Biomarker changes with systolic anterior motion of the mitral valve in cats with hypertrophic cardiomyopathy

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Abstract
Background: N-terminal pro B-type natriuretic peptide (NT-proBNP) and cardiac troponin-I (cTnI) are biomarkers commonly evaluated in cats with suspected heart disease. Many cats with hypertrophic cardiomyopathy (HCM) have systolic anterior motion of the mitral valve (SAM), but its influence on circulating NT-proBNP or cTnI concentrations is currently unknown.

Hypothesis/Objectives: Cats with HCM and SAM (HCMSAM+) have higher NT-proBNP and cTnI concentrations than do cats with HCM but without SAM (HCMSAM−).

Animals: One hundred forty cats with HCM: 70 with SAM and 70 without SAM.

Methods: Retrospective case-to-case study. Cats were recruited if diagnosed with HCM by echocardiography and results were available for NT-proBNP or cTnI concentrations or both. Cats with SAM were matched to those without SAM for clinical presentation, left atrial (LA) size and left ventricular (LV) fractional shortening.

Results: A total of 119 NT-proBNP and 123 cTnI results were available. The HCMSAM+ cats had higher median concentrations than did HCMSAM− cats for NT-proBNP (729 pmoL/L; interquartile range [IQR], 275-1467 versus 65 pmoL/L; IQR, 25-271; P < .001) and cTnI (0.27 ng/mL; IQR, 0.10-0.81 versus 0.07 ng/mL; IQR, 0.01-0.43; P = .002). In general linear models for both NT-proBNP and cTnI, the independent explanatory variables were SAM, congestive heart failure, maximal LV wall thickness, and LA size.

Conclusions and Clinical Importance: For cats with HCM and equivalent LA size and LV systolic function, those with SAM had higher NT-proBNP and cTnI concentrations than did those without SAM. Presence of SAM should be considered when interpreting biomarker concentrations in cats with HCM.

Abbreviations: ATE, aortic thromboembolism; CHF, congestive heart failure; cTnI, cardiac troponin-I; DLVOTO, dynamic left ventricular outflow tract obstruction; FS%, left ventricular fractional shortening percentage; HCM, hypertrophic cardiomyopathy; HCMSAM+, cats with hypertrophic cardiomyopathy and systolic anterior motion of the mitral valve; HCMSAM−, cats with hypertrophic cardiomyopathy but without systolic anterior motion of the mitral valve; LA : Ao, left atrium-to-aortic ratio; LAD Max, maximal left atrial diameter; LVIDd, left ventricular internal diameter in end-diastole; LVIDs, left ventricular internal diameter in end-systole; LVOT Vmax, maximal left ventricular outflow tract velocity; LVWT Max, maximal end-diastolic left ventricular wall thickness; NT-proBNP, N-terminal pro B-type natriuretic hormone; POC, point-of-care; QMHA, Queen Mother Hospital for Animals; SABP, systolic arterial blood pressure; SAM, systolic anterior motion of the mitral valve.

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Cardiac biomarkers increasingly have been used in cats with heart disease. N-terminal pro B-type natriuretic hormone (NT-proBNP) is a biomarker of myocardial stress and stretch. Its application as a cardiac biomarker includes screening for preclinical cardiomyopathy, assessing disease severity, and differentiating cardiogenic and noncardiogenic causes in cats with dyspnea. It has been proposed as being particularly useful for general practitioners without access to echocardiography. Cardiac troponin-I (cTnI) is a biomarker of myocardial injury. Its use as a cardiac biomarker includes assessing cardiomyopathy severity, differentiating cardiogenic and noncardiogenic causes in cats with dyspnea, and providing additional prognostic information independent of left atrial size and function, and left ventricular systolic function.

Hypertrophic cardiomyopathy (HCM) is a disease with considerable morphological and functional variation. For example, there are differences in segmental versus global concentric hypertrophy, presence or absence of systolic anterior motion of the mitral valve (SAM) and mid-ventricular obstruction, and papillary muscle morphology varies. Of these, SAM is encountered frequently, with a reported prevalence of approximately 30% in cats with HCM phenotype. Systolic anterior motion of the mitral valve is associated with more severe left ventricular concentric hypertrophy in cats, but whether SAM is an effect or a cause of the hypertrophy is unknown. Cardiac magnetic resonance imaging studies suggest that abnormalities of both left ventricular geometry and the mitral valve apparatus likely contribute to the origin of SAM in people. Similar abnormalities likely occur in affected cats.

Although the primary stimulus for release of NT-proBNP is myocardial stretch, other conditions that result in increased wall stress or ventricular hypertrophy have been found to increase plasma concentrations of NT-proBNP. Dynamic left ventricular outflow tract obstruction (DLVOTO) would be expected to increase myocardial wall stress, and therefore increase NT-proBNP. Furthermore, DLVOTO has been associated with an increased risk of myocardial ischemia in people with HCM, which would be expected to result in increased cTnI concentrations. Many studies have shown that NT-proBNP and cTnI concentrations are increased in cats with HCM phenotype, but whether or not the presence of SAM further increases these biomarker concentrations currently is unknown.

The first aim of our study was to compare circulating concentrations of NT-proBNP and cTnI in cats with HCM phenotype and SAM as compared to cats with HCM phenotype but without SAM. The second aim was to create multivariable models to identify factors associated with variation in NT-proBNP and cTnI concentrations. We hypothesized that cats with SAM would have higher NT-proBNP and cTnI concentrations than cats at a similar stage of HCM without SAM, and that this relationship would be independent of other factors when assessed in a multivariable model.

This study is a retrospective case-to-case study design. Ethical approval was provided by the Royal Veterinary College (URN: SR2019-0245). Electronic medical records between December 2011 and May 2018 from the Queen Mother Hospital for Small Animals (QMHA) and a cardiac screening study at rehoming centers for cats (the CatScan study) were reviewed for cats diagnosed with HCM and with NT-proBNP, cTnI, or both measured. The diagnosis of HCM was defined as left ventricular hypertrophy at any region measuring ≥6 mm on 2-dimensional (2D) imaging.

For each case, medical records corresponding with the time of biomarker measurement were reviewed. The signalment, medical history, physical examination findings, systemic arterial blood pressure (SABP) measurements using Doppler technique, presence of congestive heart failure (CHF), arrhythmias or aortic thromboembolism (ATE), and current medications were recorded. Where the intensity of a murmur varied, the highest murmur grade was recorded. Cats were excluded if they were diagnosed with dehydration, hypothyroidism, SABP ≥180 mm Hg, diabetes mellitus, cardiomyopathies with phenotype other than HCM, cardiac neoplasia, congenital heart disease, anemia (packed cell volume <20%), or had incomplete clinical records. Cats with serum creatinine concentrations ≥2.9 mg/dL (≥251 μmol/L) or ≥1.6 mg/dL (≥140 μmol/L) without SABP measurement also were excluded.

Echocardiograms matching the dates of the biomarker measurement were reviewed by a single observer (J.S.). All echocardiographic measurements were taken over 3 different cardiac cycles and averaged. After confirming the diagnosis, the presence of SAM was assessed with frame-by-frame review of the 2D imaging of a right parasternal 5-chamber view, optimized for the left ventricular outflow tract (LVOT). Systolic anterior motion of the mitral valve was defined as anterior motion of either septal or both mitral valve leaflets during systole toward the LVOT on review of the 2D cineloop. Other echocardiographic variables were measured by the same investigator (J.S.) as described in Table 1. These included: left atrium to aortic ratio (LA : Ao), maximal left atrial diameter (LAD Max), maximal left ventricular wall thickness (LVWT Max), left ventricular internal diameter in end-diastole (LVIDd) and end-systole(LVIDs), left ventricular fractional shortening (FS%), and maximal LVOT velocity (LVOT Vmax).
Dynamic left ventricular outflow tract obstruction was defined as LVOT Vmax ≥ 2.5 m/s in the absence of echocardiographic evidence of fixed aortic stenosis.\textsuperscript{15,16} Regional wall hypokinesis was defined as asynchronous motion or minimal excursion of a myocardial segment compared with the adjacent segment.\textsuperscript{12,36}

### 3.2 | Matching process

The case-to-case groups (HCM\textsuperscript{SAM+} and HCM\textsuperscript{SAM−}) were created starting with all cats without SAM (HCM\textsuperscript{SAM−}), and calculating the proportion of cats with CHF, ATE and arrhythmias, and the mean or median of the echocardiographic variables (LAD Max, LA : Ao, LVIDd, LVIDs, and FS%). Cats with SAM were introduced 1 cat at a time to a HCM\textsuperscript{SAM+} group, to create a group with characteristics matching the HCM\textsuperscript{SAM−} group. As described in the introduction, LVWT Max was expected to be higher in the HCM\textsuperscript{SAM+} group, and no attempt was made to match this variable between the groups.\textsuperscript{15}

### 3.3 | NT-proBNP and cTnI measurements

An EDTA blood sample was collected for a second-generation NT-proBNP assay. The NT-proBNP samples were processed by

| TABLE 1  | Echocardiographic variables of interest |
|-----------|----------------------------------------|
| Echocardiographic variable | View and timing | Measurement |
| LA : Ao\textsuperscript{32} | View: RPSAX at the level of the aortic valve Imaging modality: 2D Imaging Timing: Beginning of diastole, the first frame of aortic valve closure | Ao: From the blood-tissue interface at the midpoint of the right aortic sinus to the commissure between the noncoronary and left coronary aortic cusps LA: Extension of the aortic line to the blood-tissue interface of the left atrial wall, immediately lateral to the pulmonary vein |
| LAD Max\textsuperscript{33,34} | View: RPLAX4ch Imaging modality: 2D Imaging Timing: Beginning of diastole, the last frame before the mitral valve opening | Mid-interatrial septum to the leading edge of the pericardium, bisecting the left atrium parallel to the mitral valve annulus |
| LVWT Max\textsuperscript{34} | View: RPLAX4ch, RPLAX5ch, and RPSAX at the level of the papillary muscles Imaging modality: 2D Imaging Timing: End-diastole, the last frame before the aortic valve opens (RPLAX5ch), the first frame after the mitral valve closes (RPLAX4ch), or when the left ventricular internal diameter was the largest (RPSAX) | Leading edge technique avoiding the papillary muscles or false tendon attachments Only the average of 3 measurements from the area that measures the maximal thickness is used |
| LVIDd\textsuperscript{35} | View: RPSAX at the level of the papillary muscles Imaging modality: 2D Imaging Timing: End-diastole | Leading edge technique bisecting the left ventricle in end-diastole |
| LVIDs\textsuperscript{35} | View: RPSAX at the level of the papillary muscles Imaging modality: 2D Imaging Timing: End-systole | Leading edge technique bisecting the left ventricle in end-systole |
| FS%\textsuperscript{35} | – | Calculated using (LVIDd–LVIDs)/LVIDd |
| LVOT Vmax\textsuperscript{16} | View: LASch with the cursor transecting the left ventricular outflow tract. Imaging modality: Spectral Doppler (either pulse wave or continuous wave) imaging Timing: Systole, when the modal velocity was the greatest | Maximal modal velocity in systole was recorded. The measurement was not recorded when there was inadequate cursor alignment, absence of a dynamic profile, or contamination of the mitral regurgitation |
| DLVOTO\textsuperscript{15,16} | – | Defined as LVOT Vmax ≥ 2.5 m/s |
| Regional wall hypokinesis\textsuperscript{12,36} | View: Overview of the RPLAX4ch, RPLAX5ch, RPSAX, LA4ch, L5ch Imaging modality: 2D Imaging Timing: Across the entire cardiac cycle | Subjective recognition of a segment with asynchronous motion to the adjacent segment or minimal in excursion on 2D imaging |

Note: All echocardiographic measurements in this study were taken over 3 different cardiac cycles and averaged. Abbreviations: DLVOTO, dynamic left ventricular outflow tract obstruction; FS%, left ventricular fractional shortening; LA:Ao, left atrium to aortic ratio; LA4ch, left apical 4-chamber view; L5ch, and 5-chamber view; LAD Max, maximal left atrial diameter; LVIDd, left ventricular internal diameter in diastole, LVIDs, and systole; LVOT Vmax, maximal left ventricular outflow tract velocity; LVWT Max, maximal end-diastolic left ventricular wall thickness; RPLAX4ch, right parasternal long axis 4-chamber view, RPLAX5ch, and 5-chamber view; RPSAX, right parasternal short axis view.
2 different methods, both after the instructions of the reference laboratory (IDEXX Veterinary Laboratories, Wetherby, Workshire, United Kingdom): For the samples taken at the QMHA, the samples were centrifuged at 800g for 3 minutes and plasma was transferred to a separate plain tube and refrigerated at 4°C. The samples then were transported within 24 hours to the reference laboratory where the assays were performed. For samples collected at the rehoming centers, samples were centrifuged at 4000 rpm for 15 minutes. Plasma was separated and immediately frozen at ~20°C. Batches of frozen samples were transferred on dry ice to the reference laboratory and underwent batch analysis.

Cardiac troponin-I was analyzed using 2 different analyzers: For all samples from the rehoming centers and for some from the QMHA, a blood sample was collected in a plain tube and processed in an identical manner as samples for the NT-proBNP assay. These samples then were analyzed at the reference laboratory. Some cats from the QMHA had a point-of-care (POC) high sensitivity cTnI measurement performed with a handheld analyzer (VetScan i-STAT Analyzer, Abaxis, Abbott Point of Care Inc, Union City, California). Either whole blood in plain tubes or heparinized whole blood samples were used immediately or within 10 minutes after collection, respectively.

The lower and upper limits of detection for NT-proBNP were 24 and 1500 pmol/L, respectively. Results reported as <24 or >1500 pmol/L were entered as 23 or 1501 pmol/L, respectively, for statistical analysis. The high sensitivity cTnI assay from the reference laboratory had a lower limit of detection of 0.01 ng/mL and no upper limit. The POC analyzer had the same lower limit of detection but an upper limit of 50 ng/mL. Results reported as <0.01 ng/mL were entered as 0.009 ng/mL. The POC results >50 ng/mL were entered as 51 ng/mL.

4 | STATISTICAL ANALYSIS

Commercial software programs were used for statistical analysis (IBM SPSS Statistics Version 24) and generating graphs (GraphPad Prism 7 Version 7.0d). Normality of the continuous variables was tested visually and by a Shapiro-Wilk test. Normally distributed data are presented as mean (±SD) and non-normally distributed data as median (interquartile range, IQR). Continuous variables were compared using either an independent t test or Mann-Whitney test. Categorical demographic variables were compared by a Pearson chi-squared test, with posthoc analysis done by analyzing the standardized residuals. Significance was set at P < .05.

The association between the dependent variables (NT-proBNP and cTnI) and others was tested first by univariable analysis, by either Pearson's or Spearman's test for continuous variables, and either an independent t test or Mann-Whitney test for categorical variables. Significantly associated variables with P < .25 then were selected for multivariable analysis.

Multivariable analysis was performed by constructing a separate general linear model for NT-proBNP and cTnI. Both models were created in a backward stepwise manner, starting with all significant variables from the univariable analysis (P < .25) and then removing non-significant variables 1 at a time until only significant variables remained (P < .05). Variables were log-transformed if the distribution was severely skewed, and the residuals of each model were visually inspected by quantile plots, ensuring the assumption of normal distribution of linear models. Collinearity statistics were checked and multicollinearity was eliminated.

5 | RESULTS

A total of 210 cats met the initial inclusion criteria. Of these, 70 cats subsequently were excluded because of anemia (1), limited echocardiographic study (8), lymphoma (1), incomplete medical records (23), hyperthyroidism (3), diabetes mellitus (2), serum creatinine concentration ≥2.9 mg/dL (8), serum creatinine concentration ≥1.6 mg/dL with unavailable blood pressure measurement (4), and any additional cats after matching the 2 groups (20). This process resulted in a final number of 140 enrolled cats: 40 cats from rehoming centers and 100 cats from the QMHA. In total, 119 measurements were available for NT-proBNP, and 123 measurements for cTnI. These cats were divided into HCMSAM+ (70 cats) and HCMSAM− groups (70 cats; Table 2).

No significant differences in breed, sex, medications, or the proportion of cats with CHF, ATE, or arrhythmias were found between the groups. Cats in the HCMSAM+ group were significantly younger (P < .001) with lower body weight (P < .001) than cats in the HCMSAM− group. A significant difference in presenting complaints was found between the 2 groups, with most HCMSAM+ cats being presented for a heart murmur (P < .001) and most HCMSAM− cats being presented for HCM screening (P < .001). Additional demographic information is outlined in Table 2.

Based on the study design, no differences were found in LAD Max, LA : Ao, LVIDd, LVIDs, and F5%. The LVWT Max was higher in the HCMSAM− cats (P < .001). Forty-nine of 65 HCMSAM+ cats (68.4%) had DLVOTO at the time of examination, with a mean LVOT Vmax of 3.3 ± 1.2 m/s. Additional echocardiographic data are presented in Table 3.

Plasma NT-proBNP concentrations were significantly higher in the HCMSAM+ group (729 pmol/L [IQR, 275-1467] versus 65 pmol/L [IQR, 25-271]) in the HCMSAM− group; P < .001; Figure 1). Circulating concentrations of cTnI also were significantly higher in the HCMSAM+ group (0.27 ng/mL [IQR, 0.10-0.81] versus 0.07 ng/mL [IQR, 0.01-0.43]) in the HCMSAM− group; P = .002). This increase was observed in both the non-DLVOTO and DLVOTO populations of the SAM group (P = .3 for NT-proBNP and P = .6 for cTnI comparing non-DLVOTO and DLVOTO subgroups).

Results of the univariable analysis are summarized in Table S1. Using these results, the following variables were tested in a general linear model to explain the variation of NT-proBNP: source population (QMHA versus rehoming centers), presenting complaint, age, body weight, body condition score, breed, SABP, murmur grade, gallop sound, sedation, medication, urea, creatinine, CHF, ATE, arrhythmias, SAM, DLVOTO, LVOT Vmax, LVIDs, F5%, LVWT Max, LAD Max, regional wall hypokinesis, and cTnI. Similarly, the following variables...
### TABLE 2  
Demographic characteristics for the 140 cats with hypertrophic cardiomyopathy

|                          | HCM<sup>SAM+</sup> (n = 70) | HCM<sup>SAM−</sup> (n = 70) | P value |
|--------------------------|----------------------------|----------------------------|---------|
| **Population**           |                            |                            |         |
| CatScan                  | 9 (12.9%)                  | 31 (44.3%)                 | <.001   |
| QMHA                     | 61 (87.1%)                 | 39 (55.7%)                 |         |
| **Age (years)**          | 4.1 [2.4-7.0]              | 6.9 [4.0-10.6]             | <.001   |
| **Weight (kg)**          | 4.4 (±1.0)                 | 5.2 (±1.2)                 | <.001   |
| **BCS (/9)**             | 5.0 [4.0-5.0]              | 5.0 [5.0-6.4]              | .332    |
| **Presenting complaint (%)** |                        |                            | <.001   |
| Arrhythmias              | 0                          | 1 (1.4%)                   |         |
| Collapsing episodes      | 3 (4.3%)                   | 2 (2.9%)                   |         |
| Gait abnormality         | 4 (5.7%)                   | 5 (7.1%)                   |         |
| Murmur                   | 32 (45.7%)                 | 8 (11.4%)                  |         |
| Respiratory signs        | 7 (10%)                    | 9 (12.9%)                  |         |
| HCM screening            | 13 (18.6%)                 | 39 (55.7%)                 |         |
| HCM reassessment         | 11 (15.7%)                 | 6 (8.6%)                   |         |
| **Murmur (%)**           | 66/68 (94.3%)              | 42/69 (60.9%)              | <.001   |
| **Murmur grade**         | 3 [3-4]                    | 2 [0-3]                    |         |
| Gallop sound (%)         | 3/26 (11.5%)               | 8/57 (11.4%)               | 1.000   |
| **Arrhythmias<sup>a</sup>** | 5 (7.1%)                  | 8 (11.4%)                  | .562<sup>a</sup> |
| **Congestive heart failure<sup>a</sup>** | 8 (11.4%) | 13 (18.6%) | .344<sup>a</sup> |
| **Aortic thromboembolism<sup>a</sup>** | 3 (4.3%) | 3 (4.3%) | 1.000<sup>a</sup> |
| **Sex**                  |                            |                            | .590    |
| Female (%)               | 25 (35.7%)                 | 21 (30.0%)                 |         |
| Neutered : Entire        | 22:3                       | 18:3                       |         |
| Male (%)                 | 45 (64.3%)                 | 49 (70.0%)                 |         |
| Neutered : Entire        | 42:3                       | 42:7                       |         |
| **Number of pedigree cats (%)** | 15 (21.4%)              | 20 (28.6%)                 | .255    |
| Bengal                   | 2 (2.9%)                   | 2 (2.9%)                   |         |
| British Short Hair       | 3 (4.3%)                   | 2 (2.9%)                   |         |
| Domestic Long Hair       | 5 (7.1%)                   | 6 (8.6%)                   |         |
| Domestic Medium Hair     | 2 (2.9%)                   | 1 (1.4%)                   |         |
| Domestic Short Hair      | 48 (68.6%)                 | 43 (61.4%)                 |         |
| European Short Hair      | 3 (4.3%)                   | 0                          |         |
| Maine Coon               | 0                          | 1 (1.4%)                   |         |
| Norwegian Forest Cat     | 1 (1.4%)                   | 8 (11.4%)                  |         |
| Persian                  | 2 (2.9%)                   | 1 (1.4%)                   |         |
| Russian Blue/White       | 1 (1.4%)                   | 2 (2.9%)                   |         |
| Scottish Fold            | 1 (1.4%)                   | 1 (1.4%)                   |         |
| Siamese                  | 0                          | 2 (2.9%)                   |         |
| Sphynx                   | 2 (2.9%)                   | 1 (1.4%)                   |         |
| Doppler blood pressure   | 124.1 (±18.8) (n = 41)     | 129.7 (±21.6) (n = 39)     | .224    |
| **Renal markers**        |                            |                            |         |
| Urea (mg/dL)             | 27.2 [21.8-39.2] (n = 14)  | 28.3 [25.8-35.6] (n = 35)  | .603    |
| Creatinine (mg/dL)       | 1.52 (±0.28) (n = 16)      | 1.58 (±0.35) (n = 35)      | .618    |
| **Medications (number of cats)** | 6 (8.6%)                 | 4 (5.7%)                   | .512    |
| Aspirin                  | 0                          | 1 (1.4%)                   |         |
| Atenolol                 | 1 (1.4%)                   | 0                          |         |

(Continues)
TABLE 2  (Continued)

|                  | HCM^{SAM+} (n = 70) | HCM^{SAM−} (n = 70) | P value |
|------------------|----------------------|----------------------|---------|
| Benazepril       | 2 (2.9%)             | 2 (2.9%)             |         |
| Clopidogrel      | 3 (4.3%)             | 1 (1.4%)             |         |
| Diltiazem        | 1 (1.4%)             | 0                    |         |
| Furosemide       | 4 (5.7%)             | 4 (5.7%)             |         |
| Pimobendan       | 0                    | 1 (1.4%)             |         |

Note: The cats were divided in 2 groups: Hypertrophic cardiomyopathy with systolic anterior motion of the mitral valve (HCM^{SAM+}) and hypertrophic cardiomyopathy without systolic anterior motion of the mitral valve (HCM^{SAM−}).

*a Actively matched variables.

TABLE 3  Echocardiographic variables and cardiac biomarker results for the 140 cats with hypertrophic cardiomyopathy

|                  | HCM^{SAM+} (n = 70) | HCM^{SAM−} (n = 70) | P value |
|------------------|----------------------|----------------------|---------|
| SAM              | 70 (100%)            | 0                    |         |
| Sedation         | 1 (1.4%)             | 2 (2.9%)             | 1.000   |
| LVOT Vmax (m/s)  | 3.3 (±1.2) (n = 65)  | 1.1 (±0.5) (n = 29)  | <.001   |
| DLVOTO           | 49/65 (68.4%)        | 0/50 (0%)            | <.001   |
| LAD Max (mm)*    | 16.4 [14.5-17.9]     | 15.4 [13.7-17.4]     | .163*   |
| LA : Ao*         | 1.3 [1.2-1.5]        | 1.3 [1.2-1.5]        | .762*   |
| LVIDd (mm)*      | 13.9 [12.5-15.4]     | 14.2 [12.5-16.1]     | .216*   |
| LVIDs (mm)*      | 5.0 [3.9-5.9]        | 5.6 [3.8-7.2]        | .115*   |
| FS%*             | 62.4 [57.6-71.4]     | 61.9 [50.2-69.9]     | .253*   |
| Regional wall hypokinesis | 5 (7.1%) | 6 (8.6%) | 1.000   |
| LVWT Max (mm)    | 7.2 [6.6-8.2]        | 6.5 [6.1-6.8]        | <.001   |
| NT-proBNP (pmoL/L) | 729 [275-1467] (n = 61) | 65 [25-271] (n = 58) | <.001 |
| cTnl (ng/mL)     | 0.27 [0.10-0.81] (n = 59) | 0.07 [0.01-0.43] (n = 64) | .002   |
| POC cTnl         | 7/59 (11.9%)         | 7/64 (10.9%)         | 1.000   |

Abbreviations: DLVOTO, dynamic left ventricular outflow tract obstruction; FS%, fractional shortening %; LA : Ao, left atrium-to-aortic ratio; LAD Max, maximal left atrial diameter; LVIDd, LVIDs, left ventricular internal diameter in diastole and systole; LVOT Vmax, maximal left ventricular outflow tract velocity; LVWT Max, maximal left ventricular wall thickness; SAM, systolic anterior motion of the mitral valve.

*a Actively matched variables.

FIGURE 1  Box and whisker plots graphs comparing plasma concentrations of A, NT-proBNP and B, cTnl in cats with hypertrophic cardiomyopathy with and without systolic anterior motion of the mitral valve (groups HCM^{SAM+} and HCM^{SAM−}, respectively). cTnl, cardiac troponin-I; HCM^{SAM+}/HCM^{SAM−}, cats with hypertrophic cardiomyopathy and with/without systolic anterior motion of the mitral valve; NT-proBNP, N-terminal pro B-type natriuretic peptide.
NT-proBNP concentrations in the cats with SAM corresponded with a more advanced disease. We were not able to determine whether the higher concentrations sometimes are measured as a means of screening for more aggressive disease. In situations where echocardiography is unavailable, NT-proBNP concentrations are a potential alternative for both diagnostic and screening purposes.

We compared cardiac biomarker concentrations in cats with HCM and SAM to those without SAM that were otherwise similar for sex, breed, left atrial and left ventricular size, clinical signs, blood pressure, and renal markers. Concentrations of circulating NT-proBNP and cTnI were log-transformed for further analysis. 2

Results of multivariable analysis are presented in Table 4. For both models, LAD Max, CHF, and LVWT Max were the final independent variables with an adjusted $R^2$ of .52 for NT-proBNP and .49 for cTnI. The beta coefficient for SAM was higher than for LVWT Max in both models. Other variables including age, source population (QMHA versus rehoming centers), POC cTnI, creatinine, urea, SABP, and presence of regional wall hypokinesis were not significant in either model. Visual inspection of the residuals was consistent with good model fit.

| 1. log(NT-proBNP) | $B$ coefficient (95% CI) | Standardized $B$ coefficient | $P$ value |
|-------------------|-------------------------|-----------------------------|-----------|
| CHF               | 0.496 (0.209-0.783)     | 0.229                       | .001      |
| SAM               | 0.583 (0.398-0.767)     | 0.448                       | <.001     |
| LAD Max           | 0.050 (0.025-0.076)     | 0.268                       | <.001     |
| LVWT Max          | 0.137 (0.038-0.236)     | 0.208                       | .007      |

| 2. log(cTnI) | $B$ coefficient (95% CI) | Standardized $B$ coefficient | $P$ value |
|--------------|-------------------------|-----------------------------|-----------|
| CHF          | 1.060 (0.694-1.425)     | 0.407                       | <.001     |
| SAM          | 0.436 (0.188-0.685)     | 0.237                       | .001      |
| LAD Max      | 0.070 (0.038-0.102)     | 0.309                       | <.001     |
| LVWT Max     | 0.169 (0.050-0.288)     | 0.196                       | .006      |

Abbreviations: CHF, congestive heart failure; cTnI, cardiac troponin-I; LAD Max, maximal left atrial diameter; LVWT Max, maximal left ventricular wall thickness; NT-proBNP, N-terminal pro B-type natriuretic peptide; SAM, systolic anterior motion of the mitral valve.

Two multivariable models with NT-proBNP and cTnI as dependent variables after log-transformation.
Systolic anterior motion of the mitral valve usually is associated with DLVOTO, which can result in increased left ventricular wall stress and ischemia. However, although the presence of SAM was a statistically significant explanatory variable for both NT-proBNP and cTnl in our study, neither LVOT Vmax nor the presence of DLVOTO was significant in our general linear models. We defined DLVOTO as LVOT velocities >2.5 m/s, although to confirm the site of dynamic obstruction, color Doppler imaging is necessary to show variance and aliasing in the LVOT and an eccentric jet of mitral regurgitation. Dynamic LVOT obstruction is known to be labile, and is affected by loading conditions and sympathetic tone. Provocative maneuvers often are used to provoke a gradient across the LVOT in people with HCM, and auditory stimulation sometimes is used in cats with HCM for this purpose. It is difficult to standardize echocardiographic conditions in cats, and the echocardiographic examination itself could be considered a provocative maneuver, with variable effects in different cats. The magnitude of LVOT gradient that should be considered clinically relevant in cats with HCM has not been established, nor whether or not this conclusion should be based on a resting or provoked gradient. It is not clear why SAM has a closer association with NT-proBNP and cTnl concentrations than with presence of DLVOTO or LVOT Vmax. It might reflect greater ease of recording SAM compared with DLVOTO or LVOT Vmax, or that SAM is more consistently present in cats with intermittent LVOT obstruction.

Our study had a number of limitations, many of which were a consequence of its retrospective nature. First, the NT-proBNP samples were processed by 2 different methods according to the instructions provided by the reference laboratory. However, no significant effect was seen when using the general linear model with the variable “population source.” Similarly, cTnl concentrations were measured using 2 different analyzers, following the instructions provided by either the reference laboratory or the manufacturer of the POC cTnl analyzer. Although this POC cTnl assay has not been validated in cats, cTnl is well conserved across species and statistical analysis with or without these cats showed no effect of including this unvalidated assay in our results, and no significant effect of the POC cTnl was found in the final models. We elected to include the cats with cTnl analyzed on the POC assay because doing so resulted in better balancing of the 2 groups. The HCMSAM cats were younger and had higher LVWT Max. Systemic hypertension previously has been shown to result in left ventricular hypertrophy in cats. Although strict exclusion criteria were followed, systemic blood pressure recordings were not available in all cats, and some cats with systemic hypertension inadvertently may have been included. However, SAM still had a significant influence on both NT-proBNP and cTnl concentrations independent of wall thickness, and the influence of age was not significant in the final multivariable model. Finally, no attempt was made to evaluate outcome. Doing so was not an aim of our study, and therefore we cannot determine whether increased NT-proBNP and cTnl concentrations in cats with SAM are associated with a worse prognosis regardless of left atrial size.

7 | CONCLUSIONS

Cats with HCM and SAM had higher NT-proBNP and cTnl concentrations than did cats without SAM. The presence of SAM should be taken into consideration when interpreting NT-proBNP and cTnl results in cats with HCM phenotype.

ACKNOWLEDGMENT

The findings of this study were presented at the 2019 ACVIM Forum, Phoenix, AZ.

CONFLICT OF INTEREST DECLARATION

This study was not supported by any grant or other source of direct funding. Drs JR Payne and V Luis Fuentes previously received financial support from IDEXX Ltd for research on NT-proBNP and cTnl.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION
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