

STANDARD ARTICLE

Prognostic factors in dogs with presumed degenerative mitral valve disease attending primary-care veterinary practices in the United Kingdom

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Background: Prognostic risk factors were identified for dogs with degenerative mitral valve disease (DMVD) monitored by veterinary cardiologists. The value of these measurements has not been determined in the wider primary care setting.

Objectives: To evaluate whether plasma cardiac biomarkers and data obtained from routine history-taking and physical examination are predictive of survival in dogs with DMVD attending primary care practice.

Animals: Eight-hundred and ninety-three dogs with a presumptive diagnosis of DMVD recruited from 79 primary care veterinary practices in the United Kingdom.

Methods: Prospective cohort study. Primary care veterinary practitioners recorded clinical data. Plasma N-terminal pro B-type natriuretic peptide (NT-proBNP) and cardiac troponin I (cTnI) were measured at presentation. Cox regression models evaluated associations between risk factor variables and hazard of death (all-cause mortality and cardiac-related death). Flexible parametric models generated predicted survival probabilities for dogs with different combinations of prognostic risk factor variable values.

Results: Dogs with higher NT-proBNP and cTnI concentrations, higher heart rates, older dogs, females, and those reported to be exercise intolerant, dyspneic, and diagnosed with selected comorbidities had an increased hazard of death due to any cause. Dogs with higher concentrations of plasma biomarkers, higher heart rates, and heart murmur intensities, those with exercise intolerance and those receiving potent diuretics had a higher hazard of cardiac-related death.

Conclusions and Clinical Importance: Cardiac biomarkers and key clinical findings identified in this study can help primary care veterinary practitioners identify dogs with DMVD that are at highest risk of death.

KEYWORDS

canine, cardiac biomarker, epidemiology, mortality, natriuretic peptide, risk stratification, survival

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Abbreviations: BPM, beats per minute; CHF, congestive heart failure; CKCS, Cavalier King Charles Spaniel; cTnI, cardiac troponin I; DMVD, degenerative mitral valve disease; EPR, electronic patient record; HR, hazard ratio; IQR, interquartile range; NT-proBNP, N-terminal pro B-type natriuretic peptide; PH, proportional hazards; PO, proportional odds; RAAS, renin-angiotensin-aldosterone system; RPM, respirations per minute; RR, respiratory rate; SD, standard deviation; SNS, sympathetic nervous system; SRR, sleeping respiratory rate; VetCompass, Veterinary Companion Animal Surveillance System.

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1 | INTRODUCTION

Heart murmurs consistent with degenerative mitral valve disease (DMVD) are frequently found in dogs presented to primary care veterinary practices.¹ The clinical progression of DMVD varies considerably among individual dogs, with only a proportion of them experiencing clinical signs or dying as a result of their cardiac disease.^{2,3} Risk stratification based on disease severity could guide clinical decision-making

when managing dogs with DMVD in practice.^{4,5} Furthermore, owners of dogs with cardiac disease can experience high levels of concern and anxiety regarding uncertainty in their pets' likely longevity.⁶ Identifying prognostic factors thus could aid veterinary practitioners when managing dogs with DMVD and help inform dog owners of their pets' likely outcome. In addition, risk stratification based on prognostic information could help identify dogs for inclusion into large-scale clinical trials.⁷

Echocardiographic and radiographic indices of cardiac enlargement and mitral regurgitation severity have prognostic value in dogs with DMVD.^{3-5,8-15} However, the equipment and expertise required to perform these procedures and interpret the findings might not be readily available in primary care practice.^{9,16} Moreover, inter-observer variation might influence the results of radiography and echocardiography,^{17,18} distressed animals might not tolerate being restrained for diagnostic imaging¹⁹ and the procedures might pose some risk to the patient, should sedation or anesthesia be required.²⁰ It would, therefore, be desirable to identify prognostic risk factors that can be readily measured in the primary care setting, particularly if diagnostic imaging is not available. Cardiac biomarker blood tests and data derived from history taking and physical examination findings have shown promise as predictors of outcome.^{5,9,13,21} However, previous studies analyzed clinical data recorded by veterinary cardiologists, and it is unclear whether the results are applicable to first opinion practice.

Our aim was to prospectively follow dogs with presumptive DMVD attending primary care practices in the United Kingdom to evaluate the prognostic value of key clinical and biochemical measurements in this setting.

The objectives of this study were to:

1. Determine whether plasma cardiac biomarker concentrations are associated with all-cause mortality and/or cardiac-related death in dogs diagnosed with DMVD attending primary care practices; and,
2. Evaluate whether biomarker data can be used in combination with clinical signs and physical examination findings to risk stratify affected animals.

It was hypothesized that circulating plasma concentrations of N-terminal pro B-type natriuretic peptide (NT-proBNP) and cardiac troponin I (cTnI) would be associated with an increased hazard of all-cause and cardiac-related death in dogs with DMVD attending primary care practices. Furthermore, it was hypothesized that historical and physical examination factors also would be associated with the hazard of death and would thus provide complementary prognostic information.

2 | MATERIALS AND METHODS

A prospective cohort design was used to evaluate associations between explanatory (risk factor) variables and mortality in dogs with a presumptive diagnosis of DMVD. A convenience sample of UK primary care veterinary practices was recruited by publication of letters

and articles in the veterinary press and social media. Furthermore, veterinary societies, canine welfare organizations, the UK Kennel Club,²² and veterinary cardiologists promoted the study. Letters inviting participation were sent to 294 practices. Recruitment efforts also were directed toward practices that took part in the Veterinary Companion Animal Surveillance System (VetCompass) program²³ and practices that were part of large veterinary groups by emails, newsletters, a continuing professional development event and an internal conference. Ethics approval for the study was obtained from the Royal Veterinary College's Ethics and Welfare Committee (URN 2012 1144).

Dogs diagnosed with DMVD were recruited to the study by veterinarians during episodes of care or by letters inviting participation. Cases were defined as dogs with a veterinary diagnosis of DMVD attending collaborating primary care practices in the United Kingdom. Diagnosis could be presumptive and based on clinical findings alone (left apical systolic heart murmur in a dog of typical signalment). Confirmation by echocardiography was not an inclusion criterion. Dogs with any stage of the disease were eligible for inclusion. Age and breed restrictions were not imposed so as to maximize the external validity of the results. At recruitment, veterinarians obtained written owner consent, recorded clinical data on a specially designed form and collected a 2 mL venous blood sample for cardiac biomarker measurement from the dog. Clinical data collected included heart rate, heart rhythm, and heart murmur intensity and the presence or absence of coughing, dyspnea, and exercise intolerance. Veterinarians were instructed to ask dog owners to record the pet's sleeping respiratory rate (SRR) as the respiratory rate when the dog appeared to be sleeping deeply in the home environment, when it was neither too hot nor too cold.²⁴ Plasma NT-proBNP was measured using a second generation ELISA test (Cardiopet[®] proBNP) and cTnI was measured using a 2-site immunoenzymatic sandwich assay (Beckman Access 2 Troponin assay) at a commercial laboratory (IDEXX laboratories, Wetherby, The United Kingdom). Electronic patient records (EPRs) for recruited cases were either uploaded to the VetCompass database, if participating in the program,²³ or otherwise shared by email, post, or facsimile. Date of birth, breed, sex, neuter status, and body weight were extracted from the EPRs and entered into a relational database (Access 2010, Microsoft Corporation, Redmond, Washington). The diagnostic tests and therapeutic interventions used to investigate and treat the dogs' heart disease also were extracted. Any documented comorbidities, respiratory rates (RRs) and whether the dog was insured also were recorded in the database whenever reported in the EPRs.

In DMVD cases that died during the study, the date, modality (euthanasia versus natural) and cause of death were extracted where available. If a dog was not reported to have died, it was censored on the date that it was last known to be alive. Cardiac-related death was defined as spontaneous death or euthanasia primarily because of clinical signs consistent with heart disease or sudden death. Sudden death described unexpected natural death, without evidence of dyspnea, in a dog with no history or indication of another condition that could have caused death. Dogs considered to have died as a result of both renal and cardiac disease were classified as experiencing cardiac-related death if the dog received a loop diuretic before developing renal disease, because congestive heart failure (CHF) therapy might

contribute to the development of renal disorders.²⁵ Otherwise, these dogs were classified as experiencing non-cardiac death if the dog did not receive a loop diuretic before developing renal disease. If cause of death was not specified at the time the dog died, but there was documented disease progression or treatment for CHF, in the absence of evidence of comorbidity, deaths were classified as cardiac. The time of entry into the study was the date of recruitment. Analysis time ended on the date of death or censoring. For the analysis of cardiac-related deaths, dogs dying because of non-cardiac causes were censored on the date of death.

2.1 | Statistical methods

Data were exported to spreadsheets for verification (Excel 2010, Microsoft Corporation, Redmond, Washington). Statistical analyses were undertaken using commercially available software (Stata version 14.1, Stata Corporation, TX).

2.1.1 | Descriptive statistics

Continuous data were assessed graphically for normality and presented as mean and standard deviation (SD) or median and interquartile range (IQR) as appropriate. For categorical variables, the number and percentage within each category were presented. For time-to-event analyses, the median time at risk was defined as the median time from recruitment until experiencing an event or censoring.

2.1.2 | Cox proportional hazard and flexible parametric models

The following explanatory variables were evaluated in the time-to-event analyses: age (years), body weight (kg), sex, neuter status, breed, evidence of insurance, plasma NT-proBNP, plasma cTnI, presence or absence of cough, dyspnea and exercise intolerance, heart rhythm, heart rate (beats per minute [bpm]), heart murmur intensity, current cardiac treatments, and comorbidities grouped into disorder categories. Plasma concentrations of the cardiac biomarkers and observations recorded on the data collection form were explanatory variables of a priori interest.

Crossbred dogs and breeds including greater than 20 dogs within the dataset were evaluated as individual categories within a multiple breed variable in the analyses. Other breeds were included in an "other purebred" category. In addition, binary breed variables, Cavalier King Charles Spaniel (CKCS)—yes/no, and crossbred—yes/no, were created as these represented the most frequent breed types. The cardiac biomarker data were highly skewed, with many values below the lower limit of detection, and therefore these variables were included as categorical variables. Cardiac troponin I was categorized into rounded terciles and NT-proBNP categories were based on the interpretive criteria reported by the laboratory. Other continuous variables were retained as untransformed or log transformed variables if linear associations with the outcome were identified by likelihood ratio tests. If there was insufficient evidence for a linear association, continuous data were included as categorical variables in the multivariable analysis. Treatment variables related to whether dogs received the following cardiac medications at the time of recruitment: pimobendan, angiotensin-converting enzyme (ACE) inhibitors, spironolactone and potent diuretics (furosemide, torasemide, amiloride with hydrochlorothiazide). Comorbidities

TABLE 1 Diagnostic terms included in disorder categories evaluated as co-morbidity explanatory variables in survival analyses of dogs with degenerative mitral valve disease

Disorder category	Diagnostic terms
Renal disorders	Renal/kidney disease, protein-losing nephropathy, polycystic kidney disease, azotemia
Respiratory disorders	Brachycephalic airway obstruction syndrome/elongated soft palate, bronchitis, chronic airway disease, pulmonary fibrosis, pulmonary hemorrhage, tracheal collapse, unspecified pulmonary disorder
Musculoskeletal disorders	Arthritis, chronic musculoskeletal pain, intervertebral disc degeneration, intervertebral disc disorder, lame (chronic), osteoarthritis, paresis/paralysis, spinal disorder, spondylosis, stiff
Infectious or inflammatory	Cholangiohepatitis, immune mediated skin disorder, hepatitis, pancreatitis, pneumonia, pyometra
Neoplasia	Haemangiosarcoma (splenic), liposarcoma, lymphoma, mass lesion (abdominal), mass lesion (hepatic), mass lesion (mammary), mass lesion (splenic), squamous cell carcinoma (oral)
Endocrinopathies	Diabetes mellitus, hyperadrenocorticism, hypoadrenocorticism

reported to be associated with the explanatory variables of a priori interest^{26–30} and those considered by the attending veterinarian to have contributed to the death of at least 1 dog in the cohort were grouped together into disorder categories (Table 1). Dogs without a documented diagnosis of the listed disorders at the time of or preceding recruitment and those with a history of disease that had resolved before recruitment were classified as not having the disorder. Pairwise correlations between all explanatory variables were assessed to identify potential collinearity. When pairs of highly correlated variables were found, the variable of greatest a priori interest, considered to be most reliably measured or with the most complete data, was selected.³¹

Univariable Cox proportional hazard models evaluated associations between each explanatory variable and the hazard of all-cause mortality and cardiac-related death. Explanatory variables significant at the 20% level in the univariable analysis were evaluated in the multivariable models. A manual backward stepwise elimination method was used to identify variables with an independent association with the outcome (5% significance level).³¹ Eliminated variables were subsequently added back into the model to check for confounding by observing the magnitude of changes in the model coefficients. Interactions between explanatory variables included in final multivariable models were evaluated. Veterinary clinic was evaluated as a shared frailty term. The overall model fit was assessed by evaluating the distribution of Cox-Snell residuals.^{31,32} The proportional hazards (PH) assumption was evaluated by log-cumulative hazard and Kaplan-Meier Cox plots and the Schoenfeld residuals test of the assumption of PH.^{31,32} The predictive ability of the models was evaluated using Harrell's C concordance statistic.³¹ Martingale residuals were used to assess the functional form of the relationship between continuous variables and the outcome.³² To identify outliers and individuals with a disproportionate influence, deviance residuals and likelihood displacements measures were plotted, respectively.^{31,32} Changes in model coefficients were assessed when

individuals with the greatest deviance residuals and influence were excluded from the models. Dogs with missing data for any of the final model variables were excluded from the multivariable models.

Royston-Parmar flexible parametric models were used to predict survival probabilities (for cardiac-related death) and explore alternative approaches to modeling time-dependent effects (eg, where hazard ratios [HR] for explanatory variables changed over time and did not meet the PH assumption).³³⁻³⁵ Flexible parametric model selection was directed by model fit, which was assessed by graphical examination of different parametric distributions, the Akaike information criterion and Bayesian information criterion.³⁴ Continuous variables were categorized in the multivariable parametric model for ease of interpretation and risk stratification. Selection of explanatory variables and evaluation of confounding and interaction were as described for the Cox regression models. Clustering within veterinary practices was not evaluated because shared frailty terms cannot be included in flexible parametric models. Forward selection was used to identify whether explanatory variables included in the multivariable model had time-dependent effects.³⁴ After specification of the multivariable model, predicted survival probabilities (percentage of dogs not experiencing cardiac-related death) within 1-year follow-up were estimated for a range of combinations of explanatory variable values.^{33,35}

2.1.3 | Evaluating respiratory rate as an explanatory variable

Due to missing data, RR was not evaluated as an explanatory variable in the multivariable models. However, univariable Cox regression models were constructed to explore the associations between both SRR measured in the home environment and RR measured at the veterinary practice and mortality.

2.1.4 | Sample size calculations

A priori sample size calculations estimated that approximately 100 deaths would be required to detect a HR of 2 for a variable to which 20% of individuals were exposed, at a confidence level of 95% and power of 80%. It was estimated that 650-700 dogs would need to be recruited to the study over an 18-month accrual period and a minimum 6 months follow-up, assuming a 10% loss to follow-up.³⁶

3 | RESULTS

3.1 | Study population

Eight-hundred and ninety-three dogs were recruited between 19th December 2013 and 1st March 2016. Seventy-nine primary care veterinary practices participated in the study, enrolling a median of 6 dogs per practice (IQR, 2-13.5). The breed most frequently recruited to the study was the CKCS ($n = 316$, 35.9%). The mean age at recruitment was 9.6 years (SD, 3.1 years) and the median body weight was 10.4 kg (IQR, 8.0-14.5 kg). Four-hundred and eighty-three (54.6%) dogs were male (Table 2). Three-hundred and twelve dogs (35.3%) had evidence of having undergone radiography and 268 (30.3%) had evidence that echocardiography had been performed. Fifteen dogs were identified as not having DMVD after recruitment (10 dogs were diagnosed with

an alternate disorder and 5 did not have a documented heart murmur). These animals were included in analyses because the sample recruited to the study consisted of dogs that primary care veterinarians presumptively diagnosed with DMVD, which was the intended population to which the study results apply. Excluding these cases from the multivariable analyses had minimal impact on the model coefficients. Fourteen dogs had no follow-up data or had an unknown date of death and were excluded from the survival analysis.

For dogs included in the survival analysis, the total analysis time was 11 783 months. Median time at risk was 13.12 months (range, 0.03-30.81 months). Three-hundred and fifty-three (40.2%) dogs died during the study period. One-hundred and ninety deaths (53.8%) were primarily attributable to cardiac disease, of which 134 dogs (70.5%) were euthanized. Of the 163 dogs that died due to non-cardiac causes, 142 (87.1%) were euthanized.

3.2 | Prognostic risk factors associated with all-cause mortality

The following explanatory variables were evaluated in multivariable Cox models because there was some evidence for associations ($P < .2$) between these variables and hazard of death (all-cause mortality) in univariable analysis: age, sex, breed, insurance status, plasma NT-proBNP, plasma cTnI, cough, dyspnea, exercise intolerance, heart rhythm, heart rate, heart murmur intensity, cardiac treatments prescribed, and comorbidities. The final multivariable model included the following explanatory variables: age, sex, plasma NT-proBNP, plasma cTnI, presence of dyspnea and exercise intolerance, heart rate, and documented diagnosis of selected musculoskeletal disorders, neoplasia, or renal disease (Table 3). There was an interaction between NT-proBNP and age, such that the association between NT-proBNP and mortality was stronger in dogs ≤ 9.5 years old compared with older dogs ($P = .003$). Veterinary clinic was significant when included as a shared frailty term, indicating clustering of data at the practice level. No major violations of the PH assumption were identified and Cox-Snell residuals indicated that the model fit the data reasonably well. Harrell's C concordance statistic was 0.79, suggesting the discriminatory ability of the model was good. Martingale residuals supported that heart rate could be included as an untransformed linear variable. Changes in coefficients were minimal when individuals with the greatest deviance residuals and influence were excluded from the model.

3.3 | Prognostic risk factors associated with cardiac-related mortality

3.3.1 | Cox regression analysis for variables associated with cardiac-related death

The following explanatory variables had some evidence ($P < .2$) of an association with cardiac-related death in univariable analysis and were taken forward for consideration in the multivariable model: age, body weight, breed, plasma NT-proBNP, plasma cTnI, cough, dyspnea, exercise intolerance, heart rhythm, heart rate, heart murmur intensity, cardiac treatments prescribed, and diagnosis of an endocrinopathy. In the multivariable model, higher plasma NT-proBNP and cTnI concentrations, higher murmur intensities and heart rates, exercise intolerance, and

TABLE 2 Descriptive statistics for 893 dogs diagnosed with DMVD attending primary-care veterinary practices in the United Kingdom

Variable	Category	Number (%), mean (SD) ^a , median (IQR) ^b	Missing values, n (%)
Breed	CKCS ^c	316 (35.9)	12 (1.3)
	Crossbred	126 (14.3)	
	Jack Russell Terrier	54 (6.1)	
	Shih Tzu	33 (3.8)	
	King Charles Spaniel	32 (3.6)	
	Yorkshire Terrier	31 (3.5)	
	Cocker Spaniel	23 (2.6)	
	Border Collie	23 (2.6)	
	Chihuahua	22 (2.5)	
	Other purebred	221 (25.1)	
Age (years)	Mean (SD)	9.6 (3.1)	12 (1.3)
Sex/neuter status	MN ^d	298 (33.7)	9 (1.0)
	ME	185 (20.9)	
	FN	320 (36.2)	
	FE	81 (9.2)	
Body weight (kg)	Median (IQR)	10.4 (8.0-14.5)	52 (5.8)
Plasma NT-proBNP (pmol/L)	Median (IQR)	761 (377-1681)	10 (1.1)
Plasma cTnI (ng/mL)	Median (IQR)	0.02 (0.01-0.04)	8 (0.9)
Cough	No	540 (62.8)	33 (3.7)
	Yes	320 (37.2)	
Dyspnea	No	712 (84.5)	50 (5.6)
	Yes	131 (15.5)	
Exercise intolerance	No	574 (67.9)	48 (5.4)
	Yes	271 (32.1)	
Heart rhythm	Sinus arrhythmia	206 (25.2)	76 (8.5)
	Sinus rhythm	569 (69.6)	
	Other arrhythmia	42 (5.1)	
Heart rate (bpm) ^e	Mean (SD)	124 (26.0)	47 (5.3)
Heart murmur intensity	Soft (grade I/II)	274 (31.4)	21 (2.4)
	Moderate (III)	274 (31.4)	
	Loud (IV)	230 (26.4)	
	Thrilling (V/VI)	94 (10.8)	
Sleeping respiratory rate (rpm) ^f	Median (IQR)	19 (16-26)	793 (88.8)
Receiving potent diuretic	No	700 (79.1)	8 (0.9)
	Yes	185 (20.9)	
Renal disease	No	870 (98.3)	8 (0.9)
	Yes	15 (1.7)	
Respiratory disease	No	837 (94.6)	8 (0.9)
	Yes	48 (5.4)	
Musculoskeletal disorder	No	752 (85.0)	8 (0.9)
	Yes	133 (15.0)	
Infectious/inflammatory disorder	No	874 (98.8)	8 (0.9)
	Yes	11 (1.2)	
Neoplasia	No	865 (97.7)	8 (0.9)
	Yes	20 (2.3)	
Endocrinopathy	No	862 (97.4)	8 (0.9)
	Yes	23 (2.6)	

^a Standard deviation.^b Interquartile range.^c Cavalier King Charles Spaniel.^d MN, male, neutered; ME, male, entire; FN, female, neutered; FE, female, entire.^e Beats per minute.^f Respirations per minute.

TABLE 3 Multivariable cox regression model for variables associated with hazard of death (all-cause mortality) in dogs diagnosed with degenerative mitral valve disease attending primary-care practice

Variable	Category	Hazard ratio (95% CI ^a)	P-value
NT-proBNP in dogs ≤9.5 years old (pmol/L) (interaction)	<900	Baseline	.003
	900-1800	1.97 (1.14-3.40)	
	>1800	3.89 (2.38-6.34)	
NT-proBNP in dogs >9.5 years old (pmol/L)	<900	Baseline	
	900-1800	1.09 (0.75-1.59)	
	>1800	1.47 (1.04-2.07)	
Cardiac troponin I (ng/mL)	<0.02	Baseline	<.001
	0.02-0.03	1.44 (1.02-2.03)	
	>0.03	2.66 (1.88-3.75)	
Dyspnea	No	Baseline	.044
	Yes	1.39 (1.02-1.91)	
Exercise intolerance	No	Baseline	.004
	Yes	1.51 (1.15-1.99)	
Heart rate (1.007-1.017)	Continuous	1.012 ^b	<.001
Sex	Female	Baseline	.010
	Male	0.73 (0.58-0.93)	
Musculoskeletal disease	No	Baseline	.010
	Yes	1.49 (1.11-2.01)	
Neoplasia	No	Baseline	<.001
	Yes	3.83 (2.15-6.79)	
Renal disease	No	Baseline	.012
	Yes	2.32 (1.28-4.21)	
Veterinary clinic (shared frailty)		Theta 0.10	.012

Observations for 775 individuals.

^a Confidence interval.^b Increase in hazard ratio per heart beat per minute.

receiving potent diuretics at recruitment were independently associated with increased hazards of cardiac-related death (Table 4). Veterinary clinic was included as a shared frailty term, indicating clustering of data within practices. The Schoenfeld residuals test was statistically significant, providing evidence that the PH assumption was violated and cTnI was found to have a statistically significant time-dependent effect (the magnitude of the HR between the highest and lowest tertiles of the biomarker decreased over time, violating the PH assumption). Some evidence for an interaction was found between heart murmur intensity and

diuretic administration ($P = .032$). It is possible that the interaction was related to data sparsity within certain categories and this finding was not statistically significant in the flexible parametric models, and thus the interaction was not included in the final model. Harrell's C concordance statistic (0.86) suggested good model discrimination. Martingale residuals supported that heart rate could be included as an untransformed linear variable. The measures of effect reported were similar when dogs with the greatest deviance residuals and influence were excluded from the model.

3.3.2 | Multivariable flexible parametric model for factors associated with cardiac-related mortality

The flexible parametric model that best fit the data was a proportional odds (PO) model with 2 interior knots. Explanatory variables included in the multivariable model were as for Cox regression analysis, with the exception of omission of veterinary clinic (Table 5). On the PO scale, the HR for the association between categories converges toward 1 over

TABLE 4 Multivariable cox regression model for variables associated with hazard of cardiac-related death in dogs diagnosed with degenerative mitral valve disease attending primary-care practice

Variable	Category	Hazard ratio (95% CI ^a)	P-value
NT-proBNP (pmol/l)	<900	Baseline	<.001
	900-1800	2.86 (1.71-4.80)	
	>1800	5.41 (3.31-8.87)	
Cardiac troponin I (ng/ml)	<0.02	Baseline	<.001
	0.02-0.03	1.67 (0.99-2.82)	
	>0.03	7.14 (3.16-16.15)	
Heart murmur intensity	Soft (grade I/II)	Baseline	.010
	Moderate (grade III)	2.26 (1.23-4.15)	
	Loud (grade IV)	2.53 (1.38-4.65)	
	Thrilling (grade V/VI)	2.52 (1.30-4.89)	
Heart rate (1.007-1.020)	Continuous	1.013 ^b	<.001
Exercise intolerance	No	Baseline	.020
	Yes	1.55 (1.07-2.25)	
Receiving potent diuretic ^c	No	Baseline	.027
	Yes	1.61 (1.06-2.44)	
Time-dependent effect Cardiac troponin I (ng/mL)	>0.03 vs <0.02	-0.56 ^d (-0.89 to -0.24)	.001
Clinic as shared frailty term		Theta 0.11	.048

Cardiac troponin I interacted with log(time). Observations for 784 individuals.

^a Confidence interval.^b Increase in hazard ratio per heart beat per minute.^c Furosemide, torsemide, and amiloride with hydrochlorothiazide.^d The log(HR) for the highest versus lowest tertiles of cTnI decreased by 0.56 for each unit increase in log(time).

TABLE 5 Royston-Parmar flexible parametric proportional odds models with two interior knots for variables associated with hazard of cardiac-related death in dogs with degenerative mitral valve disease attending primary-care practice in the United Kingdom

Variable	Category	Odds ratio (95% CI ^a)	P-value
NT-proBNP (pmol/l)	<900	Baseline	<.001
	900-1800	3.12 (1.74-5.59)	
	>1800	8.29 (4.72-14.55)	
Cardiac troponin I (ng/ml)	<0.02	Baseline	<.001
	0.02-0.03	1.58 (0.87-2.88)	
	>0.03	3.88 (2.09-7.19)	
Heart murmur intensity	Soft (grade I/II)	Baseline	.021
	Moderate (grade III)	2.22 (1.12-4.40)	
	Loud (grade IV)	2.48 (1.24-4.97)	
	Thrilling (grade V/VI)	3.06 (1.43-6.55)	
Heart rate (bpm ^b)	≤120	Baseline	<.001
	>120	2.46 (1.60-3.80)	
Exercise intolerance	No	Baseline	.004
	Yes	1.95 (1.24-3.06)	
Receiving potent diuretic ^c	No	Baseline	.016
	Yes	1.87 (1.13-3.10)	

Observations for 784 individuals.

^a Confidence interval.

^b Beats per minute.

^c Furosemide, torasemide, and amiloride with hydrochlorothiazide.

time (ie, the magnitude of effect decreased with time).^{34,36} Likelihood ratio tests did not identify any time-dependent effects on the PO scale. Figures 1 and 2 present predicted point estimates for the percentage of dogs not experiencing cardiac death within the following year for different combinations of explanatory variable values. Figure 1 relates to dogs not receiving treatment with a potent diuretic (furosemide, torasemide, amiloride with hydrochlorothiazide) at recruitment and Figure 2 relates to dogs receiving diuretics. Combinations of different variable values produced a wide range of predicted survival probabilities, suggesting that the spread of prognoses for dogs with DMVD varied widely. Take for example a hypothetical group of dogs with DMVD not receiving diuretics, with biomarker concentrations in the lowest categories, soft intensity heart murmurs, heart rates > 120 bpm, and no reported exercise intolerance. The model predicted that 98.5% (95% CI, 96.6-99.3) of dogs with these characteristics would survive (not experience cardiac death) in the following year. In contrast, for a group of dogs with DMVD receiving diuretics, with biomarker concentrations in the highest categories, loud murmurs, heart rates > 120 bpm and exercise intolerance, 18.0% (95% CI, 11.8%-26.5%) of dogs would be predicted to survive the following year.

3.4 | Prognostic value of respiratory rates

Sleeping respiratory rate was recorded for 100 dogs. Hazard of death (all-cause) increased 1.06 times (95% CI, 1.03-1.08) for each unit increase in SRR (respiration per minute [rpm]; Table 6). Hazard of cardiac-related death increased 1.06 times (95% CI, 1.04-1.09) for each unit increase in SRR (Table 7). Veterinarians recorded respiratory rate during episodes of care for 226 cases. Eleven dogs had no follow-

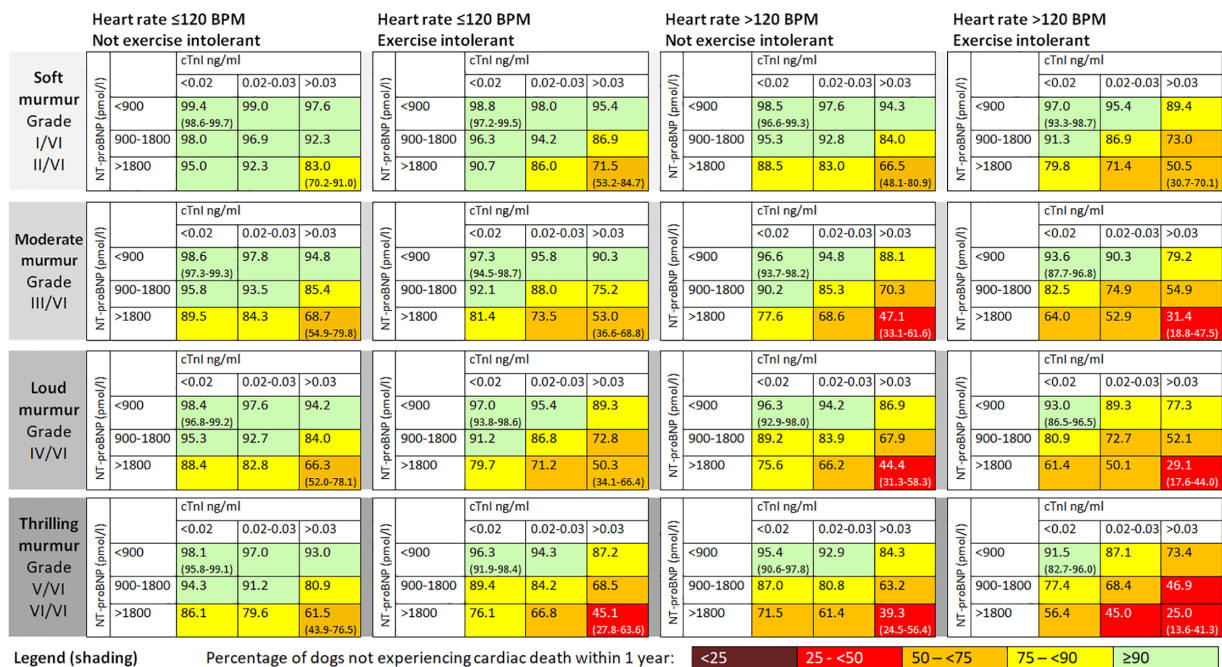


FIGURE 1 Risk estimates for the percentage of dogs with degenerative mitral valve disease not experiencing cardiac death within 1 year for each combination of variable values for dogs not receiving treatment with a potent diuretic at the time of evaluation. 95% confidence intervals are presented in parentheses for dogs with the lowest and highest cardiac biomarkers in each table. BPM, beats per minute; cTnI, cardiac troponin I. The multivariable model used to derive this figure included 784 observations, 617 dogs did not receive a potent diuretic at recruitment

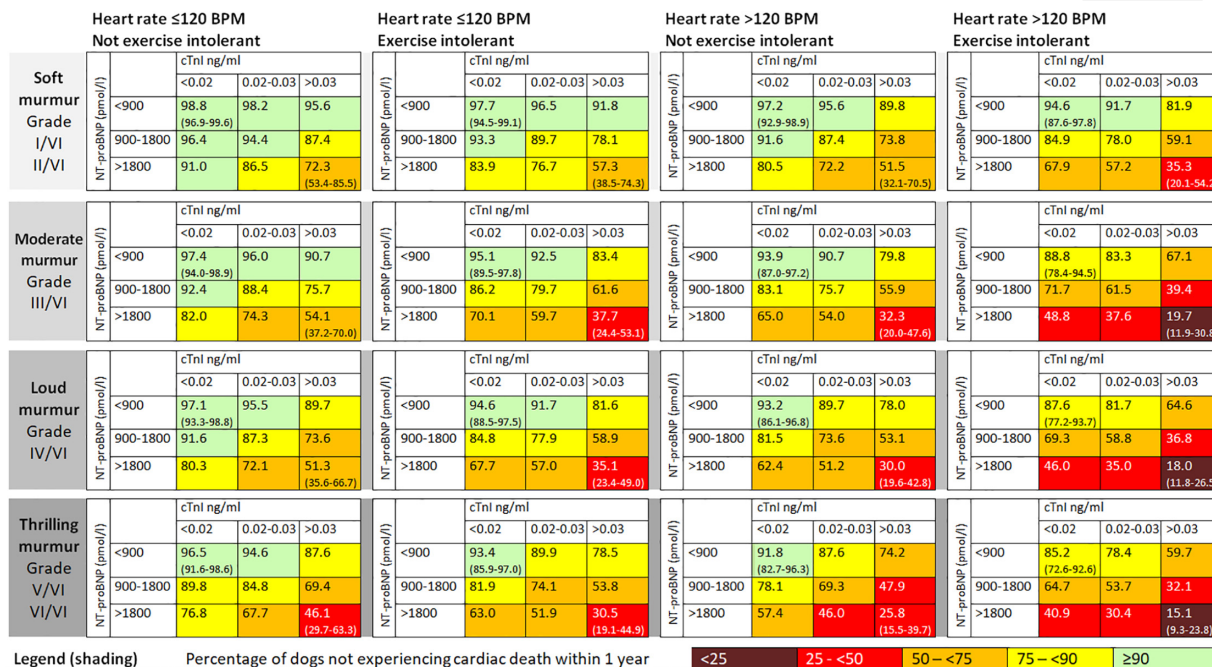


FIGURE 2 Risk estimates for the percentage of dogs with degenerative mitral valve disease not experiencing cardiac death within 1 year for each combination of variable values for dogs receiving treatment with a potent diuretic at the time of evaluation. 95% confidence intervals are presented in parentheses for dogs with the lowest and highest cardiac biomarkers in each table. BPM, beats per minute; cTnI, cardiac troponin I. The multivariable model used to derive this figure included 784 observations, 167 dogs received a potent diuretic at recruitment

up, and were excluded from analyses. Hazard of death (all-cause) increased 1.03 times (95% CI, 1.02-1.04) for each unit increase in RR (rpm; Table 6). The hazard of cardiac-related death increased 1.04 times (95% CI, 1.03-1.05) for each unit increase in RR (Table 7).

4 | DISCUSSION

We identified a number of clinical findings that were associated with survival in a heterogeneous cohort of dogs with presumed DMVD evaluated at primary care veterinary practices in the United Kingdom.

TABLE 6 Univariable cox regression analysis

Variable	Category	N (%)	Hazard ratio (95% CI) ^a	P-value
SRR ^b	Continuous		1.06 ^c (1.03-1.08)	<.001
	<20	54 (54.0)	Baseline	<.001
	20-29	32 (32.0)	2.00 (1.10-3.64)	
	30-39	6 (6.0)	3.53 (1.19-10.47)	
	≥40	8 (8.0)	9.45 (3.84-23.22)	
RR ^d	Continuous		1.03 (1.02-1.04)	<.001
	<20	23 (10.2)	Baseline	<.001
	20-29	74 (32.7)	1.72 (0.76-3.89)	
	30-39	51 (22.6)	3.09 (1.36-7.03)	
	≥40	78 (34.5)	5.17 (2.34-11.43)	

Association between respiratory rate and hazard of death (all-cause mortality) in dogs with degenerative mitral valve disease.

^a Confidence interval.

^b Sleeping respiratory rate.

^c Increase in hazard ratio per respiration per minute.

^d Respiratory rate.

The prognostic utility of a combination of measurements was greater than that of any measurement in isolation. Taking a history, performing a physical examination, and measuring plasma cardiac biomarkers when evaluating dogs with presumed DMVD thus could aid prognostication and direct further management of cases, particularly if echocardiography or thoracic radiography are unavailable.

4.1 | Cardiac biomarkers

Plasma concentration of NT-proBNP was among the strongest predictors of all-cause and cardiac-related mortality. These findings concur

TABLE 7 Univariable cox regression analysis

Variable	Category	N (%)	Hazard ratio (95% CI) ^a	P-value
SRR ^b	Continuous		1.06 ^c (1.04-1.09)	<.001
	<20	54 (54.0)	Baseline	<.001
	20-29	32 (32.0)	2.48 (1.17-5.29)	
	30-39	6 (6.0)	5.54 (1.75-17.59)	
	≥40	8 (8.0)	13.01 (4.69-36.06)	
RR ^d	Continuous		1.04 (1.03-1.05)	<.001
	<20	23 (10.2)	Baseline	<.001
	20-29	74 (32.7)	1.62 (0.55-4.80)	
	30-39	51 (22.6)	4.09 (1.41-11.83)	
	≥40	78 (34.5)	7.32 (2.62-20.49)	

Association between respiratory rate and hazard of death (cardiac-related mortality) in dogs with degenerative mitral valve disease.

^a Confidence interval.

^b Sleeping respiratory rate.

^c Increase in hazard ratio per respiration per minute.

^d Respiratory rate.

with previous studies indicating that NT-proBNP is associated with survival in dogs with DMVD.^{5,9,37,38} Circulating NT-proBNP is a marker of myocardial wall stress as a result of volume or pressure overload.³⁹ High concentrations of the marker therefore might reflect more advanced DMVD and a higher risk of death in dogs. An interaction was found between age and NT-proBNP in the model for all-cause mortality, with the association between the biomarker and hazard of death being attenuated in dogs > 9.5 years old. Cox regression models specifying non-cardiac death as the outcome event of interest identified strong associations between age and death, but failed to find an association between NT-proBNP and hazard of non-cardiac mortality (data not shown). It therefore can be speculated that the ability of NT-proBNP to predict all-cause mortality in a cohort of dogs with DMVD is chiefly attributable to the strong association between the biomarker and cardiac-related death. When evaluating all-cause mortality in older dogs, the association between NT-proBNP and mortality might therefore be diluted by the increased hazard of death due to non-cardiac disease in geriatric animals. This interaction also might have occurred as a result of a positive correlation between age and NT-proBNP, independent of disease severity, as has been reported in humans.⁴⁰ However, to our knowledge, this association has not been reported in dogs, and an interaction between age and NT-proBNP was not observed in the model evaluating factors associated with cardiac-related mortality.

In contrast, cardiac troponins are released into the circulation after myocyte injury, which can be due to primary heart disease or secondary to non-cardiac disorders.²⁹ Circulating cTnI is a sensitive and specific marker of cardiac injury (regardless of the underlying cause) and increased concentrations have been reported in dogs with many cardiac and non-cardiac diseases.²⁹ These findings are consistent with those of the current study, which identified strong evidence for associations between plasma cTnI and both all-cause mortality and cardiac-related death. Furthermore, time-to-event models specifying non-cardiac death as an outcome confirmed an association between cTnI and non-cardiac mortality (data not shown), highlighting that the prognostic utility of the marker is less specific to cardiac-related mortality than NT-proBNP. The two studied biomarkers, therefore, give different prognostic information; NT-proBNP was a more specific marker of cardiac-disease severity, whereas cTnI had a strong association with mortality, regardless of cause.

The time-dependent effect identified suggests that the ability of cTnI to predict cardiac-related death is greatest in the short- to medium-term after sampling. Moreover, flexible parametric survival models on the PO scale had a superior fit to models on the PH scale, providing further evidence that the magnitude of effect (HR) of the biomarkers is greatest in the months immediately after sampling.

Several physiological and pathological factors can influence the reported concentrations of both NT-proBNP and cTnI, potentially confounding the associations between the biomarkers and survival.^{26,27,29,38,41-56} Breed, sex, body weight, age, treatments, and comorbidities were evaluated both as explanatory variables and confounders in our study. Coefficients were only marginally different when variables excluded from the multivariable models were individually added to the final models, indicating minimal confounding. However, misclassification of potential confounders was possible and only

the most common breeds, treatments, and disorders were evaluated. Furthermore, fluctuations in circulating biomarker concentrations might have introduced measurement error because inherent biological variability in both NT-proBNP and cTnI has been reported in dogs with DMVD.^{57,58} Finally, sample handling and storage conditions might influence cardiac biomarker assay results.^{59,60} However, to be clinically useful, a diagnostic test should be evaluated in the conditions under which it will be used in practice⁶¹ and the prognostic value of NT-proBNP and cTnI was confirmed, despite potential variation in sample handling and storage.

4.2 | Presenting signs, clinical findings, and signalment

Higher heart rates were associated with increased hazards of an adverse outcome in agreement with a number of studies.^{3-5,8,12,62} Decreased cardiac output and arterial hypotension as a result of DMVD elicit a decrease in vagal tone and increase the activity of the sympathetic nervous system (SNS) and renin-angiotensin-aldosterone system (RAAS).⁶³ A major effect of these systems is catecholamine release resulting in an increase in heart rate.^{64,65} These compensatory mechanisms serve a beneficial function in the short term by maintaining cardiac output and blood pressure. However, a chronic increase in SNS and RAAS activity increases myocardial oxygen consumption and induces myocardial hypertrophy and fibrosis, thereby contributing to the progression of the disease.^{63,66} Therefore, increased heart rate might be both a risk factor for and a consequence of disease progression in dogs with DMVD, and is thus associated with a worse prognosis.

Heart murmur intensity also was associated with cardiac-related death, in agreement with a previous report.²¹ Heart murmur intensity reflects regurgitant volume and correlates with DMVD severity in small breed dogs,^{67,68} consistent with the observed associations between this measurement and hazard of cardiac-related death.

Exercise intolerance and dyspnea were associated with all-cause mortality and cardiac-related death, in agreement with other studies.^{3,12,21} The association between dyspnea and cardiac death did not persist in multivariable analysis, which might be due to the limited number of dogs with dyspnea or the lower number of events in the cardiac-related death model compared with the all-cause mortality model. Exercise intolerance and dyspnea are associated with more advanced DMVD^{12,18,21,69,70} and can have an adverse impact on quality of life,^{6,71,72} which is an important factor when dog owners are considering euthanasia.^{6,73} Because the majority of dogs with DMVD that died were euthanized, factors perceived to affect quality of life, therefore, are likely to be associated with hazard of death. The presence of a cough was significantly associated with both all-cause and cardiac-related mortality in univariable analysis. This association did not persist in multivariable analysis in contrast to the findings of another study of UK dogs with DMVD.²¹ This discrepancy might be due to inclusion of different variables in the multivariable models (the latter study did not evaluate cardiac biomarkers and included some clinical measurements not included in our study).

Respiratory rate showed promise as a predictor of mortality in univariable analysis. The magnitude of effect was stronger for SRR

than RR measured at the practice, which might reflect that the latter measurement is more likely to be influenced by non-cardiac factors, such as stress or excitement related to visiting the veterinary practice. To our knowledge, the prognostic value of RR in dogs with DMVD has not previously been described, although there is evidence supporting that this measurement is an indicator of heart disease severity.^{18,74,75}

Advancing age was associated with an increased hazard of death due to all-cause mortality in dogs with DMVD, consistent with previous reports.^{3,5,8,9} In contrast to previous studies,^{3,5,8,9} sex was an independent risk factor for all-cause mortality, with males having a lower hazard of death than females. This association might reflect sex differences in the risk of death due to non-cardiac disorders or type I error.

4.3 | Comorbidity and treatment

Diagnoses of neoplasia, renal disease, and musculoskeletal disease were significant risk factors in the multivariable model for all-cause mortality. These disorders have been reported to be major causes of mortality in the wider population of dogs attending primary care practice.⁷⁶ Hematology and biochemistry were not routinely performed and adjunct diagnostic tests were performed at the discretion of the attending veterinarian. Under-reporting and misclassification of evaluated comorbidities therefore was possible, which might have attenuated confounding effects or biased associations with hazard of death toward the null.

Receiving a potent diuretic was associated with an increased hazard of experiencing cardiac-related death. It is likely that these treatments are a proxy for dogs with a history of CHF, which have more advanced disease and thus an increased hazard of death. However, the rationale behind prescribing potent diuretics was not explored and some dogs without CHF might have inappropriately received diuretics. An alternative approach would have been to classify dogs based on their heart disease stage or whether they had a documented diagnosis of CHF. However, DMVD severity grading schemes require diagnostic imaging to stage the disease⁷⁷ and these procedures were not routinely performed. Administration of potent diuretics was evaluated rather than a documented diagnosis of CHF, because it was considered that prescribed treatments essential for dogs with CHF⁷⁷ would be more reliably recorded in electronic patient records than diagnoses of CHF.

4.4 | General strengths and limitations

Our study benefitted from a prospective cohort design, which provides the highest strength of evidence of single observational studies.³¹ Furthermore, 893 dogs were recruited by 79 practices, which, to our knowledge, is the largest prospective study of DMVD in dogs under the care of first opinion practitioners. Nonetheless, several limitations should be acknowledged. The practitioners' diagnoses of DMVD were accepted to be correct and strict eligibility criteria were not imposed. Although this pragmatic approach maximizes the external validity of the results,⁷⁸ it is possible that dogs erroneously diagnosed with DMVD were recruited to the study. However, almost all (260/268, 97.0%) of dogs that underwent echocardiography in our

study had findings consistent with DMVD. In addition, participating clinicians were provided with information on typical presentation and risk factors for DMVD to improve the accuracy of presumptive diagnoses. Clinicians therefore might have more confidence making a diagnosis of DMVD in dogs of certain breeds or ages presenting with heart murmurs typical of mitral regurgitation. Recruited cases largely consisted of small- to medium-sized, older dogs, a population known to have a high prevalence of DMVD.^{79,80} Furthermore, 98.7% of dogs referred to a DMVD research clinic by primary care veterinarians were later confirmed to have DMVD by a veterinary cardiologist,²¹ supporting that a presumptive diagnosis of DMVD based on detecting a murmur consistent with DMVD carries a high positive predictive value for the disease. Misclassification or measurement error might have occurred when recording explanatory variables. Evaluating the presence or absence of clinical signs is inherently subjective and depends on the owners' perception and the attending veterinarians' clinical acumen. Auscultatory findings are subject to inter-observer variation and can be influenced by exercise, emotional state and how easy a dog is to auscultate.⁸¹ However, despite data being collected under different conditions by a large number of practitioners, several clinical findings remained predictors of mortality. Moreover, these variables were measured before the outcome occurring, so the impact of misclassification is likely to be non-differential and bias associations toward the null. Echocardiography and radiography were not routinely performed, so it was not possible to compare the prognostic value of the explanatory variables evaluated in our study with parameters obtained from diagnostic imaging. Another limitation of our study is that selection bias might have arisen because of censoring and missing data. An assumption of time-to-event models is that censoring is independent of the outcome of interest.³¹ It is possible that right-censored dogs had different survival experiences as compared with those with more complete follow-up. Complete case analysis was used in the multivariable models, so bias might have been introduced if data were not missing randomly.³¹ Also, the outcome of cardiac-related death is subjective and was largely based on what was recorded in EPRs. Cause of death can be complex and might variably involve heart disease. Study participants were not blinded to plasma cardiac biomarker concentrations and it is possible that lack of blinding influenced the assessment of whether clinical signs leading to death were cardiac-related, thus introducing observer bias.⁸² Furthermore, management of DMVD cases was not standardized across practices and differences in treatment protocols might have affected outcome and survival. The frailty term included in the multivariable models accounts for clustering of data within practices, and suggests that practice level factors (such as the management of cases) can influence survival.

Survival predictions derived from flexible parametric models demonstrated that the prognostic value of the cardiac biomarkers was improved when evaluated with other explanatory variables, underscoring the importance of interpreting the results of these tests in conjunction with historical and physical examination findings. For example, when considering the predicted survival of dogs with biomarker concentrations in the highest categories, the percentage of dogs not experiencing cardiac-related death in the following year varied between 15.1% (95% CI, 9.3%-23.8%) and 83.0% (95% CI, 70.2%-91.0%), depending on the values of other explanatory variables.

Furthermore, important limitations arise when applying results of a population study to individual patients,⁸³ and the outcome for an individual dog will be uncertain. Although risk can be estimated based on the values of prognostic factors identified in our study (eg, the percentage of dogs not experiencing cardiac-related death in the following year), it is not possible to determine whether an individual dog will fall into the survivor or non-survivor group.

In summary, our study provided strong evidence for clinically important associations between circulating concentrations of cardiac biomarkers and mortality in a heterogeneous population of dogs with a presumptive diagnosis of DMVD managed by primary care practitioners. Furthermore, historical and physical examination findings provided complementary prognostic information. These findings could help veterinarians risk stratify dogs with DMVD and optimize diagnostic and treatment strategies to maximize patient welfare and longevity.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

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

Ethics approval for the study was obtained from the Royal Veterinary College's Ethics and Welfare Committee (URN 2012 1144). This manuscript has been approved by the Royal Veterinary College's publications approval system, to comply with Good Research Practice Policy on Publications (manuscript number PPS_01757).

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