult cardiomyopathy; negative predictive value 94%	NTproBNP SNAP (IDEXX)	Machen[21]	P;M;C;B;E

342

343

344 Table 4: Summary of the studies reporting the use of cardiac biomarkers in patients with noncardiac

345 disease

346 *cTnI, cardiac troponin I; NTproANP, N-terminal pro atrial natriuretic peptide; NTproBNP, N-terminal pro B-type natriuretic peptide; P,*

347 *prospective study design; M, multicenter recruitment; C, control group appropriate; B, blinding specified in manuscript; E,*

348 *echocardiography on all cats; RAI, radioactive iodine treatment; BP, blood pressure; PCV, packed cell volume*

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Test	**Number of cats**	**Conclusions**	**Assay**	**Citation**	**Study design**
cTnI	23 hyperthyroid cats (18 post-treatment with RAI)	cTnI higher in cats with higher total thyroxine concentration. Trend towards reduction in cTnI observed, but not statistically significant.	Immulyte (Diagnostic Products Co.)	Connolly[24]	P;B;E
NTproBNP cTnI	23 hyperthyroid cats (12 post-treatment with RAI), 17 cats with HCM, 19 controls	cTnI was increased in 46% and NTproBNP was increased in 38% hyperthyroid cats with no echocardiographic abnormalities. Both biomarkers normalised in most hyperthyroid cats after RAI treatment.	Cardiopet NTproBNP (IDEXX), Stratus CS Stat cTnI (Dade Behring)	Sangster[26]	P;C;B;E
NTproANP NTproBNP	85 hyperthyroid cats at baseline, 61 post-treatment with RAI	Hyperthyroidism was associated with a significant but modest elevation of both natriuretic peptides, which reduced after RAI treatment.	Cardiopet NTproBNP (IDEXX)	Menaut[25]	P;M
cTnI	14 cats with CKD	cTnI higher in azotemic patients, but no correlation with creatinine concentration	Stratus CS Stat cTnI (Dade Behring)	Porciello[27]	

NTproANP NTproBNP	58 cats: 22 normal, 13 CKD normotensive, 23 CKD hypertensive	NTproBNP higher in cats with severe azotemia, but no correlation with creatinine. NTproBNP was higher in hypertensive CKD than non-hypertensive CKD. Also correlated with systolic BP and age. NTproBNP reduced after treatment of hypertension. NTproANP similar, but did not reduce after treatment.	NTproANP 1-98, (Biomedica Gruppe), VETSIGN Feline Cardio-SCREEN proBNP, (Guildhay Ltd)	Lalor[28]	M;C
cTnI	49 cats: 18 anemic, 31 non-anemic, unwell controls	cTnI higher in anemic cats than controls, but no correlation with PCV	Immulite (Siemens)	Lalor[29]	P;C

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351 Table 5: Summary of the studies reporting prognostic capability of cardiac biomarkers in cats

352 *cTnI, cardiac troponin I; NTproANP, N-terminal pro atrial natriuretic peptide; NTproBNP, N-terminal pro B-type natriuretic peptide; P,*
353 *prospective study design; M, multicenter recruitment; C, control group appropriate; B, blinding specified in manuscript; E,*
354 *echocardiography on all cats*

Test	**Number of cats**	**Conclusions**	**Assay**	**Citation**	**Study design**
NTproANP	68 cats: 25 heart disease/CHF, 26 heart disease/NO-CHF, 17 controls	Increased NTproANP was associated with reduced survival time in univariable analysis, but lost significance in multivariable analysis when included with LA size.	proANP 1-98 (Biomedica Group, Immundiagnostik AG)	Zimmering[39]	P;B;C;E
cTnI cTnT	36 cats with HCM (10 cardiac death) 23 healthy controls	cTnI and cTnT were both higher in non-survivors than survivors. cTnI correlated with LVFW thickness at diagnosis	ADVIA Centaur CP TnI-ultra (Siemens), Elecsys hs-TnT (Roche)	Langhorn[40]	P;B;E
NTproBNP cTnI	41 cats with HCM (21 cardiac death)	cTnI provided prognostic information, independent of heart failure status and the presence of left atrial dilation. NTproBNP was significantly associated with prognosis only if heart failure status or LA size was not accounted for	Cardiopet NTproBNP (2nd Gen; IDEXX), AccuTnI (Beckman Coulter)	Borgeat[41]	P;B;E

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356 **Footnotes**

357 [a] http://www.ncbi.nlm.nih.gov/pubmed

358 [b] http://wok.mimas.ac.uk/

359 [c] http://scholar.google.com /

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References

1. Oyama MA. Using cardiac biomarkers in veterinary practice. *Vet Clin North Am Small Anim Pract*. 2013;43:1261-72, vi.
2. Oyama MA, Boswood A, Connolly DJ, Ettinger SJ, Fox PR, Gordon SG, Rush JE, Sisson DD, Stepien RL, Wess G and Zannad F. Clinical usefulness of an assay for measurement of circulating N-terminal pro-B-type natriuretic peptide concentration in dogs and cats with heart disease. *J Am Vet Med Assoc*. 2013;243:71-82.
3. Connolly DJ, Cannata J, Boswood A, Archer J, Groves EA and Neiger R. Cardiac troponin I in cats with hypertrophic cardiomyopathy. *J Feline Med Surg*. 2003;5:209-16.
4. Herndon WE, Rishniw M, Schrope D, Sammarco CD, Boddy KN and Sleeper MM. Assessment of plasma cardiac troponin I concentration as a means to differentiate cardiac and noncardiac causes of dyspnea in cats. *J Am Vet Med Assoc*. 2008;233:1261-4.
5. Connolly DJ, Brodbelt DC, Copeland H, Collins S and Fuentes VL. Assessment of the diagnostic accuracy of circulating cardiac troponin I concentration to distinguish between cats with cardiac and non-cardiac causes of respiratory distress. *Journal of veterinary cardiology : the official journal of the European Society of Veterinary Cardiology*. 2009;11:71-8.
6. Wells SM, Shofer FS, Walters PC, Stamoulis ME, Cole SG and Sleeper MM. Evaluation of blood cardiac troponin I concentrations obtained with a cage-side analyzer to differentiate cats with cardiac and noncardiac causes of dyspnea. *J Am Vet Med Assoc*. 2014;244:425-30.
7. Fox PR, Oyama MA, Reynolds C, Rush JE, DeFrancesco TC, Keene BW, Atkins CE, Macdonald KA, Schober KE, Bonagura JD, Stepien RL, Kellihan HB, Nguyenba TP, Lehmkuhl LB, Lefbom BK, Moise NS and Hogan DF. Utility of plasma N-terminal pro-brain natriuretic peptide (NT-proBNP) to distinguish between congestive heart failure and non-cardiac causes of acute dyspnea in cats. *Journal of veterinary cardiology : the official journal of the European Society of Veterinary Cardiology*. 2009;11 Suppl 1:S51-61.
8. Connolly DJ, Soares Magalhaes RJ, Fuentes VL, Boswood A, Cole G, Boag A and Syme HM. Assessment of the diagnostic accuracy of circulating natriuretic peptide concentrations to distinguish between cats with cardiac and non-cardiac causes of respiratory distress. *Journal of veterinary cardiology : the official journal of the European Society of Veterinary Cardiology*. 2009;11 Suppl 1:S41-50.
9. Hassdenteufel E, Henrich E, Hildebrandt N, Stosic A and Schneider M. Assessment of circulating N-terminal pro B-type natriuretic peptide concentration to differentiate between cardiac from noncardiac causes of pleural effusion in cats. *J Vet Emerg Crit Care (San Antonio)*. 2013;23:416-22.
10. Humm K, Hezzell M, Sargent J, Connolly DJ and Boswood A. Differentiating between feline pleural effusions of cardiac and non-cardiac origin using pleural fluid NT-proBNP concentrations. *J Small Anim Pract*. 2013;54:656-61.
11. Janda S and Swiston J. Diagnostic accuracy of pleural fluid NT-pro-BNP for pleural effusions of cardiac origin: a systematic review and meta-analysis. *BMC pulmonary medicine*. 2010;10:58.
12. Cortes R, Rivera M, Salvador A, Bertomeu V, de Burgos FG, Rosello-Lleti E, Portoles M, Paya R, Martinez-Dolz L and Climent V. Variability of NT-proBNP plasma and urine levels in patients with stable heart failure: a 2-year follow-up study. *Heart*. 2007;93:957-62.
13. Herndon WE, Kittleson MD, Sanderson K, Drobatz KJ, Clifford CA, Gelzer A, Summerfield NJ, Linde A and Sleeper MM. Cardiac troponin I in feline hypertrophic cardiomyopathy. *J Vet Intern Med*. 2002;16:558-64.
14. Hsu A, Kittleson MD and Paling A. Investigation into the use of plasma NT-proBNP concentration to screen for feline hypertrophic cardiomyopathy. *Journal of veterinary cardiology : the official journal of the European Society of Veterinary Cardiology*. 2009;11 Suppl 1:S63-70.

15. Wess G, Daisenberger P, Mahling M, Hirschberger J and Hartmann K. Utility of measuring plasma N-terminal pro-brain natriuretic peptide in detecting hypertrophic cardiomyopathy and differentiating grades of severity in cats. *Vet Clin Pathol*. 2011;40:237-44.
16. Fox PR, Rush JE, Reynolds CA, Defrancesco TC, Keene BW, Atkins CE, Gordon SG, Schober KE, Bonagura JD, Stepien RL, Kellihan HB, Macdonald KA, Lehmkuhl LB, Nguyenba TP, Sydney Moise N, Lefbom BK, Hogan DF and Oyama MA. Multicenter evaluation of plasma N-terminal probrain natriuretic peptide (NT-pro BNP) as a biochemical screening test for asymptomatic (occult) cardiomyopathy in cats. *J Vet Intern Med*. 2011;25:1010-6.
17. MacLean HN, Abbott JA, Ward DL, Huckle WR, Sisson DD and Pyle RL. N-terminal atrial natriuretic peptide immunoreactivity in plasma of cats with hypertrophic cardiomyopathy. *J Vet Intern Med*. 2006;20:284-9.
18. Hori Y, Yamano S, Iwanaga K, Kano T, Tanabe M, Uechi M, Kanai K, Nakao R, Hoshi F and Higuchi S. Evaluation of plasma C-terminal atrial natriuretic peptide in healthy cats and cats with heart disease. *J Vet Intern Med*. 2008;22:135-9.
19. Connolly DJ, Magalhaes RJ, Syme HM, Boswood A, Fuentes VL, Chu L and Metcalf M. Circulating natriuretic peptides in cats with heart disease. *J Vet Intern Med*. 2008;22:96-105.
20. Zimmering TM, Meneses F, Nolte IJ and Simon D. Measurement of N-terminal proatrial natriuretic peptide in plasma of cats with and without cardiomyopathy. *Am J Vet Res*. 2009;70:216-22.
21. Machen MC, Oyama MA, Gordon SG, Rush JE, Achen SE, Stepien RL, Fox PR, Saunders AB, Cunningham SM, Lee PM and Kellihan HB. Multi-centered investigation of a point-of-care NT-proBNP ELISA assay to detect moderate to severe occult (pre-clinical) feline heart disease in cats referred for cardiac evaluation. *Journal of Veterinary Cardiology*. 2014;http://dx.doi.org/10.1016/j.jvc2014.09.002.
22. Langhorn R, Willesen JL, Tarnow I and Kjelgaard-Hansen M. Evaluation of a high-sensitivity assay for measurement of canine and feline serum cardiac troponin I. *Vet Clin Pathol*. 2013;42:490-8.
23. Tang WH, Francis GS, Morrow DA, Newby LK, Cannon CP, Jesse RL, Storrow AB, Christenson RH, Apple FS, Ravkilde J and Wu AH. National Academy of Clinical Biochemistry Laboratory Medicine practice guidelines: Clinical utilization of cardiac biomarker testing in heart failure. *Circulation*. 2007;116:e99-109.
24. Connolly DJ, Guitian J, Boswood A and Neiger R. Serum troponin I levels in hyperthyroid cats before and after treatment with radioactive iodine. *J Feline Med Surg*. 2005;7:289-300.
25. Menaut P, Connolly DJ, Volk A, Pace C, Luis Fuentes V, Elliott J and Syme H. Circulating natriuretic peptide concentrations in hyperthyroid cats. *J Small Anim Pract*. 2012;53:673-8.
26. Sangster JK, Panciera DL, Abbott JA, Zimmerman KC and Lantis AC. Cardiac biomarkers in hyperthyroid cats. *J Vet Intern Med*. 2014;28:465-72.
27. Porciello F, Rishniw M, Herndon WE, Birettoni F, Antognoni MT and Simpson KW. Cardiac troponin I is elevated in dogs and cats with azotaemia renal failure and in dogs with non-cardiac systemic disease. *Australian veterinary journal*. 2008;86:390-4.
28. Lalor SM, Connolly DJ, Elliott J and Syme HM. Plasma concentrations of natriuretic peptides in normal cats and normotensive and hypertensive cats with chronic kidney disease. *Journal of veterinary cardiology : the official journal of the European Society of Veterinary Cardiology*. 2009;11 Suppl 1:S71-9.
29. Lalor SM, Gunn-Moore DA, Cash R, Foot A, Reed N and Mellanby RJ. Serum Cardiac Troponin I concentrations in cats with anaemia - a preliminary, single-centre observational study. *J Small Anim Pract*. 2014;55:320-2.
30. Wagner T, Fuentes VL, Payne JR, McDermott N and Brodbelt D. Comparison of auscultatory and echocardiographic findings in healthy adult cats. *Journal of veterinary cardiology : the official journal of the European Society of Veterinary Cardiology*. 2010;12:171-82.
31. Fernandes CJ, Jr., Akamine N and Knobel E. Cardiac troponin: a new serum marker of myocardial injury in sepsis. *Intensive care medicine*. 1999;25:1165-8.

32. Langhorn R, Oyama M, King L, Machen M, Trafny D, Thawley V, Willesen JL, Tarnow I and Kjelgaard-Hansen M. Canine Cardiac Troponin I Significantly Complements Established Prognostic Composite Score in Dogs with Systemic Inflammation. *Journal of Veterinary Internal Medicine*. 2012;26:713-713.
33. Langhorn R, Oyama MA, King LG, Machen MC, Trafny DJ, Thawley V, Willesen JL, Tarnow I and Kjelgaard-Hansen M. Prognostic importance of myocardial injury in critically ill dogs with systemic inflammation. *J Vet Intern Med*. 2013;27:895-903.
34. Moreno V, Hernandez-Romero D, Vilchez JA, Garcia-Honrubia A, Cambronero F, Casas T, Gonzalez J, Martinez P, Climent V, de la Morena G, Valdes M and Marin F. Serum levels of high-sensitivity troponin T: a novel marker for cardiac remodeling in hypertrophic cardiomyopathy. *Journal of cardiac failure*. 2010;16:950-6.
35. Kubo T, Kitaoka H, Okawa M, Yamanaka S, Hirota T, Baba Y, Hayato K, Yamasaki N, Matsumura Y, Yasuda N, Sugiura T and Doi YL. Combined measurements of cardiac troponin I and brain natriuretic peptide are useful for predicting adverse outcomes in hypertrophic cardiomyopathy. *Circulation journal : official journal of the Japanese Circulation Society*. 2011;75:919-26.
36. Kubo T, Kitaoka H, Yamanaka S, Hirota T, Baba Y, Hayashi K, Iiyama T, Kumagai N, Tanioka K, Yamasaki N, Matsumura Y, Furuno T, Sugiura T and Doi YL. Significance of high-sensitivity cardiac troponin T in hypertrophic cardiomyopathy. *J Am Coll Cardiol*. 2013;62:1252-9.
37. Geske JB, McKie PM, Ommen SR and Sorajja P. B-type natriuretic peptide and survival in hypertrophic cardiomyopathy. *J Am Coll Cardiol*. 2013;61:2456-60.
38. Coats CJ, Gallagher MJ, Foley M, O'Mahony C, Critoph C, Gimeno J, Dawnay A, McKenna WJ and Elliott PM. Relation between serum N-terminal pro-brain natriuretic peptide and prognosis in patients with hypertrophic cardiomyopathy. *Eur Heart J*. 2013;34:2529-37.
39. Zimmering TM, Hungerbuhler S, Meneses F, Nolte I and Simon D. Evaluation of the association between plasma concentration of N-terminal proatrial natriuretic peptide and outcome in cats with cardiomyopathy. *J Am Vet Med Assoc*. 2010;237:665-72.
40. Langhorn R, Tarnow I, Willesen JL, Kjelgaard-Hansen M, Skovgaard IM and Koch J. Cardiac Troponin I and T as Prognostic Markers in Cats with Hypertrophic Cardiomyopathy. *J Vet Intern Med*. 2014.
41. Borgeat K, Sherwood K, Payne JR, Luis Fuentes V and Connolly DJ. Plasma cardiac troponin I concentration and cardiac death in cats with hypertrophic cardiomyopathy. *Journal of Veterinary Internal Medicine*. 2014;in press.
42. 2015 ACVIM Forum Research Abstract Program. *Journal of Veterinary Internal Medicine*. 2015;29:1122-1256.