Open Veterinary Journal, (2024), Vol. 14(9): 2237-2249
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)
Research Article
DOI: *10.5455/OVJ.2024.v14.i9.11*

Submitted: 09/05/2024 Accepted: 23/08/2024 Published: 30/09/2024

Alteration of clinical parameters before mortality and prognostic outcomes of pulmonary hypertension in dogs with myxomatous mitral valve disease

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ABSTRACT

Background: Myxomatous mitral valve degeneration (MMVD) is a prevalent canine heart condition often accompanied by pulmonary hypertension (PH). Echocardiography is a valuable diagnostic tool for MMVD, but its accessibility is limited in small veterinary clinics.

Aim: This study aimed to identify clinical parameters and biochemistry and cardiac biomarkers as prognostic indicators for cardiac mortality in MMVD dogs with and without PH.

Animals: Ninety-nine MMVD dogs and nineteen normal dogs.

Methods: In a five-year longitudinal study, data including clinical and laboratory measurements as well as echocardiographic parameters were collected every 6 months. Dogs were monitored until death or loss to follow-up, and the cause of death was determined when possible. Statistical analysis was performed to identify factors that predicted death.

Results: Alterations in body condition score, total protein, fractional shortening percentage, and mean corpuscular volume were predictive of impending cardiac mortality. High blood urea nitrogen-to-creatinine ratio, heart rate, and low hemoglobin levels were associated with an increased risk of death. N-terminal pro-B-type natriuretic peptide was also a significant predictor of cardiac-related mortality, with higher levels indicating increased risk. Moreover, MMVD dogs with PH had a significantly lower survival rate than those with MMVD without PH. However, no significant difference in survival was observed between MMVD stage C and D with PH and MMVD stage C and D without PH groups.

Conclusion: These findings provide valuable insights into the monitoring of MMVD progression in dogs using clinical parameters and biomarkers, especially when echocardiography cannot be performed.

Keywords: Biomarkers, Echocardiography, Longitudinal study.

Introduction

Myxomatous mitral valve degeneration (MMVD) is a condition that affects the mitral valve in dogs. It is the most common type of heart disease in dogs, accounting for approximately 75% of cases (Keene *et al*., 2019). Pulmonary hypertension (PH) results from abnormally increased pulmonary vasculature pressure and has been defined as a mean pulmonary arterial pressure of ≥25 mmHg at rest. Postcapillary PH, a type of PH, is caused by left-sided heart disease. It develops as a result of the high blood pressure in the left atrium and increases the workload on the right ventricle, indirectly leading to high systolic pressures in the right ventricle (Reinero *et al*., 2020). The outcomes of mortality prediction in MMVD and MMVD with PH have been previously reported (Borgarelli *et al*., 2015; Sargent *et al*., 2015; Baron Toaldo *et al*., 2018). Echocardiographic parameters, including the dimension and function of the left side of the heart, mitral valve pathology, increase in peak transmitral blood flow velocity, and

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tricuspid regurgitation pressure gradient (TRPG), are valuable predictors of cardiac mortality in dogs with MMVD and PH (Borgarelli *et al*., 2015; Sargent *et al*., 2015; Baron Toaldo *et al*., 2018). However, advanced imaging measurements require facilities and techniques necessitating expert staff, which makes it difficult for small hospitals or private practices to monitor the clinical progression of MMVD dogs.

Therefore, tools other than echocardiography can be used as clinical monitors and prognostic factors for MMVD progression. Complete blood count (CBC) indices showed potential as biomarkers for MMVD severity using differential count ratio of mononuclear panels (Jung and Kim, 2023) and red cell distribution width (RDW). The blood urea nitrogen-to-creatinine ratio (BUN/Cr) served as a prognostic indicator in humans with acute and chronic heart failure (Zhu *et al*., 2020; Wang *et al*., 2023). BUN/Cr elevation in patients with heart failure is due to treatment with intensive diuretics causing hypovolemia owing to the low cardiac output resulting in prerenal hypoperfusion (Zhu *et al*., 2020). However, information on BUN/Cr as a prognostic factor in dogs with MMVD or congestive heart failure (CHF) is insufficient. A recent study reported that BUN/Cr is not an effective clinical marker of gastrointestinal bleeding in dogs (Stiller *et al*., 2021). In recent decades, many cardiac biomarkers have been reported to play an important role as diagnostic tools in MMVD. N-terminal pro–B-type natriuretic peptide (NTproBNP) is a protein that can be measured in the blood to diagnose heart disease and heart failure in dogs. It is released from the heart when the myocardium is stressed or strained due to volume overload. This marker can identify dogs at risk for developing heart problems, even before clinical signs or symptoms appear (de Lima and Ferreira, 2017). Moreover, NTproBNP is useful in detecting heart failure in dogs with MMVD, particularly when echocardiographic equipment is unavailable (Wolf *et al*., 2013; de Lima and Ferreira, 2017; Chanmongkolpanit *et al*., 2024). Overall, many laboratory tests provide a simple, inexpensive, and effective way to monitor clinical symptoms in dogs with MMVD.

Therefore, this study aimed to investigate the alterations in CBC, biochemistries, and cardiac biomarkers during the follow-up period until cardiac mortality and identify prognostic indicators of cardiac mortality in MMVD dogs with and without PH.

Materials and Methods

The dogs were enrolled at Prasu Arthorn Veterinary Teaching Hospital, Mahidol University, Thailand, after obtaining informed consent from their owners. The study protocol was approved by the Mahidol University-Institute Animal Care and Use Committee. The study period was from July 2017 to June 2023. All dogs, regardless of sex, breed, or weight, were included, provided that they were at least 5 years old. The dogs underwent physical examinations and cardiorespiratory evaluations, including heart murmur assessments. Furthermore, their histories were recorded.

Dogs diagnosed with MMVD, both with and without PH, and devoid of concomitant systemic diseases, were enrolled in the study. Clinical data were collected at an interval of 6 months, during which comprehensive assessments including physical examinations, CBC, serum biochemistry, cardiac biomarkers, thoracic radiography, echocardiography, and electrocardiography were performed.

Approximately 5 ml of blood was collected from each dog; separated into portions for CBC, serum biochemistry, and cardiac biomarker testings; and stored at −80°C for later analysis. Thoracic radiographs in the right lateral and ventrodorsal positions were taken to assess for cardiac and lung abnormalities.

Electrocardiograms were recorded for each dog using a standard six-lead system, including limb leads I, II, III, aVR, aVL, and aVF. Dogs with arrhythmia underwent further lead II recording for rhythm evaluation, with measurements of heart rate (HR), intervals, amplitudes, and vasovagal tone index (VVTI). The standard six-lead electrocardiograms were recorded for each dog for approximately 10 seconds (Moonarmart *et al*., 2013; López-Alvarez *et al*., 2014). If the arrhythmias were present, additional one-minute lead II recordings were used to evaluate the cardiac rhythm. The measurements, including HR, intervals, amplitudes, and VVTI, were derived from lead II.

Echocardiographic examinations were performed in awake, unsedated dogs using a GE Vivid E9 ultrasound machine (GE Healthcare, Wauwatosa, Wisconsin, USA) equipped with a multifrequency sector transducer (4.5–12-and 4–8-MHz probes for small- and medium-sized dogs, respectively). Continuous ECG recordings were maintained throughout the procedure. The measurements were averaged over at least three consecutive cardiac cycles.

A two-dimensional (2D) examination from the right parasternal (RPS) long-axis view assessed the presence of mitral valvular lesions and mitral regurgitation (MR) semiquantitatively using color flow Doppler. The left and right ventricular end-diastolic diameters were compared in the 2D RPS four-chamber view. The left atrial-to-aorta (LA:Ao) ratio was calculated from the 2D short-axis view measurements. Pulmonary artery (PA) size and presence of pulmonary regurgitation (PR) were assessed in the RPS short-axis view using color flow Doppler.

Short-axis M-mode echocardiography at the level of the left ventricle (LV) measured the LV dimensions, including the LV internal diameter in the diastolic phase (LVIDd) and LV internal diameter in the systolic phase (LVIDs). The fractional shortening percentage (FS) was calculated from the short-axis M-mode view at the LV level. The normalized LV internal diameter in the diastolic phase (NLVIDd) and normalized LV

internal diameter in the systolic phase (NLVIDs) were determined based on body weight using the following formulas: NLVIDd = LVIDd (cm)/(BW (kg))$^{0.294}$ and NLVIDs = LVIDs (cm)/(BW (kg))$^{0.315}$, where BW refers to body weight (Cornell *et al*., 2004).

Doppler echocardiography was performed to assess the maximal flow velocity of the tricuspid regurgitation (TR) jet from the following three different views: left parasternal (LPS) apical 4-chamber view, LPS long-axis of the right auricle view, and LPS cranial transverse view of the tricuspid valve. This measurement was then applied to estimate the systolic pressure gradient across the tricuspid valve, representing systolic PA pressure (PAP). A diagnosis of PH was made when the peak TR jet velocity exceeded 3 m/s. In cases in which the TR flow was absent, the maximal PR jet velocity was used to identify PH, and its measurement was applied to estimate the mean PAP, with a peak PR jet velocity of ≥2.2 m/s, indicating PH (Johnson *et al*., 1999; Schober and Baade, 2006; Kellum and Stepien, 2007; Kellihan and Stepien, 2010; Riengvirodkij *et al*., 2021).

Pulmonary flow profiles were recorded from the RPS short-axis and/or LPS cranial long-axis of the right ventricular outflow tract view by placing the Doppler sample volume distally just below the pulmonic valve. A pressure gradient across the pulmonic valve of <1.5 m/s was considered normal. Dogs with evidence of other cardiac diseases based on echocardiographic examination were excluded from the study. The enrolled dogs were evenly distributed into the following five groups according to the American College of Veterinary Internal Medicine (ACVIM) classification from the guidelines for the diagnosis and treatment of mitral valve disease in dogs (Keene et al., 2019) Group MMVD B1, dogs with MMVD but without signs of CHF (asymptomatic) or radiographic evidence of MR; Group MMVD B2, dogs with MMVD, asymptomatic, and without radiographic signs of CHF; however, they exhibited significant hemodynamic changes, leading to echocardiographic evidence of left atrial dilatation (LA:Ao > 1.6) and LV enlargement (NLVIDd > 1.7); Group MMVD C, dogs with MMVD, symptomatic, showing echocardiographic MMVD evidence, and clinical signs of CHF, identified through clinical examination and radiographic evidence of pulmonary edema or venous congestion; Group MMVD D, dogs with end-stage MMVD, which are unresponsive to standard treatment of CHF and MMVD. Group MMVD-PH, dogs with MMVD and secondary PH, identified using Doppler echocardiography with a peak tricuspid regurgitant velocity of ≥3 m/s or a pulmonary regurgitant velocity of ≥2.2 m/s; and Group NC, normal healthy control dogs without heart disease or PH, with no history or clinical signs of cardiorespiratory issues, no heart murmurs, no lung crackles, and normal heart conditions, as observed through thoracic radiography, electrocardiogram, and echocardiography.

Longitudinal clinical parameter monitoring

The dogs were monitored from the first visit until the endpoint, which is either death or loss to follow-up despite phone reminders. The changes in clinical variables, signalments, physical examinations, CBC, serum biochemistry, and echocardiographic parameters were monitored over time.

Survival monitoring

All MMVD dogs were regularly followed up. If owners declined to continue with the study, the dog was considered lost to follow-up. In the case of death, the cause was determined, distinguishing between cardiac-related and spontaneous deaths. Cardiac-related death results from heart disease progression, whereas spontaneous death occurs during sleep or activity. The cause of death was verified through telephone communication with the owners of dogs that passed away within their home environment, and the conditions leading to cardiac-related death were determined based on their clinical histories, including symptoms such as dyspnea, tachypnea, open-mouth breathing, collapse, and syncope. Survival time was recorded from the first visit to the endpoint or censoring due to the development of disorders affecting the cardiovascular system or being lost to follow-up. A survival analysis focusing on cardiac-related mortality was conducted.

Statistical analysis

Statistical analysis used computerized software R Core Team (2023) (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/). The results are presented as mean ± standard deviation and median with interquartile ranges (IQRs) for normally and non-normally distributed variables, respectively. A significance level of $p < 0.05$ was applied. Descriptive statistics employed the Kruskal–Wallis and Fisher's exact tests for nonparametric and categorical data, respectively. Univariable Cox proportional hazards analysis identified potential death predictors and calculated hazard ratios (HRs) and 95% confidence intervals (95% CIs). The significant factors from the univariable Cox proportional hazard analysis ($p < 0.05$) were included in the multivariable Cox proportional hazards analysis. The risk of death was compared between the deceased and surviving dogs using cumulative hazard function plotting and tested with the log-rank test. Cumulative hazard time was measured from the first examination, and the study endpoint was the dogs' death. Each variable was examined before and after death in the deceased dogs' group using the Wilcoxon-signed rank test for non-normally distributed variables. Kaplan–Meier survival curves were used to compare the median survival time among the groups.

Ethical approval

The animal ethics for this study was approved by the Faculty of Veterinary Science Animal Care and Use

Committee (FVS-ACUC), Mahidol University (COA Nos. MUVS-2018-06-32, MUVS-2019-05-29, and MUVS-2021-11-49).

Results

All 118 client-owned dogs with a median age of 10.88 years (IQR 8.00–12.96 years) and a median body weight of 5.4 kg (IQR 4.03–6.45 kg) were examined between July 2017 and June 2023. Most dogs were Chihuahua (*n* = 33), Poodle (*n* = 33), Shih-Tzu (*n* = 19), and Pomeranian (*n* = 14). The remaining 20 dogs were of six other breeds, Jack Russell (n=1), Miniature (n=2), Mixed (*n*=13), Terrier (*n*=1), Pug (*n*=1), and Yorkshire (*n* = 1). According to the ACVIM classification, 19, 33, and 47 dogs were in stages B1, B2, and C–D, respectively. There are 19 dogs in the NC group. Stage B2 dogs received only pimobendan, while stage C–D dogs received pimobendan, furosemide with a median dose of 3.62 mg/kg/day (IQR 2.72–4.17 mg/kg/day), and angiotensin-converting enzyme inhibitor (ACEi). Three types of ACEi were administered: benazepril (*n* = 9) with a median dose of 0.44 mg/kg/day (IQR 0.40–0.45 mg/kg/day), enalapril (*n* = 9) with a median dose of 0.74 mg/kg/day (IQR 0.60–0.91 mg/kg/day), and ramipril (*n* = 28) with a median dose of 0.18 mg/kg/day (IQR 0.15–0.21 mg/kg/day).

The baseline characteristics of the all-sample population are reported in Table 1. PH was identified in 34/118 dogs (29%). Among these 34 dogs with PH, 7, 24, and 3 dogs were in stages B2, C, and D, respectively. Of the original 118 dogs, 17 were examined only at the first visit and were not further included in the survival analyses. Death occurred in 22 of the 101 dogs that were included in the analysis, and all cases were of cardiac-related mortality with spontaneous death. The dogs that died had stage B2 (*n* = 6), C (*n* = 14), and D (*n* = 2) diseases according to the ACVIM guideline. The median time until death from cardiac-related issues was 546 days (range: 41–1,134 days). When differentiated by stage, the median times until death were 590 (177–985 days) for stage B2, 399 days (41–1,134) for stage C, and 100 days (99–102 days) for stage D cases. The median time of follow-up in the dogs that were censored because it was unclear whether they were still alive or had been lost to follow-up was 447 days (28–1,095 days).

Table 1. Descriptive statistics for all 118 dogs, dogs without pulmonary hypertension (PH), and dogs with PH.

	All (*n* = 118)	Normal (*n* = 19)	B1 (*n* = 19)	B2 (*n* = 33)	CD (*n* = 47)	*p*-Value
Age (years) (*n* = 118)	10.88 [8.00–12.96]	5.17 [5.00–8.5] [a,b,c]	10.75 [7.00–12.08][a]	12.00 [9.25–14.00][b]	11.08 [10.00–13.04][c]	<0.001
Sex (F/M) (*n* = 118)	54/64	12/7	7/12	13/20	22/25	0.74
Weight (kg) (*n* = 118)	5.40 [4.03–7.15]	4.50 [3.35–6.90]	5.80 [4.40–7.4]	5.40 [4.50 - 7.00	5.40 [4.25–7.35]	0.35
PH (*n* = 40)	40	0/19 (0%)	6/19 (31.57%)	7/33 (21.21%)	27/47 (54.54%)	0.003
LA:Ao (*n* = 118)	1.84 [1.38–2.15]	1.17 [1.13–1.28] [a,b,c]	1.37 [1.25–1.49][a,d,e]	1.82 [1.70–2.05][b,d,f]	2.19 [1.97–2.64][c,e,f]	<0.001
NLVIDd (*n* = 117)	1.67 [1.45–1.94]	1.30 [1.22–1.40] [a,b,c] (19/19)	1.55 [1.36–1.62][a,d,e] (19/19)	1.73 [1.56–1.86][b,d,f] (32/33)	1.96 [1.76–2.21][c,e,f] (47/47)	<0.001
E value (*n* = 113)	1.14 [0.84–1.35]	0.73 [0.64–0.79] [b,c] (18/19)	0.83 [0.71–0.92][d,e] (16/19)	1.11 [1.00–1.18][b,d,f] (33/33)	1.36 [1.21–1.66][c,e,f] (46/47)	<0.001
TRPG (mmHg) (*n* = 57)	43.8 [31.81–61.15]	-	36.00 [34.81–1.73] (9/19)	35.05 [28.64–44.04][f] (15/33)	52.85 [43.30–70.69][f] (32/47)	0.007

The statistically significant differences within each group were tested using Kruskal–Wallis test and Mann–Whitney-U test
[a]Statistically significant difference compared with the normal controls and MMVD stage B1 dogs ($p < 0.01$).
[b]Statistically significant difference compared with the normal controls and MMVD stage B2 dogs ($p < 0.01$).
[c]Statistically significant difference compared with the normal controls and MMVD stage C and D dogs ($p < 0.01$).
[d]Statistically significant difference compared with MMVD stage B1 dogs and MMVD stage B2 dogs ($p < 0.01$).
[e]Statistically significant difference compared with MMVD stage B1 dogs and MMVD stage C and D dogs ($p < 0.01$).
[f]Statistically significant difference compared with MMVD stage B2 dogs and MMVD stage C and D dogs ($p < 0.01$).
p-value refers to the difference between the dogs with stage B1, B2, and C–D disease and normal dogs. PH = pulmonary hypertension, LA:Ao = left atrial-to-aorta ratio, NLVIDd = normalized left ventricular internal dimension diameter, E Value = early diastolic transmitral flow velocity, TRPG = tricuspid regurgitation pressure gradient.

Table 2. Changes in variables between visit before death and previous visit (median 128.6 days), as assessed using Wilcoxon-signed rank method.

	n	Previous Visit	Last visit (visit before death)	*p*-value
Albumin	21	3.30 [3.00–3.40]	3.20 [2.93–3.30]	0.79
ALP	21	88 [61–163]	85.5 [53.75–160.50]	0.862
ALT	21	78 [41–171]	54 [43–145]	0.627
BUN	21	30 [20–43]	36 [23.25–48.25]	0.108
BUN/Cr	22	21.43 [18.14–33.56]	25.65 [18.52 – 34.48]	0.157
Creatinine	22	1.36 [1.13–1.64]	1.47 [1.14–1.55]	0.566
TP (mg/dl)	21	7.1 [6.90–7.50]	6.85 [6.33–7.38]	**0.02**
Ao_{vel}	17	1.50 [1.27–1.62]	1.31 [1.26–1.42]	0.134
E value	21	1.35 [1.19–1.63]	1.62 [1.29–1.95]	0.394
EA ratio	17	1.39 [1.23–1.82]	1.33 [1.10–1.81]	0.847
EPSS	22	1.56 [0.90–2.38]	1.35 [0.90–2.70]	0.578
FS (%)	22	46.42 [44.33–50.18]	50.62 [44.30–56.55]	**0.046**
LA:Ao	22	2.25 [1.94–2.72]	2.35 [2.13–2.74]	0.129
MR PG	21	128.14 [112.36–143.04]	127.69 [103.63–139.71]	0.927
MR velocity	21	5.66 [5.3–5.98]	5.65 [5.09–5.91]	1
NLVIDd	22	19.56 [17.12–21.72]	20.12 [18.98–22.71]	0.463
NLVIDs	22	9.76 [8.02–11.76]	9.54 [7.81–10.78]	0.775
MR PG	21	128.14 [112.36–143.04]	127.69 [103.63–139.71]	0.927
MR velocity	22	7.07 [6.38–7.40]	6.32 [5.75–6.88]	0.088
NLVIDd	22	19.56 [17.12–21.72]	20.12 [18.98–22.71]	0.463
NLVIDs	22	9.76 [8.02–11.76]	9.54 [7.81–10.78]	0.775
PV PG	14	3.61 [2.592–4.928]	2.50 [1.54–2.86]	0.056
TR PG	17	52.42 [34.11–73.96]	49.14 [35.65–65.05]	0.737
VVTI	20	8.01 [7.42–9.31]	7.67 [6.63–9.54]	0.985
Heart rate (ECG)	21	149 [133–159]	150 [121–159]	0.681
Amplitude	21	0.4 [0.4–0.4]	0.4 [0.2–0.6]	0.950
Amplitude Q	21	0.1 [0–0.6]	0 [0–0.4]	0.864
P wave duration	21	0.04 [0.04–0.04]	0.04 [0.04–0.04]	0.280
PR interval	21	0.08 [0.08–0.1]	0.08 [0.08–0.1]	0.453
QRS duration	21	0.04 [0.04–0.04]	0.04 [0.04–0.04]	0.612
R wave	21	2.2 [1.6–3]	2 [1.6–2.4]	0.111
S wave	21	0 [0–0.2]	0 [0–0.1]	0.635
VHS	15	11.5 [10.9–12.65]	11.55 [11–13.15]	0.329
BCS	22	3 [3–3.4]	3 [2.6–3]	**0.019**
BW	22	5.45 [4.15–6.6]	5.3 [3.975–7]	0.088
Heart rate (bpm)	22	148 [140–166]	160 [128–170]	0.931
Pulse rate	15	158 [140–177]	168 [150–180]	0.384

ALP: alkaline phosphatase; ALT: alanine aminotransferase; Ao_{vel}: aortic velocity; BCS: body condition score; BUN: blood urea nitrogen; BUN/Cr: blood urea nitrogen-to-creatinine ratio; BW: body weight; E value:early diastolic transmitral flow velocity; E/A: early to late diastolic transmitral flow velocity; EPSS: E-point septal separation; BUN: blood urea nitrogen; FS: fractional shortening; LA:Ao: left atrium-to-aorta ratio; NLVIDd: left ventricular internal end-diastolic diameter normalized by body weight; NLVIDs: left ventricular internal end systolic diameter normalized by body weight; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; MCV: mean corpuscular volume; MR: mitral regurgitation; PA/Ao: pulmonary artery-to-aorta ratio; PG: pressure gradient; PV: pulmonic velocity; RBC: red blood cell; TP: total protein; TR: tricuspid regurgitation; VHS: vertebral heart score; VVTI: vasovagal tonus index; WBC: white blood cell.

Bold values are statistically significant with a p value < 0.05.

Changes in variables between visit before death and previous visit

Out of the 22 dead dogs, 6 were in stage B2, 14 were in stage C, and 2 were in stage D. Their median treatment duration before the last two visits was 185.5 days (IQR 78.75–372.75 days) and the median time from the last visit to cardiac death was 59.5 days (IQR 34.5–157 days). Compared to their previous visit (median duration: 128.6 days), the last examination before the dog's death revealed a tendency for a decrease in body condition score (BCS) (p = 0.019) and total protein (TP) (p = 0.02) (Table 2). Conversely, there was an indication of an increase in FS (p = 0.046) and mean corpuscular volume (MCV) (p = 0.032).

Cox proportional hazards analysis of the development of cardiac-related mortality without echocardiographic parameter model.

Univariable analysis results of the potential predictive values for cardiac mortality of continuous and categorical variables are shown in Table 3. In the multivariable analysis, BUN/Cr, HR, and Hb were independently associated with cardiac-related mortality; for a 1-unit increase in BUN/Cr, the hazard of cardiac-related mortality increased 1.06 times (95% CI: 1.02 to 1.12, p = 0.008), and for a 10-beat per minute increase in HR, the hazard of cardiac-related mortality increased 1.26 times (95% CI: 1.07 to 1.49, p = 0.006). Moreover, in a 1-g/dl increase of Hb, the hazard of cardiac-related mortality decreased 0.75 times (95% CI: 0.60 to 0.95, p = 0.015).

Cox proportional hazards analysis of the development of cardiac-related mortality with NTproBNP and without echocardiographic parameter model.

The NTproBNP values were only available from 72 dogs, of which 21 reached the endpoint of cardiac-related mortality. In the multivariable analysis, BUN/Cr, HR, NTproBNP, and age at the time of the first visit were independently associated with cardiac-related mortality; for a 1-unit increase in BUN/Cr, the hazard of cardiac-related mortality increased 1.08 times (95% CI: 1.00 to 1.15, p = 0.043); for a 10-beat per minute increase in HR, the hazard of cardiac-related mortality increased 1.36 times (95% CI: 1.10 to 1.68, p = 0.005); and for a 100-pmol/l increase in NTproBNP level, the hazard of cardiac-related mortality increased 1.04 times (95% CI: 1.01 to 1.06, p = 0.002). Moreover, for a 1-year increase in the age at the first visit, the hazard of cardiac-related mortality decreased 0.77 times (95% CI: 0.60 to 0.98, p = 0.033).

Kaplan–Meier survival curves for cardiac-related mortality

Kaplan–Meier survival curves with respect to cardiac-related mortality of three different groups of dogs stratified according to the disease stage are illustrated in Fig. 1(a). These curves demonstrated the survival of 118 dogs categorized as stages C and D (47 dogs) dogs, stage B (52 dogs) dogs, and normal (19 dogs) dogs with the endpoint of cardiac mortality. The median survival time in the normal and stage B dogs was not determined, as >50% of dogs in this stratum survived during the observation period. Dogs with stage B disease had a survival time of 177–985 days, whereas dogs with stages C and D disease had a median survival time of 580 days (range 41–1,134 days). The dogs with stages C and D disease at entry had a significantly greater probability of death (log-rank test, $p < 0.001$) than those with stage B and normal dogs.

The Kaplan–Meier survival curves for dogs with MMVD stage C and D and PH (stage CD-PH), MMVD stages C and D (stage CD), and nonclinical signs (NC and stage B) at entry are illustrated in Fig. 1(b). These curves demonstrated the survival of 118 dogs categorized into dogs with stage CD-PH (27 dogs), stage CD (20 dogs), and nonclinical signs (71 dogs) with the endpoint of cardiac-related mortality. The median survival time in dogs with no signs could not be determined as >50% of dogs in this stratum survived during the observation period. Dogs with stage CD-PH disease had a median survival time of 532 days (range: 41–1,134 days) and those with stage CD disease had a median survival time of 605 days (152–1,001 days). Although the dogs with nonclinical signs had mortality from 177 to 985 days, dogs with stage CD and CD-PH disease at entry had a significantly greater probability of cardiac-related deaths (log-rank test, both $p < 0.001$) than those with nonclinical signs. However, there was no statistically significant difference in the probability of cardiac mortality between the stage CD and stage CD-PH groups.

The Kaplan–Meier survival curves for dogs with and without PH at entry are illustrated in Fig. 1(c). These curves depicted the survival of 99 dogs classified into the MMVD-PH (40 dogs) and MMVD (59 dogs) groups. The dogs with MMVD had a median survival time of 1,001 days (range: 152–1,001 days), whereas the dogs with MMVD-PH had a median survival time of 777 days (range: 41–1,134 days). The dogs with MMVD-PH had a significantly greater probability of cardiac-related death (log-rank test, p = 0.045) than those with MMVD.

Discussion

The major findings of our study found the alteration of the last assessment performed prior to the dog's death showed a propensity for a decline in TP and BCS when compared to their previous visit. On the other hand, MCV and FS appeared to be increasing. Additionally, we identified key factors associated with increased cardiac mortality risk, including BUN/Cr ratio, HR, NTproBNP levels, and age at the first entry through Cox proportional hazards analysis.

Several clinical parameters may be used to monitor patients for impending cardiac mortality. In the dogs that died of cardiac disease, the median BCS decreased from that recorded at the previous visit (128.6 days earlier). This finding contradicts previous observations

Table 3. Results of cox proportional hazard univariable analysis on 118 dogs with myxomatous mitral valve disease.

	n	HR	95% confidence intervals of HR	*p*-value
Albumin (mg/dl)	116	0.29	0.07–1.77	0.070
ALP	116	1.00	1.00–1.00	0.096
ALT	116	1.00	1.00–1.00	0.337
BUN	116	1.04	1.02–1.07	**<0.001**
BUN/Cr	114	1.06	1.02–1.11	**0.010**
Creatinine (×10)	114	1.20	1.05–1.35	**0.008**
Total protein	116	0.61	0.31–1.20	0.154
NTproANP (/100)	46	1.00	1–1	0.113
Cardiac troponin I	72	1.00	0.96–1.04	0.837
NTproBNP (/100)	72	1.04	1.02–1.05	**<0.001**
Basophil (cells/µl)	114	1.04	0.97–1.12	0.243
Eosinophil (cells/µl)	115	1.00	1.00–1.00	0.792
Hb	115	0.73	0.59–0.90	**0.003**
Hct (%)	115	0.90	0.84–0.98	**0.010**
Lymphocyte (cells/µl)	115	1.00	1–1	0.411
MCH	115	1.16	0.81–1.66	0.429
MCHC	115	0.79	0.53–1.20	0.278
MCV	115	1.10	0.98–1.24	0.111
Monocyte (cells/µl)	115	1.00	1–1	0.091
Neutrophil (cells/µl)	115	1.00	1–1	0.910
Neutrophil/lymphocyte	115	1.06	0.98–1.15	0.163
Platelet (×1,000)	115	0.99	1–1	0.999
RBC (cells/µl)	116	0.53	0.34–0.82	**0.004**
WBC (cells/µl)	118	1.00	0.99–1	0.356
Amplitude	106	6.49	0.50–84.06	0.153
Amplitude Q	106	4.36	1.67–11.4	**0.003**
P wave duration (sec)	106	0.00	7.04×10^{-58}–3.00×10^{28}	0.512
PR interval (sec)	106	6.26	0.67–58.21	0.107
QRS duration (sec)	106	258.03	3.21×10^{5}–2.07×10^{11}	0.398
R (mv)	106	1.74	0.85–3.56	0.127
S (mv)	105	1.42	0.45–4.48	0.550
Ao_{vel} (m/s)	108	0.56	0.15–2.14	0.396
E value (m/s)	113	17.81	6.16–51.53	**<0.001**
EA ratio	100	2.67	1.21–5.86	**0.015**
EPSS	112	1.03	0.73–1.47	0.836
FS (%)	118	1.01	0.97–1.05	0.624
LA:Ao (×10)	118	1.22	1.14–1.31	**<0.001**
MR pressure gradient	85	1.00	0.98–1.02	0.923
MR velocity	85	0.88	0.34–2.25	0.790
NLVIDs	117	1.46	1.19–1.79	**<0.001**
PA/Ao	118	1.47	0.37–5.84	0.584
PV pressure gradient	98	0.81	0.56–1.19	0.289

(*Continued*)

Table 3. *Continued*

	n	HR	95% confidence intervals of HR	*p*-value
TR pressure gradient	57	1.00	1.00–1.03	0.080
VVTI	105	0.85	0.66–1.10	0.211
NLVIDd	117	1.45	1.25–1.69	**<0.001**
Pulse rate	108	1.03	1.01–1.05	**0.001**
VHS	47	2.68	1.42–5.071	**0.002**
Age (year)	118	1.18	1.02–1.354	**0.026**
BCS	118	0.64	0.29–1.38	0.252
Body weight	118	1.11	0.97–1.27	0.135
Clinical group (non-clinical baseline)	118	9.43	3.58–24.84	**<0.001**
HR (/10)	118	1.33	1.13–1.58	**0.001**
Sex (female baseline)	118	0.46	0.19–1.11	0.084

HR and 95% confidence interval (CI) of the variables entered into univariable analysis using Cox proportional hazard. ALP: alkaline phosphatase; ALT: alanine aminotransferase; $Ao_{vel:}$ aortic velocity; BCS: body condition score; BUN: blood urea nitrogen; BUN/Cr: blood urea nitrogen-to-creatinine ratio; $E_{vel:}$ early diastolic transmitral flow velocity; E/A: early to late diastolic transmitral flow velocity; EPSS: E-point septal separation; FS: fractional shortening; Hb: hemoglobin; Hct: hematocrit; HR: heart rate; LA:Ao: ratio of left atrial diameter to aortic diameter from 2D image; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; MCV: mean corpuscular volume; MMVD: myxomatous mitral valve degeneration; MR: mitral regurgitation; NLVIDd: left ventricular internal dimension at end diastole normalized by body weight; NLVIDs: left ventricular internal dimension at end systole normalized by body weight; NTproANP: N-terminal pro-atrial natriuretic peptide; NTproBNP: N-terminal pro-brain natriuretic peptide; PA/Ao: pulmonary artery-to-aorta ratio; PV: pulmonary vein; RBC: red blood cell; TR: tricuspid regurgitation; VHS: vertebral heart score; VVTI: vasovagal tonus index; WBC: white blood cell.

Bold values are statistically significant with a p value < 0.05.

that indicated no association between BCS and cardiac mortality in dogs (Slupe *et al*., 2008; Kim *et al*., 2017). In the present study, the median body weight also decreased, although this change was not statistically significant (p = 0.088). A previous study has linked a low BCS to cardiac cachexia, which is associated with a significantly shorter survival time (Ineson *et al*., 2019). The present study was limited owing to the small number of dogs with cardiac mortality included and the nonassessment of muscle loss or cachexia from each subject.

A decline in TP levels before death can be observed in both human mortalities related to sepsis and chronic heart failure (Hu *et al*., 2021; Tan *et al*., 2024). This is likely because protein levels are influenced by nutritional status and chronic inflammation. However, the results of this study were not comparable to those obtained in previous human studies that report an alteration in albumin levels and their association with cardiac mortality rather than with the TP levels. Hypoalbuminemia is a strong predictive factor for an increase in all-cause and cardiovascular mortality in humans (Padkins *et al*., 2021; Manolis *et al*., 2022). Nonetheless, in the present study, the median albumin concentration was also decreased, but it was not statistically significant (p = 0.79). Although the increase in the MCV levels in our study was consistent with that in previous human studies reporting that macrocytosis is an independent predictor of all-cause and cardiovascular deaths in patients with acute decompensated heart failure, increasing MCV levels also increased the HRs when the increase is accompanied by an increase in RDW in both anemic and nonanemic patients since the varying risk for anisocytosis and its negative consequences (Lam *et al*., 2013; Ueda *et al*., 2013). A possible explanation for MCV is that it is merely a biomarker of malnutrition in humans (Ueda *et al*., 2013). Therefore, BCS, TP, and MCV may be associated with cachexia in dogs. More than 50% of dogs with heart failure exhibited cachexia (Freeman *et al*., 1998), with a median survival time of 233 days (Ineson *et al*., 2019). The pathophysiology of cachexia is not fully understood; however, the mechanism that could explain this could be the imbalance of energy intake and energy requirement, poor nutrient absorption, metabolic alterations, and the presence of inflammatory cytokines. Dogs with MMVD can have their clinical progression monitored using the combination of BW, BCS, and MCV which can help ensure that dogs receive early treatment, ultimately improving their quality of life and survival time.

The increase in FS appears as a hyperdynamic chamber, especially in dogs with MR and chronic heart failure

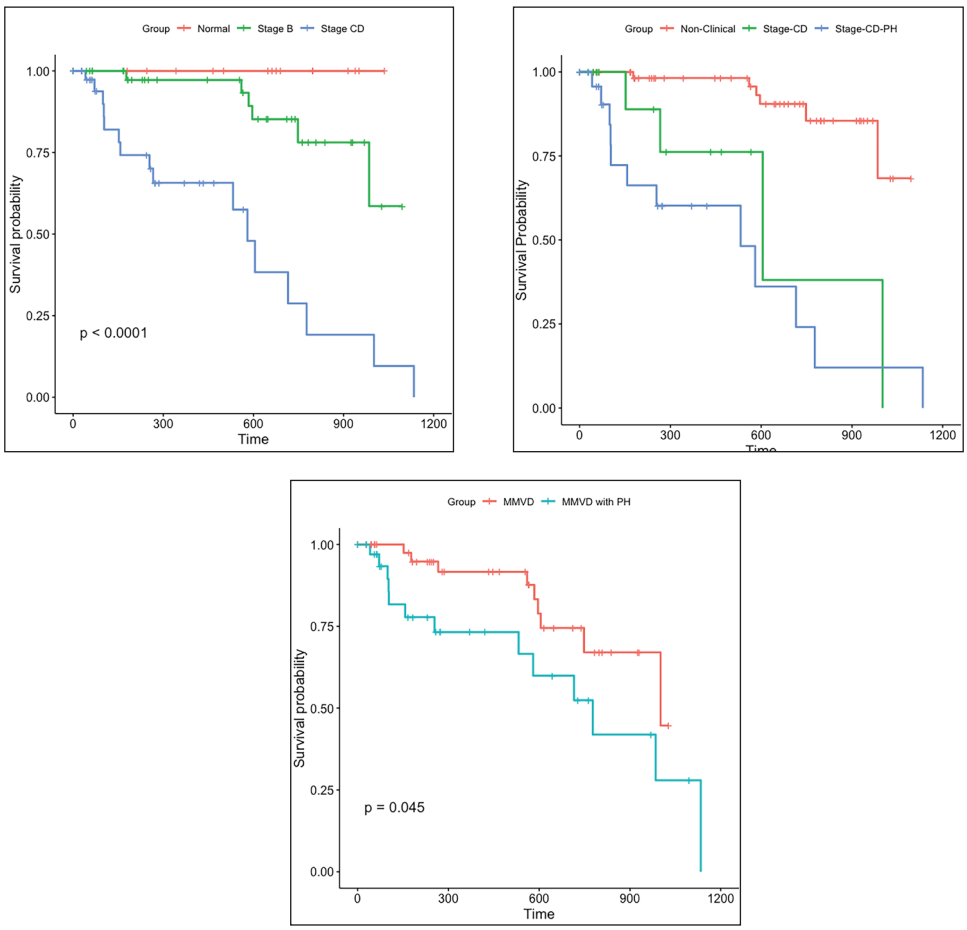

Fig. 1. (a) Kaplan–Meier survival curves with respect to cardiac-related mortality of three different groups of dogs stratified according to the disease stage. (b) Kaplan–Meier survival curves of dogs with MMVD stage CD and PH (stage-CD-PH), MMVD stage CD (stage-CD), and nonclinical signs (Non-Clinical). (c) Kaplan–Meier survival curves of dogs with MMVD and MMVD-PH.

due to the development of eccentric hypertrophy and volume overload (Borgarelli *et al*., 2007; Bonagura and Schober, 2009). The higher FS and MCV in the last visit before death may be associated with disease progression.

In the present study, a multivariable analysis of clinical parameters without echocardiographic parameters revealed that BUN/Cr, HR, and Hb were independently associated with an increased risk of cardiac mortality. We created a simplified model of this analysis for use in primary care settings, where echocardiography is not routinely available. Numerous human studies have revealed an elevation of BUN/Cr as a well-characterized poor prognostic indicator among patients with heart failure (Sood *et al*., 2015; Matsue *et al*., 2017; Sujino *et al*., 2019; Zhu *et al*., 2020). The patients with cardiovascular comorbidities had higher median BUN/Cr than the general population without cardiovascular risk. Moreover, a higher BUN/Cr was an independent predictor for cardiovascular or renal hospitalization or death (Matsue *et al*., 2017). Contrarily, a group with lower BUN/Cr was found to have a lower cumulative survival rate than a group with higher BUN/Cr (Zhu *et al*., 2020). A decline in the estimated glomerular filtration rate (eGFR) of >25% in humans with a higher mean BUN/Cr is indicative of worsening of kidney function. Abnormalities in hemodynamics, inflammatory response, neurohormonal mechanism, intrinsic tubular damages, and therapeutic management are possible factors affecting BUN/Cr alterations in patients with cardiovascular diseases (Núñez *et al*., 2015). In the field of small animal medicine, BUN/Cr in dogs is used as a diagnostic tool for gastrointestinal bleeding (Stiller *et al*., 2021). Moreover, our results have shown that BUN/Cr can serve as a predictive indicator in dogs with MMVD and a marker of high risk for heart failure.

NTproBNP, BUN/Cr, HR, and age at the first entry were significantly associated with shorter survival in our study. Our study data demonstrated that a 100-pmol/l increase in NTproBNP increased the HR by 4%, whereas a 10-beat-per-minute increase in

HR increased the HR by 36%. NTproBNP is a rapid diagnostic tool for the early detection of myocardial cell abnormalities in dogs and cats (de Lima and Ferreira, 2017). Meanwhile, an elevation in HR is associated with disease and adverse events in humans due to worsening myocardial ischemia and reduced diastolic perfusion time (Verrier and Tan, 2009). Despite the result of a previous study, a 1-year increase in age at the first entry decreased the HRs by 23% in this study. A previous study identified that early diagnosis of MMVD tends to manifest at an earlier age in Cavalier King Charles Spaniels (CKCSs) than in other canine breeds in relation to clinical progression (Haggstrom *et al*., 2004), leading to an increased risk of cardiac death at a younger age. However, our study population did not include CKCS. We hypothesized that the younger median age of clinical dogs (11 years) compared with that of nonclinical dogs (11.5 years) in the survival analysis population may be a hazard. Nevertheless, the small difference in median age between the two groups should be further investigated in future research with a larger sample size to confirm its significance. Finally, although these small percentage changes in HRs for many factors are statistically significant in this study, they may not be substantial enough to be noticeable in clinical practice.

The median survival time of dogs in the symptomatic stage (stages C and D) with cardiac mortality in the present study was longer than that reported in previous studies (Häggström *et al*., 2008; Mizuno *et al*., 2017; Brložnik *et al*., 2023). In previous studies, the median survival time was 267 (Häggström *et al*., 2008), 334 (Mizuno *et al*., 2017), and 345 days (Brložnik *et al*., 2023), whereas in our study, it was 580 days. The variations in the study's time points, the nature of the owners, and the facilities of veterinary medicine may affect the survival time and make it incomparable for each study. All dogs with stages B2, C, and D diseases were receiving pimobendan as part of their treatment. The first report on the use of pimobendan in dogs with mild-to-moderate heart failure from MMVD was published in 2005 (Smith *et al*., 2005). Pimobendan has been shown to prolong the preclinical period and decrease the heart size of dogs with MMVD (Boswood *et al*., 2016; Boswood *et al*., 2018). The impact of triple therapy on the median survival time in stages C-D dogs remains uncertain. The combined use of pimobendan, furosemide, and enalapril holds the potential for restoring typical autonomic nervous system function in canines diagnosed with stage C MMVD (Pirintr *et al*., 2023). However, the efficacy of triple therapy remains controversial, as the VALVE study reported no discernible difference in survival time among dogs receiving furosemide and pimobendan and those receiving triple therapy (Wess *et al*., 2020). The other potential factors that may contribute to the longevity of dogs are the differences in study populations, criteria for defining cardiac stages, follow-up protocols, and the fact that the study samples consisted solely of dogs with cardiac morbidities, which could also play a role. This approach ensures that the present study was not confounded by other systemic diseases or health problems. In addition, all dogs with cardiac mortality in this study died naturally without euthanasia, as all of their owners refused euthanasia due to cultural reasons and instead were committed to ensuring the best possible quality of life for their dogs.

In the present study, no statistically significant difference in the median survival time was observed between dogs with MMVD stage C disease and those with MMVD stage C-PH disease, possibly due to the disparity in the number of cardiac mortalities in each group. In particular, only 4 out of the 20 dogs with stage C disease died due to a cardiac cause, whereas 12 out of the 27 dogs with stage C-PH had cardiac mortality. If the sample size was larger, potential differences between the two groups might become more apparent. However, in our study, the median survival time of dogs with MMVD stage B2 and C disease with PH was longer (715 days) than that previously reported (456 days) (Borgarelli *et al*., 2015). The longer survival could be attributed to cardiac deaths due to natural causes and without euthanasia as an endpoint in this study, which was also mentioned in the previous paragraph. Another possible reason for the difference in the survival time between studies was the difference in the study design used. The previous study had a retrospective design, whereas the present study had a prospective design, which could reduce the risk of uncontrolled systematic errors compared with studies with a retrospective design.

The present study had several limitations. First, there was a potential for bias in the identification of cardiac mortality because some of the deaths did not occur in the hospital, and the cause of death was determined via making a phone call to the owners and asking them about the clinical problems that occurred before death. The possibility of bias could arise from the owners' clinical explanations. Second, the inclusion criteria for dogs with PH in this study did not align with the current guideline (Reinero *et al*., 2020) because the study was designed before the guideline was established. Third, since this study focused solely on dogs with MMVD, it is possible that the observed parameter alterations might also occur in dogs with other diseases, such as kidney disease. Consequently, incorporating urinalysis, abdominal imaging, and monitoring of urine production could be beneficial for confirming the diagnosis. Therefore, further studies are warranted to investigate this in different disease groups and compare the findings to this current project. Finally, the small number of mortality cases (22 out of 118) could limit the generalizability of the findings. Hence, further investigation with a larger number of dogs with cardiac mortality is warranted in the future.

Conclusion

Alterations in clinical parameters, including BCS, TP, FS, and MCV, can be used as indicators for detecting the end-of-life period in MMVD dogs. BUN/Cr, in conjunction with significantly high NTproBNP level and HR in dogs with MMVD, is considered a novel parameter for predicting cardiac mortality. In addition, this study demonstrated that the presence of PH in dogs with MMVD is associated with a shorter survival time.

Acknowledgments

The authors would like to thank the following for their support and contributions to this study: Scarecrow Inc (Japan), T.J. Animal Health Company Ltd. (Thailand), and BestAgro Companion Company Ltd. (Thailand) for supporting the canine NT-proBNP immunoassay test (Bionote®), Vcheck cTnI immunoassay test kit (Bionote®), and ANP enzyme immunoassay kit (Detect X®); staff at Prasu Arthorn Veterinary Teaching Hospital, Faculty of Veterinary Science, Mahidol University, Nakhon Pathom, Thailand; and all dog owners who participated in this study.

Authors' contributions

Conceptualization, research design, methodology, investigation, data curation, data analysis, interpretation and discussion the results, P.C., W.S., K.C., P.K., W.B., Y.J., R.P., M.T, and N.R.; Validation and resources, P.C., W.S., K.C., R.P., Y.J., M.T., and N.R.; Writing—original draft preparation, P.C., W.S., and N.R.; Writing—review and editing, P.C., W.S., K.C., P.K., W.B., Y.J., R.P., M.T, and N.R.; Supervision, W.S and N.R.; Project administration, W.S.; Funding acquisition, W.S. All authors have read and agreed to the published version of the manuscript.

Funding

This study was supported by the Faculty of Veterinary Science, Mahidol University (Thailand), the Scarecrow Inc. (Japan), the T.J. Animal Health Company Ltd. (Thailand), and Pharmalink International Ltd. (Hong Kong).

Conflict of interest

The authors declare no conflicts of interest.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

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