Journal of Veterinary Internal Medicine

ACVIM
American College of
Veterinary Internal Medicine

Open Access

STANDARD ARTICLE

Factors associated with disease progression in dogs with presumed preclinical degenerative mitral valve disease attending primary care veterinary practices in the United Kingdom

Madeleine J. Mattin [ID] | David C. Brodbelt [ID] | David B. Church | Adrian Boswood [ID]

Royal Veterinary College, University of London, London, United Kingdom

Correspondence
Madeleine Mattin, Royal Veterinary College, Hawkshead Lane, North Mymms, Hertfordshire, AL9 7TA, United Kingdom.
Email: mmattin@rvc.ac.uk

Background: Factors associated with disease progression in dogs with preclinical (stage B) degenerative mitral valve disease (DMVD) have not been evaluated previously in primary care veterinary practice.

Objectives: To evaluate whether plasma cardiac biomarkers, clinical signs, and physical examination findings are associated with clinical progression (reaching the composite endpoint of initiation of treatment with a potent diuretic or cardiac death) in dogs presumed to have stage B DMVD.

Animals: Six-hundred and eighty-four dogs diagnosed with DMVD recruited from 73 primary care practices in the United Kingdom. Dogs were not receiving potent diuretics at recruitment.

Methods: Prospective cohort study design. Primary care veterinarians recorded the presence or absence of clinical signs and physical examination findings. Baseline plasma N-terminal pro B-type natriuretic peptide (NT-proBNP) and cardiac troponin I were measured. Cox regression models measured associations between risk factor variables and clinical progression. Flexible parametric models generated predicted probabilities of reaching the composite endpoint for dogs with different combinations of prognostic risk factor variables.

Results: Plasma NT-proBNP, heart rate, heart murmur intensity, presence of a cough, being a Cavalier King Charles Spaniel, and being prescribed pimobendan were associated with clinical progression to initiation of treatment with a potent diuretic or cardiac-related death.

Conclusions and Clinical Importance: Dogs with stage B DMVD identified as having a high risk of disease progression might benefit from more frequent monitoring or further diagnostic evaluation. The prognostic factors identified could facilitate risk stratification of dogs presenting with preclinical DMVD.

KEYWORDS

cardiac biomarker, natriuretic peptide, risk stratification, survival

Abbreviations: ACVIM-HD, American College of Veterinary Internal Medicine consensus statement, heart disease severity grading scheme; bpm, beats per minute; CHF, congestive heart failure; CKCS, Cavalier King Charles Spaniel; cTnI, cardiac troponin I; DMVD, degenerative mitral valve disease; EPIC, Evaluation of Pimobendan In dogs with Cardiomegaly; EPR, electronic patient record; HR, hazard ratio; IQR, interquartile range; NT-proBNP, N-terminal pro B-type natriuretic peptide; PH, proportional hazards; SD, standard deviation; VetCompass, Veterinary Companion Animal Surveillance System.

1 | INTRODUCTION

Dogs with degenerative mitral valve disease (DMVD) are on a diverse spectrum of disease severity. The American College of Veterinary Internal Medicine consensus statement, heart disease severity grading scheme (ACVIM-HD) describes 4 stages of heart disease and failure in

dogs with DMVD.[1] Stage A describes dogs at high risk of DMVD that are yet to develop structural heart disease (eg, dogs of breeds predisposed to DMVD that do not have heart murmurs). Stage B denotes the preclinical stages of DMVD, whereby dogs have heart murmurs as a result of mitral regurgitation but no current or previous history of congestive heart failure (CHF). Stages C and D describe dogs with DMVD that has progressed to CHF. Until recently, there has been no strong evidence for the benefit of treatment in dogs with stage B DMVD. Distinguishing between ACVIM-HD stages B1 (preclinical DMVD with a normal heart size) and B2 (preclinical DMVD with cardiac chamber enlargement), therefore, might not have altered the management of these cases. However, because the EPIC study reported the benefit of administering pimobendan to dogs with echocardiographic and radiographic evidence of advanced stage B2 disease, there is greater interest and clinical merit in identifying dogs with more advanced preclinical DMVD.[2]

Previous observational studies and randomized controlled trials have evaluated factors associated with disease progression in dogs with stage B DMVD.[2–8] Natriuretic peptides,[3,4,6] radiographic and echocardiographic measurements,[2–5,7] presenting signs and physical examination findings,[2,5–8] age,[7] systolic arterial blood pressure,[2] and pimobendan treatment[2] have been reported to be associated with outcome in dogs with preclinical DMVD. However, the samples of dogs included in these studies were restricted to those attending teaching or referral hospitals or a single breed. These samples might be poorly representative of the spectrum of dogs with preclinical DMVD attending primary care veterinary practice. Furthermore, prognostic factors measured by specialist veterinary cardiologists might not be clinically useful in primary care practice, where the level of expertise and availability of equipment required to obtain these measurements might not match those found in referral centers.[9] Prognostic factors that can be readily measured in the primary care setting could help first opinion veterinarians assess the likely disease severity in dogs with preclinical DMVD. The ability to do so could aid prognostication when evaluating the likely outcome for a dog and identify those dogs that might benefit most from frequent monitoring, diagnostic imaging, or evaluation by a veterinary cardiologist.

Our aim was to prospectively follow dogs presumed to have stage B DMVD and to evaluate whether key measurements were associated with disease progression in the UK primary care setting.

Our objectives were to:

- Determine whether plasma cardiac biomarker concentrations are associated with clinical progression to initiation of treatment with a potent diuretic or cardiac-related death in dogs with presumed stage B DMVD attending primary care veterinary practices.
- Evaluate whether physical examination findings and the presence or absence of clinical signs provide additional prognostic information to risk stratify affected animals.

2 | MATERIALS AND METHODS

A prospective cohort design was used to evaluate factors associated with disease progression in dogs presumed to have stage B DMVD attending UK primary care veterinary practices. Animals included were a subset of a heterogeneous sample of dogs with any stage of DMVD described previously.[10] 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 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 of the diagnosis by echocardiography was not an inclusion criterion. Participating practices were provided with information on typical presentation and risk factors for DMVD to improve the accuracy of presumptive diagnoses. Age and breed restrictions were not imposed in order to maximize the extent to which our results could be generalized to the wider population of dogs with DMVD attending primary care practice. The subset included in our study was restricted to dogs diagnosed with presumed DMVD that did not receive a potent diuretic (furosemide, torasemide, and amiloride with hydrochlorothiazide) before or within 14 days of recruitment to the study. These dogs herein are referred to as having presumed ACVIM-HD classification stage B DMVD (dogs that have a heart murmur typical of mitral regurgitation, but no history of clinical signs caused by CHF[1]). Dogs starting diuretics within 14 days of recruitment were not included to avoid erroneously classifying dogs with preexisting CHF as stage B cases and to limit misclassification bias arising from veterinarians starting diuretic treatment based on the results of the findings at the initial visit and cardiac biomarker assay results.

The composite endpoint was reached when treatment with a potent diuretic was initiated or death attributed to cardiac disease was documented. Although thoracic radiography generally is considered to be the clinical gold standard for diagnosing CHF,[11] it was not consistently performed, and starting treatment with a potent diuretic was used as a proxy for clinical progression to stage C DMVD. This article has been approved by the Royal Veterinary College's publications approval system to comply with Good Research Practice Policy on Publications (manuscript number PPS_01758).

To recruit a dog to the study, veterinarians obtained written owner consent, recorded clinical data on a specially designed form, and collected a 2 mL blood sample to measure circulating plasma N-terminal pro B-type natriuretic peptide (NT-proBNP) and cardiac troponin I (cTnI) concentrations. Descriptors of each heart murmur intensity grade were provided in an effort to standardize how veterinarians assessed this variable and thus improve interobserver reliability. 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 either were uploaded to the Veterinary Companion Animal Surveillance System (VetCompass) database,[12] or 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 and whether the dog was insured also were extracted.

The date, modality (euthanasia versus unassisted), and cause of death were identified where applicable. 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 the unexpected natural death, without evidence of dyspnea, in a dog with no history or indication of another condition that could have caused death. If a dog was not reported to have died because of its heart disease or started diuretic treatment, it was censored on the date it was last known to be alive or the date of noncardiac death. Dogs prescribed furosemide to treat noncardiac conditions and dogs thought to have developed CHF because of doxorubicin toxicity also were censored. If potent diuretics were prescribed and then discontinued without any adverse effects during the follow-up period, dogs were not classified as having reached the endpoint because administering potent diuretics was considered essential for preserving life in dogs with stage C and D DMVD.[1,13] The time of entry into the study was the date of recruitment. Analysis time ended on the date diuretic treatment was initiated, the date the dog died because of its cardiac disease (if diuretics were not prescribed), or on the date of censoring.

2.1 | Statistical methods

Data were exported from the database to spreadsheets for cleaning and checking (Excel 2010, Microsoft Corporation). Statistical analyses were undertaken by commercially available software (Stata version 14.1, Stata Corporation, Texas).

2.1.1 | Descriptive statistics

Continuous data were assessed graphically for normality and presented as mean and 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 starting treatment with a potent diuretic, experiencing cardiac-related death or censoring.

2.1.2 | Cox proportional hazard and flexible parametric models

The following explanatory (risk factor) variables were evaluated in the time-to-event analyses: age (years), body weight (kg), sex, neuter status, breed, evidence of insurance, plasma NT-proBNP concentration, plasma cTnI concentration, 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 (presence or absence of clinical signs and physical examination findings) were explanatory variables of *a priori* interest. The cardiac biomarker data were highly skewed, with many results below the lower limit of detection, and thus these variables were included as categorical variables. Other continuous variables were retained as untransformed or log transformed variables if linear associations with the outcome were identified by likelihood ratio tests. If insufficient evidence for a linear association was present, continuous data were evaluated as categorical variables. Treatment variables related to

whether dogs received the following cardiac medications at the time of recruitment: pimobendan - yes/no; angiotensin converting enzyme (ACE) inhibitors - yes/no; and spironolactone - yes/no. Pairwise correlations among all explanatory variables were assessed to identify potential collinearity.[14]

Univariable Cox proportional hazard models evaluated associations between each explanatory variable and clinical progression to initiation of treatment with a potent diuretic or cardiac-related death. Explanatory variables significant at the 20% level in the univariable analysis were evaluated in the multivariable models.[14] Manual backward stepwise elimination model construction was used to identify variables with an independent association with the outcome (5% significance level). Each eliminated variable subsequently was 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 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.[14,15] The predictive ability of the models was evaluated by Harrell's C concordance statistic.[14] Martingale residuals were used to assess the functional form of the relationship between continuous variables and outcome.[15] To identify outliers and individuals with disproportionate influence, deviance residuals and likelihood displacements measures were plotted, respectively.[14,15]

Royston-Parmar flexible parametric models were used to predict survival probabilities and explore alternative approaches to modeling time-dependent effects (eg, where hazard ratios [HRs] for explanatory variables changed over time and did not meet the PH assumption).[16–18] 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.[17] Continuous variables were categorized in the multivariable parametric model for ease of interpretation and risk stratification. Selection of explanatory variables was as described for the Cox regression models, although variables dependent on diagnostic imaging (eg, pimobendan administration) were excluded as the flexible parametric model was developed to evaluate whether dogs could be risk stratified using data routinely obtained from taking a history, performing a physical examination, and measuring cardiac biomarker concentrations. 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.[17] After specification of the multivariable model, predicted survival probabilities (percentage of dogs not reaching the composite endpoint of initiation of diuretic treatment or cardiac-related death) at 1-year follow-up were estimated for different combinations of explanatory variable values in the model.[16,18]

2.1.3 | Sample size calculation

A priori sample size calculations estimated that approximately 100 events 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%. Using data derived from the VetCompass database (data not shown), it was estimated that 650-700 dogs would need to be recruited to the study for over an 18-month accrual period and a minimum 6 months follow-up, assuming a 10% loss to follow-up.[19]

3 | RESULTS

3.1 | Study population

Six-hundred and eighty-four dogs presumptively diagnosed with DMVD that did not receive a potent diuretic before or within 14 days after recruitment were included in the study. The breed most frequently recruited was the Cavalier King Charles Spaniel (CKCS; n = 231, 33.9%). The mean age at recruitment was 9.3 years (SD, 3.1 years) and the median body weight was 10.5 kg (IQR, 8.1-14.9 kg). Three-hundred and seventy (54.3%) dogs were male (Table 1). The median plasma concentration of NT-proBNP was 600 pmol/L (IQR, 324-1141 pmol/L) and the median plasma concentration of cTnI was 0.02 ng/mL (IQR, 0.01-0.03 ng/mL).

Five dogs were excluded from the time-to-event analysis because no follow-up data were available. For the 679 dogs included, the total analysis time was 9163 months. Median time at risk was 13.26 months (range, 0.07-30.74 months). One hundred and forty-three (21.1%) dogs reached the composite endpoint. The majority of dogs reaching an endpoint started treatment with a potent diuretic (n = 123, 86.0%). Twenty dogs died because of their heart disease without starting diuretic treatment (12 were euthanized and 8 died spontaneously).

3.2 | Prognostic risk factors for dogs with presumed stage B DMVD

3.2.1 | Cox regression analysis for variables associated with progression to initiation of potent diuretic treatment or cardiac death in dogs with presumed stage B DMVD

The following explanatory variables had some evidence of an association (P < .2) with the outcome and were evaluated in multivariable Cox regression models: age, body weight, breed, insurance status, plasma NT-proBNP, plasma cTnI, presence of cough or dyspnea, heart rhythm, heart rate, heart murmur intensity, administration of pimobendan, administration of an ACE inhibitor, administration of spironolactone, and having a musculoskeletal disorder. In the multivariable model, higher concentrations of plasma NT-proBNP, presence of cough, higher heart murmur intensities and heart rates, receiving pimobendan, and being a CKCS were independently associated with clinical progression (Table 2). Variables included in the final multivariable model also had strong associations (P < .001) with the outcome in univariable analysis. The Schoenfeld residuals test was statistically significant, suggesting that the PH assumption was violated. Plasma NT-proBNP was identified as having a time-dependent effect (P < .001). The magnitude of the HR between the highest and lowest categories of the biomarker decreased with time after sampling (ie, there was a weakening of the association between NT-proBNP and the outcome over time). Veterinary clinic was not

TABLE 1 Descriptive statistics for 684 dogs with presumed stage B degenerative mitral valve disease (DMVD) attending primary care veterinary practices in the United Kingdom

Variable	Category	Number (%), mean (SD), median (IQR)	Missing values, n (%)
Breed	CKCS	231 (33.9)	3 (0.4)
	Crossbred	104 (15.3)	
	Jack Russell Terrier	41 (6.0)	
	Shih Tzu	25 (3.7)	
	Cocker Spaniel	20 (2.9)	
	Border Collie	20 (2.9)	
	King Charles Spaniel	19 (2.8)	
	Chihuahua	17 (2.5)	
	Yorkshire Terrier	17 (2.5)	
	Other purebred	187 (27.5)	
Age (years)	Mean (SD)	9.3 (3.1)	3 (0.4)
Sex/neuter status	MN	232 (34.0)	2 (0.3)
	ME	138 (20.2)	
	FN	251 (36.8)	
	FE	61 (8.9)	
Bodyweight (kg)	Median (IQR)	10.5 (8.1-14.9)	37 (5.4)
Plasma NT-proBNP (pmol/L)	Median (IQR)	600 (324-1141)	6 (0.9)
Plasma cTnI (ng/mL)	Median (IQR)	0.02 (0.01-0.03)	5 (0.7)
Cough	No	486 (73.6)	24 (3.5)
	Yes	174 (26.4)	
Dyspnea	No	601 (93.2)	39 (5.7)
	Yes	44 (6.8)	
Exercise intolerance	No	503 (77.9)	38 (5.6)
	Yes	143 (22.1)	
Heart rhythm	Sinus arrhythmia	174 (27.9)	61 (8.9)
	Sinus rhythm	422 (67.7)	
	Other (non-sinus) arrhythmia	27 (4.3)	
Heart rate (bpm)	Mean (SD)	119 (23.1)	37 (5.4)
Heart murmur intensity	Soft (grade I/II)	256 (38.3)	15 (2.2)
	Moderate (III)	230 (34.4)	
	Loud (IV)	142 (21.2)	
	Thrilling (V/VI)	41 (6.1)	

Abbreviations: bpm, beats per minute; CKCS, Cavalier King Charles Spaniel; cTnI, cardiac troponin I; FE, female, entire; FN, female, neutered; IQR, interquartile range; NT-proBNP, N-terminal pro B-type natriuretic peptide; ME, male, entire; MN, male, neutered.

included in the final model because clustering was not significant. Harrell's C concordance statistic (0.79) suggested good model discrimination. Martingale residuals supported that heart rate could be included as an untransformed linear variable. When individuals with the largest deviance and influence were excluded from the model, the HRs changed, but the direction of associations and conclusions remained for all variables.

TABLE 2 Multivariable Cox regression model for variables associated with dogs with presumed stage B degenerative mitral valve disease (DMVD) reaching a composite endpoint of initiation of a potent diuretic or cardiac-related death. A time-dependent effect for plasma N-terminal pro B-type natriuretic peptide (NT-proBNP) (>1800 pmol/L versus <900 pmol/L) is included. Observations for 614 individuals

Variable	Category	Hazard ratio (95% CI)	P value
NT-proBNP (pmol/L)	<900	Baseline	<.001
	900-1800	1.83 (1.17-2.87)	
	>1800	10.60 (5.41-20.75)	
Cough	No	Baseline	.004
	Yes	1.75 (1.21-2.53)	
Heart rate (bpm)	Continuous	1.013[a] (1.005-1.021)	.002
Murmur intensity	Soft (grade I/II)	Baseline	<.001
	Moderate (III)	2.23 (1.32-3.76)	
	Loud (IV)	2.76 (1.60-4.78)	
	Thrilling (V/VI)	4.01 (2.14-7.50)	
CKCS	No	Baseline	.009
	Yes	1.60 (1.12-2.27)	
Pimobendan	No	Baseline	.001
	Yes	2.43 (1.50-3.94)	
Time-dependent effect NT-proBNP	>1800 pmol/L versus <900 pmol/L	−0.12[b] (−0.17 to −0.05)	<.001

Abbreviations: bpm, beats per minute; CI, confidence interval; CKCS, Cavalier King Charles Spaniel.
[a] Increase in hazard ratio per heartbeat per minute.
[b] log (HR) for the highest versus lowest levels of NT-proBNP decreased by 0.12 for each unit increase in time (month).

TABLE 3 Multivariable flexible parametric proportional hazards model for variables associated with dogs with presumed stage B degenerative mitral valve disease (DMVD) reaching a composite endpoint of initiation of a potent diuretic or cardiac-related death. A time-dependent effect for plasma N-terminal pro B-type natriuretic peptide (NT-proBNP) (>1800 pmol/L versus <900 pmol/L) is included. Observations for 614 individuals

Variable	Category	Hazard ratio (95% CI)	P value
NT-proBNP (pmol/L)	<900	Baseline	<.001
	900-1800	1.76 (1.12-2.77)	
	>1800	5.41 (3.47-8.41)	
Cough	No	Baseline	.005
	Yes	1.72 (1.18-2.49)	
Heart rate (bpm)	<110	Baseline	.02
	110-140	1.35 (0.85-2.15)	
	>140	1.94 (1.18-3.20)	
Murmur intensity	Soft (grade I/II)	Baseline	<.001
	Moderate (III)	2.38 (1.41-4.00)	
	Loud (IV)	2.70 (1.56-4.68)	
	Thrilling (V/VI)	4.45 (2.37-8.34)	
CKCS	No	Baseline	.01
	Yes	1.54 (1.08-2.19)	
Time-dependent effect NT-proBNP	>1800 pmol/L versus <900 pmol/L		<.001

Abbreviations: bpm, beats per minute; CI, confidence interval; CKCS, Cavalier King Charles Spaniel.

3.2.2 | Multivariable flexible parametric proportional hazards model: Factors associated with progression to initiation of potent diuretic treatment or cardiac death in dogs with presumed stage B DMVD

The flexible parametric model that best fit the data was a PH model with no interior knots. Explanatory variables significant in a multivariable flexible parametric PH model were as for the Cox regression model. With the exception of pimobendan administration, all significant variables were included in the parametric PH model. In agreement with the Cox regression model, a time-dependent effect for NT-proBNP was identified in the flexible parametric model (*P* < .001; Table 3). Figure 1 presents model predictions for the percentage of dogs not reaching the composite endpoint within 1 year for each combination of prognostic variables. Based on the values of explanatory variables, a range of prognoses was predicted for different hypothetical patient profiles. For example, a hypothetical group of dogs with DMVD that were not CKCSs, with moderate intensity heart murmurs, heart rates <110 bpm, no cough, and NT-proBNP <900 pmol/L had a low risk of disease progression; 93.7% (95% CI, 89.9%-96.0%) of dogs with these characteristics were predicted not to start diuretics or die because of their heart disease during the next 12 months. In contrast, for a hypothetical group of CKCSs with heart murmurs and thrills, heart rates >140 bpm, no cough, and NT-proBNP >1800 pmol/L, only

17.7% (95% CI, 4.6%-37.6%) were predicted not to reach these endpoints over the next 12 months.

4 | DISCUSSION

In our study, higher plasma NT-proBNP concentrations, heart rates and heart murmur intensities, being a CKCS, presence of cough, and receiving pimobendan were associated with a higher hazard of dogs with presumed stage B DMVD progressing to initiation of treatment with a potent diuretic or experiencing cardiac-related death.

4.1 | Cardiac biomarkers

A strong association was observed between plasma concentration of NT-proBNP and clinical progression in dogs with presumed stage B DMVD. This finding concurs with previous studies, which demonstrated that higher circulating NT-proBNP concentration was associated with developing CHF in dogs with preclinical DMVD.[3,4] In our study, NT-proBNP concentration had a time-dependent effect, whereby there was a weakening of the association with the outcome over time ie, the hazard ratio between categories including dogs with the highest [>1800 pmol/L] and lowest [<900 pmol/L] NT-proBNP concentrations decreased over time after sampling. This finding suggests that the biomarker provides the most useful information regarding prognosis in the initial months after sampling in dogs with preclinical DMVD, with less ability to predict outcome later on, thereby supporting the recommendation of serial sampling of individuals.[20-22]

Soft murmur Grade I/VI II/VI

Not CKCS, no cough

NT-proBNP (pmol/l)	Heart rate (bpm) <110	110-140	>140
<900	97.3 (95.1-98.5)	96.3	94.8
900-1800	95.3	93.7	91.0
>1800	87.8	83.9	77.6 (61.8-87.6)

Not CKCS, cough reported

NT-proBNP (pmol/l)	Heart rate (bpm) <110	110-140	>140
<900	95.4 (91.4-97.6)	93.8	91.2
900-1800	92.0	91.2	85.1
>1800	80.0	74.0	64.8 (43.8-79.6)

CKCS, no cough

NT-proBNP (pmol/l)	Heart rate (bpm) <110	110-140	>140
<900	95.8 (92.4-97.8)	94.4	92.1
900-1800	92.8	90.4	86.5
>1800	81.8	76.3	67.7 (47.7-81.5)

CKCS, cough reported

NT-proBNP (pmol/l)	Heart rate (bpm) <110	110-140	>140
<900	93.0 (86.2-96.5)	90.6	86.8
900-1800	88.0	84.1	77.9
>1800	70.9	62.8	51.2 (26.7-71.2)

Moderate murmur Grade III/VI

Not CKCS, no cough

NT-proBNP (pmol/l)	Heart rate (bpm) <110	110-140	>140
<900	93.7 (89.9-96.0)	91.5	88.1
900-1800	89.1	85.6	79.9
>1800	73.4	65.9	54.8 (36.9-69.6)

Not CKCS, cough reported

NT-proBNP (pmol/l)	Heart rate (bpm) <110	110-140	>140
<900	89.4 (82.1-93.8)	85.9	80.4
900-1800	82.1	76.67	68.1
>1800	58.8	48.8	35.6 (17.7-54.1)

CKCS, no cough

NT-proBNP (pmol/l)	Heart rate (bpm) <110	110-140	>140
<900	90.4 (84.5-94.1)	87.3	82.2
900-1800	83.7	78.7	70.8
>1800	62.1	52.5	39.6 (22.0-56.8)

CKCS, cough reported

NT-proBNP (pmol/l)	Heart rate (bpm) <110	110-140	>140
<900	84.1 (72.3-91.2)	79.2	71.5
900-1800	73.7	66.3	55.3
>1800	44.1	33.2	20.4 (6.3-40.1)

Loud murmur Grade IV/VI

Not CKCS, no cough

NT-proBNP (pmol/l)	Heart rate (bpm) <110	110-140	>140
<900	92.8 (87.9-95.8)	90.4	86.5
900-1800	87.7	83.8	77.5
>1800	70.3	62.2	50.5 (31.7-66.6)

Not CKCS, cough reported

NT-proBNP (pmol/l)	Heart rate (bpm) <110	110-140	>140
<900	88.0 (79.4-93.2)	84.2	78.0
900-1800	79.9	73.8	64.6
>1800	54.7	44.3	30.9 (14.4-49.1)

CKCS, no cough

NT-proBNP (pmol/l)	Heart rate (bpm) <110	110-140	>140
<900	89.2 (81.8-93.7)	85.7	80.0
900-1800	81.7	76.1	67.5
>1800	58.2	48.1	34.9 (17.9-52.4)

CKCS, cough reported

NT-proBNP (pmol/l)	Heart rate (bpm) <110	110-140	>140
<900	82.1 (68.8-90.2)	76.7	68.2
900-1800	70.7	62.6	51.0
>1800	39.5	28.5	16.4 (4.8-34.2)

Thrilling murmur Grade V/VI VI/VI

Not CKCS, no cough

NT-proBNP (pmol/l)	Heart rate (bpm) <110	110-140	>140
<900	88.5 (78.5-94.0)	84.8	78.8
900-1800	80.6	74.7	65.7
>1800	56.1	45.8	32.5 (11.9-55.1)

Not CKCS, cough reported

NT-proBNP (pmol/l)	Heart rate (bpm) <110	110-140	>140
<900	81.0 (66.1-89.9)	75.3	66.5
900-1800	69.1	60.7	48.7
>1800	37.0	26.2	14.5 (3.3-33.7)

CKCS, no cough

NT-proBNP (pmol/l)	Heart rate (bpm) <110	110-140	>140
<900	82.8 (69.6-90.7)	77.5	69.3
900-1800	71.7	63.9	52.4
>1800	41.0	30.0	17.7 (4.6-37.6)

CKCS, cough reported

NT-proBNP (pmol/l)	Heart rate (bpm) <110	110-140	>140
<900	72.4 (52.6-84.9)	64.6	53.3
900-1800	56.6	46.3	33.0
>1800	21.7	12.7	5.1 (0.6-18.0)

Legend (shading): Percentage of dogs not reaching composite endpoint within 1 year — <25 | 25 - <50 | 50 - <75 | 75 - <90 | ≥90

FIGURE 1 Risk estimates for the percentage of dogs with presumed stage B degenerative mitral valve disease (DMVD) not reaching the composite endpoint (cardiac death or initiation of a potent diuretic) within 1 year for each combination of explanatory variables. The 95% confidence intervals are presented in parentheses for dogs with the lowest and highest estimates for each table. CKCS, Cavalier King Charles Spaniel; bpm, beats per minute

Although marginal evidence was found for an association between plasma cTnI concentration and clinical progression in the univariable analysis, insufficient evidence was found for an association in the multivariable analysis. In contrast, previous studies identified strong associations between cTnI and mortality in cohorts including dogs with CHF caused by DMVD.[10,21,23] The subset of dogs in our study was likely to represent a group with less severe cardiac disease (on average) than cohorts that also included dogs prescribed CHF treatment. Serial measurements of cTnI disclosed an increase in this biomarker within the 6 months preceding death in dogs that died because of their cardiac disease,[21] highlighting that cTnI is increased in the most advanced stages of DMVD. Because cTnI is not a specific biomarker for cardiac disease, the increase in this marker in presumed stage B dogs was possibly more likely to be a result of noncardiac causes than advanced cardiac disease, thus explaining a lack of association between cTnI and DMVD clinical progression in our study.

4.2 | Presenting signs, clinical findings, and signalment

Having a higher heart rate was associated with an increased hazard of presumed stage B dogs progressing to initiation of potent diuretic treatment or cardiac-related death. This finding concurs with previous studies that observed associations between increased heart rate and adverse cardiac outcome in dogs with preclinical DMVD.[2,6] Moreover, studies evaluating the prognostic value of heart rate in dogs with a wider range of DMVD severity (including those diagnosed with CHF)[10,21,24,25] reported that having a higher heart rate was associated with an increased hazard of adverse outcome. Although having an increased heart rate is associated with more advanced DMVD,[26] this finding is not specific for cardiac disease and can be influenced by a number of factors including emotional state and noncardiac disease.[27–29]

In our study, dogs with heart murmur intensities grade III or above had a higher hazard of clinical progression than those with softer murmurs. This observation concurs with the findings of a randomized controlled trial that observed an association between higher heart murmur intensity and shorter time until developing CHF in CKCSs with preclinical DMVD.[7] In agreement, another study reported that CKCSs with heart murmur intensities ≥III/VI had an increased hazard of developing CHF than did those with lower intensity murmurs.[6] Moreover, heart murmur intensity has been found to correlate with DMVD disease severity[30,31] and was associated with cardiac-related death in another cohort of dogs with DMVD attending a UK research clinic.[32] Dogs with higher intensity murmurs in our study thus might represent dogs with more advanced preclinical DMVD that were more likely to subsequently receive potent diuretics or die of their heart disease. Although descriptors of heart murmur intensity grades were provided on data collection forms, grading heart murmur intensity is subjective and can be influenced by factors such as body condition, emotional state, and how easy a dog is to auscultate.[33] Our study evaluated heart murmur intensity measured on a single occasion for each dog, thus analyses of intraobserver and interobserver variation were not performed.

The presence of cough also was associated with increased hazard of clinical progression. In agreement, a previous study reported an association between presence of cough at initial examination and subsequent progression of ACVIM-HD class in dogs with stage B DMVD.[5] Coughing can be triggered by bronchial compression as a result of atrial enlargement in more advanced preclinical DMVD, preceding the onset of CHF.[34] Alternatively, the observed association might have occurred if veterinarians elected to start treatment with a potent diuretic

because of the presence of a cough, without confirming the diagnosis of CHF. A cough in a dog with a murmur is not considered sufficient reason to start CHF treatment, and radiographic evaluation is recommended to avoid inappropriate use of diuretics.[1,13,34] However, only 71 (57.7%) dogs starting diuretic treatment had evidence that radiography was undertaken, showing that veterinary practitioners frequently prescribed diuretics without radiographic confirmation of CHF.

Cavalier King Charles Spaniels had a higher hazard of starting diuretic treatment or experiencing cardiac-related death compared with a category including all other breeds. This observation may be a result of breed differences in disease progression. Alternatively, knowledge of breed predispositions for DMVD might influence the likelihood of dog owners seeking veterinary care or veterinarians prescribing CHF treatment for symptomatic CKCSs compared to other breeds showing similar clinical signs.

4.3 | Treatment

Pimobendan administration was associated with increased hazard of reaching the composite endpoint in our study. Strong evidence indicates that pimobendan is beneficial in extending survival in a subset of dogs with advanced preclinical DMVD and cardiomegaly confirmed by diagnostic imaging (ie, left atrial-to-aortic ratio ≥1.6, normalized left ventricular internal diameter in diastole ≥1.7, and vertebral heart sum >10.5).[2] Results of our study were therefore likely to reflect that pimobendan was more frequently prescribed to dogs with more advanced stage B DMVD and a worse prognosis. Echocardiographic and radiographic assessment of cardiac size currently is required to determine whether a dog would benefit from pimobendan.[2] This factor is reflected in our study, because exploratory analyses identified a strong association between pimobendan administration and undergoing diagnostic imaging in presumed stage B dogs (Chi squared test, $P < .001$). Because we aimed to identify the predictive value of factors not dependent on diagnostic imaging, pimobendan administration was excluded from the flexible parametric model.

4.4 | General strengths and limitations

Our study used a prospective cohort design to provide further insights into factors associated with disease progression in dogs with presumed stage B DMVD. Several clinical measurements identified in our study (NT-proBNP, heart rate, and heart murmur intensity) also were predictive of mortality in cohorts of dogs with different stages of DMVD,[10,21,25,32,35] supporting the prognostic value of these measurements. Some prognostic factors in our study might reflect changes occurring earlier in the clinical course of the disease, whereas prognostic factors associated with mortality in heterogeneous cohorts (but not with clinical progression in presumed stage B dogs) might reflect changes occurring during the later stages of DMVD. For example, a strong association was found between plasma NT-proBNP concentration and clinical progression in presumed stage B dogs, but a significant association between cTnI and this outcome was not observed. Conversely, associations between both biomarkers and mortality have been reported for cohorts including dogs with CHF because of DMVD.[10,21,23] This is consistent with the findings of

another study, in which NT-proBNP measurements increased at an earlier stage in the natural progression of the disease than did circulating cTnI.[21]

The predictions derived from the flexible parametric model highlight that using information from a number of factors is more informative when forming a prognosis than measuring a single factor. The prognostic factors identified in our study, therefore, should be evaluated in combination rather than in isolation. Risk stratification based on disease severity could facilitate decision-making and advice to owners when managing dogs with DMVD in practice.[2,21] For example, dogs identified as having more advanced presumed stage B DMVD might receive the greatest benefit from further diagnostic investigations and subsequent treatment, whereas a "watch and wait" approach might be more appropriate for a dog belonging to a low risk group. In addition, dogs predicted to have a high risk of clinical progression might benefit from more vigilant monitoring (eg, measuring sleeping respiratory rate). Although risk can be estimated based on the values of factors identified in our study (eg, the percentage of dogs with those values not reaching the composite endpoint within 12 months), it is not possible to say whether an individual dog will reach the composite endpoint and uncertainty will remain in the prognosis for an individual dog.

Our study analyzed data from a large cohort of dogs attending 73 primary care practices located across the United Kingdom. However, it is unclear whether this sample of dogs was representative of all dogs with DMVD attending UK primary care practices. For example, clinicians might have more confidence making a presumptive diagnosis of DMVD in dogs of certain breeds that have presented with heart murmurs typical of mitral regurgitation because of preconceived knowledge of breed predispositions. Furthermore, the results of our study might not be generalizable to populations of dogs with presumed DMVD in other geographical locations.

To allow the results of our study to be applied to settings in which diagnostic imaging is not routinely performed, echocardiographic confirmation of the presumptive DMVD diagnosis was not an inclusion criterion. It is therefore possible that dogs erroneously diagnosed with DMVD were included in our study. However, participating clinicians were provided with information on typical presentation and risk factors for DMVD to improve the accuracy of presumptive diagnoses and recruited cases largely consisted of small to medium-sized older dogs, a population known to have a high prevalence of DMVD.[36,37] Moreover, the breed, body weight, and age distributions of the dogs in our study were similar to those reported in previous studies restricted to dogs with DMVD confirmed by echocardiography.[21,32] Our study included dogs that were not receiving potent diuretics at recruitment and that were assumed to have stage B DMVD, defined as "patients with structural heart disease (e.g. the typical murmur of mitral valve regurgitation is present), but that have never developed clinical signs caused by heart failure."[1] Because potent diuretics are essential for dogs with CHF,[1] it is unlikely that dogs included in our study had stage C or D DMVD at recruitment. However, the rationale behind prescribing diuretics was not explored and some dogs without CHF may have been inappropriately prescribed diuretics and excluded from the study.

Misclassification or measurement error of explanatory variables also was possible, and evaluating physical examination findings and the presence or absence of clinical signs is inherently subjective. One measurement for each explanatory variable, recorded at recruitment, was used in our study. However, some variables, such as plasma biomarker concentrations, might have changed over time (time-varying risk factors). An alternative approach would have been to record serial measurements of explanatory variables and to develop a Cox regression model including time-varying covariates.[38] However, there might be practical difficulties associated with collecting multiple measurements of variables at specified time points. Another limitation of our study is that selection bias might have arisen because of censoring and missing data. Right-censored dogs may have had different outcomes than those with more complete follow-up. Complete case analysis was used in the multivariable models (dogs with missing data for any of the final model variables were excluded), and thus bias might have been introduced if data were not missing randomly.[14]

In addition, limitations relating to the outcome of interest should be borne in mind when interpreting our results. The majority of presumed stage B dogs reaching an endpoint started treatment with a potent diuretic, rather than dying of their heart disease without initiation of diuretic treatment. It is unclear whether the composite endpoint was a reliable proxy for advancing disease severity, and it is possible that some explanatory variables (eg, presence of cough or loud heart murmur) were the reason that veterinarians started treatment with diuretics, rather than being predictive of future onset of CHF or cardiac death. This possibility could have resulted in differential misclassification and an overestimate in the measure of association. However, dogs starting diuretics within 14 days of recruitment were excluded, thereby limiting misclassification bias associated with clinicians initiating diuretic treatment because of clinical findings at the initial visit. Although guidelines for the management of DMVD recommend performing thoracic radiography to diagnose CHF,[1] this procedure was not consistently undertaken before initiating diuretic treatment, and starting treatment with a potent diuretic was based on the clinicians' perceptions of heart disease progression in many cases. Radiographic confirmation of CHF would have improved the validity of the results, but the lack of these data reflects the reality of primary care practice. Another UK study also reported that diagnostic imaging was not undertaken often when evaluating dogs with suspected CHF in primary care practice.[39] It is unclear why thoracic radiography was not performed more frequently in these cases, and future research exploring clinical decision making when managing dogs with DMVD and the reliability of CHF diagnoses in primary care practice is warranted. Factors that may deter clinicians from performing thoracic radiographs might include owner inability to pay for diagnostic tests and a reluctance to sedate uncooperative patients for radiography in a country where manual restraint for radiography is discouraged. Finally, relevant treatment data might not be captured in EPRs if owners obtained diuretics from online pharmacies or other veterinary practices. However, despite limitations relating to the outcome, several key findings were consistent with those reported in previous studies that used radiography to assess clinical progression in dogs with preclinical DMVD.[2-4,6,7]

Our results cannot be used to differentiate stages B1 and B2 DMVD, or be used to make treatment recommendations. However, it might be possible to develop a scoring system based on variables obtained from routine history, physical examination, and cardiac biomarker concentrations to help distinguish between the preclinical stages of DMVD and even potentially select animals that are most likely to benefit from treatment.[40] The prognostic variables identified in our study, that are likely to reflect more advanced disease, could be worthy candidates to evaluate in such work.

As described above, it is unclear whether the evaluated composite endpoint was a reliable proxy for advancing DMVD. The observed associations between explanatory variables and the outcome could have arisen if veterinarians elected to start treatment with a potent diuretic because of preconceptions relating to breed predispositions, historical, or physical examination findings (eg, the presence of cough and high intensity murmur), rather than these factors being predictive of future disease progression. Validation of the prognostic model in a different population would therefore be of value,[17] particularly if disease progression was supported by diagnostic imaging in an independent test set.

In conclusion, data obtained from taking a history and performing a physical examination could be used in combination with NT-proBNP concentrations to risk stratify dogs with presumed stage B DMVD managed by primary care practitioners. Dogs identified as having a high risk of disease progression might benefit from more frequent monitoring, further diagnostic evaluation, or referral to a veterinary cardiologist.

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_01758).

HUMAN ETHICS APPROVAL DECLARATION

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

ORCID

Madeleine J. Mattin ⓘ https://orcid.org/0000-0003-0346-3767
David C. Brodbelt ⓘ https://orcid.org/0000-0001-5628-4194
Adrian Boswood ⓘ https://orcid.org/0000-0003-1795-4364

REFERENCES

1. Atkins C, Bonagura J, Ettinger S, et al. Guidelines for the diagnosis and treatment of canine chronic valvular heart disease. *J Vet Intern Med*. 2009;23:1142-1150.
2. Boswood A, Haggstrom J, Gordon SG, et al. Effect of pimobendan in dogs with preclinical myxomatous mitral valve disease and cardiomegaly: the EPIC study—a randomized clinical trial. *J Vet Intern Med*. 2016;30:1765-1779.
3. Reynolds CA, Brown DC, Rush JE, et al. Prediction of first onset of congestive heart failure in dogs with degenerative mitral valve disease: the PREDICT cohort study. *J Vet Cardiol*. 2012;14:193-202.
4. Chetboul V, Serres F, Tissier R, et al. Association of plasma N-terminal pro-B-type natriuretic peptide concentration with mitral regurgitation severity and outcome in dogs with asymptomatic degenerative mitral valve disease. *J Vet Intern Med*. 2009;23:984-994.
5. Borgarelli M, Crosara S, Lamb K, et al. Survival characteristics and prognostic variables of dogs with preclinical chronic degenerative mitral valve disease attributable to myxomatous degeneration. *J Vet Intern Med*. 2012;26:69-75.
6. Eriksson AS, Haggstrom J, Pedersen HD, et al. Increased NT-proANP predicts risk of congestive heart failure in Cavalier King Charles spaniels with mitral regurgitation caused by myxomatous valve disease. *J Vet Cardiol*. 2014;16:141-154.
7. Kvart C, Haggstrom J, Pedersen HD, et al. Efficacy of enalapril for prevention of congestive heart failure in dogs with myxomatous valve disease and asymptomatic mitral regurgitation. *J Vet Intern Med*. 2002; 16:80-88.
8. Gordon SG, Saunders AB, Hariu CD, Boggess MM, Miller MW. Retrospective review of carvedilol administration in 38 dogs with preclinical chronic valvular heart disease. *J Vet Cardiol*. 2012;14:243-252.
9. Oyama MA. Using cardiac biomarkers in veterinary practice. *Vet Clin North Am Small Anim Pract*. 2013;43:1261-1272, vi.
10. Mattin MJ, Boswood A, Church DB, Brodbelt DC. Prognostic factors in dogs with presumed degenerative mitral valve disease attending primary-care veterinary practices in the United Kingdom. *J Vet Intern Med*. 2018. [epub ahead of print].
11. Schober KE, Hart TM, Stern JA, et al. Detection of congestive heart failure in dogs by Doppler echocardiography. *J Vet Intern Med*. 2010; 24:1358-1368.
12. VetCompass. Veterinary Companion Animal Surveillance System; 2018. Available at: http://www.rvc.ac.uk/VetCompass. Accessed March 1, 2018.
13. Borgarelli M, Haggstrom J. Canine degenerative myxomatous mitral valve disease: natural history, clinical presentation and therapy. *Vet Clin North Am Small Anim Pract*. 2010;40:651-663.
14. Dohoo IR, Martin SW, Stryhn H. *Veterinary Epidemiologic Research*. 2nd ed. Charlotte, PEI: VER, Inc.; 2009.
15. StataCorp. *Stata: Release 14. Statistical Software*. College Station, TX: StataCorp LP; 2013.
16. Royston P, Parmar MK. Flexible parametric proportional-hazards and proportional-odds models for censored survival data, with application to prognostic modelling and estimation of treatment effects. *Stat Med*. 2002;21:2175-2197.
17. Royston P, Lambert PC. *Flexible Parametric Survival Analysis Using Stata: Beyond the Cox Model*. College Station, TX: Stata Press; 2011:xxvi, 347 p.
18. Lambert PC, Royston P. Further development of flexible parametric models for survival analysis. *Stata J*. 2009;9:265-290.
19. Collett D. *Modelling survival data in medical research*. 2nd ed. Boca Raton, FL: Chapman & Hall/CRC; 2003.
20. Winter RL, Saunders AB, Gordon SG, Buch JS, Miller MW. Biologic variability of N-terminal pro-brain natriuretic peptide in healthy dogs and dogs with myxomatous mitral valve disease. *J Vet Cardiol*. 2017; 19:124-131.
21. Hezzell MJ, Boswood A, Chang YM, Moonarmart W, Souttar K, Elliott J. The combined prognostic potential of serum high-sensitivity cardiac troponin I and N-terminal pro-B-type natriuretic peptide concentrations in dogs with degenerative mitral valve disease. *J Vet Intern Med*. 2012;26:302-311.
22. Ruaux C, Scollan K, Suchodolski JS, Steiner JM, Sisson DD. Biologic variability in NT-proBNP and cardiac troponin-I in healthy dogs and dogs with mitral valve degeneration. *Vet Clin Pathol*. 2015;44:420-430.
23. Linklater AKJ, Lichtenberger MK, Thamm DH, Tilley L, Kirby R. Serum concentrations of cardiac troponin I and cardiac troponin T in dogs with class IV congestive heart failure due to mitral valve disease. *J Vet Emerg Crit Care (San Antonio)*. 2007;17:243-249.
24. Haggstrom J, Boswood A, O'Grady M, et al. Effect of pimobendan or benazepril hydrochloride on survival times in dogs with congestive heart failure caused by naturally occurring myxomatous mitral valve disease: the QUEST study. *J Vet Intern Med*. 2008;22:1124-1135.
25. Borgarelli M, Savarino P, Crosara S, et al. Survival characteristics and prognostic variables of dogs with mitral regurgitation attributable to myxomatous valve disease. *J Vet Intern Med*. 2008;22:120-128.
26. Uechi M, Shimizu A, Mizuno M. Heart rate modulation by sympathetic nerves in dogs with heart failure. *J Vet Med Sci*. 2002;64: 1023-1029.
27. Katayama M, Kubo T, Mogi K, Ikeda K, Nagasawa M, Kikusui T. Heart rate variability predicts the emotional state in dogs. *Behav Processes*. 2016;128:108-112.
28. Lopez-Alvarez J, Boswood A, Moonarmart W, et al. Longitudinal electrocardiographic evaluation of dogs with degenerative mitral valve disease. *J Vet Intern Med*. 2014;28:393-400.
29. Hezzell MJ, Dennis SG, Humm K, et al. Relationships between heart rate and age, bodyweight and breed in 10,849 dogs. *J Small Anim Pract*. 2013;54:318-324.
30. Haggstrom J, Kvart C, Hansson K. Heart sounds and murmurs: changes related to severity of chronic valvular disease in the Cavalier King Charles spaniel. *J Vet Intern Med*. 1995;9:75-85.
31. Ljungvall I, Rishniw M, Porciello F, Ferasin L, Ohad DG. Murmur intensity in small-breed dogs with myxomatous mitral valve disease reflects disease severity. *J Small Anim Pract*. 2014;55:545-550.
32. Lopez-Alvarez J, Elliott J, Pfeiffer D, et al. Clinical severity score system in dogs with degenerative mitral valve disease. *J Vet Intern Med*. 2015;29:575-581.
33. Pedersen HD, Haggstrom J, Falk T, et al. Auscultation in mild mitral regurgitation in dogs: observer variation, effects of physical maneuvers, and agreement with color Doppler echocardiography and phonocardiography. *J Vet Intern Med*. 1999;13:56-64.
34. Ferasin L, Crews L, Biller DS, Lamb KE, Borgarelli M. Risk factors for coughing in dogs with naturally acquired myxomatous mitral valve disease. *J Vet Intern Med*. 2013;27:286-292.
35. Moonarmart W, Boswood A, Luis Fuentes V, et al. N-terminal pro B-type natriuretic peptide and left ventricular diameter independently predict mortality in dogs with mitral valve disease. *J Small Anim Pract*. 2010;51:84-96.

36. Serfass P, Chetboul V, Sampedrano CC, et al. Retrospective study of 942 small-sized dogs: prevalence of left apical systolic heart murmur and left-sided heart failure, critical effects of breed and sex. *J Vet Cardiol*. 2006;8:11-18.

37. Parker HG, Kilroy-Glynn P. Myxomatous mitral valve disease in dogs: does size matter? *J Vet Cardiol*. 2012;14:19-29.

38. Dekker FW, de Mutsert R, van Dijk PC, Zoccali C, Jager KJ. Survival analysis: time-dependent effects and time-varying risk factors. *Kidney Int*. 2008;74:994-997.

39. Davies T, Everitt S, Cobb M. Variation in the management of congestive cardiac failure in dogs. *Vet Rec*. 2015;176:435.

40. Boswood A. Ten-minute chat. *Vet Rec*. 2017;180:i-ii.