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The predictive value of clinical, radiographic, echocardiographic variables and cardiac biomarkers for assessing risk of the onset of heart failure or cardiac death in dogs with preclinical myxomatous mitral valve disease enrolled in the DELAY study

M. Borgarelli, DVM, PhD[a,*], L. Ferasin, DVM, PhD[b], K. Lamb, PhD[c], D. Chiavegato, DVM, PhD[d], C. Bussadori, DVM, MD, PhD[e], G. D'Agnolo, DVM[f], F. Migliorini, DVM[g], M. Poggi, DVM[h], R.A. Santilli, DVM, PhD[i], E. Guillot, DVM[j], C. Garelli-Paar, Pharm.D, MS[j], R. Toschi Corneliani, DVM, MS[k], F. Farina, DVM, MS[l], A. Zani, DVM[m], M. Dirven, DVM[n], P. Smets, DVM, PhD[n], C. Guglielmini, DVM, PhD[o], P. Oliveira, DVM[p], M. Di Marcello, DVM, PhD[q], F. Porciello, DVM[r], S. Crosara, DVM, PhD[s], P. Ciaramella, DVM[t], D. Piantedosi, DVM, PhD[t], S. Smith, DVM[u], S. Vannini, DVM[e], E. Dall'Aglio, DVM[v], P. Savarino, DVM, PhD[w], C. Quintavalla, DVM, PhD[x], M. Patteson, DVM, PhD[y], J. Silva, DVM[e], C. Locatelli, DVM, PhD[z], M. Baron Toaldo, DVM, PhD[aa]

[a] *Department Small Animal Clinical Science, Virginia Maryland College of Veterinary Medicine, Blacksburg, VA, USA*
[b] *Specialist Veterinary Cardiology Consultancy Ltd, Alton, Hampshire, UK*
[c] *Lamb Statiscal Consulting and Scientific Writing LLC, West St. Paul, MN, USA*
[d] *Clinica Veterinaria Arcella, Padova, Italy*
[e] *Clinica Veterinaria Gran Sasso, Milan, Italy*
[f] *Trieste, Italy*

* Corresponding author.
E-mail address: mborgarelli@gmail.com (M. Borgarelli).

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[g] *Ospedale Veterinario Croce Azzura, Roma, Italy*
[h] *Centro Veterinario Imperiese, Imperia, Italy*
[i] *Clinica Veterinaria Malpensa, Samarate Varese, Italy*
[j] *Ceva Santé Animale, Libourne, France*
[k] *Ospedale Veterinario San Francesco, Milan, Italy*
[l] *Ambulatorio Veterinario del Parco Margherita, Naples, Italy*
[m] *Clinica Cardiovet, Livorno, Italy*
[n] *Dierenkliniek Rijen, Rijen, the Netherlands*
[o] *Dipartimento di Medicina Animale, Produzione e Salute, Università di Padova, Legnaro, Padova, Italy*
[p] *Davies Veterinary Specialists Ltd, Higham Gobion, Hertfordshire, UK*
[q] *Centro Veterinario Cellatica, Cellatica, Brescia, Italy*
[r] *Dipartimento di Medicina Veterinaria, Università degli Studi di Perugia, Perugia, Italy*
[s] *Clinica Veterinaria CMV, Varese, Italy*
[t] *Dipartimento di Medicina Veterinaria e Produzioni Animali, Università degli Studi di Napoli Federico II, Naples, Italy*
[u] *Sarah Smith Cardiology Ivy Court, Willington, UK*
[v] *Clinica Veterinaria Milano Sud, Peschiera Borromeo, Milano, Italy*
[w] *Facoltà di Medicina Veterinaria di Torino, Ospedale Didattico Veterinario della Facoltà, Sezione Clinica Medica, Grugliasco, Turin, Italy*
[x] *Dipartimento di Scienze Mediche Veterinarie, Università di Parma, Parma, Italy*
[y] *Heartvets, The Animal Hospital Stinchcombe, Dursley, UK*
[z] *Dipartimento di Medicina Veterinaria, Università degli Studi di Milano, Milan, Italy*
[aa] *Dipartimento di Scienze Mediche Veterinarie, Alma Mayor Studiorum, Università di Bologna, Ozzano Emilia, Italy*

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KEYWORDS
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Abstract *Objectives:* To identify the predictive value on time to onset of heart failure (HF) or cardiac death of clinical, radiographic, and echocardiographic variables, as well as cardiac biomarkers N-terminal pro brain natriuretic peptide (NT-proBNP) and cardiac troponin I in dogs with preclinical myxomatous mitral valve disease (MMVD).
Animals: One hundred sixty-eight dogs with preclinical MMVD and left atrium to aortic root ratio $\geq$1.6 (LA:Ao) and normalized left ventricular end-diastolic diameter $\geq$1.7 were included.
Methods: Prospective, randomized, multicenter, single-blinded, placebo-controlled study. Clinical, radiographic, echocardiographic variables and plasma cardiac biomarkers concentrations were compared at different time points. Using receiving operating curves analysis, best cutoff for selected variables was identified and the risk to develop the study endpoint at six-month intervals was calculated.
Results: Left atrial to aortic root ratio >2.1 (hazard ratio [HR] 3.2, 95% confidence interval [95% CI] 1.9—5.6), normalized left ventricular end-diastolic diameter > 1.9 (HR: 6.3; 95% CI: 3.3—11.8), early transmitral peak velocity (E peak) > 1 m/sec (HR: 3.9; 95% CI: 2.3—6.7), and NT-proBNP > 1500 ρmol/L (HR: 5.7; 95% CI: 3.3—9.5) were associated with increased risk of HF or cardiac death. The best fit model to predict the risk to reach the endpoint was represented by the plasma NT-proBNP concentrations adjusted for LA:Ao and E peak.
Conclusions: Logistic and survival models including echocardiographic variables and NT-proBNP can be used to identify dogs with preclinical MMVD at higher risk to develop HF or cardiac death.

List of abbreviations

95% CI	confidence interval
DELAY	DELay of Appearance of sYmptoms of canine degenerative mitral valve disease treated with spironolactone and benazepril
E peak	early transmitral peak velocity
HF	heart Failure
HR	hazard ratio
LA:Ao	left atrium to aortic root ratio
LVEDDn	normalized left ventricular end diastolic diameter
MMVD	mxomatous mitral valve disease
NT-proBNP	N-terminal pro brain natriuretic peptide
ROC	receiver operating characteristic

Introduction

Dogs affected by mitral regurgitation caused by preclinical myxomatous mitral valve disease (MMVD) represent a heterogeneous group of patients with different degree of cardiac remodeling and variable prognosis [1]. Several studies have reported the value of clinical, radiographic, and echocardiographic variables in identifying MMVD dogs at higher risk to develop heart failure (HF) or cardiac death [1—5]. Cardiac biomarkers, such as N-terminal pro brain natriuretic peptide (NT-proBNP) and cardiac troponin I, have also been associated with disease severity and increased risk of developing HF in dogs with MMVD [6—12]. However, there are only a few controlled studies that have prospectively investigated the prognostic values of clinical, radiographic, and echocardiographic variables and cardiac biomarker concentrations in identifying the risk for dogs affected by preclinical MMVD to develop HF or cardiac death [5]. The 'DELay of Appearance of sYmptoms of canine degenerative mitral valve disease treated with spironolactone and benazepril (DELAY) study' was a prospective, multicenter, single-blinded, randomized, placebo-controlled study aimed to assess the efficacy of the combined treatment on delaying onset of HF or cardiac death in dogs with preclinical MMVD and cardiomegaly [13]. Dogs enrolled in the DELAY study had to be rechecked at established time points after initial enrollment, and for this reason, this population was ideal to assess the prognostic value of clinical and diagnostic variables in identifying dogs with higher risk to die of cardiac disease or to progress into HF. The aim of this study was to investigate the prognostic value of clinical, radiographic, and echocardiographic variables, as well as NT-proBNP and cardiac troponin I concentrations to identify the risk to the development of HF or experience cardiac death at 6, 12, 18, 24, 30, 36, and 42 months after enrollment in this population of dogs with preclinical MMVD and cardiomegaly.

Animals, material, and methods

Study design

The DELAY study was a prospective, multicenter, single-blinded, randomized, placebo-controlled study [13]. Complete and detailed description of the study design is reported in the published article [13]. Briefly, dogs with MMVD and left atrial to aortic root ratio (LA:Ao) $\geq$ 1.6 and normalized left ventricular end diastolic diameter (LVEDDn) $\geq$ 1.7 were included. Dogs were excluded if they had cardiovascular disease other than MMVD, significant concurrent disease, systemic or pulmonary hypertension, atrial fibrillation, previous treatment with cardiovascular drugs lasting more than 2 weeks or in the 2 weeks before inclusion. Dogs with hemodynamically insignificant tricuspid regurgitation were eligible for enrollment in the study. Eight mandatory visits were scheduled every 6 months from day 0 to 42 months. Additional visits were allowed for individual medical concerns. At each visit, dogs underwent a complete clinical evaluation and thoracic radiographs. In addition, a urine sample for measurement of urinary aldosterone-creatinine ratio and a blood sample were collected for assessment of creatinine, NT-proBNP, and cardiac troponin. An echocardiographic examination was mandatory for visit 1, 3, 5, 7, and 8. However, the investigators had the option to perform an additional examination at the other visits, and most dogs included in the study had these additional examinations performed. The population of this study included animals that were enrolled in both the treatment and placebo group of the DELAY study, as there was no significant difference identified on the primary endpoint between groups.

Clinical evaluation

At inclusion, dogs' characteristics (breed, age, sex, body weight, and body condition score) were recorded. Clinical history and physical findings were documented at each visit.

Thoracic radiographs

Thoracic radiographs were performed for assessing cardiac size by the vertebral heart scale method [14]. They were also taken, whenever possible, for confirming the presence of pulmonary edema at the time dogs presented onset of clinical signs. Radiographic evidence of cardiogenic pulmonary edema was defined by the presence of pulmonary venous congestion and unstructured interstitial pattern or alveolar pattern that could not be explained by other medical conditions (e.g. pneumonia).

Echocardiography

Echocardiographic examinations were performed on unsedated dogs as per the established standard for veterinary cardiology [15]. All measurements were taken from at least three consecutive cardiac cycles, and the mean was recorded. The following measurements were taken from the right parasternal short-axis view: LA:Ao obtained in two-dimensional view as described by Hanson et al. 2002 [16], and left ventricular diameter measured in M-mode from the short axis with the leading edge to inner edge method at the level of the tips of papillary muscles. Left ventricular normalized dimensions were calculated as described in the study by Cornell et al. [17]. Early (E peak) and late A transmitral inflow velocities were assessed by spectral pulsed-wave Doppler from the left four-chamber apical view with the Doppler gate positioned at the tip of the mitral valve leaflets.

Endpoint

The endpoint was the time to cardiac death or first occurrence of HF defined by the presence of either dyspnea and/or tachypnea ($\geq$36 breaths/min at rest) that could not be explained by another disease based on clinical judgment by the investigator [18]. Radiographs were performed at the last visit if the dog was presented at the investigator clinic and if its health condition allowed it. They were used as a basis to either confirm pulmonary edema or to exclude any other reason associated with the observed clinical signs. To confirm the first onset of HF, radiographs were first evaluated by the investigator, and in case of detection of pulmonary edema, this had to be confirmed by the lead investigator (M.B.) within 7 days. In case of discrepancy between the two evaluators (investigator and M.B.), a third opinion from a certified boarded radiologist, blinded to dog clinical status, had to be requested.

In case where it was impossible to obtain radiographs because of critical health conditions or refusal by the owner to perform this examination, or if the dog was not directly seen by the investigator because of a sudden clinical deterioration (death at home, visit to their general practitioner), diagnosis of HF was determined by the investigator based on observed clinical signs and/or information reported by the owner or by other veterinarians. Details about validation of the primary endpoint were previously published [13].

Statistical methods

The statistical analyses were composed of four main components carried out in order, with one building on the previous model. These are briefly described as (1) ordinary Cox model, (2) extended longitudinal Cox model with interval censoring, (3) receiver operating characteristic (ROC) curve analysis to define categorical variable cutoffs based on continuous variables used in the longitudinal Cox model, and (4) the longitudinal Cox model retaining the full follow-up survival time in the study for all time intervals, but right censoring events at 6-month intervals, providing event-free survival analysis after a specified time period. Analyses were performed with statistical software[ab], where a $p < 0.05$ was deemed significant.

Conventional Cox model

The conventional Cox model time-to-event analyses were carried out exclusively in univariable by way of Kaplan—Meier product limit estimates and Cox semiparametric regression models used to generate unadjusted survival curves and hazard ratios (HRs). In this approach, the date of study entry was the time of the enrollment visit on a single visit as previously published [13], and data obtained at this time were evaluated in what is referred to as baseline analyses. The extended longitudinal Cox model was built on the ordinary Cox model. While continuing to use the existing endpoints from the ordinary Cox model, the approach was extended to control for individual change of variables over time by the way of interval censoring. The surveillance time (survival) was the time between the entrance of the study to the composite endpoint (HF or cardiac death) or censoring.

[ab] PLAN procedure in SAS, SAS Institute, Cary NC, 2016.

Extended Cox model

Because some patients were examined at times between scheduled clinic visits, neither the conventional Cox model nor a series of logistic regressions could address varying intervals; hence, an extended Cox model with interval censoring was used. This model allows for the most up-to-date variable value, while still using instantaneous risk of the same composite endpoint associated with the Cox model approach. For example, in contrast to the fixed variable (e.g. the variable NT-proBNP), in the baseline analysis, the value of the variable may change over the course of the study at different visits which may not be measured at consistent times (e.g. repeated measurements of the variable NT-proBNP). Specifically, although in most cases 'visit 2' may be at 12 months exactly for most patients, there could be some deviation or missed visits. The extended Cox model (interval censoring) was used to accommodate possible deviation and congruency of visit number to time. To address this nuance of time-varying covariates, two models were constructed, one evaluating hazards of baseline values (baseline HR) based on a single visit (previously described) and the currently described longitudinal approach assessing the HR with change in time and measured values of respective variables (longitudinal HR) for both univariable and multivariable in terms of selection, as well as goodness of fit, as described by Eriksson [19]. The multiple visits with refreshed or updated data are hereafter referred to as the longitudinal analyses, where the covariates are the same throughout the longitudinal analysis, but the HR takes into account the changes in serially measured covariates and ultimately analyzes the most up-to-date value. In summary, the baseline analysis only considers the initial baseline visit for survival time, whereas the longitudinal considers multiple visits and associates the survival time with the respective covariate value. Notably, the baseline analysis is only measured and presented in univariable, whereas the longitudinal analysis is presented as both univariable and multivariable. Covariates found significant in longitudinal univariable were used in conjunction with treatment (spironolactone and benazepril) and other candidate variables for multivariable models. The multivariable models were analyzed using main effect and interaction terms preceded by an iterative enter method of selection to avoid over-specification of the model which may be a risk in certain stepwise approaches when observations and events are limited. Models being evaluated were subsequently compared with each other by Chi-squared goodness of fit 1 degree of freedom based on the Akaike information criterion when a variable was added or removed from the model returning the probability of difference between the models as determined from the central Chi-squared distribution. The Chi-square probability function $P(x, df)$ suggests that an observation from a Chi-square distribution, with degrees of freedom df, is less than or equal to x. In our case, x is defined as a numeric constant, variable, or expression that specifies the value of a random variable; df is a numeric constant, which is defined as the Akaike information criterion difference between the model variable while df defines the degrees of freedom parameter which in this case is 1 as there are only two models (n-1) being compared.

Significant covariates from the multivariable Cox model were used to generate adjusted survival curves presented in concert with unadjusted survival curves for presentation. Specifically, regarding adjusted survival curves, the longitudinal population mean value of the significant covariate was imputed (held constant) over both strata of the respective survival curve (e.g. stratified NT-proBNP of $\geq$1500 ρmol/L vs. $<$1500 ρmol/L incorporated population mean LA:Ao and E peak velocity, both statistically significant in multivariable, to the Cox model generating the adjusted survival probability).

Multicollinearity was assessed by variance inflation factor analysis and deemed acceptable. Covariates for the multivariable model were presented as both continuous and categorical. Tests for proportionality were carried out by visual inspection of Schoenfeld residuals, negative log estimated survival distribution function, and formal hypothesis testing of covariate by log (time) interactions followed by Wald Chi-squared statistics and deemed proportional. Statistical differences between groups for time to event were analyzed by survival time analyses. Time to event represented the time from the first day of enrollment in the study to the end date. Cases lost to follow-up or remaining alive were censored. The effect of time-to-event and change in HR with a given variable was modeled using the spline approach as previously described.[ac]

Receiver operating characteristic

Continuous demographic and echocardiographic variables were categorized into two discrete (dichotomized) values by the way of a generalized

[ac] Allignol A, Latouche A. CRAN task view: survival analysis, http://cran.r-project.org/web/views/Survival.html; 2012.).

linear logistic model using ROC curve analysis and by inspection of HR change in the value of a given variable exhibited by the spline approach. The ROC curve analysis was based on the interval-censored data set used by the extended Cox model where the last known value before congestive HF or right censoring was used as the value of the given variable analyzed for the ROC curve calculation. The optimal cut point was assigned by a Youden statistic as previously described [19]. Specifically, a variable such as NT-proBNP, which may have demonstrated a range of 200–1700 ρmol/L, may have been found to have an optimal cut point associated with congestive HF calculated to be 1500 ρmol/L. The same ROC approach was applied to remaining echocardiographic and radiographic variables. Dichotomized covariates defined by ROC analysis were subject to further analysis in univariable and multivariable in conjunction with other categorized and continuous variables.

The longitudinal Cox model with right censoring The longitudinal analysis with updated covariate values was explored further by retaining each patient's time in the study in place but investigating a modified calculation of survivorship at 6-month intervals. This univariable analysis was right censored for both time and composite endpoint events at months 6, 12, 18, 24, 30, 36, and 42. Briefly, the full population is analyzed, but frozen in 'snapshots' of 6-month intervals building on each other. Specifically, a given variable was analyzed (e.g. LA:Ao) with survival time followed for the duration of the study. Seven right censored evaluation periods were constructed (months 6, 12, 18, 24, 30, 36, and 42), whereas subsequent Cox models independent of each other were carried out based on each window, for each variables evaluated, by right censoring events at 6-month intervals to determine the effect of full follow-up coupled with serially censored events. For example, in the 6-month censored analysis, if a patient were to have survival time and corresponding HF event date of 6 months and 1 day, this event would be censored for the 6-month analysis because that event occurs after 6 months and the survival time would have a maximum time of 6 months. In this 6-month example, while all patients' comprehensive time in the study is considered, only events occurring in this 6-month window are considered. In contrast, using the 12-month window analysis, this same patient's full follow-up and event of 6 months and 1 day would be recorded for the 12-month window. Extending further with the example for the purpose of completeness, this patient would not be included for the 18, 30, 36, 42, and full follow-up because the patient would be censored with event at 6 months and 1 day. In summary, seven different censored windows (months 6, 12, 18, 24, 30, 36, and 42) were constructed with the respective set limit of survival time but censoring potential events if the event occurred after that given period. Pointedly, the 42-month analysis would be nearly the same as the full follow-up of the study because the study was only three years (48 months) long. This approach is hereafter referred to as the longitudinal right censored analysis. The ROC curve dichotomized variables which remained statistically significant in the longitudinal multivariable models were further analyzed for survivorship at the specified 6-month intervals in this longitudinal right censored analysis.

Results

One hundred and sixty-eight dogs constitute the per protocol population and were used for analysis in this study. The median age of patients enrolled per investigator was 7.5 years (range 5–13 years). Eighteen different breeds were included, with mixed-breeds being the most represented (n = 72; 40%), followed by Cavalier King Charles spaniels (n = 34; 19%), poodles (n = 12; 7%), dachshunds (n = 12; 7%), Chihuahuas (n = 7; 4%), Jack Russell terriers (n = 5; 3%), and bichon frise (n = 5; 3%). No differences were observed between the two groups at inclusion.

The median time in the study was 561 days (95% confidence interval [95% CI]: 142–977 days), and 75 dogs (44.9%) reached the composite endpoint. Forty-seven dogs developed HF, and 28 died of a cardiac cause. The median survival time based on the Kaplan–Meier product limit estimate considering censored events, in the study of dogs reaching the composite endpoint, was 1045 days (95% CI: 766 days–not available). Ninety-three dogs were censored from the analysis of endpoint, including 36 dogs that were still asymptomatic at the end of the study.

In the univariable Cox proportional hazard analysis that included the baseline (single visit) values of covariates, four variables were associated with an increased hazard to reach the composite endpoint at the baseline (Fig. 1). Covariates found significant were used in conjunction with treatment (spironolactone and benazepril) for multivariable models. However, treatment remained non-significant. In the univariable longitudinal Cox proportional analysis, seven variables, using the most up-to-date covariate value associated with a given time interval,

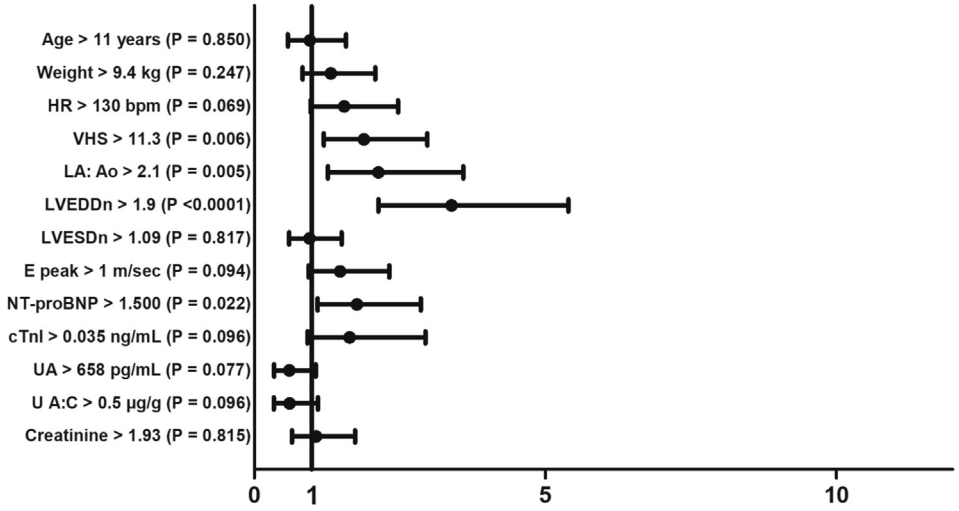

Fig. 1 Hazard ratios and 95% confidence intervals obtained from univariate Cox proportional hazard analysis at the baseline. BPM: beats per minute; cTnI: cardiac troponin I; E peak: early transmitral peak velocity; HR: heart rate; LA:Ao: left atrium to aortic root ratio; LVEDDn: normalized left ventricle end-diastolic diameter; LVESDn: normalized left ventricular end-systolic diameter; NT-proBNP: N-terminal pro brain natriuretic peptide; SB treatment: spironolactone and benazepril treatment; U A: urine aldosterone; U A:C: urinary aldosterone-creatinine ratio; VHS: vertebral heart score.

were associated with an increased risk for reaching one of the composite endpoints (Table 1, Fig. 2). The ROC curve analysis demonstrated optimal Youden statistic cut points of LA:Ao $>$ 2.1, LVEDDn $>$ 1.9, E peak $>$ 1 m/sec, and NT-proBNP $>$ 1.500 ρmol/L. Based on the longitudinal data univariable analysis, the risk of reaching the composite endpoint based solely on NT-proBNP $\pm$ 1500 ρmol/L (parameter estimate: 1.717, HR: 5.57, 95% CI: 3.3—9.5, $p < 0.001$) was greater than the risk of reaching the composite endpoint for NT-proBNP $\pm$ 1500 ρmol/L (parameter estimate: 0.999, HR: 2.71, 95% CI: 1.45—5.1, $p = 0.0018$) when in the presence of continuous variables LA:Ao*10 (parameter estimate: 0.105, HR: 1.1, 95% CI: 1.01—1.2, $p = 0.02$) and E peak*10 (parameter estimate: 0.168, HR: 1.18, 95% CI: 1.06—1.3, $p = 0.0026$). On the aforementioned parameter estimates, the following equation was used to generate the adjusted survival curves: NT-proBNP $\pm$ 1500 ρmol/L (parameter estimate: 0.999) + LA:Ao*10 * (parameter estimate: 0.105) + E peak*10 (parameter estimate: 0.168). Specifically, the adjusted survival curves were generated based on the significant variables in multivariable in the presence of NT-proBNP $\pm$ 1500 ρmol/L stratifying on NT-proBNP $\pm$ 1500 ρmol/L coupled with imputing or holding constant the mean longitudinal population value for LA:Ao and E peak. For purposes of meaningful clinical parameterization, both LA:Ao and E peak were multiplied by ten to present a 0.1-unit increase. Specifically, the actual longitudinal population means of LA:Ao and E peak were 1.91 and 1.01, respectively, but they were parameterized on values of 19.1 and 10.1, to allow for a meaningful clinical 1-unit increase for the regression when applying the model in a clinical setting.

The adjusted model suggests that the risk of reaching the endpoint for dogs with [NT-proBNP] ρmol/L $>$ 1500 ρmol/L is 2.71 times higher than the risk of dogs with the lower NT-proBNP. In the presence of the dichotomized NT-proBNP ρmol/L $>$ 1500 ρmol/L variable, the risk of achieving the combined endpoint increases by 11% per 0.1-unit increase of LA:Ao and 18.4% per 0.1-unit increase of E peak. The mentioned unadjusted and adjusted survival curves based on this

Table 1 Probability to reach the primary endpoint in Cox longitudinal proportional univariate analysis.

	Hazard ratio	95% CI	*p* value
HR $>$ 130 bpm	2.289	1.43—3.66	<0.001
VHS $>$ 11.3	2.566	1.57—4.19	<0.001
LA:Ao $>$ 2.1	3.253	1.88—5.64	<0.001
LVEDDn $>$ 1.9	6.272	3.34—11.76	<0.001
E peak $>$ 1 m/sec	3.909	2.27—6.73	<0.001
NT-proBNP $>$ 1.500 (ρmol/L)	5.567	3.26—9.51	<0.001
cTnI $>$ 0.35 (ng/mL)	3.371	1.92—5.91	<0.001

bpm: beats per minute; cTnI: cardiac troponin I; E peak: early transmitral peak velocity; HR: heart rate; LA:Ao: left atrium to aortic root ratio; LVEDDn: normalized left ventricle end-diastolic diameter; NT-proBNP: N-terminal pro brain natriuretic peptide; VHS: vertebral heart score.

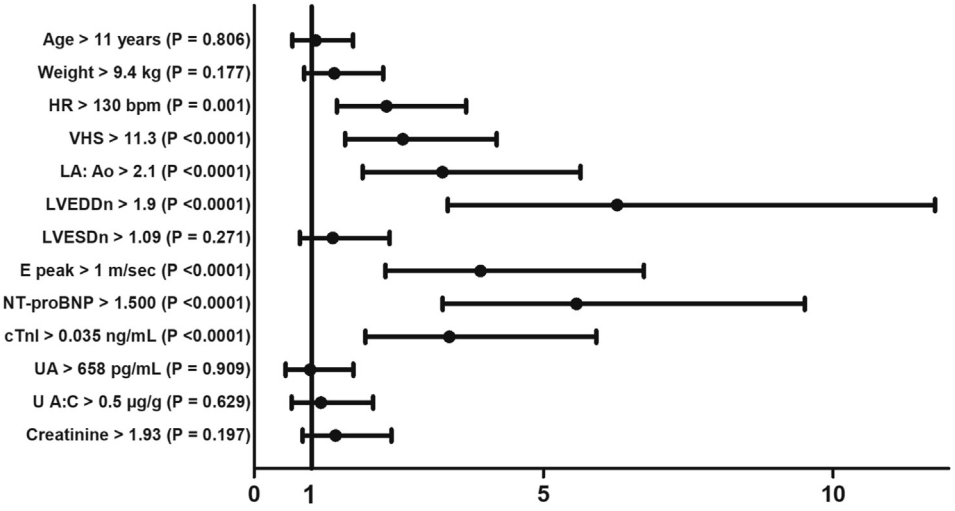

Fig. 2 Hazard ratios and 95% confidence intervals obtained from longitudinal univariate Cox proportional hazard analysis. BPM: beats per minute; cTnI: cardiac troponin I; E peak: early transmitral peak velocity; HR: heart rate; LA:Ao: left atrium to aortic root ratio; LVEDDn: normalized left ventricle end-diastolic diameter; LVESDn: normalized left ventricular end-systolic diameter; NT-proBNP: N-terminal pro brain natriuretic peptide; SB treatment: spironolactone and benazepril treatment; U A: urine aldosterone; U A:C: urinary aldosterone-creatinine ratio; VHS: vertebral heart score.

longitudinal analysis are presented in Fig. 3. In observing the unadjusted survival in Fig. 3, median survival of NT-proBNP $\geq$ 1500 ρmol/L was 16 months, whereas NT-proBNP $<$ 1.500 ρmol/L was not able to be estimated. However, the 25th percentile can be found in Fig. 3 to be 10 and 36 months. In observing the effect of the population mean value being imputed for LA:Ao and E peak

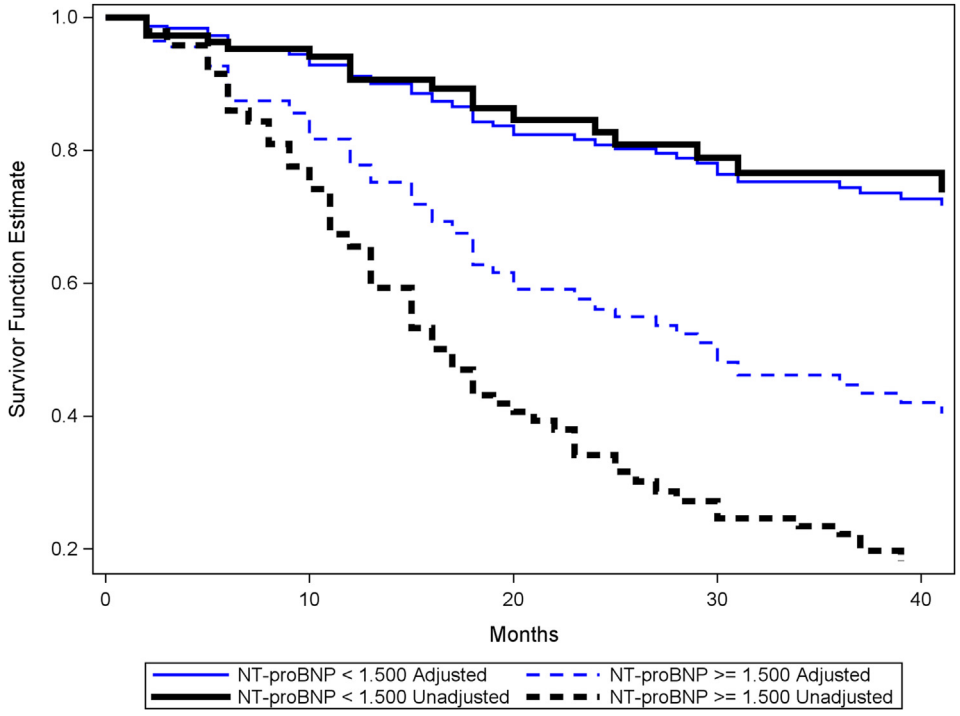

Fig. 3 Unadjusted and adjusted Kaplan–Meier survival curves as per N-terminal pro brain natriuretic peptide plasma concentration (NT-proBNP). The survivor function of NT-proBNP in univariate (NT-proBNP ± 1500 ρmol/L unadjusted) by definition does not include the effect of LA/Ao and peak transmitral E wave velocity. By adjusting for these values (NT-proBNP ± 1500 ρmol/L adjusted), a truer survival fit is achieved.

for the adjusted model survival curves, the median survival for NT-proBNP $\geq$ 1500 ρmol/L was 29 months, whereas the NT-proBNP $<$ 1500 ρmol/L was still non-estimable as indicated to the very similar survivorship to the unadjusted NT-proBNP $<$ 1.500 ρmol/L, suggesting that imputing the population mean has a minimal effect on this group. In contrast, imputation of the mean value of LA:Ao and E peak improves survivorship with the NT-proBNP $\geq$ 1.500 ρmol/L population, suggesting contribution of the statistically significant covariates LA:Ao and E peak to predicting survivorship, as opposed to the NT-proBNP $\pm$ 1.500 ρmol/L being the sole predictor. In a sensitivity analysis with the minimum LA:Ao = 1.14 and E Peak = 0.48 m/sec imputed as opposed to the mean values presented, neither the median nor the 25th percentile survivorships could be estimated. In contrast, in a similar sensitivity analysis using the population maximum values of LA:Ao = 3.18 and E Peak = 1.81 m/sec imputed, as opposed to the presented mean imputation, the median survival of the NT-proBNP $\geq$ 1.500 ρmol/L population was 3 months while the NT-proBNP $<$ 1.500 ρmol/L population was 6 months (minimum and maximum imputed survivorships not shown on Fig. 3). Although the longitudinal unadjusted survival for NT-proBNP $\pm$ 1.500 ρmol/L presented a larger HR between groups, the multivariable longitudinal analysis stratifying on NT-proBNP $\pm$ 1.500 ρmol/L in conjunction with LA:Ao and E peak as continuous variables provided the best model fit.

The risk for reaching the composite endpoint for dogs with LA:Ao $>$ 2.1, LVEDDn $>$ 1.9, E peak $>$ 1 m/sec, and NT-proBNP $>$ 1.500 ρmol/L based on the longitudinal analysis right censored at 6, 12, 18, 24, 30, 36, and 42 months is reported in Table 2.

Table 2 Risk to reach the combined endpoint for selected baseline echocardiographic variables and N-terminal pro natriuretic peptide (NT-proBNP) censored at 6 through 42 months.

Visit time (months)	NT-proBNP $>$ 1.500 ρmol/L		E peak $>$ 1 m/sec		LA:Ao $>$ 2.1		LVEDD $>$ 1.9	
	HR (95% CI)	p_value	HR (95% CI)	p_value	HR (95% CI)	p_value	HR (95% CI)	p_value
6	3.04 (0.96, 9.59)	0.0580	14.71 (1.9, 113.98)	0.0100	2.97 (0.94, 9.38)	0.0630	7.86 (2.13, 29.06)	0.0020
12	4.28 (1.94, 9.47)	<0.001	7.1 (2.36, 21.37)	<0.001	2.97 (1.15, 7.67)	0.0250	6.29 (2.24, 17.66)	<0.001
18	5.71 (2.95, 11.03)	<0.001	7.99 (3.51, 18.19)	<0.001	3.03 (1.56, 5.9)	0.0010	6.35 (2.98, 13.53)	<0.001
24	5.65 (3.08, 10.38)	<0.001	5.81 (2.94, 11.48)	<0.001	3.1 (1.67, 5.77)	<0.001	5.7 (2.85, 11.39)	<0.001
30	5.59 (3.17, 9.85)	<0.001	4.14 (2.31, 7.44)	<0.001	3.25 (1.82, 5.79)	<0.001	6.03 (3.13, 11.63)	<0.001
36	5.46 (3.14, 9.47)	<0.001	3.87 (2.21, 6.77)	<0.001	2.94 (1.67, 5.2)	<0.001	5.77 (3.06, 10.9)	<0.001
42	5.53 (3.23, 9.44)	<0.001	3.87 (2.25, 6.67)	<0.001	3.23 (1.86, 5.61)	<0.001	6.15 (3.28, 11.54)	<0.001

CI: confidence interval; E peak: early transmitral peak velocity; HR: hazard ratio; LA:Ao: left atrium to aortic root ratio; LVEDDn: normalized left ventricle end-diastolic diameter; NT-proBNP: N-terminal pro brain natriuretic peptide.

Discussion

This study demonstrates that NT-proBNP and some selected echocardiographic variables can predict the risk for the first onset of HF or cardiac death in dogs with preclinical MMVD and cardiac remodeling. Previous studies have assessed and reported the value of NT-proBNP to assess the risk for progression or death in patients with both preclinical and clinical MMVD. For example, Moonarmart et al. (2010) [6] reported that a 100 ρmol/L increase of NT-proBNP was associated with an increased risk of all-cause mortality in a population of dogs with both preclinical and clinical MMVD. In another study, Wolf et al. (2012)

[7] reported that NT-proBNP plasma concentrations < 965 ρmol/L 7–30 days after initiating treatment of HF were associated with a higher survival time in a population of dogs with clinical MMVD. Furthermore, Mattin et al. (2019) [20] reported that plasma NT-proBNP > 1.800 ρmol/L was strongly associated with the higher risk for clinical progression in dogs affected by preclinical MMVD. In this study, NT-proBNP plasma concentrations > 1.500 ρmol/L represented the best predictor for reaching the predefined composite endpoint. Reynolds et al. (2012) [11] reported that the same cutoff for NT-proBNP was an independent predictor of onset of HF in a population of preclinical MMVD with an LA:Ao > 1.6. In the same study, they also reported that, similar to our study, the best predictive model included the NT-proBNP concentration and the left atrial dimension.

In this study, in addition to NT-proBNP concentration, left atrial and ventricular dimensions and E peak of transmitral flow were identified as useful independent predictors for reaching the endpoint. The prognostic value of echocardiographic variables in preclinical MMVD has been previously reported in several studies. Left atrial enlargement assessed by different methods, or LA:Ao increment by 0.1 unit, represented the most common identified echocardiographic predictor for the progression of the preclinical disease [1,4,13,21]. An LVEDDn > 1.7 and the rate of change of left ventricular end-diastolic dimensions were reported to be associated with an increased hazard for dogs with preclinical MMVD [3,4,13,21]. In our study, the optimal cutoff values to identify dogs with an increased risk for progression of the disease were 2.1 for LA:Ao and 1.9 for LVEDDn. These cutoff values are higher than those previously reported [1,3,4,6]. Left atrial to left aortic ratio values > 1.6 are generally accepted for identifying dogs with left atrial enlargement [22]. However, a recent study has reported that normal dogs may present an LA:Ao up to 1.9 [23]. Normalized end-diastolic left ventricular diameter >1.7 has been found to be a predictor of outcomes [6], and this cutoff is used to identify dogs with left ventricular enlargement [17,24]. However, it should be noted that LVEDDn = 1.73 represents the upper 95% percentile for normal dogs in the study from Cornell et al. (2004) [17], whereas the upper 97.5% for normal dogs is 1.85. Therefore, the cutoff values reported in this present study identify dogs with significant cardiac enlargement and therefore that may have a higher risk of developing HF or die for their cardiac disease.

In the present study, transmitral inflow E peak velocity higher than 1 m/sec was an independent predictor for progression to a primary endpoint. This cutoff value was higher than normal values observed in normal dogs of similar age [25]. A possible explanation for this finding is the association between increased E peak velocity and increased left atrial pressure [26]. However, in patients with volume overload and normal systolic function, such as dogs with preclinical MMVD, increased E peak velocity may also reflect left ventricular volume overload and not necessarily a sole increased atrial pressure [27]. Regardless of the cause of its increased value, E peak velocity can still be considered as an indirect indicator of the severity of the disease, and it has been reported being independently associated with an increased hazard in both preclinical and clinical dogs [1,3,28].

In this study, the best predictor model for identifying dogs with increased risk of reaching the composite endpoint was a composite of NT-proBNP concentrations, LA:Ao ratio, and E peak velocity. Normalized left ventricular end-diastolic diameter was not included in this statistical model because of its strong correlation to the left atrial enlargement. However, it is of interest to notice that LVEDDn was a variable associated with an increased hazard at 6 months after inclusion in the study.

All echocardiographic variables that were found to be associated with clinical outcomes and the NT-proBNP showed an increased hazard over time. This result is not unexpected considering the long duration of the preclinical phase of MMVD in dogs [1,13,21]. This finding suggests that monitoring the progression of the disease by echocardiographic variables and NT-proBNP may represent an effective way to make appropriate therapeutic decisions and to assess the risk for a preclinical dog to develop HF or to die for the underlying cardiac disease.

Limitations

The major limitation for this study is represented by the fact that the onset of HF was not confirmed in all dogs by documenting the presence of cardiogenic pulmonary edema on thoracic radiographs. However, although thoracic radiography is often considered the gold standard for diagnosing left-sided congestive HF, interpretation is mostly subjective. Indeed, it is now generally accepted that combination of history and clinical assessment in association with response to treatment represents an acceptable way to

identify onset of HF in dogs with preclinical MMVD [20,22]. Another limitation of this study is represented by the presence of a treatment and a placebo group, which were combined and analyzed as a single population. Because the treatment in this study had no effect on primary endpoints, it is unlikely that combining the two groups into a single population affected the results of this study.

Conclusions

This study reports reliable echocardiographic and plasma NT-proBNP cutoff values that can be used to identify dogs with preclinical MMVD that are at higher risk of developing HF or experiencing cardiac death. Furthermore, the results of this study may also be useful for designing future studies to evaluate the effect of various interventions aimed at delaying the progression of MMVD.

Conflicts of Interest Statement

This project was funded by Ceva Santé Animale, and two authors, E.G. and C.G-P., are representatives of Ceva Santé Animale.

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